Determination of physicochemical characteristics and bioactive compounds in samples of pollen, geopropolis and honey from Melipona Scutellaris bee species

Determinação das características físico-químicas e compostos bioativos em amostras de polén, geoprópolis e mel de abelha Melipona Scutellaris

DOI:10.34117/bjdv6n4-353

Recebimento dos originais: 20/03/2020
Aceitação para publicação: 27/04/2020

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ABSTRACT
The interest in other products from the meliponids, such as pollen and the geopropolis, has provided a growing demand in the market due to their chemical composition and pharmacological activities. This study aimed to determine the physicochemical characteristics and bioactive compounds in honey, geopropolis and pollen of the bee species - *Melipona scutellaris*. Moisture, ashes, water activity and pH were evaluated, averaging 25%; 0.25%; 0.724; 5.52 for honey; 4.49%; 73.68%; 0.931; 3.55 for geopropolis and 49.61%; 2.31%; 0.931; 3.55 for pollen, respectively. Regarding the bioactive compounds, total phenolics, flavonoids, carotenoids and antioxidant activity (DPPH) were evaluated, with mean values of respectively 243.6 mgGAE / 100g; 22.75 mgEQ / 100g; 0.48 mg β-carotene / 100 g; 10.72% for honey, 14.1 0 mgGAE / g; 6.91 mgEQ / g; 1.55 mg β-carotene / g; 43.48% for geopropolis and 21.21 mgGAE / g; 8.52 mgEQ / g; 3.62 mg β-carotene / g and 45.92% for pollen. It is concluded that variations of these parameters suffer influence of flowering season, geographic location and bee type. The content of bioactive compounds is
relevant and can represent functional properties for consumers, and may add value not only to honey but to other products.

**Keywords**: Meliponids; stingless bee; functional properties.

**RESUMO**
O interesse por outros produtos oriundos dos meliponídeos como o pólen e a geoprópolis, tem proporcionado uma demanda crescente no mercado devido as suas composições químicas e atividades farmacológicas. O presente estudo teve o objetivo de determinar as características físico-químicas e compostos bioativos em mel, geoprópolis e pólen de uma mesma espécie de abelha - *Melipona scutellaris*. Foram avaliados os parâmetros de umidade, cinzas, atividade de água e pH obtendo-se para o mel médias de 25%; 0,25%; 0,724; 5,52, para o geoprópolis: 4,49%; 73,68%; 0,931; 3,55, e para o pólen: 49,61%; 2,31%; 0,931, 3,55, respectivamente. Com relação a análise dos compostos bioativos, avaliou-se os teores de fenólicos totais, flavonoides, carotenoides e atividade antioxidante (DPPH), com valores médios respectivamente de 243,60 mgGAE/100g; 22,75 mgEQ/100g; 0,48 mg β- caroteneno/100g; 10,72% para o mel, 14,10 mgGAE/g; 6,91 mgEQ/g; 1,55 mg β- caroteneno/g; 43,48% para o geoprópolis e 21,21 mgGAE/g; 8,52 mgEQ/g; 3,62 mg β- caroteneno/g e 45,92% para o pólen. Conclui-se que as variações dos parâmetros avaliados sofrem influência da florada, localização geográfica e tipo de abelha. O conteúdo de compostos bioativos é relevante e podem representar propriedades funcionais para os consumidores, agregando valor não só ao mel como a outros produtos.

**Palavras-chave**: Meliponídeos; abelha sem ferrão; propriedades funcionais.

**1 INTRODUCTION**

Bees of the genus *Melipona* are characterized by having a stunted form of the stinger, not having it as a defensive weapon (Silva *et al*., 2009). Popularly known as stingless (native) bees, they have an essential role in maintaining the diversity of plant species through the pollination process. Besides the production of honey, interest in other products from the meliponids, such as pollen and the geopropolis, has provided a growing demand in the market due to their chemical composition and pharmacological activities (Sousa *et al*., 2015).

The honey produced by *Melipona* bees consists of a complex mixture of water, sugars such as glucose, fructose, sucrose, maltose, minerals, proteins, organic and phenolic acids, volatile compounds and some vitamins (Khalil *et al*., 2010), which can be variable according to its botanical origin, edaphoclimatic conditions, bee species and storage conditions (Silva *et al*., 2013; Nweze *et al*., 2017).

The geopropolis is a mixture of resins, salivary secretions of bees, wax, earth and / or clay that has the function of protecting and repairing cracks in the hive in order to maintain the queen bee in aseptic conditions (Bankova *et al*., 2000). The geopropolis also has interesting bioactive
characteristics due to the presence of polyphenolic compounds, associated with antimicrobial, anti-inflammatory, antitumoral, healing and antioxidant properties (Lustosa et al., 2008; Nascimento et al., 2008).

The pollen produced by these bees, also known as saburá or samburá, has been used for a long time, mainly among supporters of natural food, as a supplement, probably due to the high content of vitamins (B, C, D, E and H) and mineral salts (Silva, 1998). It is also the primary source of nitrogen for the bees, being collected in floral sources and stored within their nests (Costa et al., 2015).

The present study aimed to determine physicochemical characteristics and bioactive compounds in honey, geopropolis and pollen samples produced by stingless bee Melipona scutellaris.

2 MATERIAL AND METHODS

2.1 MATERIALS

The samples of honey, geopropolis and pollen were collected in a meliponary located in the metropolitan region of Salvador - Bahia. All matrices were retained from the same hive and the same species of bees (Melipona scutellaris). The samples were placed in sterile high-density polyethylene bottles and transported to the Fish and Applied Chromatography Laboratory (Lapesca), located at the Faculty of Pharmacy of the Federal University of Bahia (UFBA), where they were kept under refrigeration. The tests were performed in triplicate and the results were expressed as mean ± standard deviations (SD).

2.2 PHYSICOCHEMICAL CHARACTERIZATION

For the analyses of pH, water, moisture, ashes and water activity, the analytical standards of the Adolfo Lutz Institute (2004) were followed.

2.3 CHARACTERIZATION OF BIOACTIVE COMPOUNDS

2.3.1 Elaboration of hydroalcoholic extracts of geopropolis and pollen

The pollen samples were ground and the geopropolis samples were macerated. Soon after, the extracts were prepared to obtain a 10% (1:10 m / v) extract in 80% ethanol solution. The extraction was performed under agitation at 70 °C for 1 hour, followed by centrifugation at 150 rpm / 25 °C for 5 minutes. The supernatant was removed and kept under refrigeration (Silva et al., 2012).
2.3.2 Total Phenolic Compounds

The determination of total phenolic compounds was performed using the spectrophotometric (760 nm) and Folin Ciocalteau method (Singleton, Orthofer, & Lamuela-Raventos, 1999). For honey, 70% ethanol extracts were used. The pollen and geopropolis hydroalcoholic extracts were diluted in proportion of 1:10 with 80% ethanol. Gallic acid was used as a reference standard, with solutions in the concentration range from 50 to 300 µg / mL for the construction of the standard analytical curve. The content of total phenolic were expressed as mg equivalents of gallic acid per 100 grams of honey (mg GAE. 100 g⁻¹) and mg equivalents of gallic acid per g of dry matter of pollen and geopropolis (mg EAG.g⁻¹).

2.3.3 Total flavonoids

The determination of total flavonoid content was performed according to Woisky & Salatino (1998). Aliquots of 0.5 ml of hydroalcoholic extracts of geopropolis, pollen, and ethanolic extract of honey were added to an equal volume of solution containing methanol and aluminum chloride 5% (AlCl₃). The samples were read in a spectrophotometer (Perkin Elmer UV) at 415nm.

2.3.4 Determination of carotenoid content

The determination of the total carotenoid content was performed according to the method described by Rodriguez-Amaya (1999), with adaptations. The reading was performed directly from the samples extracts (Ferreira et al., 2009) in a spectrophotometer (Perkin Elmer UV) at 450nm. The total carotenoid content was determined using an analytical curve with β-carotene as standard (40 - 200 / ml). The total carotenoid content was expressed in mg of β-carotene equivalents in 100 grams of honey, mg β-carotene for the geogropolis and µg of β-carotene.g⁻¹ of pollen dry matter.

2.3.5 Antioxidant Activity

The antioxidant activity of honey, pollen and geopropolis was measured by the DPPH radical scavenging ability, according to the method described by Meda et al. (2005) using a spectrophotometric at 517nm. The percentage of scavenging activity (% AA) was determined according to (Vinson et al. 2001), according to equation 1.

\[
%\text{ AA} = 100 - (((\text{Sample abs} - \text{White abs}) \times 100) / \text{Control abs}) \quad \text{(equation 1)}
\]
3 RESULTS AND DISCUSSION

Table 1 shows the results of the physicochemical analyses of honey, geopropolis and pollen. The moisture content for honey is above the limit specified in the national legislation for honey from *Apis mellifera*, which determines the maximum allowed content of 20% for this parameter (BRASIL, 2000). This legislation does not contemplate honey from stingless bees, and therefore cannot be used for comparison of honeys from *Melipona*. The values are consistent with Evangelista-Rodrigues et al (2005) who established mean values of moisture between 16.6 and 45.0% for *M. scutellaris* honey. According to Alves (2005) high values satisfy the basic characteristic of stingless honeys, which is high hygroscopicity. For geopropolis, moisture is higher than that found by Silva et al. (2009), which ranged from 1.94% to 2.74% for the geopropolis of *Melipona rufiventris*. As for the pollen, the moisture value is higher than the values found by Marchini et al. (2006), 16.8 to 33.18%, for *Apis mellifera*, and this fact can be explained because the pollen is a highly hygroscopic material, therefore, being greatly affected by environmental conditions.

Table 1 - Mean values with standard deviation of the physicochemical analyzes of the honey, geopropolis and pollen samples of *Melipona scutellaris*.

| Sample     | Moisture (%) | Ashes (%) | Water activity | pH        |
|------------|--------------|-----------|----------------|-----------|
| Honey      | 25.00±0.00   | 0.25±0.01 | 0.724 ±0.009   | 5.52±0.15 |
| Geopropolis| 4.49±0.01    | 73.68±0.75| 0.801±0.002    | 4.33±0.11 |
| Pollen     | 49.61±0.02   | 2.31±0.01 | 0.931±0.006    | 3.55±0.03 |

The value found for ashes in honey meets the reference value established in the Legislation of a maximum of 0.6%. Holland et al. (2012) found in the honeys of *Melipona fasciculata* a variation from 0.02% to 0.57%. The ashes content expresses the richness of honey in minerals and is a characteristic widely used to check its quality. The value of the geopropolis is above the values found by Garcia et al. (2001), 3.56% and 3.20%. These analyzed propolis of *Apis mellifera* had lower ashes content, since geopropolis is a mixture of clay / earth and resin, consequently its ash content is higher. For pollen, the ashes value were close to the results collected by Almeida-Muradian et al. (2004) with an average value of 2.87%.
The value of water activity for honey is higher than that reported by Souza et al. (2009), who determined the water activity in 15 samples of *Melipona Scutellaris* honey from the region of Porto de Sauipe-Ba, (0.722 and 0.743). For geopropolis, values were higher than those found by Monfort (2002) ranging from 0.730 to 0.750 for *Apis mellifera* in the region of Paraná. The values for this parameter for pollen are greater than Almeida-Muradian et al. (2004), from 0.570 to 0.605 for *Apis mellifera*. The relevance of this characteristic is related to the fact that water is the main component of many foods and has an influence on its biochemical and microbiological stability.

For pH, Souza et al. (2006) reported values close to honey, ranging between 3.15 and 4.66 for *Melipona subnitida*, in the rural municipality of Mossoro. Similar values for geopropolis were reported by Garcia et al., (2001), 4.5 to 5.2 for *Apis mellifera*, in Paraná. The pollen values were higher than those found by Almeida-Muradian et al. (2004), 4.6 to 5.9, for *Apis mellifera*, in the southern region of Brazil. This parameter is considered an important antimicrobial factor, promoting greater stability to the product regarding the development of microorganisms.

### Table 2 - Mean values with standard deviation for bioactive compounds analyzes of honey, geopropolis and pollen of *M. scutellaris*

| Samples  | Phenolics (mg GAE / g) | Flavonoids (mg EQ / g) | Carotenoids (mg β-carotene / g) | % DPPH radical sequestration |
|----------|------------------------|------------------------|---------------------------------|-------------------------------|
| Honey    | 242.60±0.01*           | 22.75±0.00*            | 0.48±0.00*                      | 10.72±0.01                   |
| Geopropolis | 14.10±0.01             | 6.91±0.01              | 1.55±0.01                       | 43.48±0.00                   |
| Pollen   | 21.21±0.11             | 8.52±0.02              | 3.62±0.01                       | 45.92±0.00                   |

*(mg GAE/100g)*

The value found for phenolic compounds in honey was higher than the values reported by Lira (2004), whose results varied between 101.68 to 105.59 mg GAE / 100g of honey. This change happens because these compounds are very variable, and in honey, they are presented according to the floral source that originated them (Nordin et al, 2018). In relation to flavonoids, the value found was higher than those obtained by Liberato et al. (2011) from 0.25 to 8.38 mgQE / 100 g for *Apis mellifera* bee honey, in the northeast region of Brazil. For Sant'Ana et al. (2012) the greater the quantification of these phenolic compounds, the darker the color of honey, thus showing that there is a correlation between the color and these substances.
It is observed that the content of carotenoids found in honey is below the results described by Rizelio (2011), who, when analyzing this same compound in *Melipona scutellaris* honeys, in Santa Catarina, found values that varied from 0.63 to 1.17 mg β-carotene / 100g. These results can be justified because these compounds may be influenced by the area and botanical origin. Carotenoid can be correlated with coloration, since authors found small concentrations of carotenoids in dark colored honeys (10mg β-carotene.kg-1) and virtually none in clear colored honeys (Liberato et al., 2011).

The results found for honey in relation to the potential for the sequestration of the DPPH radical proved to be relevant, since inferior results were presented by Spath (2013), analyzing the honey of *M. scutellaris* in the State of Bahia, (1.81% and 8.78%). According to Sant’Ana et al. (2012) and Meda et al. (2005), the main responsible for the antioxidant effect of honey are phenolic and flavonoid acids, although they are not the only ones. This occurs due to the presence of phenolic hydroxyls in the structures of these substances, forming the intermediate phenoxy radicals, which are stable and act by reacting with other free radicals, blocking the oxidation propagation reactions.

The phenolic content found in the geopropolis is below the values reported by Guo et al. (2011), who, when analyzing the propolis extracts of *Apis mellifera* in China, found 200 to 400mg GAE / g. According to Cunha et al. (2009), in the honeycomb structure, *Meliponini* added vegetable fibers, seeds and ground propolis, shaping the product into the geopropolis, then the values compared with propolis are smaller. Regarding the flavonoids quantified in geopropolis sample, a higher value than the required by law Brazilian propolis was found (from 1.0 to 2.0 mgEQ / g) (Brazil, 2001). According to Dutra et al. (2008), analyzing geopropolis, found values lower than this study (2.67 to 0.61mg / g). This variation is influenced due to climatic conditions, botanical origin and region (Gomes-Caravaca et al., 2006).

The antioxidant activity for the geopropolis showed lower values than Alves et al., (2013), 80.55 to 92.56% of geopropolis of *M. scutellaris*. According to Silva et al. (2006) the strong antioxidant activity of propolis can come from flavonoids, such as quercetin, flavones, isoflavones, flavonones, anthocyanins, catechins and isocatequinas. However, phenolics can participate together with flavonoids in determining these activities. The content of total carotenoids detected in the geopropolis sample showed results inferior to those of Santos et al. (2003), with values between 3.1 and 3.6 mg β-carotene / g of *M. scutellaris*. This parameter is also influenced by the presence of total phenolic compounds, by the variability of the chemical composition and origin of the material collected by each bee species.
For the results for pollen, the content for phenolic compounds was similar to the results of Carpes (2009), which ranged from 19.80 to 48.90 GAE / g of pollen of *Apis Mellifera*. The flavonoids were superior to the results obtained by Leja et al. (2007) which ranged from 0.72 to 1.99 mg EQ / g of pollen. According to Campos et al. (2003) this variation of compounds occurs especially in relation to the plant species, among other parameters, such as environmental conditions, drying conditions, storage time and the type of pollen collected.

The carotenoids value for the pollen was higher than the values found by Almeida-Muradian et al. (2005). The authors analyzed ten samples of fresh pollen from *Apis mellifera* and found 76.33 β-carotene mg / g and DPPH radical sequestration from 37.94 to 91.81%. According to Leja et al. (2008), samples with high levels of phenolic constituents indicate high antioxidant capacity.

4 CONCLUSIONS

Through the results presented in this study, it can be seen that the characteristics of these matrices are influenced by botanical origin, soil type, geographical position and vegetation visited by the producing bees. The total phenolic compounds, total flavonoids, antioxidant activity and total carotenoids results showed significant content of bioactive compounds that may present functional properties for consumers, adding value not only for honey but for other products, contributing to the development of the meliponiculture.

THANKS

The authors would like to thank FAPESB for the financial support and Meliponário Costa do Sauípe for providing the samples.

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