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Standardization proposal to quality control of propolis extracts commercialized in Brazil : A fingerprinting methodology using a UHPLC-PDA-MS/MS approach

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ABSTRACT

Propolis is a rich source of known and largely explored bioactive compounds with many pharmacological properties. It is used in several commercialized products, such as propolis-enriched honey, candies, mouth and throat sprays, soaps, toothpaste, and skin creams. However, the great diversity of propolis products and different types make the standardization of realistic quality control procedures challenging. Moreover, the extraction of propolis bioactive compounds depends on the technique and the solvent used. In Brazil, the Ministry of Agriculture, Livestock, and Supply (MAPA) set standards to establish commercialized propolis extracts' identity and quality. In addition, according to legislation, propolis extracts must present the main classes of phenols at 200 and 400 nm on the UV spectrum. Still, it is not specified which analysis method should be used to guarantee feasible quality control of the commercialized samples. For this, we proposed a new fast UHPLC-PDA-MS/MS method for analysis and quantification of propolis phenolic compounds. Moreover, we hypothesize that there is no efficient monitoring regarding the quality of the propolis extracts sold in Brazilian stores. Therefore, the present study aimed to perform quality control of 17 Brazilian propolis extracts produced in the Southeast region (green or brown – the most representative samples). The dry extract content (% g/mL), oxidation index (seconds), total flavonoids, and phenolics (% m/m) of each sample were compared with legislation. We conclude that using the UHPLC-PDA method and the investigation that allowed the comparison with the current legislation efficiently practical problems in the commercialization of propolis extracts. However, of the 17 analyzed samples, 6 did not meet the desired the recognized standards, denoting a lack of supervision and efficient quality control, which highlights a dangerous situation regarding the commercialization of this critical product used in several industrial fields, mainly in the food and pharmaceutical sector.

1. Introduction

Propolis, or bee glue, belongs to natural substances widely used in traditional and alternative medicine. Propolis is produced by honeybees mixing plant resins with wax. Beeswax was originally the only natural wax in commercial use and has been a valuable substance ever since XIV century (Saralaya, 2021). Propolis extracts are essential in the food and pharmaceutical sector since they are frequently used with other bee products, namely, honey, pollen, royal jelly (Rojczyk et al., 2020a). In alternative medicine, propolis is used due to its pharmacological properties, such as antioxidant, antimicrobial, antiviral, antitumor, antiparasitic, hepatoprotective, and immunomodulatory activities (Bhargava et al., 2018; Daikh et al., 2020; Kwon et al., 2020; Santos et al., 2019; Silva et al., 2017). These properties are related to the complex composition, being>800 organic compounds already identified in different propolis samples (Kasote et al., 2022). Specifically, propolis's most common chemical classes are fatty and phenolic acids, esters, phenolic esters, flavonoids, terpenes, β-steroids, aldehydes, aromatic alcohols, stilbene derivatives, and amino acids (Anjum et al., 2019; Gardini et al., 2018). However, the phenolic compounds are the

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protagonists in high chemical diversity and significant anti-inflammatory and antioxidant potential (Bhargava et al., 2018). Additionally, in the past couple of years, the pandemic of COVID-19 highlighted the role of propolis extracts as a possible adjuvant as conventional treatment for infected patients. This reinforces the need to guarantee that the propolis extract used for this application is rich in those compounds that display the desired biological effect (Berretta et al., 2020; Refaat et al., 2021; Silveira et al., 2021).

Artepillin C and *p*-coumaric acid are regarded as the main biomarkers from propolis samples in Brazil. Besides, these compounds have notorious biological effects (mainly antioxidant and anti-inflammatory), which justifies the high added value of the market attributed to propolis-based products (Angelo & Jorge, 2007; Anjum et al., 2019; Beserra et al., 2021; Paulino et al., 2008; Shi et al., 2012; Zhang et al., 2017). Moreover, due to the diversity of its botanical origin, propolis's physical and chemical composition variations are common, producing many types of propolis samples, mainly green and brown varieties (Lavinas et al., 2019), the most known types commercialized worldwide.

Propolis is used or consumed in several ways, such as propolis-enriched honey, candies, extracts, mouth and throat sprays, soaps, toothpaste, and skin creams. Industrially, ethanol is the primary solvent used to produce propolis extracts in large-scale, although it is common to find aqueous extracts (Abdelrazeg et al., 2020; Bankova et al., 2021; Contieri et al., 2022; Prospecting et al., 2021; Santos et al., 2020). Indeed, water is an alternative to replace ethanol-based extracts. Still, it does not display the same extraction performance as ethanol-based extracts. In addition, some populations like Muslims, pregnant women, children, and alcohol dependents are not recommended to consume ethanolic extracts. However, since the aqueous propolis extracts do not have the same chemical composition as the ethanolic extracts, they may not have the same biological effect. Furthermore, efficient quality control ensures that propolis's natural properties are preserved after industrial produced (Barreto, 2020). Also, if the propolis extract used to prepare the commercialized product is under appropriate physical–chemical conditions.

Despite this need, the great diversity of propolis products and the existence of different propolis types make the standardization of quality control procedures of the material sold to consumers challenging (Abdelrazeg et al., 2020). This happens because propolis is a complex matrix whose characteristics can be changed during collection and processing, which can compromise the quality of the final product (Barreto, 2020). Moreover, the propolis bioactive compounds extraction depends on the method used and the operational conditions performed (temperature, solid–liquid ratio, time of extraction, light absence, and so on), which can modify or at least change the yield of extraction, concentration, and the composition of the final product (Escriche & Juan-Borrás, 2018a). So, the lack of standardization extraction methods from industrial manufacturers and insufficient quality control procedures can be an obstacle to the controlled applications of propolis as a food supplement in the medicinal and nutraceutical field.

Thus, it is essential to establish standardized procedures to analyze propolis products, where the presence and quantity of the crucial constituents and biological activities can be determined (Barreto, 2020). In Brazil, the Ministry of Agriculture, Livestock, and Supply (MAPA) with the "Technical Regulation on the Identity and Quality of Propolis Extract", established in Decree n°. 03, of January 19, 2001 (https://www.gov.br/agricultura/pt-br, accessed on April 12, 2022), sets up standards to develop the identity and quality of commercialized propolis extracts (Table 1 shows some of these specifications). In addition, according to legislation, propolis extracts must present the main classes of phenols at 200 and 400 nm in the UV spectrum (Normative Instruction N. ° 3, January 19, 2001). Several methods have been developed to analyze the phenolic compounds from propolis; especially by high-performance liquid chromatography (HPLC) coupled with mass spectrometry (MS) (Gardana et al., 2007; Saftić et al., 2019; Volpi & Bergonzini, 2006); and HPLC associated with photodiode array detection (PDA) (Escriche & Juan-Borrás, 2018b; Volpi & Bergonzini, 2006). More recently, ultra-performance liquid chromatography (UPLC) has emerged as an alternative to HPLC (Caputo, 2005; Nov et al., 2006). Despite this, the legislation does not specify which analysis method should be used to evaluate the propolis extracts composition. Thus, the standardization of a single analysis method is challenging to overcome for efficient quality control of commercialized propolis extracts.

Table 1
"Technical Regulation of Identity and Quality of the Propolis Extract", present in regulation n°. 03, of January 19, 2001 of Ministry of Agriculture, Livestock and Supply (MAPA).

Physical-chemical requirements	Limits established by Brazilian legislation
Wax dry extract	Maximmum (15 % m/m)
Dry extract content	Minimum 11 % (m/v),
Phenolics compounds	Minimum 0.50 % (m/m)
Flavonoids	Minimum of 0.25 % (m/m)
Antioxidant activity	22 s maximum
Ethanol content	Maximmum 70^0 GL (v/v)
Color	Amber tones, reddish and greenish

* Adapted (https://www.gov.br/agricultura/pt-br accessed on April 12, 2022).

Given the challenges presented, it is possible to speculate that there is no efficient quality control of propolis extracts sold in Brazil. This scenario could also be the reality of other countries that usually produce and consume propolis extracts. This aspect is mainly due to the lack of standardized processes for extracting bioactive compounds from the biomass and the divergences of the original matrix. Thus, the product quality control is aggravated by the absence of a single procedure for analyzing the phenolic composition of commercial propolis samples. So, given the importance of propolis and the search for the quality of products sold, the present study aimed to perform quality control of 17 Brazilian propolis samples (green or brown) produced in Southeast Brazil. For this we proposed the use of a new ultra-fast UHPLC-PDA-MS/MS method for analysis and quantification propolis bioactive compounds. Furthermore, the results were compared with the legislation between commercial and control groups composed of extracts using raw samples of green and brown propolis extracted with different solvents (ethanol 70 % (v/v) and water).

2. Materials and methods

2.1. Chemicals and reagents

Acetic acid (Dinâmica, Campinas, Brazil), acetonitrile (JT Baker), and methanol (Sigma Aldrich, São Paulo, Brazil), were UHPLC grades. Ultrapure water was supplied by a Milli-Q Advantage 8 water purifier system (Purelab Elga, São Paulo, Brazil). The reference standard of *p*-coumaric crystalline (*trans*-4- hydroxycinnamic acid, ≥98.0 %) and Artepillin-C ((2E)-3-[4-Hydroxy-3,5-bis(3-methyl-2-buten-1-yl) phenyl]-2-propenoic acid, ≥90.0 %), was purchased from Sigma-Aldrich Brazil Ltda (São Paulo, Brazil). For the evaluation of the antioxidant capacity, 6-hydroxy-2,5,7,8-tetramethylchromane-2-carboxylic acid (Trolox), 2,2′-azobis(2-methylpropionamidine) dihydrochloride (AAPH), and fluorescein were purchased from Sigma-Aldrich (SP, Brazil).

2.2. Commercial samples

17 commercials propolis samples were assured from a natural products company (*Vale Verde*, Santos-SP, Brazil), being 8 green propolis ethanolic extracts (GP_{EtOH}); 4 brown propolis ethanolic extract (BP_{EtOH}); 3 green propolis aqueous extract ($GP_{aqueous}$), 1 brown propolis aqueous extract ($BP_{aqueous}$) and 2 extracts in capsule; green ($GP_{capsule}$) and brown ($BP_{capsule}$). All the commercial samples were obtained from Brazil brands located in the Southeast region. The extracts were centrifuged (Cheeselab, model Centurion, Brazil) in 15 mL conic tubes at 75 g, 20 °C for 15 min, and the supernatant was collected; These extracts were

stored in a dark compartment in a freezer (Metalfrio, model Consul, Brazil) at – 20 °C until being used as the sample. In Table 2 are the specification of each commercial sample. Commercial propolis samples were acquired in different concentration percentages according to product manufacture (0.5 %, 11 %, 12 %, 16 %, 20 %). Still, all the samples were diluted in the same final concentration for analysis, which allowed trustworthy comparisons regarding their concentration of phenolic compounds and antioxidant activity.

2.3. Control groups

As a control sample, propolis extracts obtained experimentally from raw green and brown propolis were produced. The green raw propolis was donated by Mn Propolis (Mogi das Cruzes- SP, Brazil), and the brown raw propolis was donated by Campmel (Valinhos – SP, Brazil). The samples were milled in a domestic blender (Model OSTER, 450 W 220 V) for a few seconds. The milled propolis was sifted in a steel sieve (Model Bestifer), standardizing a size sample between 0.5 mm — and 1.0 mm. The samples were stored in a dark compartment in a freezer (Metalfrio, model Consul) at – 20 °C until used.

The extraction was carried out on an ultrasonic bath (Elmasonic P60H, Elma Schmidbauer GmbH) with the following conditions: frequency 37 kHz, power 135 W, 45 °C, extraction time of 2 h, Solid Liquid Ratio (SLR) of 0.05. The extraction solvents were pure water and ethanol 70 % (v/v). After the extraction, an aliquot was collected and centrifuged (75 g, 20 °C for 15 min), and the supernatant was collected for analysis.

2.4. Dry extract content

The dry extract content was determined according to the Official Methods for Food Analysis (https://www.gov.br/agricultura/pt-br, accessed on 20/04/2020). Briefly, 1 g of the commercial propolis extracts was subjected to heat in a porcelain crucible and previous desiccation in an oven at 105 °C for 2 h. Then, it was submitted to the incineration process in a muffle at 550 °C until constant weight. The analyzes were carried out in triplicate, and the results were expressed in % (g/mL).

Table 2
Specifications of the 17 commercial samples obtained from the Brazilian Southeast.

Identification	Solvent/vehicule	Extract percentage (%)	US Dolar value (30 mL)
Green propolis			
GP_{EtOH} 1	Neutral grain alcohol	11	3.40
GP_{EtOH} 2	Neutral alcohol	11	4.26
GP_{EtOH} 3	Neutral alcohol	11	4.26
GP_{EtOH} 4	Neutral alcohol and purified water	16	5.57
GP_{EtOH} 5	Cereal alcohol,	20	5.82
GP_{EtOH} 6	Neutral grain alcohol	11	5.64
GP_{EtOH} 7	Neutral alcohol	11	3.79
GP_{EtOH} 8	Neutral alcohol	11	3.84
$GP_{aqueous}$ 1	Deionized water	11	6.15
$GP_{aqueous}$ 2	Deionized water	11	3.61
Brown propolis			
BP_{EtOH} 1	Neutral grain alcohol	11	3.87
BP_{EtOH} 2	Neutral grain alcohol	11	
BP_{EtOH} 3	Neutral grain alcohol	11	4.25
BP_{EtOH} 4	Deionized alcohol	11	5.90
$BP_{aqueous}$ 1	Deionized water	11	5.70
Capsules			
$GP_{capsule}$ 1	n.a	n.i	5.76
$GP_{capsule}$ 2	n.a	0.5	7.51

n.a.: not applicable; **n.i.:** non information.

2.5. Oxidation index

The oxidation index was determined according to the Official Methods for Food Analysis (https://www.gov.br/agricultura/pt-br, accessed on 20/04/2020). First, 0.2 g of the commercial propolis extracts were dissolved in 5 mL of ethyl alcohol and integrated into rest for 60 min at room temperature (27 °C). After this period, 100 mL of distilled water was allocated, and the solution was filtered on filter paper n° 3. Then, 1 mL of each sample was transferred to a 250 mL flask from the filtrate. This sample was added to 40 mL of distilled water and 1.0 mL of 20 % sulfuric acid. The mixture was stirred for 1 min, and then 5 μL of 0.1 N potassium permanganate was added. The permanganate color's time to disappear was evaluated, determining the oxidation index. The results were expressed in seconds (s).

2.6. Oxygen radical absorbance capacity (ORAC)

The oxygen radical absorbance capacity (ORAC) was determined according to Ou et al. (2013). Briefly, pure potassium phosphate buffer 75 mM (pH 7.4) was blank and diluent for samples and standard (Trolox solution: 5–25 μg/mL). Diluted sample, standard or blank (25 μL), and 150 μL fluorescein working solution (0.4 μg/mL) were added to each well of a black microplate. Afterwards, the microplate was incubated in the microplate reader (FLUOstar Omega microplate reader BMG LABTECH GmbH, Ortenberg, Germany) at 37 °C for 15 min. Then, AAPH solution (41.4 mg/mL) was added to each well (25 μL). The fluorescence decrease (excitation at 485 nm; emission at 510 nm) was measured for each 1 min during 100 min at 37 °C, and data were processed by Omega Mars 3.32R5 data analysis software. The reaction was carried out in triplicate, and the results were expressed in mg of Trolox equivalent (TE) *per* mL of propolis extract (mg TE/mL).

2.7. UPLC- PDA analysis

The analysis was carried out on a UPLC system (Waters Corp, Limeira, Brazil), using the methodology proposed by Contieri et al., 2022 (*submitted article*). The separation of compounds was carried out on a fused-core type column (Kintex, 1.3 um C_{18}, LC column 2.1 × 50 mm). The UV absorbance was monitored at 260 nm, and the injection volume was 1 μL. The software for instrument control and data acquisition was Empower® 3 (Waters Corp). The optimized gradient was performed at 55 °C, with a 0.5 mL/min flow rate and 1 min until re-equilibration. The solvents were: (A) composed of water; and (B) Acetonitrile, both acidified with 0.1 % (v/v) acetic acid. The separation gradient profile was 0 min, 12 % B; 0.5 min, 15 % B; 1.0 min, 20 % B; 1.50 min, 25 % B; 2.0 min, 29 % B; 2.50 min; 32 % B; 3.00 min, 50 % B; 3.50 min, 65 % B, 4.50, 70 % B, 5.00 70 % B; 5.50 12 % B. Being a total of 6.50 min of chromatographic methodology. The method guarantees the return to the initial solvents A and B percentages.

2.8. UHPLC-PDA-MS/MS

For characterization of the peaks obtained in the UHPLC-PDA analysis, the same chromatographic method reported above was applied hyphenated with mass spectrometry detection (UHPLC-PDA-MS/MS). Nevertheless, to provide the fragmentation profile of the compounds extracted from green propolis, flow injection analysis (FIA) was performed using a Thermo Fisher Scientific ion trap mass spectrometry (San Jose, Ca, USA) equipped with an electrospray ionization source. After this, MS, and MS/MS analysis in negative ionization (100–1500 Da) under the following operational conditions: flow rate 0.5 mL/min, capillary voltage −35 V, spray voltage 5 kV, tube lens offset 75 V, capillary temperature 250–300 °C, sheath gas (N_2) flow rate 8 (arbitrary units). Data were acquired and processed using Xcalibur software (version 2.2 SPI.48).

2.9. Content of phenolics compounds

Two molecules were used as references for quantifying the total phenolic content of the extracts, namely, *p*-coumaric acid and Artepillin C, which were identified by comparing retention times, and UV spectra of separated compounds with the authentic standard and MS/MS analysis. The quantification was done by integrating the peak areas at 260 nm using an external standard calibration curve. The standard curve was prepared with *p*-coumaric by plotting the concentration (100; 50;25; 12.50; 6.25; 3.13;1.56, 0.78 ppm) against the area of the peak, which was used to quantify ferulic acid, *p*-coumaric acid, and quinic acids derivatives (first part of the chromatogram). The Artepillin-C calibration curve was achieved by plotting the concentration (1000; 500;250;125; 100;50;25;12.5;6.25, and 3.13 ppm) against the peak area to quantification cinnamic acids and flavonoids derivatives (second part of the chromatogram (Fig. S1 and Table S1 –supplementary). The results were expressed in % (m/m).

2.10. Color analysis

For determination of the standard color of each sample (17 commercial samples and controls – brown and green), aliquots of 200 μL of each extract were placed in a 96-well microplate. Then, the absorbance between 400 and 700 nm was recorded (Synergy HT microplate reader – BioTek), and the UV–vis data was converted into color values. The CIE (Commission Inter- Nationale de l'eclairage) L* a* b* according to the 1964 standard observatory was applied by applying the Illuminant D65 spectral distribution at 10° view angle (*ColorBySpectra* software). In the end, the CIELAB values were converted into RGB scale and illustrated as color stamps by applying an online converter.

2.11. Statistical analysis

First, all variables were subjected to adherence testing to verify their approximation with the theoretical curves (Shapiro-Wilk's test). Next, the dry extract content (% g/mL), oxidation index (second), flavonoids, and phenolics totals (% m/m) of each sample were compared with legislation applying the one-sample *t*-test. Additionally, to verify the internal control, each sample and control samples (brown and green propolis) were compared (n = 3) using one-way ANOVA with Dunnet posthoc test. A multiple linear regression model was performed to determine which indicators best predict the price variation. The model's independent variables presented Pearson's correlation $r > 0.20$. The variables were included using forward selection. Finally, the model goodness-of-fit was checked through the residual analysis.

For the similarity analysis, a hierarchical cluster using the squared Euclidean distance as the composition of the samples in terms of the 23 peaks found in the UHPLC-PDA-MS/MS was the criterion for distinguishing each detected molecule to create which describes how similar the labels are in terms of common chemical markers when compared with the control groups and by each other. Then, a distance dendrogram was plotted for each analysis. The analysis compared the samples with the control extracts made in our laboratory (brown and green propolis extracts with the solvents ethanol 70 % (v/v) and pure water). The Principal Component Analysis (PCA) was used to reduce the dimensionality of sample compounds. The 23 peaks found in the UHPLC-PDA-MS/MS detector were included in this analysis. Only factors with loads above 0.50 were considered. Promax rotation with Kaiser normalization was used to maximize the difference between variance captured by each component. The Kaiser-Meyer-Olkin (KMO) was used to verify the adequacy of the model. The Bartlett test was used to check sphericity. For all variables, $p < 0.05$ was considered significant. Statistical analyses were performed using Statistical Package for Social Sciences (SPSS) v.20 (IBM Corp. Armonk – US).

3. Results

3.1. Dry extract content, oxidation Index, and antioxidant activity (ORAC)

Dry Extract Content (or dry residue content) is the parameter that quantifies the concentration of soluble solids extracted from propolis by a given solvent (Barreto, 2020; de Carvalho, 2020). Table 3 shows the values of dry extract content (% g/mL). Brazilian legislation (normative instruction N. ° 3, January 19, 2001) determines a minimum of 11 % of dry extract (g/mL). Analyzing the commercial samples, it was noticeable

Table 3
Values of dry extract content (% g/ mL); oxidation index (second); total of phenolics and flavonoids componds (% m/m) and ntioxidant activity (ORAC - mg TE/mL) and the total of phenolics and flavonoids componds (% m/m).

Sample	Dry extract content % (g/ mL) ± SD *(P value)*	Oxidation index (second) ± SD *(P value)*	Flavonoids totals % (m/m) ± SD *(P value)*	Phenolics totals % (m/m) ± SD *(P value)*	ORAC (mg TE/ mL)*
$GP_{capsule}$ 1	n.a	23.43 ± 0.45 *(0.003)*	0.64 ± 0.051 *(0.006)*	1.06 ± 0.05 *(0.003)*	0.30 ± 0.011
$GP_{capsule}$ 1	n.a	>60 sec ±	2.09 ± 0.18 *(0.003)*	2.92 ± 0.12 *(0.001)*	0.06 ± 0.002
GP_{EtOH} 1	12.17 ± 0.32 *(0.24)*	4.47 ± 0.16 *(<0.001)*	0.88 ± (0.14) *(0.521)*	0.82 ± 0.71 *(0.52)*	118.06 ± 7.81
GP_{EtOH} 2	10.07 ± 0.63 *(0.124)*	11.54 ± 1.05 *(0.003)*	0.83 ± 0.36 *(0.003)*	1.25 ± 0.36 *(0.07)*	93.08 ± 6.20
GP_{EtOH} 3	13.595 ± 3.12 *(0.286)*	7.13 ± 0.47 *(<0,001)*	1.20 ± 0.66 *(<0,001)*	1.75 ± 0.69 *(0.09)*	106.70 ± 12.75
GP_{EtOH} 4	17.18 ± 0.94 *(0.008)*	6.51 ± 0.71 *(0.001)*	0.92 ± 0.58 *(0.051)*	1.89 ± 0.12 *(0.002)*	148.15 ± 12.53
GP_{EtOH} 5	18.52 ± 0.12 *(<0.001)*	20.60 ± 0.63 *(0.06)*	0.61 ± 0.013 *(0.001)*	0.81 ± 0.006 *(<0,001)*	115.46 ± 9.85
GP_{EtOH} 6	10.35 ± 1.77 *(0.592)*	6.48 ± 0.73 *(0.001)*	1.52 ± 0.36 *(0.002)*	1.23 ± 0.36 *(0.072)*	59.33 ± 7.75
GP_{EtOH} 7	24.21 ± 0.39 *(<0.001)*	5.50 ± 0.16 *(<0.001)*	0.089 ± 0.0002 *(0.006)*	0.11 ± 0.0009 *(0.001)*	50.00 ± 3.00
GP_{EtOH} 8	12.54 ± 0.28 *(0.11)*	6.69 ± 0.27 *(<0,001)*	0.85 ± 0.045 *(0.003)*	1.41 ± 0.081 *(0.003)*	218.53 ± 9.06
BP_{EtOH} 1	10.60 ± 0.96 *(0.543)*	11.20 ± 0.80 *(0.002)*	0.40 ± 0.022 *(0.036)*	0.56 ± 0.018 *(0.028)*	50.67 ± 2.70
BP_{EtOH} 2	11.85 ± 0.24 *(0.026)*	8.24 ± 0.13 *(<0,001)*	1.30 ± 0.085 *(0.091)*	2.32 ± 0.037 (0.009)	85.44 ± 2.05
BP_{EtOH} 3	12.49 ± 0.36 *(0.019)*	12.26 ± 0.16 *(<0,001)*	0.06 ± 0.071 *(0.030)*	1.01 ± 0.093 (0.08)	113.12 ± 4.35
BP_{EtOH} 4	4.79 ± 0.23 *(<0.001)*	9.25 ± 0.30 *(<0,001)*	0.39 ± 0.044 *(0.002)*	0.62 ± 0.06 (0.077)	49.35 ± 6.10
$GP_{aqueous}$ 1	13.52 ± 0.26 (0.003)	35.53 ± 1.44 *(0.004)*	0	0.06 ± 0.003 (<0,001)	18.77 ± 0.91
$GP_{aqueous}$ 2	38.46 ± 0.26 *(<0.001)*	9.86 ± 0.30 *(<0,001)*	1.08 ± 0.059 *(0.11)*	1.96 ± 0.05 (<0,001)	99.39 ± 4.70
$BP_{aqueous}$ 1	3.90 ± 0.83 *(0.005)*	48.67 ± 2.18 *(0.002)*	0	0	7.24 ± 0.70

N = 3 for all analysis; **n.a** = not aplicable; ***ORAC**: Trolox equivalent/mL.

that 2 commercialized samples were below the reference, $BP_{aqueous}$ 1, 3.90 % (±0.83) g/mL and BP_{EtOH} 4, 4.79 % (±0.23) g/mL.

The Oxidation Index is calculated by the time required for the extract to completely reduce the added potassium permanganate (de Oliveira, 2013). The Brazilian legislation (Normative Instruction N. ° 3, January 19, 2001) limits the oxidation index to 22 s. It was observed that both commercial capsules showed the worst parameters, $GP_{capsule}$ 1, 23.43 (±0.45) seconds, and $BP_{capsule}$ 2, > 60 s. In addition, other brands also presented results above those allowed by legislation, $GP_{aqueous}$ 1, 35.53 (±1.44) seconds, and $BP_{aqueous}$ 1, 48.67 (±2.18) seconds (Table 3).

We evaluated the samples' oxygen radical absorbance capacity (ORAC) to complement the previous results. We noticed that both capsules had the lower ORAC values, $GP_{CAPSULE}$ 1, 0.30 (±0.011) mg TE/mL and $BP_{CAPSULE}$ 2, 0.06 (±0.002) mg TE/mL. In addition, like other samples that showed a longer oxidation index time than that provided for by the legislation ($GP_{aqueous}$ 1 and $BP_{aqueous}$ 1), were also samples that showed lower ORAC indices $GP_{aqueous}$ 1, 18.77 (±0.91 mg TE/mL) and $BP_{aqueous}$ 3, 7.24 (±0.70) mg TE/mL (Table 3).

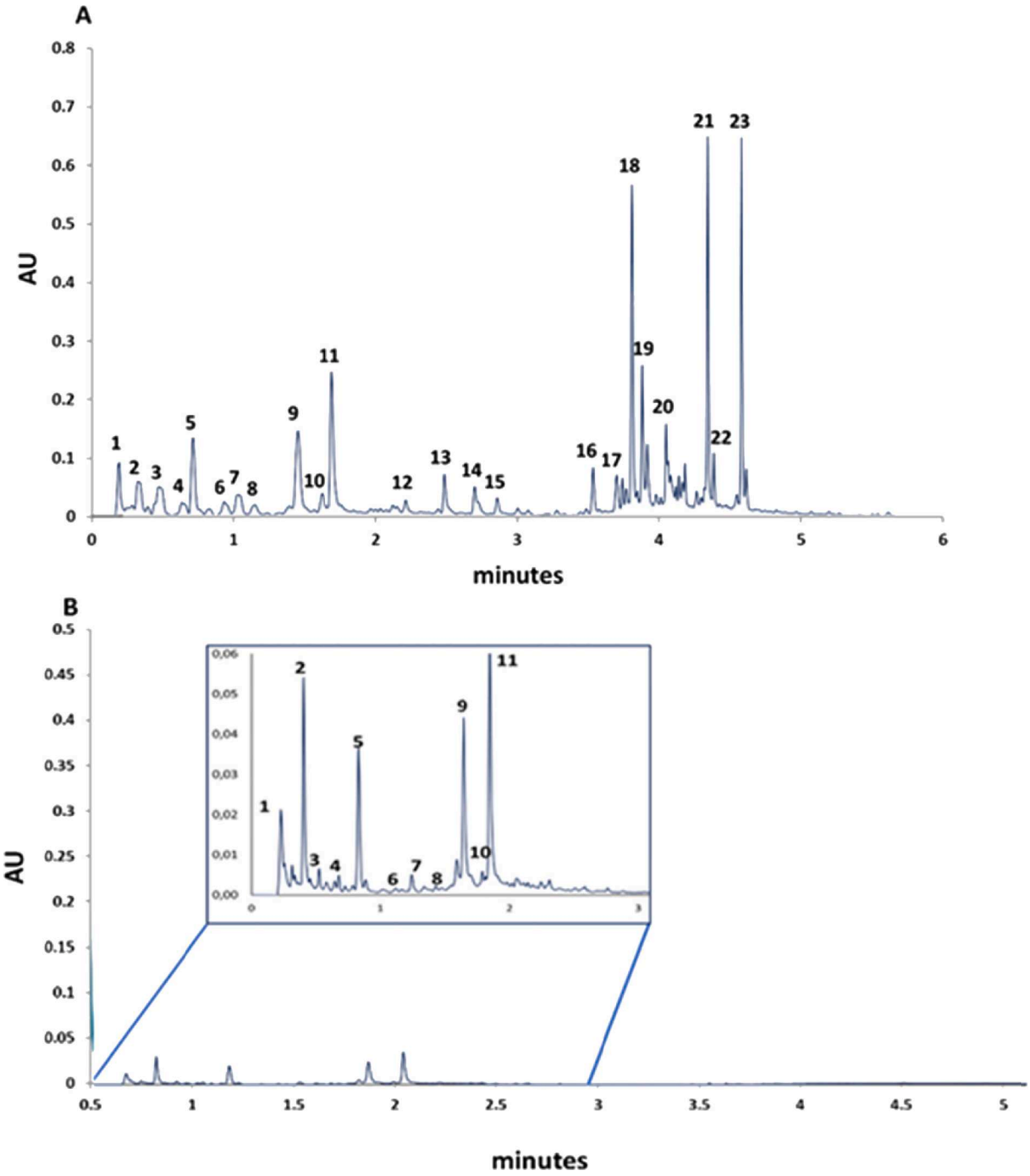

Fig. 1. Influence of the extraction solvent, performed with **(A)** Ethanol 70 % and **(B)** Water 100 %. Chromatogram recovered on 260 nm. Numbers represents the indentified peaks with UHPLC-PDA-MS/MS method: (1) Ferulic acid; (2) Feruloylquinic acid; (3) Quinic acid; (4) Caffeoylquinic acid (a); (5) p-Coumaric acid; (6) n.i; (7) Caffeoylquinic acid (b); (8) Dicaffeoylquinic acid (a); (9) Dicaffeoylquinic acid (b); (10) Dicaffeoylquinic acid (c); (11) Dicaffeoylquinic acid (d);(12) Tricaffeoylquinic acid (a); (13) Tricaffeoyquinic acid (b); (14) 3,4-dihydroxy-5-prenyl-cinnamic acid; (15) Dihydrokaempferide; (16) Quercetin; (17) Dihydrokaempferide derivative; (18) n.i.; (19) Chrysin; (20) Kaempferide; (21) Artepillin C, (22) Kaempferol-methyl-ether; (23) Baccarin.

3.2. UHPLC- PDA analysis: totals of flavonoids and phenolic compounds

Brazilian legislation (Normative Instruction N. ° 3, January 19, 2001) sets that propolis extracts contain a minimum of 0.50 % (m/m) of phenolic compounds and a minimum of 0.25 % (m/m) of flavonoids. To perform these analyses, we apply the UHPLC-PDA method described above. Initially, to understand the chromatographic profile of the aqueous and ethanolic propolis extracts, we tested the control samples (green propolis extracts made with the solvents, ethanol 70 % (v/v), and pure water). As a result, we observed that the aqueous extract had a lower concentration of phenolic compounds. It is possible to observe that cinnamic acid derivatives and flavonoids were not extracted and were absent in the chromatogram's second part (with retention times between 3.50 and 5.50 min). Otherwise, the ethanolic extract has at least five times the yield of all phenolic compounds in the biomass. Then, using the same method, we applied mass spectrometry detection (UHPLC-PDA-MS/MS) and identified 23 peaks in the ethanol extract (Fig. 1A) and only 11 in the aqueous one (Fig. 1B). Table S2, Figs. S1 and S2 – supplementary show the UHPLC-PDA-MS/MS data, the identification of the peaks, and the mass spectra of each compound (MS^n).

In general, for quantitative LC analysis with UV–vis detection, compounds should be detected at their λ^{max}. However, since propolis is a complex matrix with several minor peaks, each with different absorption spectrums, compromises must be made to allow a more straightforward, comprehensive characterization of different samples. In this study, we propose using 260 nm to retrieve peak areas for two reasons: Artepillin C and p-coumaric acid still absorb UV light at 260 nm (although less than at λ^{max}). Since they are found in high concentrations, they can be correctly quantified at this wavelength, despite the lower absorption. It is essential to highlight that the quantification of the standards was plotted using peak areas at 260 nm ($r^2 > 0.99$), indicating that quantification was performed in a linear concentration range (Fig. S3). Another reason for selecting 260 nm to integrate peaks is the lack of absorption of several peaks at higher wavelengths. As shown in Fig. S4, several peaks (indicated by arrows) can only be found in lower wavelengths, and a clean chromatogram can be obtained at 260 nm. Therefore, we believe using a single wavelength (260 nm) to retrieve peak areas is correct and produces reliable results. Alternatively, each peak can be integrated at their λ^{max} to improve detection levels, but this will complicate data processing, contrasting with the need for quality control procedures.

After knowledging this, the commercial samples were analyzed using the same UPLC-PDA method, and peak areas were obtained at 260 nm for all samples. We noticed that $GP_{aqueous}$ 1 and $BP_{aqueous}$ 1 flavonoids and cinnamic acid derivatives were not detected in the second part of the chromatogram (Fig. 2A and B). Consequently, the content of phenolic compounds was below the legislation, 0.06 (±0.003) % (mg/g) and 0 (mg/g), respectively, as well as the minimum flavonoid content. Regarding the chromatograms of these samples, it is noted that only the sample $GP_{aqueous}$ 1 presented a profile like the aqueous control extract (Fig. 2A). However, the sample $BP_{aqueous}$ 1 presented only a single peak, which was not identified in this work, contrasting with a typical propolis sample chromatographic profile (Fig. 2B). Unfortunately, the product packaging did not provide specific information about the extract's composition. On the other hand, it was noticed that despite being extracted with water, the sample $GP_{aqueous}$ 2 has the same peaks as an ethanolic extract, which should not happen since water does not extract cinnamic acids and flavonoids from propolis (Fig. 2C). The chromatogram of this sample is like the 70 % (v/v) ethanol control, extracting the compounds present in the second part of the chromatogram (between 3.50 and 5.50 min) (Fig. 2C). In this sample, the total flavonoids and phenolics indices are found within the legislation 1.08 (±0.059) % (m/m) and 1.96 (±0.05) % (m/m), respectively. Additionally, the oxidation index is inside the legislation values (9.86 ± 0.30 s) and the ORAC index (99.39 ± 4.70 mg TE/mL), like ethanolic extracts samples.

Concerning the other samples, most ethanolic commercialized samples presented the values within limits defined by the legislation, both green and brown (Figures supplementary – S4 and S5). However, the GP_{EtOH} 7, presented the total flavonoid and phenolic contents, 0.089 (±0.0002) % (mg/g) and 0.11 (±0.0009) % (mg/g), respectively below the minimum values defined by the legislation. It was possible to see low detected components in its chromatographic profile compared to the other samples (figure supplementary – S4G). But the dry extract content 24.21 (±0.39) % (g/ mL) and oxidation index 5.50 (±0.16) (seconds) was in accordance with legislation and ORAC index was similar as other ethanolic samples 50.00 (±3.00) (mg TE/mL).

3.3. Correlation and regression analyses

No statistically significant correlation was found between all the analyzed parameters (Table S3- supplementary). Additionally, linear regression was also performed to verify which indicator best represents the price variation (Table S4 - supplementary). Using the equation $Price\ R\$ = 20{,}72 - (7{,}30 \times extract) - (4{,}95 \times type\ of\ propolis) + (0{,}47 \times oxidation\ activity) - (0{,}03 \times ORAC) + (0.39 \times phenolics\ totals\ [mg])$. The explanatory power was $R^2 = 0.59$. Thus, we found 41 % of price variation that could not be explained by these variables.

3.4. Color analysis

According to the legislation, propolis commercial extracts must have amber tones, reddish or greenish (Normative Instruction N. ° 3, January 19, 2001). Considering the results depicted in Table 4 and following the RGB scale, only the brands GP_{ETOH} 5 (reddish-pink), and $BP_{aqueous}$ 1 (gray) do not match the characteristic color compared to the original samples (controls). In all the samples analyzed, the color index L* varied from 42.13 to 90.61, which means a lighter color. The negative value of the color index a* demonstrated that the samples have a green tone, which is depicted in all the control groups. It is possible to note that the highest a* value is observed in the GP_{ETOH} 5. The lower color index b* was found in $BP_{aqueous}$ 1, which reflects in a dark gray color, not representative of propolis samples.

3.5. Similarity analysis and Principal component analysis (PCA)

The hierarchical cluster analysis compared the samples and the control extracts (brown and green propolis extracts with the solvents ethanol 70 % (v/v) and pure water). We excluded both capsules and the sample $BP_{aqueous}$ 1 for these analyses because it only had a single peak. Thus, we know that only one chromatographic peak at 260 nm does not match a propolis sample. So, Fig. 3 represents the overall cluster analysis with all comparisons performed between control samples ($GP_{control\ aqueous}$, $GP_{control\ ETOH}$, $BP_{control\ aqueous}$, and $BP_{control\ ETOH}$) and commercialized ones. The clustering analysis considered the chemical composition of each extract (presence of peaks and quantification of phenolic compounds).

We used the raw green propolis extract as control, considering that it is the most complex chemical composition compared to the brown variety. Brown propolis shares several compounds with green propolis. However, the composition of the extract is a complex subject, which will depend on several factors, especially the solvent used. In this sense, samples of commercial brown propolis were also evaluated by comparing the peaks in the control groups, which was possible by the multivariate analysis (Clustering). As seen in Fig. 3, depending on the sample and solvent used, some brown samples are more closely related to other green samples. The composition of the control aqueous green propolis extract is more closely related to the control aqueous brown propolis extract than the ethanolic control extracts.

It was possible to notice that the aqueous control extracts from 2 varieties of propolis (brown and green) were grouped into a small group, together with the commercial sample $GP_{aqueous}1$, showing that this sample complies with the theoretical phenolic profiles concerning the

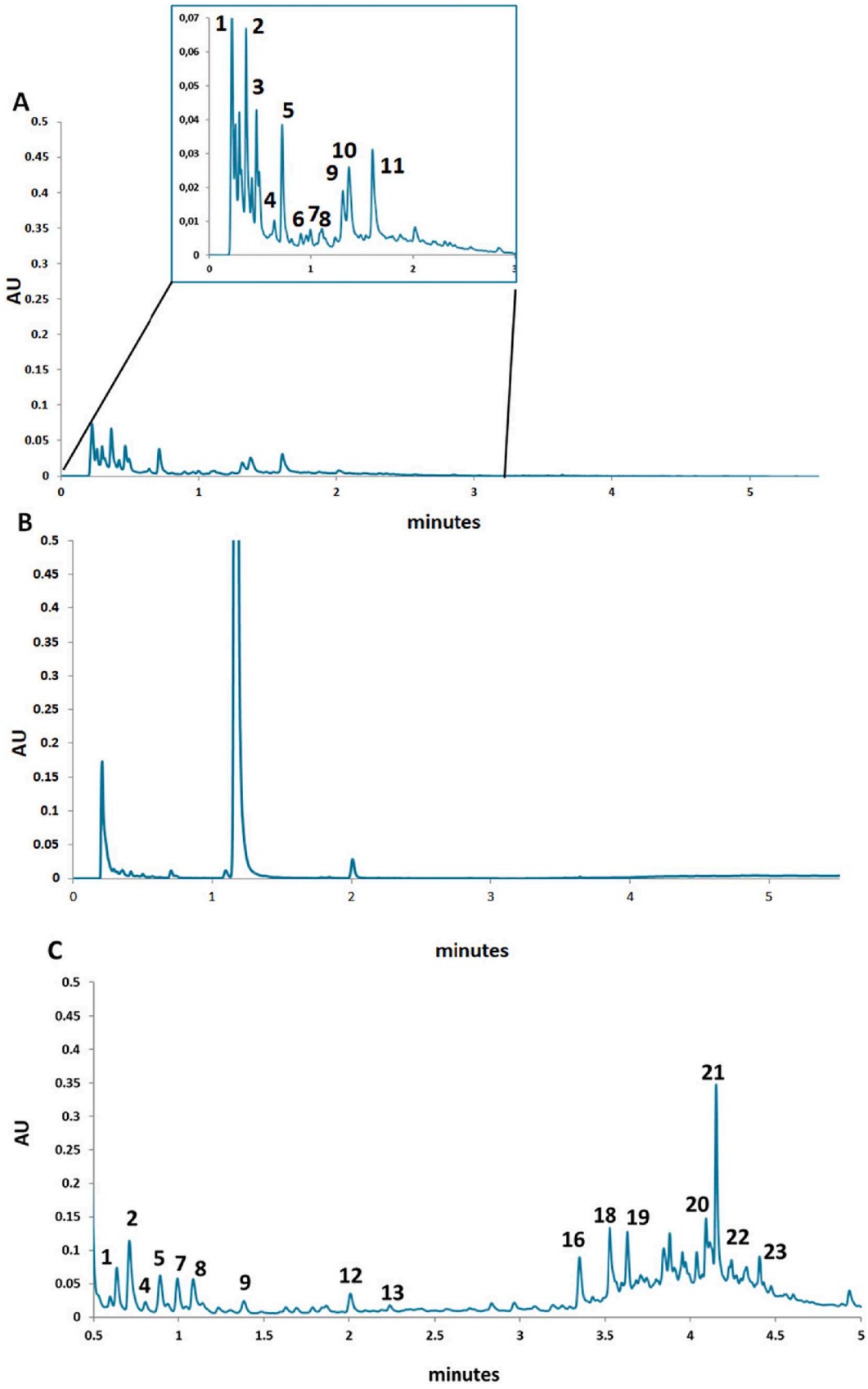

Fig. 2. Chromatographic profile of aqueous samples **(A)** $GP_{aqueous}$ 1; **(B)** $BP_{aqueous}$ 1, **(C)** $GP_{aqueous}$. Chromatogram recovered on 260 nm. Numbers represents the indentified peaks with UHPLC-PDA-MS/MS method: (1) Ferulic acid; (2) Feruloylquinic acid; (3) Quinic acid; (4) Caffeoylquinic acid (a); (5) p-Coumaric acid; (6) n.i; (7) Caffeoylquinic acid (b); (8) Dicaffeoylquinic acid (a); (9) Dicaffeoylquinic acid (b); (10) Dicaffeoylquinic acid (c); (11) Dicaffeoylquinic acid (d);(12) Tricaffeoylquinic acid (a); (13) Tricaffeoyquinic acid (b); (14) 3,4-dihydroxy-5-prenyl-cinnamic acid; (15) Dihydrokaempferide; (16) Quercetin; (17) Dihydrokaempferide derivative; (18) n.i.; (19) Chrysin; (20) Kaempferide; (21) Artepillin C, (22) Kaempferol-methyl-ether; (23) Baccarin.

Table 4
Color index of propolis extracts, CIELAB and RGB parametrers and representative color stamp.

Sample	Color cordinator			RGB scale	Representative color
	L*	a*	b*		
$GP_{control\ aqueous}$	90.61 ± 3.06	−2.37 ± 0.53	16.68 ± 0.02	#E9E5C4	
$GP_{control\ ETOH}$	87.79 ± 0.76	−8.58 ± 0.00	43.41 ± 3.68	#E2E087	
$BP_{control\ aqueous}$	88.59 ± 1.89	−0.12 ± 0.04	9.84 ± 2.50	#E4DECC	
$BP_{control\ ETOH}$	90.14 ± 0.06	−5.86 ± 034	32.34 ± 2.17	#E9E5A4	
$GP_{ETOH\ 1}$	62.55 ± 1.47	7.51 ± 0.58	38.25 ± 1.78	#B49152	
$GP_{ETOH\ 2}$	53.80 ± 0.95	9.06 ± 0.09	24.53 ± 1.10	#9A7A56	
$GP_{ETOH\ 3}$	50.21 ± 1.3	13.15 ± 0.4	14.75 ± 0.80	#936F5F	
$GP_{ETOH\ 4}$	56.43 ± 0.19	16.91 ± 0.23	25.09 ± 0.32	#AD7C5C	
$GP_{ETOH\ 5}$	54.96 ± 4.23	51.45 ± 2.27	22.58 ± 4.15	#D75860	
$GP_{ETOH\ 6}$	66.73 ± 1.29	10.36 ± 1.58	42.77 ± 3.54	#C59B54	
$GP_{ETOH\ 7}$	87.55 ± 0.45	−4.88 ± 0.72	39.12 ± 1.24	#E6DD90	
$GP_{ETOH\ 8}$	58.44 ± 0.73	5.61 ± 2.69	31.17 ± 0.73	#A38855	
$BP_{ETOH\ 1}$	88.58 ± 0.12	−5.21 ± 0.13	31.21 ± 0.78	#E5E0A2	
$BP_{ETOH\ 2}$	51.05 ± 1.07	14.93 ± 0.56	15.85 ± 1.84	#98705F	
$BP_{ETOH\ 3}$	48.98 ± 0.47	9.14 ± 0.24	12.49 ± 0.74	#896F60	
$BP_{ETOH\ 4}$	45.05 ± 0.21	8.99 ± 0.47	15.21 ± 0.78	#806552	
$GP_{aqueous\ 1}$	86.44 ± 1.01	−5.14 ± 0.18	23.01 ± 1.02	#DCDAAC	
$GP_{aqueous\ 2}$	44.78 ± 0.47	5.55 ± 0.41	37.66 ± 0.22	#806628	
$BP_{aqueous\ 1}$	42.13 ± 1.21	−0.24 ± 0.12	0.47 ± 0.05	#646363	

aqueous extracts. Also, the sample GP_{EtOH} 7 was grouped with the aqueous extract, demonstrating that it is outside the ideal parameters for ethanolic extracts (besides presenting a lower concentration of flavonoids and phenolic content). It is also possible to see that the sample $GP_{aqueous}$ 2 is not grouped with aqueous extracts, which reinforces our suspicion that it is an ethanolic extract marketed as an aqueous one. Finally, it is notorious that the commercialized GP_{ETOH} samples have a varied similarity index (dispersed in the Dendrogram). Not all the samples are grouped closely, mainly due to the diversity in the chemical composition/concentration of cinnamic acids that varied around 30 – 45 % from each other.

4. Discussion

Propolis is an effective and safe functional food supplement (Irigoiti et al., 2021), also associated with several biological activities preconized by the traditional populations (mainly for wound healing (Rojczyk et al., 2020b), improvement of oral health (da Silva Barboza et al., 2021), promoting gastroprotective effect (Costa et al., 2018). Besides, different food and nutraceutical products are based (or enriched) with commercial propolis extracts, representing the market's central niche for this raw material (Costa et al., 2018). However, the complexity and existence of many propolis types and the various production processes make it difficult to standardize the quality of products sold. Furthermore, there are mainly-four kinds of propolis in Brazil, namely green, brown, yellow, and red; being their composition associated with their botanical and geographical origin. Thus, specific chemical markers are found in each variety, like Artepillin C, usually found in the green variety which is a very pertinent chemical marker to perform reasonable quality control of the samples from Southeast Brazil (Machado et al., 2016; Zhang et al., 2017). However, in this study, the developed UPLC-PDA-MS/MS method led to a high-chromatographic resolution for green and brown varieties (the main explored commercial types), a helpful tool for quality monitoring of Brazilian brands.

Following the Brazilian legislation, some minimum requirements regarding the phenolic composition and their biological effects need to be fulfilled, such as dry extract, phenolics, flavonoid compounds, and antioxidant activity. In addition, the chromatographic profiles of

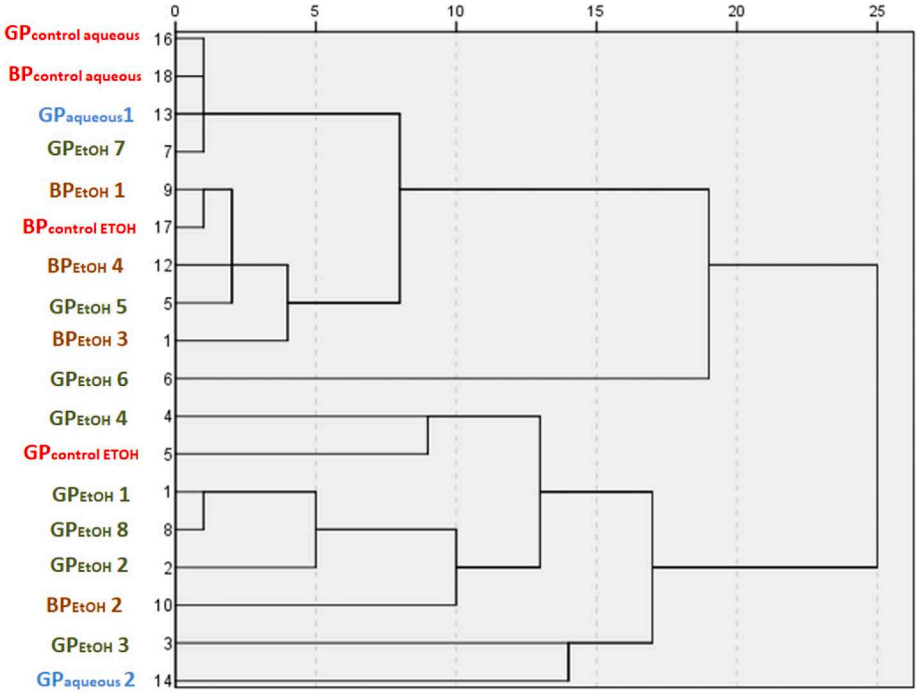

Fig. 3. Hierarchical cluster using squared euclidean distance. Compare the quantification of 23 HPLC-PDA-MS/MS identified 23 peaks (Ferulic acid; Feruloylquinic acid; Quinic acid; Caffeoylquinic acid (a); *p*-Coumaric acid; Caffeoylquinic acid (b); Dicaffeoylquinic acid (a); Dicaffeoylquinic acid (b); Dicaffeoylquinic acid (c); Dicaffeoylquinic acid (d); Tricaffeoylquinic acid (a);Tricaffeoyquinic acid (b); 3,4-dihydroxy-5-prenyl-cinnamic acid; Dihydrokaempferide;Quercetin; derivative; Chrysin; Kaempferide; Artepillin C; Kaempferol-methyl-ether and Baccarin).

commercialized extracts need to be standardized to guarantee the same (or at least similar) biological advantages (by the content of phenolic compounds) and concerning the presence of the main target compounds that display anti-inflammatory and antioxidant activities. The present study was developed from the suspicion that the commercialized Brazilian propolis extracts do not meet these minimal requirements. Our results showed that some samples are not following the recognized standards, denoting a lack of supervision and efficient quality control. Even though this work is performed using Brazilian commercialized samples, the same lack of surveillance could happen worldwide since similar problems concerning standardization are frequently reported for other traditional medicines, similarly happening with medicinal plants/herbs (Ekor, 2014).

Since legislation must be complied with, we started our analysis by comparing commercial propolis extracts with the standards recommended by Brazilian legislation (normative instruction N. ° 3, January 19, 2001). The legislation recommends at least 11 % (m/v) of dry extract to obtain the minimum number of soluble solids in the commercialized product. According to the analyses, 2 samples ($BP_{aqueous}$ 1 and BP_{EtOH} 4) did not comply with the recommended law, which could not be accepted without adequate inspection. Furthermore, although studies relate the dry extract content with a high concentration of phenolic and flavonoid and an increased oxidation index (Bastos et al., 2011), no correlation was found. Therefore, it is possible to speculate that a low concentration of phenolics and flavonoids may be related to variations in location and collection period, which may interfere with the soluble solids content (Sousa et al., 2007); or even to poor post-production storage conditions, which can lead to degradation of phenolic compounds (Ali et al., 2018).

Regarding the oxidation index, the results were even more discouraging. According to legislation, values below 22 s indicate low antioxidant capacity. Our analysis showed that both capsules ($GP_{CAPSULE}$ 1 and $BP_{CAPSULE}$ 2) and the other 2 samples ($GP_{aqueous}$ 1 and $BP_{aqueous}$ 1) showed unsatisfactory results. Although both extracts are aqueous, this does not allow affirming that water as a solvent is the main factor that influences the antioxidant activity once $GP_{aqueous}$ 2, also an aqueous extract, presented values within the legislation. These findings corroborate the ORAC index, which also showed unsatisfactory results for the same samples. Thus, supposing that the antioxidant activity is a primary indicator of positive biological potential (such as anti-inflammatory effect) and considering that the commercialized capsule samples have a higher selling price, we can affirm that the cost-benefit of consuming capsule extracts is not an advantage. However, we think that the most significant advantage of consuming capsules is the absence of the characteristic tasty, which discourages some consumers like children, such as already discussed by Bodini et al. (2013) and Yang et al. (2017).

As seen in previous articles, variations in the post-harvest period and the storage conditions of propolis extracts, specifically at higher temperature conditions, cause an increase in the oxidation index, with effects on the sample biological activity (Orientação, 2005). Thus, these explanations can be associated with the unsatisfactory values in $GP_{capsule}$, $BP_{capsule}$ 2, $GP_{aqueous}$ 1and $BP_{aqueous}$ 1. These results are significant since many industries apply propolis extracts as a natural antioxidant, for example, in food products. Therefore, that reinforces a need for greater quality control of commercialized propolis-based products to ensure that the antioxidant properties of the raw product are kept until commercialization (Cavalaro, 2020).

Still, concerning the propolis antioxidant activity, works related this property to the composition and types of phenolic compounds, mainly flavonoids, found in the samples (Cavalaro, 2020; Chon et al., 2020; de Oliveira, 2013; Šuran et al., 2021). In addition, the propolis phenolic composition is directly related to this product's properties, among which they stand out as antimicrobial and antioxidant activities. That's why the propolis phenolic composition is considered a criterion for evaluating the product quality of propolis (Anjum et al., 2019; Freires et al., 2016; Gardini et al., 2018).

Thus, we assessed the content of totals phenolics and flavonoids to understand if they relate to the above results. Our results noted that the aqueous extracts showed worse chromatographic parameters than ethanol; once water cannot extract the same compounds, like cinnamic acids and flavonoids, the main ones responsible for the therapeutic effects of hydrophilic propolis extracts. Therefore, there are no flavonoids and cinnamic acids in the aqueous extract, which impact the total phenolic content and, consequently, the biological activity of these extracts. This characteristic of aqueous propolis extracts should be repeated on commercialized samples (that is, these samples should not also present flavonoids in the UV spectrum).

However, as seen in the results, this did not occur for the sample $GP_{aqueous}$ 2, which resembled 70 % ethanolic control extract. This finding is interesting for analyzing the reliability of the solvent in the sample packaging, which suggests that in this sample, some adulteration occurred in the production process, probably using ethanol as solvent. These results are reinforced by the cluster analysis that presents this sample, $GP_{aqueous}$ 2, not grouped with the other aqueous extracts. It is worth stressing that some people do not use alcohol-based products. So, alternative methods for extracting bioactive compounds from propolis samples are urgently necessary to replace the use of ethanol. In this sense, non-volatile alternative solvents, like eutectic mixtures and ionic liquids, have been explored as safer and feasible high-performance strategies (dos Santos et al., 2021; Martins et al., 2019; Ventura & Silva, 2017). Thus, evaluating the occurrence of adulteration/contamination with ethanol in aqueous extracts is extremely important (Kubiliene et al., 2015).

Regarding the color analysis, it is notorious that the propolis color changes according to chemical composition (Salatino et al., 2005), mainly due to the concentration of flavonoids. Thus, it is expected that the colors of the commercial extracts be different from each other since they have different concentrations of phenolic compounds (varying from 0 % (m/m) - $BP_{aqueous}$ 1, to 2.32 % (m/m) – BP_{ETOH} 2). Besides, they do not have the same chemical compounds, as highlighted in the cluster analysis in Fig. 3. Interestingly, $BP_{aqueous}$ 1, the gray extract, also showed the worst results in the other evaluated parameters (mainly lowest antioxidant activity and flavonoid content), reinforcing a potential adulteration. However, color has a sizeable possible spectrum. Unfortunately, the legislation does not propose any official methodology or a specific range to verify if the commercial sample is according to the expected, impairing comparisons. Besides, the color can be easily tampered with by synthetic pigments, masking the natural color of the extract. In addition, the propolis color can be changed over oxidation and time, leading to dark colors (Vargas-Sánchez et al., 2019), as seen in most commercial samples. Therefore, we believe that to use color as one of the control parameters, more studies are still needed to make this efficient and safe.

Our results did not find a statistically significant relationship between the content of phenolic compounds and the other variables analyzed. However, we noticed that samples with lower flavonoid concentration impact the total phenolic content. For example, we saw that the aqueous propolis extracts $GP_{aqueous}$ 1 and $BP_{aqueous}$ 1 which flavonoids were not detected, present the content of phenolic compounds below the legislation reference, low values of antioxidant activity, and ORAC index. In addition, the sample $BP_{aqueous}$ 1 also has a different color scale than the control samples, suggesting a possible adulteration. Interestingly, these are the two most expensive brands sold. Concerning the commercial propolis sample price, 41 % of price variation could not be explained by any variables analyzed here. So, this variation can refer to branding, packaging, shipping, and scale costs. In addition, the price of the raw material may vary according to the quality of the propolis, botanical origin, and the target market (Barreto, 2020).

5. Conclusion

The use of the UPLC-PDA-MS/MS method and the other parameters preconized by the Brazilian MAPA allowed the comparison with the

current legislation, which was essential to observe problems in the commercialization of propolis extracts. The differential of the present study was the success of the analysis method application and the evaluation of the parameters recommended by the legislation in the accurate quality control of Brazilian propolis samples that can be applied to other species worldwide. Regarding the 17 commercial samples, 6 presents at least one unsatisfactory result compared to legislation (~35 %). We also found a possible adulteration/contamination with ethanol in one of the aqueous extracts. Furthermore, it showed that different commercialized propolis products could not be used for the same biological purpose. Even more dangerous is that one of the commercialized samples ($BP_{aqueous}$ 1) does not even have any representative chemical compounds from propolis samples. Thus, the question is: what are we consuming when we ingest this propolis extract?

Our results reinforce the need for a more rigorous inspection by the authorities to comply with the legislation. Furthermore, we support the need for a standardized analysis and quantification method for the components of the commercialized product that must be fast and easy to reproductive by other people. Therefore, a more significant partnership between the quality sector and scientific researchers is encouraged to optimize efficient quality control procedures. Mainly to amplify the quality parameters evaluated by the legislation, such as sensory characteristics, since the legislation recommends that the color (amber tones, reddish and greenish) and aroma (from mild to strong, bitter, and spicy) must be characteristic depending on the botanical origin (Decree n°. 03, of January 19, 2001 (https://www.gov.br/agricultura/pt-br, accessed on April 12, 2022), i.e. a recommendation of low specificity and difficult to control. So, techniques and standards to control sensorial parameters are lacking and inefficient since they depend on a technical panelist.

Furthermore, the contamination of antibiotics and pesticides also deserves attention. It must be explored e more directly recommended in the current legislation since this kind of contamination could decrease the biological potential of this critical raw material. Therefore, an efficient control inspection ensures that these products do not lose credibility, which is the key to ensuring that the industry sells its products safely, allowing consumers to benefit from the propolis biological properties.

CRediT authorship contribution statement

Letícia S. Contieri: Methodology, Validation, Formal analysis, Investigation, Resources, Writing – original draft. **Leonardo M. de Souza Mesquita:** Conceptualization, Methodology, Validation, Formal analysis, Investigation, Writing – original draft, Writing – review & editing, Visualization, Supervision. **Vitor L. Sanches:** Investigation, Writing – original draft. **Juliane Viganó:** Investigation, Writing – original draft. **Julian Martinez:** Investigation, Writing – original draft. **Diogo T. da Cunha:** Methodology, Validation, Formal analysis, Investigation, Writing – original draft. **Mauricio A. Rostagno:** Conceptualization, Methodology, Validation, Formal analysis, Investigation, Resources, Data curation, Writing – original draft, Writing – review & editing, Visualization, Supervision, Project administration, Funding acquisition.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data availability

Data will be made available on request.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.foodres.2022.111846.

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