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Chemical markers and antifungal activity of red propolis from Sergipe, Brazil

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Abstract

The aim of this study was to analyze the physicochemical properties and antifungal activities of the red propolis samples from Sergipe, Brazil, and also evaluate their variability throughout the year. The characterization of the hydroalcoholic extract (HPE) of the red propolis samples was performed monthly from October 2009 to September 2010. The concentrations of the bioactive compounds varied during the year, but their chromatographic profiles were similar. Four compounds were identified by comparison with authentic standards. Formononetin was one of the predominant compounds in all propolis extracts. In our study, it was observed that all the propolis samples inhibited the growth of *Candida* species. Multivariate analysis confirmed the variations in chemical composition and color of the HPEs throughout the year. The biological activities of the HPEs were statistically significant ($p<0.05$), and all samples exhibited antifungal properties.

Keywords: flavonoids; phenolic acids; formononetin; seasonality.

Practical Application: Result of significance for the commercial exploitation of propolis.

1 Introduction

Propolis is a resinous mixture of substances collected by the honey bees (*Apis mellifera*) from various plant sources (Daugsch et al., 2008). It is used as a sealant for unwanted open spaces in the hive and contains mostly sticky plant substances, beeswax and other bee secretions (Lotti et al., 2010). Numerous biological properties have been attributed to propolis, including antimicrobial, anti-inflammatory (Park et al., 2002), anticarcinogenic, antioxidant (Burdock, 1998; Kumazawa et al., 2004) antitumoural and immunomodulatory (Frozza et al., 2013), and wound healing activities (Albuquerque Junior et al., 2009). However, its chemical composition and pharmacological activity vary widely from region to region (Piccinelli et al., 2005).

In Brazil, Park et al. (1998) classified propolis into 12 groups based on their physicochemical characteristics. A new variety of propolis named red propolis was reported by Trusheva et al. in 2006. Trusheva et al., described the bioactive constituents of this Brazilian red propolis, but they did not discuss its botanical origin. Daugsch et al. (2008) studied the red propolis from the state of Alagoas (Brazil) and observed that the bees collect the reddish exudates from the surface of *Dalbergia ecastophyllum*. It was thus assumed that this was the botanical origin of the red propolis. Lotti et al. (2010) reported similar chemical profiles for red propolis from Mexico, Cuba and Brazil. According to López et al. (2014) at least two plant species are the main sources of resins for red Brazilian propolis and the relative contribution of each species to the composition of propolis varies regionally and possibly seasonally, resulting in two different types of Brazilian red propolis.

Over 300 constituents from various propolis samples have been identified and characterized. The compounds isolated from propolis are mainly flavonoids and phenolic acids, which are the components responsible for its bioactivity against various pathogenic microorganisms (Burdock, 1998).

The healing properties of Brazilian red propolis have been previously reported (Albuquerque Junior et al., 2009), suggesting an enormous biological potential for this product among them the prevention and treatment of cardiovascular diseases such as atherosclerosis (Iio et al., 2012). The goal of this study was to determine the variations in the chemical and physical characteristics of red propolis extracts throughout the year by multivariate statistical analysis. We also investigated their antifungal activities.

2 Materials and methods

2.1 Chemicals and samples

Methanol was "HPLC-gradient-grade", and the phenolic standards, formononetin, quercetin, kaempferol, pinocembrin, 3-hydroxy-7-methoxyflavone, catechin, epicatechin, rutin, propyl gallate, ferulic acid, p-coumaric acid and the Folin-Ciocalteu reagent were purchased from Sigma-Aldrich. Other chemicals

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and solvents not specified above were all of analytical grade and obtained from the local suppliers. Samples were collected from hives located in Sergipe, Brazil (S 10° 28' 25" and W 36° 26' 12"). The samples of red propolis were collected between October 2009 and September 2010 (12 samples), using propolis traps to minimize contamination by foreign substances, and frozen at –20 °C. Sample collection was performed monthly in intervals of 33±8 days.

2.2 Preparation of Hydroalcoholic Propolis Extracts (HPEs)

Propolis samples (1 g) were extracted with 70% ethanol (12.5 mL) at room temperature for 1 hour in an ultrasound bath. After extraction, the mixture was centrifuged, and the supernatant was evaporated under low pressure to produce the HPE, which was prepared at 5% w/v with 70% ethanol.

2.3 Characterisation of the HPEs

Yield

The dry-weight yields were obtained after evaporation of the solvent from the hydroalcoholic solution. The results are given as a percentage of the original weight of the crude propolis.

Color measurement

The coloration of the HPEs was measured using a Minolta Chroma Meter, CR-10 Konica Minolta. The instrument was calibrated using blank and white references prior to use. The color parameters L* (lightness), a* (redness) and b* (yellowness) were measured in triplicates.

Total polyphenolic acid and flavonoid contents

The total polyphenol content in the HPEs were determined by the Folin–Ciocalteu colourimetric method (Singleton et al., 1999). The extract solutions (0.5 mL) were mixed with 2.5 mL of the Folin–Ciocalteu reagent (1:10) and 2.0 mL of 4% aqueous solution of Na_2CO_3. Absorbance was measured at 740 nm after 2 h of incubation at room temperature. The HPEs were evaluated at a final concentration of 50 µg/mL. The total polyphenol contents were expressed as mgGAE/g (gallic acid equivalents). The flavonoid contents in the extracts were determined using a method described by Alencar et al. (2007), with minor modifications. A total of 0.5 mL of HPE (1:10), 1.5 mL of 95% ethanol, 0.1 mL of 10% $AlCl_3$, 0.1 mL of 1 M potassium acetate solution and 2.8 mL of distilled water were mixed together. After an incubation of 40 min at room temperature, the absorbance was measured at 415 nm. The total flavonoid contents were calculated as quercetin equivalents (mgQE/g) from a standard curve.

Ultra-Fast Liquid Chromatography (UFLC)

The chemical compositions of the HPEs were determined by UFLC. A reverse-phase column (XP-ODS 50 × 3 mm; particle size, 2.2 µm) with a diode array detector (Shimadzu Co.) was used according to the method described by Alencar et al. (2007) and Cabral et al. (2009), with modifications. The HPE was dissolved in methanol (50 mg/mL) and filtered with a 0.45 µm filter (Millipore). Then, 2 µL aliquots of 1% HPE (w/v) were injected into the UFLC system. The column was eluted using a linear gradient of water (solvent A) and methanol (solvent B) with a solvent flow rate of 0.4 mL/min. The gradient started at 40% B, increased to 60% B (after 22.5 min), was held at 90% B (37.3-42.3 min), and then decreased to 30% B (after 42.3 min). Chromatograms were recorded at 260 nm and processed using LC Solutions software. The following authentic standards of flavonoids and phenolic acids were used: formononetin, quercetin, kaempferol, pinocembrin, 3-hydroxy-7-methoxyflavone, catechin, epicatechin, rutin, propyl gallate, ferulic acid, and p-coumaric acid.

Biological assay

Strains were supplied by the Applied Microbiology Laboratory, Federal Universityof Sergipe (AML / UFS). The three standard fungal strains *Candida albicans* ATCC 18804, *Candida glabrata* ATCC 2001 and *Candida parapsilosis* ATCC 22019 were used. The fungal suspensions were prepared and adjusted by comparison with 0.5 McFarland turbidity standard (1.5 x 10^8cells/mL) tubes. The agar diffusion method was employed to determine the antifungal activities of the HPEs. After solidifying the plates at room temperature, wells were made in the agar (d=5 mm). Suspensions of microorganisms were then spread onto the solid media plates, and 40 µL aliquots of HPE (50 mg/mL) were added to the wells. The inoculated plates were incubated for 48 h at 28 °C. The diameters of the clear zones around the wells (inhibition zones) were measured in mm. All of the tests were performed in triplicate.

2.4 Statistical analysis

Shapiro-Wilk test was performed in order to evaluate the distribution of the values. The color parameters and yield values presented normal distribution ($p>0.05$) and one-way ANOVA followed by post-hoc Tukey test was performed to analyze these parameters (Granato et al., 2014). In other analyses, after Shapiro-Wilk test ($p<0.05$), we performed Kruskall-Wallis followed by post-hoc analysis (Duncan´s) to evaluate the differences between the groups (Granato et al., 2014). *P* values of < 0.05 were regarded as significant. These analyses were performed using the software Statistica 6.0 (Stat Soft, Inc.). The degree of the linear relationship between two variables was measured using the Pearson product moment correlation coefficient (r). Spearman correlation was carried out for analysis of non-parametric data (microbiological results). Values close to 0 indicate no linear relationship between a pair of variables, whereas r values that are close to 1 suggest a strong linear relationship. A principal component analysis (PCA) based on a correlation matrix was performed to simplify the data set and also to investigate whether the parameters were sufficient to represent the propolis samples. The PCA was done using the yield, flavonoids, phenols, propyl gallate, catechin and colorimetric (CL, Ca, Cb) values from all the 12 samples collected over a period of an year in triplicate. The results of each parameter were represented as columns and the samples as rows. Two principal components (PC1 and PC2) were used for data projection, and eigenvalues were obtained to determine the amount of variance explained by each principal component. Autoscaling was used as a pre-treatment of the results to equalize the statistical importance of all variables

(Granato et al., 2014). Statistical analysis was conducted using the PAST software 2.03 (Hammer et al., 2001).

3 Results

The samples collected during the rainy months (April to September) exhibited a lower level of productivity (45-92 g) compared to the dry season (October to March) (95-109 g), and a gradual variation in the color of the HPEs was observed. The color values L* (lightness), a* (redness), and b* (yellowness) of the HPEs differed significantly over the course of the year ($p<0.05$, Table 1).

The highest yields were obtained in April and June, producing values that were statistically similar ($p=0.661$). The samples from March, November and May produced the lowest yields ($p<0.0427$). The pluviometric index profile was similar to the yield profile over the course of the year, exhibiting a correlation coefficient of 0.74, $p=0.0065$ (Figure 1). The sample collected in March produced different results compared to other samples,

Table 1. Productivity of red propolis and color parameters of HPE*.

Samples	Productivity (g)	L	a	b	Color
Oct	100	$37.6 \pm 2.4^{c,d,e,f}$	$24.6 \pm 1.7^{a,b}$	$36.4 \pm 2.8^{a,b,c}$	
Nov	95	$36.1 \pm 1.4^{d,e,f}$	$16.0 \pm 0.4^{c,d}$	$35.2 \pm 2.1^{a,b,c}$	
Dec	103	$43.0 \pm 3.0^{a,b,c,d}$	$15.0 \pm 1.2^{d,e}$	40.0 ± 4.1^{a}	
Jan	103	32.9 ± 1.6^{f}	$20.9 \pm 0.5^{b,c}$	29.0 ± 1.5^{c}	
Feb	109	33.0 ± 2.4^{f}	$19.8 \pm 2.5^{b,c,d}$	29.2 ± 3.3^{c}	
Mar	98	47.6 ± 2.8^{a}	1.4 ± 0.6^{f}	17.2 ± 1.2^{d}	
Apr	75	$34.7 \pm 3.3^{e,f}$	26.1 ± 3.1^{a}	$30.5 \pm 3.9^{b,c}$	
May	91	$36.2 \pm 2.3^{d,e,f}$	$24.4 \pm 3.1^{a,b}$	$32.8 \pm 3.2^{a,b,c}$	
Jun	92	$38.3 \pm 2.5^{b,c,d,e,f}$	27.5 ± 2.1^{a}	$35.7 \pm 4.0^{a,b,c}$	
Jul	81	$43.8 \pm 3.1^{a,b,c}$	$17.9 \pm 1.2^{c,d}$	41.5 ± 3.5^{a}	
Aug	55	$45.5 \pm 1.6^{a,b}$	10.6 ± 0.3^{e}	40.2 ± 1.7^{a}	
Sep	45	$40.9 \pm 2.1^{a,b,c,d,e}$	$14.7 \pm 0.9^{d,e}$	$39.2 \pm 2.8^{a,b}$	

*Mean values of triplicate ± SD; identical superscript lowercase letters in the same column are statistical similar values. ANOVA and Tukey tests using 5% of significance was performed. (L) Luminosity, (a) chromaticity value for redness and (b) chromaticity value for yellowness.

exhibiting the highest lightness index and the lowest chromaticity values ("a" and "b"). The samples from October, April, May and June presented the highest values of "a" (redness) that could also be confirmed by visual analysis. The results of this study demonstrate a strong correlation between the chromaticity value ("a") and the yield, with a correlation coefficient of 0.75, p=0.005 (Figure 1).

The propolis samples displayed similar concentration of polyphenols or flavonoids among the year. However, samples from the month of December and May showed significant difference for polyphenolic concentration in HPE ($p<0.01$). In the same way, samples from the February and April were different in relation to flavonoids contents ($p<0.001$). These results were obtained using the spectrophotometric method (Table 2).

Representative UFLC chromatograms of the samples are shown in Figure 2. The compounds were identified by a comparison of their UV spectra and chromatographic behavior with external standards. The UFLC chromatograms of the various samples showed very similar profiles, but there were differences in the peak intensities. The sample from February exhibited a different pattern that included additional peaks in the chromatogram. Peaks 1, 3, 6 and 9 were identified as propyl gallate, catechin, epicatechin and formononetin, respectively. by comparison with authentic standards (Figure 2), but only the peaks for 1 and 3 were quantified (Table 2). Although formononetin was one of the major compounds in these propolis extracts, this compound and epicatechin were not quantified because they were co-eluted with other compounds. Peak 10 (which is present at a high concentration was not identified.

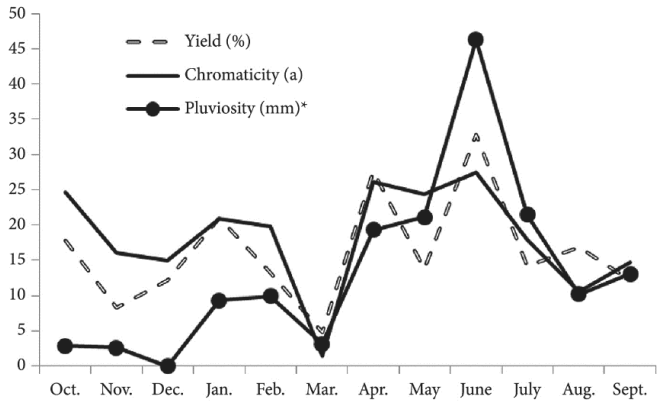

Figure 1. Global yield values (%), pluviometric index (* × 10^{-1} mm), chromaticity value ("a") of HPE (r = 0.75, $p<0.05$) along the months.

Table 2. Total polyphenol and flavonoids content in hydroalcoholic extract of red propolis*.

Sample	mg EAG/g of HPE	mg EQ/g of HPE
Oct	619.60 ± 34.58^{a,b}	0.09 ± 0.01^{d}
Nov	444.83 ± 28.80^{e}	0.03 ± 0.01^{e,f}
Dec	212.37 ± 22.11^{c}	0.06 ± 0.02^{d,e,f}
Jan	580.79 ± 34.80^{b}	0.09 ± 0.02^{d}
Feb	468.52 ± 29.46^{e,f}	0.00 ± 0.00^{f}
Mar	254.48 ± 7.33^{c}	0.08 ± 0.02^{d,e}
Apr	480.13 ± 21.32^{e,f}	0.31 ± 0.01^{a}
May	745.69 ±90.30^{d}	0.08 ±0.03^{d,e}
Jun	734.08 ± 14.71^{d}	0.15 ± 0.01^{c}
Jul	662.37 ± 7.64^{a}	0.27 ± 0.01^{a,b}
Aug	590.67 ± 12.41^{b}	0.23 ± 0.03^{b}
Sep	512.59 ± 8.59^{f}	0.07 ± 0.03^{d,e}

*Mean values of triplicate ± SD; identical superscript lowercase letters in the same column are statistical similar values. Kruskall-Wallis and Duncan´s tests using 5% of significance were performed.

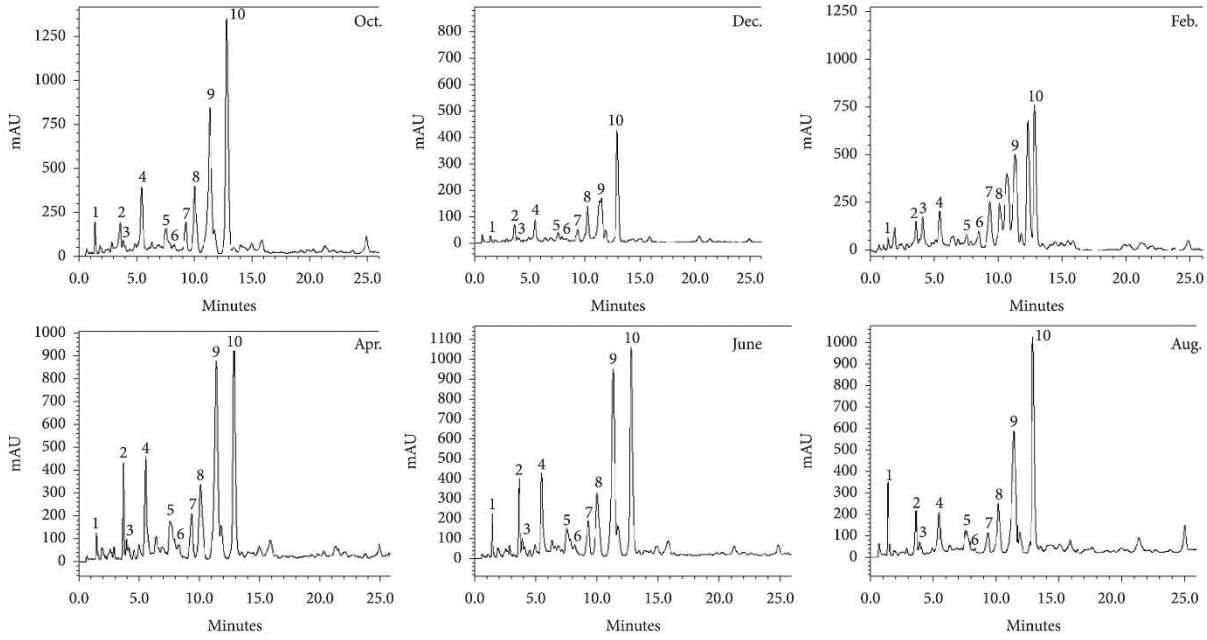

Figure 2. UFLC chromatograms of HPE. (1) Propyl gallate; (3) Catechin; (6) Epicatechin; (9) Formononetin; (2, 4, 5, 7, 8 and 10), unidentified compounds.

In our study, it was observed that all of the propolis samples inhibited the development of *Candida sp.* (Table 3), and the March samples displayed the lowest inhibitory performance for all strains ($p < 0.05$). The same behavior was observed for the other species. The HPEs were most effective against *C. glabrata*, producing inhibition zones that ranged from 18.7 mm (March) to 35.7 mm (January), Table 3.

A multivariate analysis (PCA) was performed on all the 12 samples using 8 variables to provide a partial visualization of the data set in a reduced number of dimensions (2-D). The first two principal components (PC) with eigenvalues >1 explained 73.8% of the total variance, as shown in Figure 3. The March sample exhibited a larger difference than the other samples. We observed a wide range of similar features in the samples collected in July, August and September (Brazilian winter, rainy months, and temperatures of 20-25°C). Despite the formation of clusters not very evident in the PCA, the months of October, May, June and July, in which there was increased production of phenols, were found to be grouped together.

Table 3. Antifungal activity of HPE*.

	C. albicans	*C. parapsilosis*	*C. glabrata*
Month	Inhibition zone (mm)		
Oct	16.67 ± 0.58^{a}	17.00 ± 1.00^{a}	$29.67 \pm 0.58^{a,e}$
Nov	16.67 ± 0.58^{a}	17.33 ± 1.15^{a}	$32.33 \pm 1.15^{a,b}$
Dec	$14.00 \pm 1.00^{a,b}$	$16.33 \pm 0.58^{a,b,c}$	$28.33 \pm 1.15^{a,d}$
Jan	16.67 ± 0.58^{a}	17.67 ± 0.58^{a}	35.67 ± 0.58^{b}
Feb	$14.67 \pm 1.53^{a,c}$	16.67 ± 0.58^{a}	$29.67 \pm 0.58^{a,f}$
Mar	10.33 ± 0.58^{b}	9.33 ± 0.58^{d}	18.67 ± 1.15c
Apr	$13.33 \pm 1.53^{a,b}$	$13.67 \pm 0.58^{b,e}$	$26.00 \pm 2.00^{d,e,f}$
May	$11.33 \pm 0.58^{b,c}$	$13.67 \pm 0.58^{b,e}$	$27.00 \pm 2.65^{d,e,f}$
Jun	15.67 ± 2.08^{a}	$16.00 \pm 1.73^{a,b,f}$	24.33 ± 2.08^{d}
Jul	$14.00 \pm 2.00^{a,b}$	$13.67 \pm 1.15^{c,e,f}$	$25.00 \pm 0.00^{d,e,f}$
Aug	15.33 ± 0.58^{a}	12.33 ± 1.53^{e}	$26.33 \pm 2.08^{d,e,f}$
Sep	$13.00 \pm 2.00^{a,b}$	13.00 ± 1.00^{e}	$27.33 \pm 2.52^{d,e,f}$

*Mean values of triplicate ± SD; identical superscript lowercase letters in the same column are statistical similar values. Kruskall-Wallis and Duncan´s tests using 5% of significance were performed.

4 Discussion

Propolis plays an important role in the conservation of the beehive. The continuous production of red propolis observed over the course of this study demonstrates that the production of propolis is not influenced by seasonality and also provides evidence for its direct relation to the protection of the hive. In the temperate zone of the Northern hemisphere, bees produce propolis only in the summer and early autumn, but in the tropical zone, especially the Brazilian regions, the production is continuous and independent of the seasonal variations (Torres et al., 2008). Valencia et al. (2012) found that different amounts of Mexican propolis could be collected during each season of the year. According to the authors, the largest amount was obtained during the summer, followed by fall, spring and winter. Additionally, the color, consistency and physical characteristics of the collected propolis varied widely among the samples. In the Northeast region of Brazil, there are no low temperatures, and the average annual temperature is 26 °C. There are rainy days in the winter, but this condition did not influence the production of propolis.

The hydroalcoholic extracts of the propolis samples from different months produced varying yields. These results were strongly correlated with the pluviometric index (Figure 1, r=0.74, p=0.0065) and color parameter "a" (Figure 1, r=0.75, p=0.005). Depending on the environmental conditions, the availability of the natural products around the hive changes during the year (Agüero et al., 2010), which may be responsible for different yields. The most common type of propolis is dark-brown, but red propolis

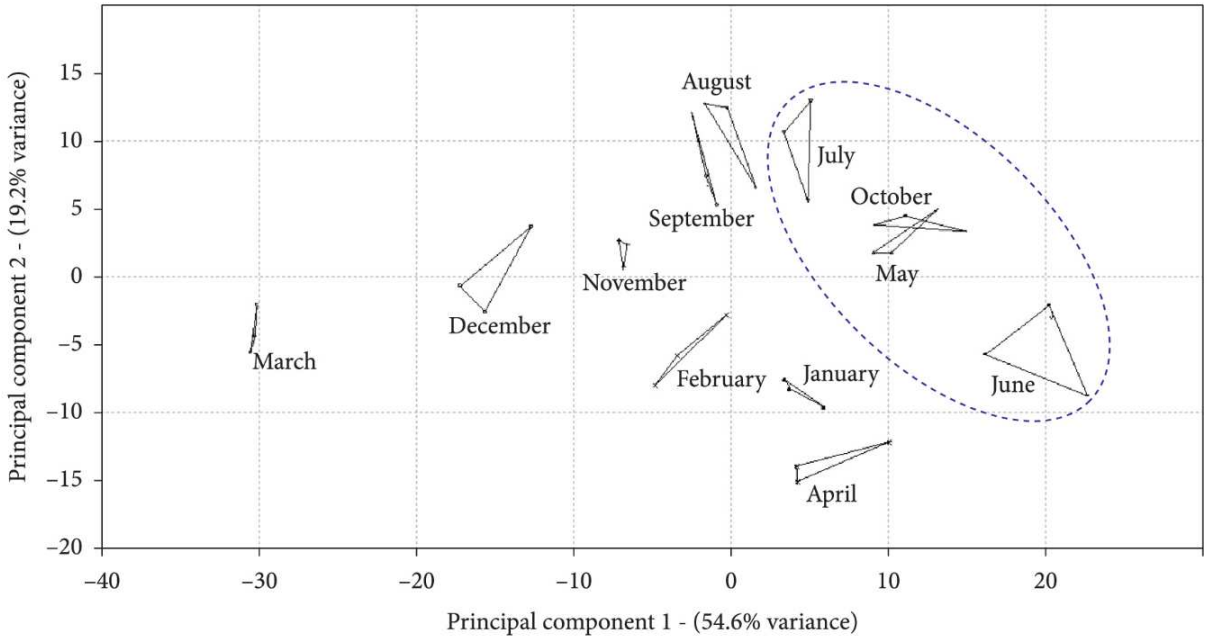

Figure 3. Principal Component Analysis applied to Yield, phenolic and flavonoid compounds, propyl gallate and catechin concentration and colorimetric parameters (L, a and b) of HPEs. The ellipse indicates the association between the months with high phenol content.

has been observed in tropical countries such as Brazil and Cuba (Lotti et al., 2010). In this study, the propolis color varied over the course of the year and exhibited a strong correlation with the yields and the extraction process. These results indicate that high concentration of hydroalcohol soluble substances directly influenced the intensity of the red color (r=0.075; p=0.005). The concentration of colored compounds (a*) did not show a strong correlation with the biological results (r= 0.345, p= 0.2973 for *C. albicans*; r=0.4718, p=0.1215 for *C. parapsilosis*; r = 0.0666; p=0.8346 for *C. glabrata*), Spearman correlation).

The composition of propolis has been reported to vary qualitatively and quantitatively with the geographical and botanical origins, seasons, genetic varieties of the queens and environmental factors (Agüero et al., 2010; Laskar et al., 2010; Miguel et al., 2010). In the temperate zone, the seasonal variations in propolis composition are predominantly quantitative, indicating that bees collect propolis from the same group of plants throughout the year (Miguel et al., 2010). However, tropical propolis samples have shown differences in their chemical compositions (Trusheva et al., 2006; Cuesta-Rubio et al., 2007). The present work showed that, despite the local biodiversity, the chemical compositions of the collected propolis did not show much qualitative variation. However, the quantities of the bioactive compounds, such as flavonoids and phenolic acids, did vary over the course of the year.

The highest contents of phenolic compounds that had previously been found in samples of Brazilian red propolis were 232 mg/g and 257.98 mg/g, which were reported by Alencar et al. (2007) and Cabral et al. (2009), respectively. However, in the present study, even larger amounts of phenolic compounds were found (745.69 mg/g). Brazilian propolis samples analyzed in this study also contained more total phenolic content than samples from other countries, such as China (302 ± 4.3 mg/g, Ahn et al., 2007, and 299 ± 0.5 mg/g, Kumazawa et al., 2004), Korea (212.7 ± 7.4 mg/g, Choi et al., 2006), Argentina (187 mg/g, Bankova et al., 2000), India (159.10 ± 0.26 mg/g, Laskar et al., 2010), Portugal (151 ± 0.01 mg/g, Moreira et al., 2008) and Thailand (31.2 ± 0.7, Kumazawa et al., 2004).

The main compounds reported to be present in propolis are phenolic acids and their esters, flavonoids (flavones, flavonones, flavonols, chalcones), terpenes, caffeic acid, cinnamic acid derivatives, and benzoic acid. These substances are attributed to the various regional plants visited by bees (Castro et al., 2009). Studies have indicated that the propolis from Europe and China contain many varieties of flavonoids and phenolic acids, but propolis samples from tropical zones generally contain low concentrations of flavonoids (Bankova et al., 2000).

A spectrophotometric analysis of the February sample did not show any flavonoids, but the total phenolic content was above the legally recommended values. The aluminium chloride spectrophotometric method based on the color reaction is frequently used to quantify flavonoids in propolis extracts and is especially useful for the rapid screening of propolis for bioactive components (Luo et al., 2011). In this study, a detailed analysis of the flavonoids was also performed using UFLC, which provides a more reliable quantification of these compounds (Alvarez-Suarez et al., 2010). The UFLC method identified the flavonoids (Figure 2) that were not identified by the spectroscopic method. The aluminium chloride spectrophotometric method does not respond equally to all flavonoid groups, and it might underestimate the flavonoid content (Alvarez-Suarez et al., 2010).

Seasonal variation did not have a significant effect on the relative abundances of the main chemical constituents in the propolis samples. The peak profiles for the samples were very similar, demonstrating a consistent chemical composition throughout the year (Figure 2) and suggesting that the main botanical source of red propolis is available all year. However, the concentrations of these compounds varied over the course of the year. Formononetin, is used as a chemical marker (Cuesta-Rubio et al., 2007) for red propolis and has only been identified in red propolis samples from Brazil (Cabral et al., 2009) and Cuba (Cuesta-Rubio et al., 2007). Trusheva et al. (2006) also observed the presence of isoflavonoids in Brazilian red propolis. The presence of isoflavonoids in propolis suggests new potential biological applications for this natural product. Isoflavonoids have been reported to possess antimicrobial, antifungal, anticancer and antioxidant activities (Alencar et al., 2007). Novak et al. (2014), enriched a fraction of the red propolis extract with formononetin making it more active than the original extract for its cytotoxic activity .

Evaluations of the biological activity of propolis, particularly its antifungal activity, have been reported previously (Herrera et al., 2010). All propolis samples, independent of their source, present antimicrobial activity because this material must protect the hive (Buriol et al., 2009). The biological activity of Brazilian propolis is mostly due to the high levels of phenolic acids (Laskar et al., 2010), whereas flavonoids are considered to be responsible for the activity of extracts from European propolis (Hegazi et al., 2000). The March sample exhibited the lowest values for antifungal activity, and a corresponding decrease in the phenolic acid and flavonoid content was also observed. The extract from this sample was yellow, suggesting that either the botanical source in this month was different or the plants did not produce a colored resin during this time of the year.

The principal component analysis (PCA) was performed to compare the red propolis samples using the following parameters: yields (V1) and concentrations of flavonoids (V2), phenolic acids (V3), propyl gallate (V4) and catechin (V5). This type of analysis is an exploratory method to evaluate a general hypothesis from the collected data. The scores of each propolis sample were examined using a two-dimensional plot of the first two principal components, which together explain 73.8% of the total variation. Some clustering was also observed. The samples from October, May, June and July appear together which must be mainly related to the high phenols content found in these samples. On the other hand, the samples from the month of March which are very different from the rest, containing low- phenols and yellow coloration were placed isolated from the rest. PCA analysis also confirmed data reproducibility. As can be seen in Figure 3, all the samples were grouped according to the month of their collection (represented by triangles), which indicates high similarity between the samples analyzed each month.

5 Conclusion

Brazilian red propolis from the state of Sergipe showed significant variations in the concentrations of its phenolic

components over the course of a year. There was a positive correlation between the yields and the pluviosity. However, the changes in color and chemical composition observed during the year did not influence the biological activity of the HPEs, showing that despite some variation in quantity, this product can be used independent of the time of year at which it was collected. The amount of total phenols were found to be much higher than those described in propolis from other countries as well as from other regions of Brazil, demonstrating higher potential of the propolis samples from the state of Sergipe, Brazil.

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