

# Chemical composition and antioxidant activity of geopropolis produced by *Melipona fasciculata* (Meliponinae) in flooded fields and cerrado areas of Maranhão State, northeastern Brazil

Marisa Cristina Aranha BATISTA<sup>1</sup>; Bruno Vinicius de Barros ABREU<sup>1</sup>; Richard Pereira DUTRA<sup>1</sup>; Mayara Soares CUNHA<sup>1</sup>; Flavia Maria Mendonça do AMARAL<sup>1</sup>; Luce Maria Brandão TORRES<sup>2</sup>; Maria Nilce de Sousa RIBEIRO<sup>1\*</sup>

<sup>1</sup> Universidade Federal do Maranhão, Departamento de Farmácia, Laboratório Farmacognosia, Avenida dos Portugueses 1966, Campus Bacanga, 65080-805, São Luís-Maranhão, Brazil.

<sup>2</sup> Instituto de Botânica, Núcleo de Pesquisa em Fisiologia e Bioquímica, Av. Miguel Stéfano 3687, Água Funda, 04301-9012, São Paulo, São Paulo, Brazil

\* Corresponding author: mnribeiro@ufma.br

## ABSTRACT

Geopropolis, a mixture of plant resin, wax, soil and salivary secretion, is produced by the stingless bee *Melipona fasciculata*. This aim of this study was to investigate the chemical composition and antioxidant activity of geopropolis collected from beehives in two phytogeographical regions, flooded fields and cerrado, in the municipalities of Palmeirândia and Fernando Falcão, Maranhão State, northeastern Brazil. The geopropolis compounds were identified by gas chromatography–mass spectrometry (GC/MS). Additionally, total phenolic content was determined with the Folin–Ciocalteu reagent and antioxidant activity was evaluated *in vitro* by 2,2-diphenyl-1-picrylhydrazyl (DPPH) and ferric reducing antioxidant power (FRAP) assays. The four geopropolis samples varied in terms of total phenolic content and antioxidant activity and the highest values were observed for geopropolis from Fernando Falcão. Triterpenes such as cycloartane, ursane and oleanane and phenolic acids (protocatechuic acid and gallic acid) were identified in the geopropolis from Palmeirândia, while the phenolic acids, gallic and ellagic acid were the main compounds in geopropolis from Fernando Falcão. The antioxidant property of geopropolis is due to its high total phenolic content and predominance of gallic acid and ellagic acid. The results showed that the plant resources in two phytogeographical regions of Maranhão used by bees for the production of geopropolis contribute to the chemical composition and bioactivity of this product.

**KEYWORDS:** stingless bee, polyphenols, triterpenes, phytogeographical regions.

## Composição química e atividade antioxidante da geoprópolis de *Melipona fasciculata* (Meliponinae) produzida em áreas de campos alagados e de cerrado no Estado do Maranhão, Nordeste do Brasil

### RESUMO

Geoprópolis, uma mistura de resinas vegetais, cera, terra e secreção salivar, produzida pela abelha sem ferrão *Melipona fasciculata*. Este estudo investigou a composição química e a atividade antioxidante da geoprópolis coletada em colmeias em duas regiões fitogeográficas de campos alagados e de cerrado, nos municípios de Palmeirândia e Fernando Falcão, no Estado do Maranhão, Nordeste do Brasil. Os compostos da geoprópolis foram identificados por cromatografia gasosa acoplada a espectrometria de massas (CG/EM). Foram determinados os teores de fenólicos totais pelo reagente de Folin–Ciocalteu, e atividade antioxidante utilizando o ensaio *in vitro* com 2,2-difenil-1-picrilhidrazil (DPPH) e capacidade redutora do ferro (FRAP). As quatro amostras de geoprópolis apresentaram variações no teor de fenólicos totais e atividade antioxidante, as geoprópolis de Fernando Falcão, apresentaram maiores teores. Nas geoprópolis do município de Palmeirândia foram identificados, triterpenos do tipo cicloartano, ursano e oleanano e ácidos fenólicos (ácido protocatecuico e ácido gálico), enquanto que na geoprópolis de Fernando Falcão, ácidos fenólicos, ácido gálico e elágico foram os principais constituintes. A propriedade antioxidante da geoprópolis pode ser atribuída aos altos teores de fenólicos totais e de ácido gálico e elágico. Os resultados demonstram que as fontes vegetais das duas regiões fitogeográficas do Maranhão, Brasil, utilizadas pelas abelhas para a produção da geoprópolis contribuem para a composição química e bioatividade deste produto.

**PALAVRAS-CHAVE:** abelhas sem ferrão, polifenóis, triterpenos, regiões fitogeográficas.

## INTRODUCTION

Stingless bees are found in Tropical and Neotropical regions and play an important role in pollination and agriculture (Slaa *et al.* 2006). In Brazil, 244 stingless bee species have been identified mainly in the northern and northeastern regions of Brazil, corresponding to about 20% of all Neotropical species of stingless bees (Pedro 2014).

In the State of Maranhão (northeastern Brazil), *Melipona fasciculata* Smith, popularly known as tiuba, is the species most frequently cultivated for honey production by rural populations, especially in flooded fields and cerrado areas, because of its high economic value and the production of wax, pollen, and geopropolis. The last product has potential applications in the fields of chemistry and biology, but few studies have been conducted (Bezerra 2002; Bankova and Popova 2007; Holanda *et al.* 2012).

In the beehives, geopropolis is produced by bees from the resinous material of buds, leaves, and plant exudates, mixed with salivary secretions, wax, and soil. Geopropolis is used to protect beehives against insects and pathogenic microorganisms, to restrict entry into the hive, to line the interior walls of the hive, to strengthen the honeycombs, and embalm animals (Nogueira-Neto 1997).

Geopropolis and its subproducts are used by the population for the treatment of inflammatory diseases, fatigue, hemorrhoids, gastritis, and cough (Kerr 1987). Several studies have demonstrated the biological properties of geopropolis, such as antimicrobial, cytotoxic, antitumor, antioxidant, antinociceptive, anti-inflammatory, immunomodulatory, and gastroprotective (Libério *et al.* 2011; Souza *et al.* 2014; Araújo *et al.* 2015).

The chemical composition of geopropolis is complex. It has been reported the presence of polyphenolic compounds (phenolic acids, flavonoids, and tannins) (Silva *et al.* 2013; Souza *et al.* 2013; Dutra *et al.* 2014), terpenes (monoterpenes, sesquiterpenes, diterpenes, and triterpenes), fatty acids, steroids, and saponins (Dutra *et al.* 2008; Cunha *et al.* 2009; Araújo *et al.* 2015). However, its chemical composition varies according to the flora visited by stingless bees, the region, and the time of collection (Bankova 2009; Ribeiro *et al.* 2013; Barth and Freitas 2015).

In view of the scarcity of studies on the chemical composition and biological activity of *M. fasciculata* products in Brazil, the aim of this study was to investigate the chemical composition of geopropolis produced by *M. fasciculata* collected from beehives of two phytogeographical regions of Maranhão, and evaluate its antioxidant activity.

## MATERIALS AND METHODS

### Geopropolis samples

Four geopropolis samples: G1 (660.5 g), G2 (496.4 g), G3 (1519.0 g) and G4 (2534.8 g) were collected from meliponaries located in two phytogeographical regions of Maranhão State, northeastern Brazil. Samples G1 and G2 were collected in two beehives of the same meliponary in the municipality of Palmeirândia (2°40'80.3"S e 44°52'66.1"W). Palmeirândia is located in the region of periodically flooded fields (lowlands of the northern micro-region of Maranhão, northeastern Brazil) with the predominance of "castanha do Pará" (*Bertholletia excelsa* Humb. & Bonpl.), "embaúba" (*Cecropia* sp.), "gameleira" (*Clusia burchellii* Engl.), "cedro" (*Cedrella fissilis* Vell.) and "babaçu" (*Orbignya phalerata* Mart.). During the rainy season, the lowlands are flooded, forming islands of dry land with occurrence of "buriti" (*Mauritia flexuosa* L.f.), "aninga" (*Montrichardia linifera* Schott), among others (Martins *et al.* 2011).

Samples G3 and G4 were collected from two beehives of the same meliponary in the municipality of Fernando Falcão (6°08'99.2"S e 44°54'99.4"W), region of cerrado of the southern micro-region of Maranhão, northeastern Brazil. This region is characterized by typical cerrado vegetation with species of great ecological and economic value, such as "barbatimão" (*Stryphnodendron barbatiman* M.), "gonçalave" (*Astronium graveolens* Jacq.), "mangabeira" (*Hancornia speciosa* Muell. Arg.), "piqui" (*Caryocar brasiliensis* Camb.), "fava d'anta" (*Dimorphandra gardneriana* L.), "candeia" (*Platymenia reticulata* Benth.), "tamboril" (*Enterolobium contortisiliquum* (Vell.) Morong), "puça" (*Mouriri pusa* Gardn.), "sucupira" (*Bowdichia virgilioides* HBK.), "murici" (*Byrsonima crassifolia* HBK.), "cagaita" (*Eugenia dysenterica* DC.) and "pau terra" (*Qualea grandiflora* Mart.) (Muniz 2002; Ribeiro *et al.* 2013).

### Preparation of hydroalcoholic extracts of geopropolis (HEG)

The geopropolis samples (500 g) were separately macerated with 1:2 (w/v) in 70% ethanol for 48 h and filtered to separate the inorganic part (soil). The extractive solutions were concentrated in a rotating evaporator (Q344B2, Quimis, São Paulo, Brazil) to yield HEG (Dutra *et al.* 2014). The extracts were codified as HEG1 and HEG2 (geopropolis collected from Palmeirândia), and as HEG3 and HEG4 (geopropolis collected from Fernando Falcão).

### Total phenolic content (TPC)

Total phenolic compounds were determined by the *Folin-Ciocalteu* reagent and 20% sodium carbonate method (Dutra *et al.* 2014). The reaction mixture was kept in the dark for 2 h at room temperature, and absorbance was measured at 760 nm using a UV-Vis spectrophotometer (Lambda 35,

Perkin Elmer Corporation, Massachusetts, USA). TPC was expressed as milligrams of gallic acid equivalent per gram of geopropolis extracts (mg GAE g<sup>-1</sup>).

### Determination of the antioxidant activity

#### DPPH radical scavenging activity

The antioxidant activity of geopropolis samples was evaluated using the *in vitro* 2,2-diphenyl-1-picrylhydrazyl (DPPH) assay (Dutra *et al.* 2014). The samples were diluted in methanol at different concentrations (1.0 to 100.0 µg mL<sup>-1</sup>) and added to a methanol solution of DPPH (40.0 µg mL<sup>-1</sup>). After 30 min of reaction at room temperature in the dark, the absorbance of each solution was read at 517 nm using a UV-Vis spectrophotometer (Lambda 35, Perkin Elmer Corporation, Massachusetts, USA). Methanol was used as the control and DPPH solution was used as the blank. Standards of gallic acid and 6-Hydroxy-2,5,7,8-tetramethylchromane-2-carboxylic acid (Trolox, Sigma) were treated under the same conditions as the samples. The percent inhibition was calculated according to equation.

$$\text{DPPH}_{\text{scavenging activity}} (\%) = 100 \times (A_{\text{control}} - A_{\text{sample}}) / A_{\text{control}}$$

where  $A_{\text{sample}}$  = absorbance of the sample after 30 min of reaction, and  $A_{\text{control}}$  = absorbance of the control. The percent of scavenging activity was plotted against the sample concentration to obtain the IC<sub>50</sub>, defined as the concentration of sample necessary to cause 50% inhibition.

#### Ferric reducing antioxidant power assay (FRAP)

The FRAP's method was used to determine the antioxidant activity based on iron reduction. FRAP measures the ferric-reducing ability of a sample in acid medium (pH 3.6), yielding an intense blue color attributable to the reduction of the ferric tripyridyltriazine (Fe<sup>III</sup>-TPTZ) complex to the ferrous (Fe<sup>II</sup>) form (Dutra *et al.* 2014). FRAP reagent was prepared immediately before analysis by mixing 25 mL of acetate buffer (300 mM, pH 3.6), 2.5 mL of TPTZ solution (10 mM TPTZ in 40 mM HCl), and 2.5 mL of FeCl<sub>3</sub>·6 H<sub>2</sub>O (20 mM) in aqueous solution. Different concentrations of 100 µL of the samples (1 to 100 µg mL<sup>-1</sup>) were added to 300 µL of distilled water and 3 mL of FRAP reagent, and the mixtures were incubated in a water bath at 37 °C for 30 min. The absorbance of the reaction mixture was read at 593 nm using a UV-Vis spectrophotometer (Lambda 35, Perkin Elmer Corporation, Massachusetts, USA), with FRAP solution as a blank. The calibration curve was constructed using different concentrations of FeSO<sub>4</sub>·7H<sub>2</sub>O (100 to 2000 µM) (r<sup>2</sup> = 0.9987) and the results are expressed as millimole of Fe<sup>II</sup> per gram of sample. Standards of gallic acid and Trolox (Sigma) were treated under the same conditions as the samples.

### UV-Vis analysis of geopropolis extracts

The UV-Vis spectra was obtained for each extract (50 µL) in methanol (3 mL), and the absorption spectra was measured at the wavelength range of 200 to 450 nm using a UV-Vis spectrophotometer (Lambda 35, Perkin Elmer Corporation, Massachusetts, USA).

### Analysis of geopropolis extracts by gas chromatography–mass spectrometry (GC/MS)

To assess geopropolis chemical composition, 1 mg of dry HEGs was added of 300 µL of pyridine and 100 µL of bis-(trimethylsilyl) trifluoroacetamide (BSTFA) including 1% of trimethylchlorosilane (TMCS) in a sealed glass tube for 1 h at 80 °C to prepare samples for gas chromatography according to Campos *et al.* (2014). The analyses were carried by gas chromatograph and mass spectrometry (GC/MS), using an Agilent GC (6890 Series gas chromatography system; Agilent Technologies, California, USA) quadrupole mass-selective detector (MSD) system (5973, Agilent Technologies, California, USA), and capillary column fused silica HP-5MS (30 m × 0.25 mm i.d., film thickness, 0.25 µm). The oven temperature was programmed to linearly increase from 70 °C to 310 °C at 5 °C min<sup>-1</sup>, with 1 min heating at 310 °C and 60 min elution time. The system was balanced for 6 min at 70 °C before automatic injection of the subsequent sample. Injector temperature was 230 °C and detector temperature was 250 °C; an ion source at 200 °C, carrier Helium gas at 1.0 mL min<sup>-1</sup>, constant pressure mode, injection volume 1 µL; split ratio 10:1. Electron-impact mass spectra (EI-MS; 70 eV) were acquired over the mass-to-charge ratios (m/z) range to 50 to 650 and a scan interval of 2 scan s<sup>-1</sup>. The identification of geopropolis compounds was based on the percentage of similarity plus comparison of mass spectra (MS) using software NIST AMIDS version 2.0 data library, with the percentage of total ion chromatograms (TIC%). Gallic acid and ellagic acid, standards, were co-chromatographed and identified on the basis of the retention times (RT) and mass spectra (MS) fragmentation.

### Statistical analysis

All analyses were performed in triplicate. The results are expressed as the mean ± standard deviation (SD) and were analyzed using the GraphPad Prism 5.0 software. Comparisons between groups were made using analyses of variance (ANOVA) followed by Tukey's test *p* value ≤ 0.05 and Pearson's correlation.

## RESULTS

Total phenolic content ranged from 126.6 to 847.5 mg GAE g<sup>-1</sup> (Table 1), and antioxidant activity (expressed as IC<sub>50</sub> in µg mL<sup>-1</sup> and as mmol Fe<sup>II</sup> g<sup>-1</sup>) of the hydroalcoholic extracts

ranged from 4.24 to 44.44  $\mu\text{g mL}^{-1}$  and 1.29 to 18.42  $\text{mmol Fe}^{\text{II}} \text{g}^{-1}$  (Table 1).

In the DPPH assay, HEG4 ( $\text{IC}_{50}$  value of 4.24  $\mu\text{g mL}^{-1}$ ) and HEG3 (5.92  $\mu\text{g mL}^{-1}$ ) had higher antioxidant activity than HEG1 and HEG2 ( $\text{IC}_{50}$  value of 19.05  $\mu\text{g mL}^{-1}$  and 44.44  $\mu\text{g mL}^{-1}$ , respectively). The FRAP assay showed that HEG3 and HEG4 exhibited the best ferric-reducing property (13.59 and 18.42  $\text{mmol Fe}^{\text{II}} \text{g}^{-1}$ , respectively), which was higher than that observed for Trolox. The correlations between the results of DPPH and FRAP assays and TPC are shown in Table 2.

A negative correlation was observed between DPPH and TPC (-0.878) and DPPH and FRAP (-0.836), in which a low  $\text{IC}_{50}$  value in the DPPH assays was correlated with a high TPC and a high FRAP value and vice versa. The correlation between TPC and FRAP was positive (0.843), indicating a high reducing power.

The UV spectra of the geopropolis extracts were also evaluated. All four extracts had absorption peak at  $\lambda_{\text{max}}$  at 268-275 nm, which was compatible with the presence of phenolic compounds.

**Table 1.** Total phenolic content (TPC) concentrations (mg GAE g<sup>-1</sup>) and antioxidant activity, 2,2-diphenyl-1-picrylhydrazyl, DPPH ( $\text{IC}_{50}$  in  $\mu\text{g mL}^{-1}$ ), and ferric reducing antioxidant power, FRAP (in  $\text{mmol Fe}^{\text{II}} \text{g}^{-1}$ ), mean values ( $\pm$  standard deviation; n = 3) of the hydroalcoholic extract of geopropolis (HEG) in two phytogeographical regions, flooded fields and cerrado, in the municipalities of Palmeirândia and Fernando Falcão, Maranhão State, northeastern Brazil. HEG1 and HEG2 are geopropolis collected at Palmeirândia; HEG3 and HEG4 are geopropolis collected at Fernando Falcão.

| Extracts (HEG) | TPC (mg GAE g <sup>-1</sup> ) | DPPH $\text{IC}_{50}$ ( $\mu\text{g mL}^{-1}$ ) | FRAP ( $\text{mmol Fe}^{\text{II}} \text{g}^{-1}$ ) |
|----------------|-------------------------------|---|---|
| HEG1           | 212.30 $\pm$ 0.290 a          | 19.05 $\pm$ 0.012 a                             | 1.78 $\pm$ 0.007 a                                  |
| HEG2           | 126.60 $\pm$ 0.840 b          | 44.44 $\pm$ 0.813 b                             | 1.29 $\pm$ 0.016 a,d                                |
| HEG3           | 847.50 $\pm$ 0.040 c          | 5.92 $\pm$ 0.120 c                              | 13.59 $\pm$ 0.430 b                                 |
| HEG4           | 348.30 $\pm$ 0.005 d          | 4.24 $\pm$ 0.015 c                              | 18.42 $\pm$ 0.210 c                                 |
| Gallic acid    | -                             | 1.83 $\pm$ 0.030 d                              | 0.73 $\pm$ 0.040 d                                  |
| Trolox         | -                             | 5.11 $\pm$ 0.040 c                              | 9.09 $\pm$ 0.100 e                                  |

Different letters in the same column indicate a significant difference by Tukey test, p < 0.05.

**Table 2.** Pearson correlation coefficient between the 2,2-diphenyl-1-picrylhydrazyl (DPPH) and ferric reducing antioxidant power (FRAP) assays and total phenolic content (TPC).

|      | DPPH   | FRAP   |
|------|--------|--------|
| TPC  | -0.878 | 0.843  |
| DPPH |        | -0.836 |
| FRAP | -0.836 |        |

The chromatograms obtained by GC/MS permitted the identification of a large number of compounds in the four geopropolis extracts (Table 3). HEG1 and HEG2 had a similar composition. Triterpenoids were the main compounds, including cycloartane- (cycloartenol, 5.39% and 4.12%), oleanane- ( $\beta$ -amyrin, 1.23% and 2.66%), and ursane-type triterpenoids (cycloursane, 1.62% and 0.83% and 3-oxo-urs-

**Table 3.** Chemical composition of the hydroalcoholic extract of geopropolis (HEG) in two phytogeographical regions, flooded fields and cerrado, in the municipalities of Palmeirândia and Fernando Falcão, Maranhão State, northeastern Brazil, expressed in percentage of total ion chromatograms (TIC%). HEG1 and HEG2 are geopropolis collected at Palmeirândia; HEG3 and HEG4 are geopropolis collected at Fernando Falcão). Each value denotes the mean of three analyses.

| Compound class | Chemical constituents        | Extracts (HEG) TIC (%) |      |       |       |
|----------------|------------------------------|------------------------|------|-------|-------|
|                |                              | HEG1                   | HEG2 | HEG3  | HEG4  |
| Fatty acids    | Palmitic                     | 0.16                   | 0.12 | 0.52  | 1.05  |
|                | Stearic                      | 0.46                   | 0.39 | 1.53  | 3.44  |
|                | Linoleic                     | 0.15                   | -    | -     | -     |
|                | Melissic                     | -                      | 1.35 | -     | -     |
|                | Octenoic                     | -                      | 0.14 | -     | -     |
| Organic acids  | Glycolic                     | -                      | 0.01 | -     | -     |
|                | Gluconic                     | 0.07                   | -    | -     | -     |
|                | Quinic                       | -                      | -    | 1.68  | 1.85  |
| Sugars         | Glucose                      | 0.08                   | 0.02 | 24.3  | 14.93 |
|                | Fructose                     | -                      | -    | 0.32  | 1.34  |
|                | Mannose                      | -                      | -    | 12.80 | -     |
|                | Arabinose                    | 0.15                   | -    | 0.13  | 1.24  |
|                | Galactose                    | 0.22                   | 0.04 | -     | -     |
|                | Fucose                       | 0.13                   | -    | -     | -     |
|                | Sorbose                      | 0.05                   | -    | -     | -     |
|                | Xylose                       | 0.45                   | -    | 0.61  | -     |
|                | Ribose                       | 0.12                   | -    | -     | -     |
|                | Aucubin                      | 0.17                   | 0.07 | -     | -     |
| Alcohols       | Erythritol                   | 0.25                   | 0.14 | -     | 0.28  |
|                | Arabitol                     | 0.06                   | -    | -     | -     |
|                | Sorbitol                     | 0.11                   | -    | -     | -     |
|                | Glycerol                     | -                      | -    | 0.22  | 0.27  |
|                | Xylitol                      | -                      | -    | 0.92  | -     |
| Phenolic acids | Inositol                     | -                      | -    | 0.10  | 0.45  |
|                | Protocatechuic               | 1.04                   | 0.38 | 0.10  | 0.13  |
|                | Gallic                       | 0.66                   | 1.03 | 22.30 | 18.90 |
| Triterpenes    | Ellagic                      | -                      | -    | 14.70 | 13.60 |
|                | Urs-12-en-24-oic acid, 3-oxo | 0.99                   | 0.61 | -     | -     |
|                | $\beta$ -Amyrin              | 1.23                   | 2.66 | -     | -     |
|                | Unknown triterpene           | 1.26                   | 1.65 | -     | -     |
|                | Cycloursane                  | 1.62                   | 0.83 | -     | -     |
| Steroids       | Cycloartenol                 | 5.39                   | 4.12 | -     | -     |
|                | Lanosterol                   | 0.18                   | 0.47 | -     | -     |
|                | Lanosterol acetate           | 0.02                   | -    | -     | -     |

12-en-24-oic acid, 0.99% and 0.61%, respectively). The extracts also contained steroids, the phenols protocatechuic acid (1.04% and 0.38%) and gallic acid (0.66% and 1.03%), fatty acids, and sugars.

The chemical composition of HEG3 and HEG4 did not contain triterpenoids or steroids, but had a high concentration of phenolic compounds such as gallic acid (22.3% and 18.9%, respectively) and ellagic acid (14.7% and 13.6%). These phenolic acids were identified and confirmed using an authentic standard based on mass-spectral fragmentation, total ion chromatograms (TIC%), and retention times (RT). Sugars, especially glucose (24.3% and 14.93%) and mannose (12.8%) were identified.

## DISCUSSION

In the state of Maranhão, meliponiculture is predominant activity in flooded fields and cerrado areas, especially in the municipalities of Palmeirândia and Fernando Falcão where the geopropolis samples were collected.

HEG3 and HEG4 contain higher levels of polyphenols than HEG1 and HEG2; thus, the determination of total phenol content has become a standard test and is usually evaluated by Folin-Ciocalteu method (Sawaya *et al.* 2011). Our findings are in line with the results of studies conducted by Cunha *et al.* (2009) on geopropolis extracts from Palmeirândia, Maranhão State, by Dutra *et al.* (2014) on geopropolis from Fernando Falcão, Maranhão State, and by Silva *et al.* (2013) on geopropolis produced by the Amazonian species *M. interrupta* and *M. seminigra*.

Two different methods, the DPPH scavenging and FRAP metal ions, were used to determine the antioxidant properties of geopropolis, which allowed us to obtain information about the activity of these extracts during different stages of the oxidation reactions (Souza *et al.* 2013).

According to Campos *et al.* (2003), extracts and natural substances are considered active at  $IC_{50} < 500 \mu\text{g mL}^{-1}$ . In the DPPH and FRAP assays, all extracts exhibited significant *in vitro* antioxidant activity. The correlations between the results of the DPPH and FRAP assays and TPC suggest that total phenols were responsible for the antioxidant activity and are consistent with results of studies investigating geopropolis produced by *M. interrupta*, *M. seminigra*, *M. fasciculata*, and *M. subnitida* (Silva *et al.* 2013; Souza *et al.* 2013; Dutra *et al.* 2014; Souza *et al.* 2014). A high phenolic content is related to antioxidant activity and all extracts exhibited high levels of polyphenols (Table 1), as confirmed by GC/MS (Table 3).

Chromatographic methods are essential for the analysis of products that contain complex mixtures, permitting the identification and quantification of biologically active compounds (Sawaya *et al.* 2011; Righi *et al.* 2013).

Geopropolis is a complex mixture of chemical substances. Thus, all extracts were subjected to GC/MS for complete analysis. MS provides information of molecular mass and structural information, and thereby the identification of the components of a mixture.

The triterpenic compounds found in our study corroborate the findings by Araújo *et al.* (2015) who analyzed the chemical composition of geopropolis collected in Palmeirândia. Triterpenes have been identified in propolis and geopropolis produced by stingless bees in Brazil and Mexico (Bankova and Popova 2007).

Protocatechuic acid has been detected in propolis produced by the stingless bee *Tetragonisca angustula* (Pereira *et al.* 2003) and in propolis (Kalogeropoulos *et al.* 2009) and pollen (Bonvehí *et al.* 2001) produced by *Apis mellifera*. However, there are no reports on the presence of this compound in geopropolis produced by *M. fasciculata*.

The chemical composition of HEG3 and HEG4 is similar, but differs from that of HEG1 and HEG2, as the former do not contain triterpenoids and steroids but exhibit high concentrations of phenolic compounds. Gallic acid and its derivatives have been identified in geopropolis and propolis of stingless bees in the Brazilian states of Maranhão (Dutra *et al.* 2014), Pernambuco, Paraná, São Paulo (Velikova *et al.* 2000), Piauí (Bankova *et al.* 1998) and Tocantins (Araújo *et al.* 2016).

HEG3 and HEG4 exhibited the highest *in vitro* antioxidant activities of the four extracts, suggesting a positive relationship between high levels of ellagic and gallic acids and antioxidant activity. Phenolic acids (gallic acid, ellagic acid, and protocatechuic acid) have been reported to be strong antioxidants (Kakkar and Bais 2014; Zhang *et al.* 2014).

The botanical sources used by stingless bees for the production of geopropolis can influence its chemical composition. Analysis of the geopropolis collected in Fernando Falcão suggests that the botanical sources are rich in phenolic compounds, particularly phenolic acids and hydrolyzable tannins found in HEG3 and HEG4, as it was also observed by Dutra *et al.* (2014).

It is noteworthy the presence of phenolic acids, triterpenoids, and steroids in geopropolis because it may predict the pharmacological properties of this natural product such as antimicrobial (Libério *et al.* 2011), antioxidant (Dutra *et al.* 2014), anticancer and immunomodulatory activities (Araújo *et al.* 2015).

## CONCLUSIONS

The geopropolis collected in Palmeirândia contained triterpene compounds of the cycloartane, ursane, and oleanane type as the main compounds, in addition to phenolic acids, protocatechuic and gallic acid. In contrast,

geopropolis collected in Fernando Falcão contained high concentrations of phenolic acids (gallic acid and ellagic acid) and exhibited high antioxidant activity, suggesting that the high levels of phenolic acids are responsible for the antioxidant property of this geopropolis. The chemical composition and antioxidant activity contribute to the identity and quality of the types of geopropolis produced by *M. fasciculata* collected in two phytogeographical regions of the Maranhão State, northeastern Brazil.

## ACKNOWLEDGEMENTS

The authors wish to thank the Coordination for the Improvement of Higher Education Personnel (Project No 925/2010), the National Council for Scientific and Technological Development (Project No. 554318/2010-5) and the Foundation for the Support of Research Scientific and Technological Development of the State of Maranhão (Project No. 00963/09) for financial support. Thanks to the beekeepers for donating the geopropolis samples.

## REFERENCES

- Araújo, M.J.A.M.; Búfalo, M.C.; Conti, B.J.; Fernandes Junior, A.; Trusheva, B.; Bankova, V.; Sforcin, J.M. 2015. The chemical composition and pharmacological activities of geopropolis produced by *Melipona fasciculata* Smith in Northeast Brazil. *Journal of Molecular Pathophysiology*, 4: 12-20.
- Araújo, K.S.S.; Santos Júnior, J.F.; Sato, M.O.; Finco, F.D.B.A.; Soares, I.M.; Barbosa R.S.; Alvim, T.C.; Ascêncio, S.D.; Mariano, S.M.B. 2016. Physicochemical properties and antioxidant capacity of propolis of stingless bees (Meliponinae) and *Apis* from two regions of Tocantins, Brazil. *Acta Amazonica*, 46: 61-68.
- Bankova, V.; Christov, R.; Marcucci, M.C.; Popov, S. 1998. Constituents of Brazilian geopropolis. *Zeitschrift für Naturforschung C*, 53: 402-406.
- Bankova, V. 2009. Chemical diversity of propolis makes it a valuable source of new biologically active compounds. *Journal of ApiProduct and ApiMedical Science*, 1: 23-28.
- Bankova, V.; Popova, M. 2007. Propolis of stingless bees: a promising source of biologically active compounds. *Pharmacognosy Reviews*, 1: 88-92.
- Barth, O. M.; Freitas, A.S. 2015. Palynology as a tool to distinguish between propolis and geopropolis: southern Brazilian samples. *Open Access Library Journal*, 2: e2217.
- Bezerra, J.M.D. 2002. Meliponicultura: Uma atividade essencial para a economia familiar do Trópico Úmido. In: Moura, E.G. (Org). *Agroambientes de transição entre o trópico úmido e o semi-árido: Atributos, alterações e uso na produção familiar*. Universidade Estadual do Maranhão, São Luís, Maranhão, p.144-203. (<http://www.iica.org.br/docs/publicacoes/publicacoesiica/agroambientestransicao.pdf>) Accessed on 20/09/2015.
- Bonvehí, J.S.; Torrentó, M.S.; Lorente, E.C. 2001. Evaluation of polyphenolic and flavonoid compounds in honeybee collected pollen in Spain. *Journal of Agricultural and Food Chemistry*, 49: 1848-1853.
- Campos, M.G.; Webby, R.F.; Markham, K. R.; Mitchell, K.A.; Cunha, A.P. 2003. Age-induced diminution of free radical scavenging capacity in bee pollens and the contribution of constituent flavonoids. *Journal of Agricultural and Food Chemistry*, 51: 742-745.
- Campos, J.F.; Santos, U.P.; Macorini, L.F.B.; Melo, A.M.M.F.; Balestieri, B.P.J.; Paredes-Gamero, E.J.; Cardoso, C.A.L.; Souza, K.P.; Santos, E.L. 2014. Antimicrobial, antioxidant and cytotoxic activities of propolis from *Melipona orbignyi* (Hymenoptera, Apidae). *Food and Chemical Toxicology*, 65: 374-380.
- Cunha, M.S.; Dutra, R.P.; Batista, M.C.A.; Abreu, B.V.B.; Santos, J.R.; Neiva, V.A.; Amaral, F.M.M.; Ribeiro, M.N.S. 2009. Padronização de extrativos de geopropolis de *Melipona fasciculata* Smith (túba). *Cadernos de Pesquisa*, 16: 31-38.
- Dutra, R.P.; Nogueira, A.M.C.; Marques, R.R.O.; Costa, M.C.P.; Ribeiro, M.N.S. 2008. Pharmacognostic evaluation of geopropolis of *Melipona fasciculata* Smith from Baixada Maranhense, Brazil. *Revista Brasileira de Farmacognosia*, 18: 557-562.
- Dutra, R.P.; Abreu, B.V.B.; Cunha, M.S.; Batista, M.C.A.; Torres, L.M.B.; Nascimento, F.R.F.; Ribeiro, M.N.S.; Guerra, R.N.M. 2014. Phenolic acids, hydrolyzable tannins, and antioxidant activity of geopropolis from the stingless bee *Melipona fasciculata* Smith. *Journal of Agricultural and Food Chemistry*, 62: 2549-2557.
- Holanda, C.A.; Oliveira, A.R.; Costa, M.C.P.; Ribeiro, M.N.S.; Souza, J.L.; Araújo, M.J.A.M. 2012. Qualidade dos méis produzidos por *Melipona fasciculata* Smith da região do Cerrado maranhense. *Química Nova*, 35: 55-58.
- Kakkar, S.; Bais, S. 2014. A review on protocatechuic acid and its pharmacological potential. *International Scholarly Research Notices Pharmacology*, 2014: ID 952943.
- Kalogeropoulos, N.; Konteles, S.J.; Troullidou, E.; Mourtzinis, I.; Karathanos, V.T. 2009. Chemical composition, antioxidant activity and antimicrobial properties of propolis extracts from Greece and Cyprus. *Journal of Agricultural and Food Chemistry*, 116: 452-461.
- Kerr, W.E. 1987. Abelhas indígenas brasileiras (meliponíneos) na polinização e na produção de mel, pólen, geopropolis e cera. *Informe Agropecuário*, 13: 15-27.
- Libério, S.A.; Pereira, A.L.A.; Dutra, R. P.; Reis, A.S.; Araújo, M.J.A.M.; Mattar, N.S.; et al. 2011. Antimicrobial activity against oral pathogens and immunomodulatory effects and toxicity of geopropolis produced by the stingless bee *Melipona fasciculata* Smith. *BioMed Central Complementary and Alternative Medicine*, 11: 108.
- Martins, A.C.L.; Rêgo, M.M.C.; Carreira, L.M.M.; Albuquerque, P.M.C. 2011. Espectro polínico de mel de túba (*Melipona fasciculata* Smith, 1854, Hymenoptera, Apidae). *Acta Amazonica*, 41: 183-190.
- Muniz, F.H. 2002. A vegetação da região de transição entre a Amazônia e o Nordeste, diversidade e estrutura. In: Moura,

- E.G. (Org). *Agroambientes de Transição entre o Trópico Úmido e o Semi-árido: Atributos, alterações e uso na produção familiar*. Universidade Estadual do Maranhão, São Luís, Maranhão, p.44-60.
- Nogueira-Neto, P. 1997. *Vida e criação de abelhas indígenas sem ferrão*. Nogueirapis, São Paulo, São Paulo, 446p.
- Pedro, S.R.M. 2014. The stingless bee fauna in Brazil (Hymenoptera: Apidae). *Sociobiology*, 61: 348-354.
- Pereira, A.S.; Bicalho, B.; Aquino-Neto, F.R. 2003. Comparison of propolis from *Apis mellifera* and *Tetragonisca angustula*. *Apidologie*, 34: 291-298.
- Ribeiro, M.H.M.; Luz, C.F.P.; Albuquerque, P.M.C. 2013. Pollen analysis of geopropolis of *Melipona (Melikerria) fasciculata* Smith, 1854 (Meliponini, Apidae, Hymenoptera) in areas of restinga, cerrado and flooded fields in the state of Maranhão, Brazil. *Grana*, 52: 81-92.
- Righi, A. A.; Negri, G.; Salatino, A. 2013. Comparative chemistry of propolis from eight Brazilian localities. *Evidence-Based Complementary and Alternative Medicine*, 2013: ID 267878.
- Sawaya, A.C.H.F.; Cunha, I.B.S.; Marcucci, M.C. 2011. Analytical methods applied to diverse types of Brazilian propolis. *Chemistry Central Journal*, 5: 27.
- Silva, E.C.C.; Muniz, M.P.; Nunomura, R.C.S.; Nunomura, S.M.; Zilse, G.A.C. 2013. Constituintes fenólicos e atividade antioxidante da geoprópolis de duas espécies de abelhas sem ferrão amazônicas. *Química Nova*, 36: 628-633.
- Slaa, E.J.; Chaves, L.A.S.; Malagodi-Braga, K.S.; Hofstede, F.E. 2006. Stingless bees in applied pollination: practice and perspectives. *Apidologie*, 37: 293-315.
- Souza, S.A.; Camara, C.A.; Silva, E.M.S.; Silva, T.M.S. 2013. Composition and antioxidant activity of geopropolis collected by *Melipona subnitida* (Jandaíra) bees. *Evidence-Based Complementary and Alternative Medicine*, 2013: ID 801383.
- Souza, S.A.; Dias, T.L.M.F.; Silva, T.M.G.; Falcão, R.A.; Moreira, M.S.A.; Silva, E.M.S.; Camara, C.A.; Silva, T.M.S. 2014. Chemical composition, antinociceptive and free radical-scavenging activities of geopropolis from *Melipona subnitida* Ducke (Hymenoptera: Apidae: Meliponini). *Sociobiology*, 61: 560-565.
- Velikova, M.; Bankova, V.; Marcucci, M.C.; Tsvetkova, I.; Kujumgiev, A. 2000. Chemical composition and biological activity of propolis from Brazilian meliponinae. *Zeitschrift für Naturforschung C*, 55: 785-789.
- Zhang, L.L.; Wang, Y.M.; Xu, M.; Wu, D.M.; Chen, J.H. 2014. Quantification of gallic acid and ellagic acid from the seed of *Cornus officinalis* by UHPLC method and their antioxidant activity. *Chemical Engineering Communications*, 201: 545-556.

Recebido em 08/01/2016

Aceito em 25/03/2016

