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Phenolic profile, antioxidant activity and palynological analysis of stingless bee honey from Amazonas, Northern Brazil

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ABSTRACT

In this study honey samples produced by *Melipona (Michmelia) seminigra merrillae*, collected in seven counties distributed in the central and southern region of Amazonas state in Brazil, were analysed for their botanical origin, content and profile of phenolic compounds, and antioxidant and antimicrobial activities. Twenty-two pollen types were identified. The total phenolic content ranged from 17 to 66 mg GAE/g of extract; the highest contents were found in honeys produced from pollen types such as *Clidemia* and *Myrcia*. The antioxidant activity was higher in the samples that contained higher quantities of phenolic compounds. In relation to the antibacterial activity, samples CAD3, CAD4 and SAD3 presented the best results. Fourteen phenolic compounds were determined. Among them, we identified the flavonoid taxifolin, which has not previously been described in honeys from stingless bees, and we report the identification of catechol in Brazilian honey samples for the first time.

1. Introduction

Honey is characterised by its complex composition, which varies with the origin of the raw material as nectar or honeydew, the bee species, the edaphoclimatic conditions, the available floral source and the storage conditions (Gheldof & Engeseth, 2002). Honey mainly consists of glucose and fructose but also contains amino acids, phenolic compounds, organic acids, vitamins, minerals, lipids, enzymes and other phytochemicals (Baltrušaityte, Venskutonis, & Čeksteryte, 2007).

In the Northern and Northeastern regions of Brazil, there are several native stingless bee species that produce honey and are known as indigenous bees, stingless or meliponini (Silva et al., 2013). In the state of Amazonas, among the several species already identified, attention should be given to *Melipona (Michmelia) seminigra merrillae* Cockerell, 1919 (Hymenoptera: Apidae: Meliponini), which is endemic to Central Amazonia and is an important pollinator of the Amazon rainforest (e.g., *Theobroma grandiflorum* Schum, *Bixa orellana* L., *Euterpe oleracea* Mart., and *Paullinia cupana* Kunth). The growing interest in the honey produced by stingless bees proceeds from its composition, which has been associated with antiseptic, antimicrobial, anticancer, anti-inflammatory, and wound-healing properties and may provide defence for and promote cell functions in erythrocytes (Alvarez-Suarez et al., 2012; Silva et al., 2006, 2013; Vit & Tomás-Barberán, 1998).

Hundreds of bioactive substances have already been found in honeys from the *Melipona* species in different countries (Oddo et al., 2008; Oliveira et al., 2012; Silva et al., 2013). Among the compounds with biological activity that are present in honeys, the compounds that display antioxidant capacity, such as phenolic acids, flavonoids and the enzymes glucose oxidase and catalase, have received special attention from research groups, due to their role in the prevention of diseases associated with oxidative stress (Aljadi & Kamaruddin, 2004). Silva et al. (2013) studied the phenolic profile of the ethyl acetate fraction of *Melipona* honey extract (*M. subnitida*) collected in Paraíba State and reported a strong relation between the results of the DPPH (1,1-diphenyl-2-picryl hydrazyl) and ABTS (2,2-azinobis-3-ethylbenzothiazoline-6-sulfonic acid) antioxidant assays and the phenolic compounds 3,4-dihydroxybenzoic acid, gallic acid, and vanillic acid.

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However, the phenolic compounds present in honey are directly related to botanical resources, such as pollens, nectars, resins and oils that are supplied to the bees, and consequently, honeys from different floral origins possess distinct bioactive properties (Aljadi & Kamaruddin, 2004). The botanical and geographical origin of honey may be evaluated through melissopalynology, which is used to assess the pollen types present in the honey and to suggest its floral source. In the Brazilian Amazonia, few melissopalynological studies have been conducted since the 1980s. Pollen foraging has been studied, especially in the genus *Melipona*; however, the pollen found in *Melipona* honey has been poorly studied in this region (Rech & Absy, 2011).

Taking into account all these aspects, the present study was undertaken with the purpose of determining the botanical origin and phenolic compound profile of honeys produced by the species *M. (Michmelia) s. merrillae* in seven counties of the Amazonas state in the Northern region of Brazil. In addition, we evaluated the honeys for antioxidant and antimicrobial activities.

2. Materials and methods

2.1. Standards and reagents

The reagents Folin–Ciocalteu, potassium persulfate, Trolox (6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid 97%), ascorbic acid, gallic acid, ABTS [2,2′-azinobis-(3-ethylbenzothiazoline-6-sulfonic acid)] and the phenolic standards were supplied by Sigma–Aldrich (St. Louis, MO). The solvents ethyl acetate, methanol, ethanol and DSMO were supplied by Cinética e Tédia (Brazil), and the Mueller–Hinton agar and the Sabouraud Dextrose Agar were purchased from Difco Laboratories (Detroit, MI).

2.2. Honey samples

The samples of honey from the species *M. s. merrillae* were collected from beehive meliponaries in seven counties from Amazonas state, Brazil. There were four counties chosen from the Central region of Amazonas state [Manaus (CAD1), Rio Preto da Eva (CAD2), Coari (CAD3) and Maués (CAD4)], and there were three counties chosen from the Southern region of Amazonas [Boca do Acre (SAD1), Pauini (SAD2) and Lábrea (SAD3)]. The collection was performed with 20-mL sterile disposable syringes, and the honey was transferred to 600-mL polyethylene bottles, which were stored at 8 °C until analysis.

2.3. Methanol extracts and ethyl acetate fractions of the honeys

The methanol extracts and the ethyl acetate fractions of the honey samples were prepared following the methodology previously described by Andrade, Ferreres, and Amaral (1997). Initially, 50 g of honey, 250 mL of water acidified with hydrochloric acid (pH 2) and 100 g of Amberlite XAD-2 resin were mixed. After homogenisation using a magnetic stirrer for 30 min, the mix was transferred to a glass column (42 × 3.2 cm) and was washed with 250 mL of acidified water (pH 2), followed by 300 mL of distilled water. The elution was performed with 300 mL methanol. To obtain the extract, the solvent was removed at 40 °C under reduced pressure in a rotary evaporator.

Fractionation with ethyl acetate for the removal of sugars was performed utilising 1 g of the methanol extract, to which 5 mL of distilled water and 5 mL of ethyl acetate were added. The contents were transferred to a separating funnel, and the acetate fraction was collected and concentrated at 40 °C under reduced pressure in a rotary evaporator.

2.4. Melissopalynological analysis

Ten grams of each honey sample were taken, diluted in tepid water and 95% ethanol, centrifuged, de-hydrated with anhydrous acetic acid, submitted to the acetolysis method with acetic anhydride and sulfuric acid (9:1) and successively centrifuged (Erdtman, 1960). After the acetolysis process, slides containing glycerinated gelatin were prepared for the mounting of the pollen grains, which were later examined and identified by optical microscopy. The frequency classes were established from counting at least 300 pollen grains for each honey sample. The classification was based upon the following criteria: predominant pollen type (DP, >45%), secondary pollen type (SP, 16–45%), important minor pollen (IMP, 3–15%) and minor pollen (MP, <3%). The identification of pollen types found in each sample was based on pollen catalogues and comparison with the slide collection of the pollen libraries from the Federal University of the West of the Pará (PUFOPA) and the State University of the Santana Fair (PUEFS).

2.5. Total phenolic content analysis

The determination of total phenolic content of the honey samples and the ethyl acetate fractions (EtOAct) was conducted by the colorimetric Folin–Ciocalteu method (Slinkard & Singleton, 1977). A 300-μL aliquot of methanol extract (5 mg mL^{-1} in MeOH) was transferred to a test tube containing 60 μL of the Folin–Ciocalteu reagent and 2.46 μL of distilled water. The mixture was stirred for 1 min before 180 μL of Na_2CO_3 (15%) were added. The contents were stirred for an additional 0.30 min to obtain a final extract concentration of 0.2 mg mL^{-1}. The samples were kept in the dark for 2 h prior to analysis using a UV–Vis spectrophotometer at 760 nm. The total phenolic content (TFC) was determined by interpolation of the sample absorbance against a calibration curve built with gallic acid standards (0.001–0.015 mg mL^{-1} in ethanol) and expressed as milligrams of gallic acid equivalents per gram of extract (mg GAE/g). All the analyses were performed in triplicate.

2.6. Antioxidant activity analysis

2.6.1. $ABTS^{\cdot+}$ cation radical scavenging

The ABTS test was performed according to the methodology reported by Re et al. (1999). The cation radical $ABTS^{\cdot+}$ was synthesised by the reaction of a 7 mM ABTS solution with a 2.45 mM potassium persulfate solution. The mixture was kept at 23 °C in the dark for 16 h. Afterwards, the $ABTS^{\cdot+}$ solution was diluted with ethanol until an absorbance (A) of 0.7 at 734 nm was achieved in a UV–Vis spectrophotometer. Aliquots of 2.7 mL from the $ABTS^{\cdot+}$ solution were added, immediately after being prepared, to the sample solutions diluted in methanol (MeOH) to reach final concentrations between 0.1 and 0.5 mg mL^{-1}. After 10 min, the percentage inhibition of absorbance at 734 nm was calculated for each concentration, relative to the blank absorbance (ethanol). The scavenging capability of the $ABTS^{\cdot+}$ radical (%AS) was calculated using the following equation:

$$\%\ AS = 100(A_{\text{control}} - A_{\text{sample}})/A_{\text{control}}$$

where A_{control} is the control absorbance obtained from the $ABTS^{\cdot+}$ radical alcoholic solution, and A_{sample} is the radical absorbance in the presence of the sample or the trolox standard. The results were expressed as $CE_{50} \pm$ sd, where CE_{50} represents the sample concentration required to obtain half the $ABTS^{\cdot+}$ radical scavenging activity and sd is the calculated standard deviation.

2.7. HPLC analysis

The chromatographic analyses were conducted on a Shimadzu (Kyoto, Japan) high-performance liquid chromatograph (HPLC). The chromatograph was equipped with an automatic Rheodyne 7125i injector with a 20-μL loop and a diode array detector. The columns used were a Shimadzu LC-18 column (25 cm × 4.6 mm from Supelco, Bellefonte, PA), a Rexchrom LC-18 column (15 cm × 4.6 mm; Supelco) and a Shimadzu pre-column C-18 ODS. For the analysis of the phenolic acids, the elution system was composed of 5% formic acid (solvent A) and MeOH (solvent B). The elution conditions were: 0.01–15 min 20–30% B, 15–20 min 30% B, 20–30 min 30–40% B and 40–50 min 100% B, at a flow rate of 1.0 mL min^{-1}. For the monitoring, the wavelengths of 254 and 290 nm were employed.

For the determination of flavonoids, the elution system used 1% formic acid (solvent A) and MeOH (solvent B) for the mobile phase. The elution conditions were as follows: 0.01–3 min 40% B, 5–15 min 45% B, 17–25 min 50% B, 27–35 min 55% B and 40 min 40% B. For the monitoring, the wavelength of 320 nm was utilised. The flow rate of the mobile phase was 1 mL min^{-1}, and the oven temperature of the column was fixed at 35 °C. The identification of phenolic compounds was based on the retention times, the UV-spectra and chromatographic comparison (co-injection) with authentic markers (Silva et al., 2013).

Based on compounds previously found in honeys, the phenolic standards selected for comparison were as follows: apigenin, isorhamnetin, kaempferol, luteolin, myricetin, quercetin, tricetin, taxifolin, naringenin, ferulic acid, 3-hydroxy-4-methoxycinnamic acid, caffeic acid, *p*-coumaric acid, cinnamic acid, sinapic acid, 4-methoxycinnamic acid, chlorogenic acid, 3,4,5-trihydroxybenzoic acid, 3,4-dihydroxybenzoic acid, 4-hydroxybenzoic acid, syringic acid, (*trans*–*trans*)-abscisic acid, (*cis*–*trans*)-abscisic acid, salicylic acid, catechol, gallic acid and vanillic acid.

2.8. Determination of the antibacterial and antifungal activities

Strains of *Staphylococcus aureus*: ATCC 25923, *S. epidermidis* ATCC 12228, *Pseudomonas aeruginosa* and *Escherichia coli* were kept in Müller–Hinton agar (bacteria) at 4 °C, and strains of *Candida albicans* ATCC 6645, *C. tropicalis* ATCC 13803 and *C. krusei* LM 13 were kept in Sabouraud Dextrose Agar at 35 °C. All the strains were obtained from the Pharmaceutical Sciences Department, São Paulo University, Adolfo Lutz Institute, Brazil.

For the evaluation of the antimicrobial activity, the EtOAc fractions were solubilised in 10% dimethyl sulfoxide (DMSO) and were tested at concentrations ranging from 0.032 to 1.024 mg mL^{-1}. For each microorganism, a suspension at 10^6 CFU mL^{-1} (0.5 McFarland Scale) was prepared. Nutrient broth was used for the bacteria and Sabouraud Dextrose Agar for the yeasts. The determination of the minimal inhibitory concentration (MIC) was conducted by broth microdilution, with the microplates sealed and incubated at 35 °C for 24–72 h. The MIC was defined as the smallest concentration able to inhibit the growth of microorganisms. The result was expressed as the average of three separate tests (Souza, Stamford, Lima, & Trajano, 2007).

The antibacterial and antifungal activities were interpreted based on the following parameters: from no growth to 0.5 mg mL^{-1}, excellent/optimal activity; from no growth to 0.6–1.5 mg mL^{-1}, moderate activity; from no growth to over 1.6 mg mL^{-1}, low activity (Houghton, Howes, Lee, & Steventon, 2007). Chloramphenicol (0.1 mg mL^{-1}) and nystatin (100 IU mL^{-1}) were used for the negative control, and for the positive control, the inoculation was performed using only DMSO.

2.9. Statistical analysis

The analyses were made in triplicate and the results expressed as the average ± standard deviation. The analyses of correlations ($p \leqslant 0.05$) between the pollen, phenolic compounds and ABTS were investigated by multivariate statistical analysis in PAST 2.17.

3. Results and discussion

3.1. Melissopalynological analyses of honeys

A total of 22 pollen types, belonging to 16 different botanical families, were identified in the honey samples (Table 1). Five pollen types that were lacking an established botanical affinity were named "Undetermined". The Fabaceae family stood out in the pollen spectrum with six recognised pollen types. The high pollen diversity found in the honeys reflects the flora diversity of Amazonas state, a feature that favours the production of honeys with different characteristics.

The pollen type *Clidemia* from the Melastomataceae family was identified in six of the seven samples analysed. It is present in both state regions in which the honey samples were collected, with the smallest occurrence (1.34%) in CAD3 and the largest occurrence (90.96%) in CAD4 (Table 1). These data show that the bees *M. s. merrilae* collect material from species of the Melastomataceae family; however, plants from this family are often polliniferous and have a low nectar production. *Clidemia* and *Miconia* (Melastomataceae) constitute important protein sources for Meliponini, and their pollen grains are harvested by several stingless bee species in the Amazon. Moreover, Melastomataceae is typically found in vegetable formations in the Amazon rain forest. Its flowers show poricidal anthers, and they are therefore visited primarily by bees able to vibrate the anthers in a phenomenon known as buzz pollination, which is characteristic of bees such as *Bombus* and *Xylocopa* (Renner, 1989).

The honey samples collected in SAD1, CAD2 and CAD4, representing the two state regions analysed had *Clidemia* pollen in quantities greater than 65% of the overall identified pollen. In the analysed honey samples, no secondary pollen types were found, and the percentages of the important minor pollen and minor pollen were low. Nevertheless, we consider CAD2 and SAD1 as multifloral honeys due to the high frequency of pollen types related to polliniferous plants; counterpart CAD4 was classified as a monofloral honey, with more than 90% of *Clidemia* pollen and just two minor pollen types (Table 1). As an important contribution to the understanding of the botanical distribution over the geographical region, in CAD3, the *Clidemia* pollen type was identified only as minor pollen, and in SAD2 and CAD1, it was identified as a secondary pollen (Table 1). The distribution of the *Clidemia* pollen may be associated with features that limit the bee's access to flowers of this genus, or its incidence may not be prevalent in these counties. In spite of presenting the *Serjania* (Sapindaceae) as the predominant pollen type, SAD2 displayed *Clidemia* as a secondary pollen, however it corresponding to 33.8%.

In the honey collected in SAD3, the *Myrcia*, Myrtaceae family, pollen type was identified with a 77.6% occurrence rate, and SAD3 was classified as monofloral. Pollen from the genus *Myrcia* is often found in palynological analyses of *Melipona* bee products in the Amazon (Marques-Souza, 1996). The *Clidemia* pollen type was not found, but the collection location was away from urban areas and located in the Ituxi-Lábrea Indian extractive reserve, which displays characteristic native vegetation. CAD1 and CAD3 were classified as multifloral because they contained two and four secondary pollen types, respectively (Table 1).

Table 1
Pollen spectra, percentages and frequency classes of the pollen types in honeys produced by *Melipona (Michmelia) seminigra merrilae* (Apidae, Meliponini) from seven areas in Amazonas State, northern Brazil.

Plant family	Pollen type	Honey samples						
		SAD1	SAD2	SAD3	CAD1	CAD2	CAD3	CAD4
Anacardiaceae	*Tapirira guianensis*	–	–	–	–	–	22.52 (sp)	–
Araliaceae	*Schefflera morototoni*	–	2.01 (mp)	–	1.08 (mp)	–	27.08 (sp)	–
Arecaceae	*Maximiliana maripa*	–	–	–	0.36 (mp)	–	–	–
Arecaceae	*Euterpe*	–	–	–	–	–	6.17 (imp)	0.53 (mp)
Burseraceae	*Protium*	–	–	0.32 (mp)	–	5.17 (imp)	–	0.27 (mp)
Bignoniaceae	*Tabebuia*	–	–	1.26 (mp)	–	–	3.22 (imp)	–
Dilleniaceae	*Tetracera*	–	–	1.58 (mp)	–	–	–	–
Euphorbiaceae	*Alchornea triplinervia*	–	–	–	–	–	0.54 (mp)	
Fabaceae – Caesalpinioideae	*Chamaecrista*	–	–	11.36 (imp)	–	–	–	–
Fabaceae – Caesalpinioideae	*Mora paraensis*	4.44 (imp)	–	–	–	–	28.15 (sp)	–
Fabaceae – Caesalpinioideae	*Senna*	–	0.29 (mp)		–	4.86 (imp)		–
Fabaceae – Mimosoideae	*Mimosa pudica*	–	0.57 (mp)	–	37.91 (sp)	–	–	–
Fabaceae – Mimosoideae	*Stryphnodendron pulcherrimum*	–	3.44 (imp)	–	–	–	0.54 (mp)	–
Fabaceae – Papilionoideae	*Zornia echinocarpa*	–	–	0.95 (mp)	–	–	–	–
Malpighiaceae	*Byrsonima*	–	–	–	–	–	6.70 (imp)	–
Melastomataceae	*Clidemia*	86.98 (dp)	33.81 (sp)	–	38.63 (sp)	69.00 (dp)	1.34 (mp)	90.96 (dp)
Moraceae	*Brosimum*	0.30 (mp)	–	–	–	3.34 (imp)	–	–
Myrtaceae	*Myrcia*	–	7.16 (imp)	77.60 (dp)	–	1.22 (mp)	–	–
Sapindaceae	*Serjania*	0.59 (mp)	47.28 (dp)	–	14.08 (imp)	–	–	–
Sapotaceae	*Pouteria*	–	–	–	–	–	3.75 (imp)	–
Solanaceae	*Solanum paniculatum*	1.48 (mp)	–	6.94 (imp)	–	0.30 (mp)	–	–
Urticaceae	*Cecropia*	–	3.72 (mp)	–	–	–	–	–
Undetermined type 1		4.14 (imp)	–	–	–	–	–	–
Undetermined type 2		2.07 (mp)	0.86 (mp)	–	–	–	–	–
Undetermined type 3		–	0.86 (mp)	–	7.94 (imp)	14.29 (imp)	–	–
Undetermined type 4		–	–	–	–	1.82 (mp)	–	–
Undetermined type 5		–	–	–	–	–	–	8.24 (dp)
Undetermined type 6								
TOTAL		100	100	100	100	100	100	100

The samples were taken from the following municipalities: Boca do Acre (SAD1), Pauini (SAD2), Lábrea (SAD3), Manaus (CAD1), Rio Preto da Eva (CAD2), Coari (CAD3) and Maués (CAD4). Frequency classes: predominant pollen (DP, >45%), secondary pollen (SP, 16–45%), important minor pollen (IMP, 3–15%), and minor pollen (MP, <3%).

The analyses of correlation based on the pollen type results showed a dendrogram of similarity with a distribution of four clusters (A, B, C and D). The cluster A including the closely related samples SAD1, CAD2 and CAD4, where CAD4 and SAD1 showed more similarity among themselves than with sample CAD2. The similarity observed in this group is a consequence of the predominance of the *Clidemia* pollen type (Table 1). The samples SAD2 and CAD1 formed cluster B, which showed weak similarity with cluster A and high degree of differences with the samples CAD3 and SAD3, distributed in clusters C and D, respectively. In the cluster B the *Clidemia* pollen type also was found, however in minor quantities when compared to the cluster A. The negative correlation of CAD3 with all other samples probably is because only in this sample was identified the pollen from *Tapirira guianensis* (Anacardiaceae); this same behaviour in SAD3 could be explained by the identification of *Myrcia* pollen as predominant only this sample.

In the Brazilian Amazon, Meliponini species, including *M. s. merrillae*, have exploited floral sources such as *Byrsonima*, *Euterpe*, *Maximiliana*, *Mimosa*, *Myrcia*, *Schefflera* and *Solanum* (Marques-Souza, 1996). In a palynological work focused on pollen stored by 23 Meliponini species along the Rio Negro channel, Amazonas (Rech & Absy, 2011), the species cited as having been exploited by *M. s. merrillae* (and the other Meliponini) included related *Alchornea*, *Byrsonima* (subsp. *Cephalotrigona*), *Cecropia* (subsp. *Oxytrigona*, *Ptilotrigona* and *Tetragonisca*) and *Mora* (subsp. *Aparatrigona*). Melissopalynological analyses of the present study identified pollen grains of these same floral sources in the analysed honeys. However, even these data showed a diversity of pollen collected by Meliponini. Stingless bees may change their trophic niche throughout the year due to several factors. The availability of floral resources (pollen, nectar and resin), climatic oscillations, distance between the colony and the flowering plant species, and competition exerted by exotic and other native bee species represent some factors that contribute to oscillations.

3.2. Total phenolic content and antioxidant activity

The total phenolic content of the methanol extracts of the honey samples ranged from 17 to 66 mg GAE/g of extract (Table 2). These figures are related to the honey floral source, because the phenolic compounds are related to the botanical origin of the nectar and pollen and to the species of the honey-producing bees (Gheldof & Engeseth, 2002). The samples with predominance of a single pollen type, CAD4, SAD3 and CAD2, presented the highest quantities of total phenolic contents, and the lowest total phenolic contents were observed for the honeys SAD2 and SAD1.

Table 2
Total phenolic content (TFC) and $ABTS^{\cdot+}$ radical scavenging activity of the methanol extract of the samples of honey produced by bees from the species *Melipona (Michmelia) seminigra merrillae* from Amazonas state.

Methanol extract	TFC (mg GAE /g[a])	$ABTS^{\cdot+}$ (CE_{50}) (mg/mL)
SAD1	26.5 ± 0.05	0.3 ± 0.01
SAD2	17.0 ± 0.02	0.3 ± 0.02
SAD3	64.0 ± 0.03	0.2 ± 0.03
CAD1	34.0 ± 0.01	0.2 ± 0.01
CAD2	43.0 ± 0.02	0.2 ± 0.02
CAD3	36.0 ± 0.01	0.2 ± 0.03
CAD4	66.0 ± 0.05	0.2 ± 0.01

Manaus, CAD1; Rio Preto da Eva, CAD2; Coari, CAD3; Maués, CAD4; Boca do Acre, SAD1; Pauini, SAD2; Lábrea, SAD3; CE_{50}, sample concentration required to obtain half of the $ABTS^{\cdot+}$ radical scavenging activity.
[A] Milligrams of gallic acid equivalent per gram of extract (method detection).

In relation to the $ABTS^{\cdot+}$ cation radical-scavenging activity, the methanol extracts of the honeys showed activities in which the CE_{50} varied from 210 ± 0.25 to 337 ± 3.17 mg mL^{-1} (Table 2). Among the honeys analysed, the samples collected at CAD4, SAD3 and CAD2 showed the highest antioxidant capacity, most likely as a consequence of their higher total phenolic content compared with the remaining samples, because antioxidant activity can be increased by the synergetic interaction between compounds that have the capacity to scavenge free radicals, such as phenolic compounds.

SAD1 and SAD2 showed the smallest total phenolic contents and displayed the smallest antioxidant activities. The results of the present paper agree with previous works that report the correlation between total phenolic contents and antioxidant activity (samples displaying smaller total phenolic contents also showed smaller antioxidant responses) (Aljadi & Kamaruddin, 2004; Alvarez-Suarez et al., 2012; Bertoncelj, Doberšek, Jamnik, & Golob, 2007).

The similarity between the honeys considering the results of total phenolic content and antioxidant activity showed a dendrogram with four clusters (A, B, C and D). The cluster A included the related samples CAD1, CAD3 and CAD2, which showed intermediary values for phenolic content and radical scavenging activity (Table 2). The cluster A had weakly correlation with cluster C, formed by SAD1 and SAD2; these two samples showed the lowest antioxidant activity, probably as a consequence of the lower phenolic compounds content when compared to the others samples (Table 2). The clusters A, C and B had no correlation with the cluster D. This cluster (D) included the samples SAD3 and CAD4, which possessed the highest antioxidant activity and the highest phenolic content (Table 2). Although the samples distribution in the cluster of phenolic content and antioxidant activity differed from that observed due to pollen type, it is interesting to note that the two samples with the highest antioxidant activity were monofloral honeys (Table 1).

3.3. Phenolic profile

The chromatography data showed the presence of 14 different phenolic compounds in the EtOAc fraction of the studied honeys (Table 3)

The phenolic compounds present in honey come from the nectar of flowers, pollen and propolis and are typically composed of benzoic and cinnamic acid and their esters, and some flavonoids (Estevinho, Pereira, Moreira, Dias, & Pereira, 2008; Silva et al., 2013). In the samples SAD1, SAD2, CAD2 and CAD1, gallic acid, 3,4-dihydroxybenzoic acid, 4-hydroxybenzoic acid, catechol and the isomers *trans–trans* abscisic acid and *cis–trans* abscisic acid were identified. In these samples, the predominant pollen type was the same (*Clidemia*), which reinforces the fact that the floral source may determine the phenolic profile in honeys.

The isomers *trans*,*trans*-abscisic acid and *cis*,*trans*-abscisic acid were found in high quantities in all the honey samples analysed, with the exception of CAD3. These two isomers of floral origin (Ferreres, Andrade, & Tomás-Barberán, 1996) were already described for honeys collected in New Zealand and Australia (Yao et al., 2003), in Slovenia (Bertoncelj, Polak, Kropf, Korošec, & Golob, 2011) and in Northeastern Brazil (Silva et al., 2013). Taking into account that abscisic acid regulates aspects related to plant physiology in response to water stress (Jiang & Hartung, 2008), its presence in the studied honeys is most likely a consequence of water stress suffered by botanic species in the Amazon region, which possesses an equatorial climate with elevated temperature. The absence of the isomers *trans*,*trans*-abscisic acid and *cis*,*trans*-abscisic acid in the CAD3 honey sample may be due to the botanical origin of the region, because the resources to be utilised by the bees depend on their availability in the collection area (Bertoncelj et al., 2011).

The occurrence of 1,2-dihydroxybenzene, also known as catechol, in the honey samples was similar to that of the abscisic acid isomers. This is, to the best of our knowledge, the first report of the presence of this compound in honeys.

The flavonoid taxifolin was found in all the analysed honeys, independent of the predominant pollen type or geographical localization. This is the first report of taxifolin in honeys produced by stingless bees, although taxifolin has been described in honeys from *Apis mellifera*. Phenolic compounds may be considered in determining the origin and authenticity of honey (Alvarez-Suarez et al., 2012; Tomás-Barberán, Martos, Ferreres, Radovic, & Anklam, 2001); however, other factors, in addition to the floral source, could be related to the presence of taxifolin in these honeys. Taxifolin is characterised by the presence of several hydroxyls that confer strong antioxidant activity. Additionally, several other biological activities have been ascribed to this flavonoid (Sendra, Sentandreu, & Navarro, 2007). The antioxidant activity found for the honeys in the present study most likely resulted from the interaction between taxifolin and the other identified phenolic compounds.

Gallic acid was also found in all the honey samples in quantities ranging from 18.2 to 92.7 μg/100 g. Indeed, the presence of gallic acid has been reported in honeys from several countries including Portugal (Andrade et al., 1997), New Zealand (Yao et al., 2003),

Table 3
Phenolic compounds present in the EtOAc fraction of the samples of honey produced by bees of the species *Melipona (Michmelia) seminigra merrillae* in Amazonas state.

Phenolic compounds	SAD1 (mg/100 mL)	SAD2 (mg/100 mL)	SAD3 (mg/100 mL)	CAD1 (mg/100 mL)	CAD2 (mg/100 mL)	CAD3 (mg/100 mL)	CAD4 (mg/100 mL)
Gallic acid	0.02	0.02	0.05	0.09	0.06	0.06	0.03
3,4-Dihydroxybenzoic acid	0.03	0.01	nd	0.20	0.01	nd	nd
4-Hydroxybenzoic acid	0.04	0.19	nd	0.42	0.15	0.06	0.16
Vanillic acid	0.03	nd	0.10	0.24	nd	nd	nd
Salicylic acid	0.02	nd	nd	0.17	0.06	nd	0.08
Syringic acid	nd	nd	nd	1.51	nd	0.29	0.40
Coumaric acid	nd	nd	nd	0.15	nd	0.04	0.14
Cinnamic acid	nd	nd	nd	0.11	nd	0.02	nd
Catechol	0.21	0.14	8.76	0.13	0.11	nd	0.17
trans,*trans*-Abscisic acid	0.67	0.57	0.71	1.60	1.06	nd	0.75
cis,*trans*-Abscisic acid	0.40	0.29	0.40	1.58	0.89	nd	0.18
Taxifolin	15.40	11.60	54.00	54.00	67.00	3.80	58.20
Naringenin	nd	nd	1.30	nd	nd	nd	nd
Luteolin	nd	nd	2.26	nd	nd	nd	nd

nd, not detected.

Table 4
Minimal inhibitory concentration (*MIC* μg mL^{-1}) of the acetate fraction of the honeys produced by bees of the species *Melipona (Michmelia) seminigra merrillae* in Amazonas state.

Samples	*MIC* (μg mL^{-1})						
	S. aureus ATCC 25923	*S. epidermidis* ATCC 12228	*C. tropicalis* ATCC 13803	*C. krusei* LM 13	*C. albicans* ATCC 6645	*P. aeruginosa* ATCC 25853	*E. coli* ATCC 11105
SAD1	+	+	+	+	+	+	+
SAD2	+	+	+	+	+	+	+
SAD3	256	256	512	512	512	512	512
CAD1	+	+	+	+	+	+	+
CAD2	+	+	+	+	+	+	+
CAD3	512	256	512	512	512	512	512
CAD4	256	256	512	512	512	512	512
NC	+	+	+	+	+	+	+
PC	−	−	−	−	−	−	−

+, microorganism growth; −, no growth of microorganism; NC, negative control; PC, positive control (chloramphenicol for bacteria and nystatin for fungi).

Australia (Yao, Jiang, Singanusong, Datta, & Raymont, 2004) and Brazil (Silva et al., 2013).

3.4. Antibacterial and antifungal activities

The results of the antimicrobial activity of the honey samples CAD1, CAD2 CAD3, CAD4, SAD1, SAD2 and SAD3 are presented in Table 4. Among the studied samples, the acetate fractions corresponding to CAD4, SAD3 and CAD3 were active against *S. aureus*, *S. epidermidis*, *P. aeruginosa*, *E. coli*, *C. krusei*, *C. tropicalis* and *C. albicans* with *MIC* values (minimal inhibitory concentration) ranging from 256 to 512 μg mL^{-1}.

The samples that showed the best antimicrobial activities also had the highest total phenolic contents. The antimicrobial activity of phenolic compounds has been reported by several research groups in studies on Gram+ and Gram− bacteria, as well as yeasts (Estevinho et al., 2008; Kačániová et al., 2011). Two of the three honey samples that showed the highest antimicrobial activity (CAD3; CAD4) had similar phenolic profiles that were distinct from the third sample (SAD3). However, other factors, in addition to the phenolic composition, like the presence of hydrogen peroxide, catalase and glucose oxidase, which are known to be present in honeys of diverse origins (Weston, Brocklebank, & Lu, 2000), may have contributed to the antimicrobial activity of the studied honeys. Moreover, the presence of a high content of catechol in SAD3 could contribute to its bioactivity.

4. Conclusions

The honeys CAD2, CAD4 and SAD3 showing showed a high frequency of the *Clidemia* (Melastomataceae) and *Myrcia* (Myrtaceae) pollen types and together with CAD3 showed the highest total phenolic contents. In the evaluation of the antioxidant activity, the highest ABTS$^{\cdot+}$ cation radical scavenging capacity was observed for the samples that displayed the highest total phenolic contents. In the antimicrobial activity tests, the best results were ascribed to samples CAD4, SAD3 and CAD3. We report the presence of the flavonoid taxifolin in honeys from stingless bees and the presence of catechol in Brazilian honey samples for the first time.

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