Received: 19 December 2017 | Revised: 5 February 2018 | Accepted: 14 February 2018

DOI: 10.1111/jfbc.12536

FULL ARTICLE

WILEY Journal of Food Biochemistry

Phenolic profile by HPLC-MS, biological potential, and nutritional value of a promising food: Monofloral bee pollen

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Funding information
State of São Paulo Research Foundation (FAPESP), Grant/Award Number: Process 2011/51741-5 (AAM De-Melo); Scholarship granted, Grant/Award Numbers: Process 2011/11746-8 and 2013/23179-6; National Council for Scientific and Technological Development (CNPq), Grant/Award Number: Process 304067/2013-0 (OM Barth); Postdoctoral fellowship financed by POPH-QREN, Grant/Award Number: SFRH/BPD/97049/2013 (MM Moreira)

Abstract

The objective of this study was to determine physicochemical and phenolic profiles as well the antioxidant and antimicrobial capacities of monofloral bee pollen samples from Brazil. Traditional methods were used. The phenolic profile was determined by high-performance liquid chromatography-mass spectrometry (HPLC-MS). The protein (10.6–33.9 g/100g), lipids (3.2–8.3 g/100g), ashes (2.6–3.8 g/100g), total phenolic (5.6–29.7 mg GAE/g), and total flavonoid (0.3–19.0 mg QE/g) values were variable, even between products with the same botanical origin. The minerals analyzed were found in amounts varying between 10 mg/kg and 9.1 g/kg. Each product presented specific color parameters. *Myrcia* bee pollen presented the greater antioxidant capacity, and *Mimosa scabrella* bee pollen from Santa Catarina state was the most efficient in inhibiting bacteria and yeasts. Among the compounds identified by HPLC-MS, flavonoid 3-O-glycosides predominated. The physicochemical and phenolic profiles of each sample were distinct, and there was no pattern between monofloral products of the same pollen type.

Practical applications

This manuscript reports physicochemical parameters, the biological potential, and the phenolic profile from HPLC-MS of some unknown monofloral bee pollen produced in Brazil. In addition to the possibility of the identification of phenolic compounds using a reliable technique, the current study helps to clarify one of the main questions of apicultural product researchers: Would monofloral bee pollens of the same botanical origin have similar profiles? Besides this, these results can increase consumer interest in this product and thus have positive impacts on apiculture in Brazil.

KEYWORDS

antimicrobial capacity, antioxidant capacity, bee products, mass spectrometry, physicochemical, polyphenols

1 | INTRODUCTION

Apiculture is one of the few agricultural activities that serve the triple bottom line, that is, that have a positive economic, social, and environmental impact. It is extremely important to encourage it, and the most effective approach to doing this is to increase the commercial value of bee products. Bee pollen, one of the main products from apiculture, is the result of the agglomeration of hundreds and even thousands of pollen grains harvested by *Apis mellifera* L. bees, which use their salivary secretions as well as nectar and/or honey to agglutinate the grains (Campos et al., 2008; Komosinska-Vassev, Olczyk, Kaźmierczak, Mencner, & Olczyk, 2015). It is common for bee pollen's physicochemical parameters to vary according to botanical origin because it is mainly composed of pollen grains (Barth et al., 2010; Campos et al., 2008).

Bee pollen contains macro- and micro-nutrients as well as compounds with biological potential, such as polyphenols. Antioxidant, antifungal, antibacterial, antiviral, anti-inflammatory, anti-osteoporosis, anti-allergic, anti-atherosclerosis, antimutagenic, immunostimulatory, hepatoprotective, and cardioprotective actions have already been attributed to these substances (Komosinska-Vassev et al., 2015). The nutritional and biological potential of bee pollen has attracted consumers looking for food supplements that are also capable of assisting in the prevention of certain diseases (Campos et al., 2008; De-Melo & Almeida-Muradian, 2017).

The composition of bee pollen has also awakened researchers' interest; however, the variety of products marketed is so great that the physicochemical properties of many remain unknown. Additionally, the determination of the phenolic profile of bee pollen by mass spectrometry remains a little-investigated area, and compounds of high biological potential could still be discovered. Therefore, considering the nutraceutical potential of this food, the need for more information regarding bee pollen produced in Brazil and the importance of precisely identifying the phenolic compounds present in this product, the aim of the current study was to determine the physicochemical profile, as well as the phenolic profile by HPLC-MS and the antioxidant and antimicrobial capacities, of monofloral bee pollen samples collected in different locations in Brazil.

2 | MATERIAL AND METHODS

2.1 | General

Reagents were purchased from Synth (São Paulo, SP, Brazil), and standards from Sigma-Aldrich (St. Louis, MO). The following equipment was used: nitrogen distiller (Tecnal, São Paulo, Brazil); digester block (Quimis, São Paulo, Brazil); Soxhlet extractor (Tecnal, São Paulo, Brazil); oven (Quimis, São Paulo, Brazil); ICP-OES spectrometer (Ciros Vision EOP, Spectro, Kleve, Germany); ColorQuest XE (Hunterlab, serial number: CQX 2329, Reston, VA); rotary evaporator with a vacuum system (Buchi, RE-111 and B-461, Flawil, Switzerland); spectrophotometer Shimadzu UV 1650 PC (Kyoto, Japan) for total phenolic, total flavonoid, and antioxidant capacity; spectrophotometer Varian UV–Visible Spectrophotometer Cary 50 Scan (California) for antimicrobial capacity; mass spectrometer LTQ Orbitrap XL (Bremen, Germany); as well as Shimadzu HPLC system (Software LabSolutions, Kyoto, Japan), with a column oven (CTO-10AS VP), an injection system (LC-20AD), an autosampler (SIL-20A HT), and a photodiode array detector (PDA) (SPD-M20A).

2.2 | Bee pollen samples

Eight monofloral bee pollen samples were produced by *Apis mellifera* bees in seven Brazilian states from April 2012 to September 2013 and subsequently collected and processed by beekeepers. Processing of the samples involved a dehydration step, in which, according to the producers, the temperatures ranged from 40 to 50°C. The products were sent to the Food Analysis Laboratory (FAL) of the Faculty of Pharmaceutical Sciences at the University of São Paulo (São Paulo, Brazil), where they were coded.

A portion of each sample was sent to the Palynology Laboratory (PL) of the Institute of Geosciences at the Federal University of Rio de Janeiro (Rio de Janeiro, Brazil) to confirm its botanical origin; another portion was sent to the Microbiology Laboratory (ML) of the Agrarian School at the Polytechnic Institute of Bragança (Bragança, Portugal) to determine its antimicrobial capacity as well as to identify and quantify the phenolic compounds by HPLC-MS; and a third portion was sent to the Agricultural and Environmental Technology Center (AETC) (Porangatu, Brazil) to analyze its mineral content. The samples were transported at room temperature and then stored at −4°C (FAL and ML) or at room temperature (PL and AETC). Before each analysis, the samples were crushed and sieved through a 0.595 mm sieve. It was not necessary to crush the bee pollen used to prepare methanolic extracts.

The botanical origin of the bee pollen was confirmed using the methodology proposed by Barth et al. (2010). All samples were classified as monofloral, since they had more than 90% of a unique pollen type. The code, the location, date of collection, and the botanical origin of the eight bee pollen samples from seven Brazilian states are presented in Table 1.

2.3 | Physicochemical parameters

Nitrogen concentration was determined by the micro-Kjeldahl method, and a factor of 6.25 was used to convert the value into protein. Ethereal extract (lipids) was determined by Soxhlet extraction method using diethyl ether as a solvent. Ashes were determined by the incineration of samples in an oven at 550°C for 8 hr (Almeida-Muradian, Arruda, & Barreto, 2012).

The minerals were extracted from a 500 mg sample by acid digestion with nitric acid and perchloric acid (2:1 v/v) in an open system. Then, the iron, copper, calcium, magnesium, zinc, manganese, sodium, and potassium concentrations were determined by inductively coupled plasma optical emission spectrometry (ICP-OES) as described by Morgano et al. (2012). The following operating parameters were used: forward power of 1.45 kW; cooling air flow rate of 12 L/min; auxiliary argon flow of 1 L/min; nebulizer air flow rate of 1 L/min; and sample flow rate of 2 mL/min. The wavelengths were specific for each element

TABLE 1 Geographical origin, collection date and frequency of the pollen types of eight monofloral bee pollen samples produced in seven Brazilian states

Sample/code	Geographical origin	Collection date	Pollen types (frequency)
Ala	Ribeirão Preto, São Paulo	Jun to Jul/13	***Alternanthera*** (94.2%)
Ana	Ribeirão Preto, São Paulo	Aug to Sep/13	***Anadenanthera*** (95%), *Schinus* (3.4%)
Cna	Una, Bahia	Sep/12	***Cocos nucifera*** (97.5%)
Mca	Neópolis, Sergipe	Apr to May/12	***Mimosa caesalpiniaefolia*** (96.3%), *Cocos nucifera* (3.7%)
Mya	Arvorezinha, Rio Grande do Sul	Nov/12	***Myrcia*** (99.5%)
MsaSC	Fraiburgo, Santa Catarina	Sep/12	***Mimosa scabrella*** (95.5%)
MsaRN	Taipu, Rio Grande do Norte	Oct/12	***Mimosa scabrella*** (98.7%)
MsaMT	Cáceres, Mato Grosso	Sep/13	***Mimosa scabrella*** (100%)

and a calibration curve was prepared with different concentrations of each mineral.

The instrumental color parameters were determined by reflectance using ColorQuest XE equipment operating CIE Lab [L^*: 0–100, a^*: (−) blue to (+) red, and b^*: (−) green to (+) yellow] with Universal Software V4.10. The analyses were performed in a 50 mm quartz cuvette and the results were expressed as $L^*a^*b^*$ color parameters.

2.4 | Preparation of extracts

The ethanolic extracts (ETE) and the methanolic extracts (MEE) were obtained using traditional methods (Carpes et al., 2008; De-Melo et al., 2016; Morais, Moreira, Feás, & Estevinho, 2011). For ETE, an ethanol solution (ethanol : distilled water, 70:30 v/v) was used, and only methanol was used for the MEE. All extractions were performed in triplicates, and the extracts were stored in an amber glass at −4°C until analysis.

2.5 | Total phenolic and total flavonoid contents

The ETE were analyzed according to Carpes et al. (2008), with the modifications proposed by De-Melo et al. (2016). All readings were performed with a spectrophotometer operating at 20°C. For the total phenolic content, a gallic acid standard curve was constructed to express the results in mg of gallic acid equivalents (GAE) per g of the sample. For the total flavonoid content, a quercetin standard curve was constructed, and the results were expressed in mg of quercetin equivalents (QE) per g of the sample.

2.6 | Antioxidant capacity

Two methods were used to determine the antioxidant capacity of the ETE. 2,-dipheny-1-picrylhydrazyl (DPPH) assay was performed according to Brand-Williams, Cuvelier, and Berset (2005) while considering the modifications proposed by De-Melo et al. (2016), and the oxygen radical absorbance capacity (ORAC) assay was conducted according to the procedure described by Huang, Ou, and Prior (2005), with the modifications also proposed by De-Melo et al. (2016). Different concentrations of Trolox were prepared to construct a standard curve, and the results were expressed in μmol of Trolox equivalents (TE) per gram of the sample.

2.7 | Antimicrobial capacity

The MEE were tested against American Type Culture Collection (ATCC) strains (LGC Standards S.L.U., Barcelona, Spain): *Escherichia coli* 25922^{TM}, *Klebsiella* BAA1705^{TM}, *Streptococcus pyogenes* 12344^{TM}, *Staphylococcus aureus* 25923^{TM}, and *Candida albicans* 60193^{TM}. The extracts were also tested against bacteria and yeasts isolated from biological fluids and identified in the Northeast Hospital Centre, Bragança, Portugal: *E. coli* ESA72, *Klebsiella* ESA61, *S. pyogenes* ESA12, *S. aureus* ESA54, and *C. albicans* ESA109. The microorganisms were stored and prepared according to the conditions described by De-Melo et al. (2016).

For analysis, the inoculum was transferred to an Erlenmeyer flask containing a sodium chloride solution (0.85 g/100 mL of distilled water). Then, the solution was adjusted to 0.5 on the MacFarland scale, confirmed by a spectrophotometric reading at 580 nm for bacteria and 640 nm for yeasts. Cell suspensions were finally diluted to 10^4 CFU/mL, and the tests were carried out according to the method described by Morais et al. (2011). Fluconazole and gentamicin were used as positive controls. The results were expressed as minimum inhibitory concentration (MIC), that is, the lowest concentration of MEE that inhibited visible growth of microorganisms.

2.8 | Identification and quantification of phenolic compounds

The ETE were analyzed to determine their phenolic profile using a mass spectrometer equipped with an electrospray ionization source and controlled by software LTQ Tune Plus 2.5.5 as well as Xcalibur 2.1.0. The instrument was operated in a negative-ion mode, the capillary (sprayer) voltage was set at 2,800 V, and the capillary temperature was set at 275°C. The flow rates of the sheath gas and the auxiliary gas (both nitrogen) were adjusted to 40 and 10 (arbitrary units), respectively. Electrospray ionization was performed at a capillary voltage of −28 V and a tube lens voltage of −150 V. The full scan covered the

TABLE 2 HPLC-MS (retention time, m/z fragments) and HPLC-PDA [calibration curves, limit of detection (LOD), and limit of quantification (LOQ)] parameters of standards used for identification and quantification of phenolic compounds in monofloral bee pollen produced in seven Brazilian states

	HLPC-MS					HPLC-DAD			
				MS/MS fragments					
Compound	R_t (min)	M_W	MS [M–H]$^-$ (*m/z*)	MS^2 (*m/z*)	MS^3 (*m/z*)	$m \pm \Delta m$*	$b \pm \Delta b$**	LOD (mg/L)	LOQ (mg/L)
Gallic acid	6.9	170	169	125	125, 97, 81	50,706 ± 214	−6,217 ± 5,928	0.55	1.83
Protocatechic acid	11.6	154	153	109	109	33,664 ± 661	−21,592 ± 19,863	2.77	9.24
Catechin	16.2	290	289	245, 205, 179	227, 203, 187, 161	13,105 ± 138	2,406 ± 3,573	1.28	4.27
Chlorogenic acid	19.2	354	353	191	173, 127, 111, 93, 85	56,066 ± 556	−32,375 ± 14,629	1.23	4.09
Vanillic acid	20.6	168	167	153,123	108	35,314 ± 321	−931 ± 7,773	1.03	3.45
Caffeic acid	22.4	180	179	135	135	10,6608 ± 566	−16,156 ± 13,030	0.57	1.91
Epicatechin	22.9	290	289			69,040 ± 491	−25,251 ± 12,141	0.83	2.75
β-resorcylic acid	23.2	154	153	109	109, 65	12,308 ± 49	−423 ± 1,368	0.52	1.74
Syringic acid	23.7	198	197	182,166	153	21,490 ± 574	16,716 ± 14,077	3.08	10.3
p-coumaric acid	30.3	164	163	119	119	127,784 ± 937	−3,060 ± 23,418	0.86	2.87
Ferulic acid	32.1	194	193	178, 149, 134	134	107,272 ± 902	−9,853 ± 22,762	1.00	3.32
Synapic acid	32.7	224	223	208, 179, 164	193, 164, 149	95,532 ± 1417	−8,888 ± 36,837	1.81	6.04
Naringin	40.1	580	579	459, 313, 271, 235	441, 357, 339, 271, 235	33,168 ± 49	616 ± 1,247	0.18	0.59
Rutin	43.5	610	609	301	273, 179, 151	31,543 ± 79	1,460 ± 1,971	0.29	0.98
Cinnamic acid	48.3	148	147			163,683 ± 393	19,094 ± 11,379	0.33	1.09
Naringenin	52.6	272	271	177, 151	107	62,404 ± 494	12,483 ± 12,394	0.93	3.11
Quercetin	53.6	302	301	273, 179, 151	151	64,868 ± 1312	−42,618 ± 31,783	2.30	7.67
Kaempferol	58.8	286	285	229, 151	185	73,460 ± 1244	14,914 ± 30,308	1.94	6.46

*m: slope ± standard deviation ($n = 5$) expressed in μV min/mg L.
**b: intercept ± standard deviation ($n = 5$) expressed in μV min.

TABLE 3 Nutritive value (dry-matter basis) and instrumental color parameters of monofloral bee pollen samples produced in seven Brazilian states

Parameters	Samples[1]							
	Ala	Ana	Cna	Mca	Mya	MsaSC	MsaRN	MsaMT
Proteins (g/100 g)	10.6 ± 0.4^{g}	16.0 ± 0.1^{d}	11.8 ± 0.3^{f}	17.6 ± 0.1^{c}	22.2 ± 0.1^{b}	14.4 ± 0.2^{e}	11.7 ± 0.1^{f}	33.9 ± 0.1^{a}
Lipids (g/100 g)	6.3 ± 0.6^{c}	3.2 ± 0.2^{e}	8.3 ± 0.3^{a}	4.9 ± 0.3^{d}	$7.8 \pm 0.7^{a,b}$	$7.0 \pm 0.3^{b,c}$	$6.8 \pm 0.1^{b,c}$	5.1 ± 0.4^{d}
Ashes (g/100 g)	3.1 ± 0.2^{d}	$3.0 \pm 0.1^{d,e}$	3.5 ± 0.0^{b}	3.8 ± 0.1^{a}	2.6 ± 0.0^{f}	2.8 ± 0.0^{e}	$3.2 \pm 0.1^{c,d}$	$3.4 \pm 0.0^{b,c}$
Minerals								
Ca (g/kg)	0.9 ± 0.1^{e}	0.8 ± 0.0^{e}	3.9 ± 0.1^{a}	3.2 ± 0.2^{b}	1.1 ± 0.0^{e}	1.7 ± 0.1^{c}	1.4 ± 0.1^{d}	1.4 ± 0.1^{d}
K (g/kg)	8.8 ± 0.2^{b}	7.5 ± 0.1^{c}	7.4 ± 0.1^{c}	9.1 ± 0.1^{a}	5.7 ± 0.1^{e}	6.6 ± 0.1^{d}	9.1 ± 0.1^{a}	7.6 ± 0.1^{c}
Mg (g/kg)	0.9 ± 0.0^{e}	$1.5 \pm 0.1^{c,d}$	2.3 ± 0.1^{a}	$1.6 \pm 0.0^{b,c}$	1.3 ± 0.1^{d}	1.3 ± 0.1^{d}	0.9 ± 0.1^{e}	1.8 ± 0.1^{b}
Na (mg/kg)	41.4 ± 1.4^{e}	40.8 ± 2.3^{e}	52.3 ± 1.3^{d}	148.5 ± 2.2^{c}	43.9 ± 1.3^{e}	38.0 ± 1.1^{e}	98.1 ± 1.6^{c}	279.3 ± 2.0^{a}
Zn (mg/kg)	50.8 ± 2.7^{d}	99.0 ± 2.7^{a}	72.6 ± 2.1^{c}	88.7 ± 2.7^{b}	67.6 ± 3.0^{c}	72.6 ± 2.7^{c}	65.6 ± 2.8^{c}	72.5 ± 2.0^{c}
Fe (mg/kg)	$1{,}017.5 \pm 5.1^{a}$	466.3 ± 5.0^{b}	109.0 ± 1.6^{e}	79.4 ± 0.6^{g}	142.2 ± 3.8^{d}	187.7 ± 1.9^{c}	94.5 ± 2.2^{f}	78.9 ± 0.5^{g}
Mn (mg/kg)	50.8 ± 4.3^{e}	89.1 ± 2.8^{c}	94.7 ± 2.8^{c}	69.3 ± 3.3^{d}	43.5 ± 1.6^{e}	112.3 ± 6.9^{b}	86.4 ± 2.2^{c}	314.0 ± 5.9^{a}
Cu (mg/kg)	10.0 ± 0.6^{e}	$11.3 \pm 0.6^{b,c,d}$	$11.6 \pm 0.0^{b,c}$	$10.8 \pm 0.0^{b,c,d,e}$	17.1 ± 0.6^{a}	11.8 ± 0.0^{b}	$10.2 \pm 0.6^{c,d,e}$	$11.5 \pm 0.6^{b,c}$
Inst. Color								
L^* (0–100)	51.6 ± 0.1^{h}	66.8 ± 0.4^{c}	71.7 ± 0.4^{a}	68.0 ± 0.5^{b}	$62.7\ 0.2^{e}$	59.8 ± 0.0^{f}	65.0 ± 0.3^{d}	58.6 ± 0.3^{g}
a^* (− or +)	9.0 ± 0.1^{b}	2.8 ± 0.2^{f}	1.1 ± 0.1^{g}	4.1 ± 0.1^{d}	5.9 ± 0.1^{c}	9.1 ± 0.0^{b}	3.7 ± 0.2^{e}	10.0 ± 0.1^{a}
b^* (− or +)	48.8 ± 0.7^{b}	41.2 ± 0.1^{c}	31.5 ± 0.2^{g}	35.4 ± 0.3^{f}	48.3 ± 0.1^{b}	54.5 ± 0.4^{a}	36.9 ± 0.3^{e}	39.0 ± 0.4^{d}

[1]**Ala,** *Alternanthera*; **Ana,** *Anadenanthera*; **Cna,** *Cocos nucifera*; **Mca,** *Mimosa caesalpiniaefolia*; **Mya,** *Myrcia*; **MsaSC,** *Mimosa scabrella* from Santa Catarina; **MsaRN,** *Mimosa scabrella* from Rio Grande do Norte; **MsaMT,** *Mimosa scabrella* from Mato Grosso.
Values correspond to the average ± standard deviation of three replicates. Averages followed by the same letter are not significantly different ($p > .05$).

mass range from *m/z* 100 to 1,000. Fragmentation experiments were performed using the iron trap with helium as the collision gas. The compounds were identified by comparing the spectra with phenolic standards and MS database (literature). The data generated by the analysis of the phenolic standards are presented in Table 2.

The quantification of phenolic compounds was carried out based on the procedure described by Rubilar, Pinelo, Shene, Sineiro, and Nuñez (2007), with some modifications. The HPLC system used was a Shimadzu equipped with *Phenomenex* Gemini C_{18} reversed phase column (250 × 4.6 mm, 5 μm) (Torrance, The United States). The ETE were filtered using a 0.22 μm nylon filter (Millipore, São Paulo, Brazil), and 20 μL of them was injected into the HPLC system. The mobile phase was composed of water (solvent A) and methanol (solvent B) that were both acidified with 0.1% formic acid, filtered using a 0.45 μm nylon filter (Whatman, Maidstone, The United Kingdom) and degassed in a sonicator for 10 min. The mobile phase flow rate was 1 mL/min, and the elution in the gradient mode occurred as follows: 0 min, 15% B in A; 20 min, 30% B in A; 40 min, 45% B in A; 45 min, 50% B in A; 50 min, 55% B in A; 65 min, 70% B in A; and 75–80 min, 100% B. Then, the system returned to the initial conditions to stabilize the column, whose temperature was maintained at 25°C.

The concentrations of some compounds were determined from a standard curve constructed with different concentrations of the standards in a methanol solution (methanol : water, 50:50 mL:mL). The standards were chosen based on the literature. The parameters of the calibration curves, limit of detection (LOD) and limit of quantification (LOQ) are presented in Table 2. The correlation coefficient (r^2) was at least .99. Detection was performed by scanning between 190 and 600 nm, and quantification was carried out at 280, 320 as well as 360 nm depending on the maximum absorption of the identified compound. The results were expressed as mg of a compound per 100 g of a sample.

2.9 | Statistical analyses

All analyses were performed in triplicate, and the results were presented as means ± standard deviation. ANOVA followed by Student's *t* test were used to identify differences at a significance level of 5%. All statistical analyses were performed using the SAS software (version 9.0, SAS Institute, Inc., Cary, NC).

3 | RESULTS AND DISCUSSION

3.1 | Physicochemical parameters

The physicochemical parameters of the eight bee pollen samples from seven Brazilian states are presented in Table 3. The Fe content in the *Alternanthera* (Ala) sample (1,017.5 ± 5.1 mg/kg) was 2–12 times greater than values observed in the other samples (78.9 ± 0.5 to 466.3 ± 5.0 mg/kg). In bee pollen, the Fe level can vary from 11.1 to 1,290 mg/kg, according to the botanical origin of the samples, the season and, principally, the soil characteristics of where the plant grew (De-Melo & Almeida-Muradian, 2017; Morgano et al., 2012). To the best of our knowledge, high Fe levels in Brazilian bee pollen have never been related to environmental pollution; therefore, the Fe content in the eight samples can be considered nutritionally interesting, since in a portion of only 25 g, 2–25 mg of this mineral was found.

The Zn levels in samples were similar to those observed in *Zea mays* monofloral bee pollen from Australia (48 mg/kg) and from Egypt (80 mg/kg), in heterofloral bee pollen from Argentina (23–106 mg/kg)

(De-Melo & Almeida-Muradian, 2017), and in *Brassica* monofloral bee pollen samples produced in Brazil (64.1–76.2 mg/kg) (De-Melo, 2015). The highest Zn level was found in the *Anadenanthera* (Ana) sample (99.0 ± 2.7 mg/kg), and the lowest level was in the Ala sample (50.8 ± 2.7 mg/kg). In the case of Ana and Ala samples, we suggest that all the differences observed in their compositions are related to botanical origin, because both were produced in the same apiary, same soil conditions, during winter, and with identical processing practices.

The highest Ca (3.9 ± 0.1 g/kg), Mg (2.3 ± 0.1 g/kg), and L^* parameter (71.7 ± 0.4) values were observed in the *Cocos nucifera* (Cna) sample. These results corroborate those observed by De-Melo (2015), who, using an exploratory multivariate approach to physicochemical parameters of 56 samples from different regions of Brazil, identified the tendency for *Cocos nucifera* monofloral bee pollen to have elevated levels of Ca, Mg, and L^*. One of the highest lipid values (8.3 ± 0.3 g/100 g) were also identified in the Cna sample. In bee pollen, this parameter can reach 22 g/100 g, and there is a strong influence of botanical origin on the values (De-Melo & Almeida-Muradian, 2017); however, the dehydration techniques adopted by the beekeeper can influence the extraction of these compounds at the time of analysis and, consequently, the result (De-Melo et al., 2016).

The highest ashes (3.8 ± 0.1 g/100 g) and *K* values (9.1 ± 0.1 g/kg) were observed in the *Mimosa caesalpiniaefolia* (Mca) sample. The *K* level in bee pollen can vary from 1.4, as observed by Morgano et al. (2012) in a heterofloral bee pollen produced in Brazil, to 38 g/kg, a value quantified in a monofloral bee pollen from *Asphodelus fistulosus* produced in Australia (De-Melo & Almeida-Muradian, 2017). Melo, Freitas, Barth, and Almeida-Muradian (2009) observed a negative correlation between the pollen type *M. caesalpiniaefolia* and the lipid content in bee pollen from Brazil, which could explain the fact that one of the lowest levels of this nutrient was found in the Mca sample (4.9 g/100 g).

The highest Cu content was found in the *Myrcia* (Mya) sample (17.1 ± 0.6 mg/kg) as well as the lowest values for ashes (2.6 ± 0.0 g/100 g), Ca (1.1 ± 0.0 g/kg), and Mn (43.5 ± 1.6 mg/kg). Generally, this food contained elevated levels of Cu, varying from 3 to 42 mg/kg (De-Melo & Almeida-Muradian, 2017). In monofloral samples from *Eucalyptus bridgesiana* collected in Australia, the average content for this mineral was 16.5 mg/kg, whereas it varied from 5.6 to 23.9 mg/kg in Poland (De-Melo & Almeida-Muradian, 2017) and oscillated between 3.2 and 25.4 mg/kg in other studies with Brazilian samples (Morgano et al., 2012).

The physicochemical parameters of the *Mimosa scabrella* monofloral samples varied between harvesting regions, possibly due to the pollen composition of the plants of this species being variable. In the *M. scabrella* from Rio Grande do Norte (MsaRN) sample, the K content was more pronounced (9.1 ± 0.1 g/kg). The *M. scabrella* from Santa Catarina (MsaSC) sample had the highest b^* value (54.5 ± 0.4), that is, a more intense tonality of yellow, whereas in the *M. scabrella* from Mato Grosso (MsaMT) sample, the a^* value (10.0 ± 0.1) was the highest among the samples, indicating a more intense tonality of red. The MsaMT sample also had the highest values of Mn (314.0 ± 5.9 mg/kg), Na (279.3 ± 2.0 g/kg), and protein (33.9 ± 0.1 g/100 g) among the three *M. scabrella* monofloral bee pollen samples. In bee pollen, protein levels can vary from 10 to 40 g/100 g, depending on the botanical origin (Campos et al., 2008); thus, the values observed not only in Msa samples but also in the others are within the expected range.

The Mn content in the Msa samples (86.4 ± 2.2 to 314 ± 5.9 mg/kg) should be emphasized. In bee pollen, the content of this mineral can reach 429.8 mg/kg, which is the value cited by De-Melo and Almeida-Muradian (2017) in samples collected in China. As far as we know, in Brazil, the highest Mn content observed until then was that found in the present study. Furthermore, all the samples contain a significant quantity of this mineral.

Based on the results of the present study and the literature, it is not possible to establish a physicochemical profile that would be characteristic of *M. scabrella* monofloral bee pollen produced in Brazil. This is possibly because there are differences in pollen composition, which can be related to genotypic variations and/or different environmental conditions during plant development.

The fact that all samples had low sodium content should be highlighted. All over the world, there exists a preoccupation with an excessive sodium intake, frequently correlated with the consumption of processed and industrialized foods. A portion of 25 g of the analyzed sample provides only approximately 0.05–0.3% of the maximum 2 g/day intake of Na recommended by the OMS (WHO, 2012).

3.2 | Biological potential

The biological activity indicators are presented in Table 4. The more elevated phenolic and total flavonoid levels were observed in the Mya bee pollen: 29.7 ± 0.3 mg GAE/g and 19.0 ± 0.6 mg QE/g, respectively. At the same time, the lowest values were observed in the Cna (total phenolics: 5.6 mg ± 0.0 GAE/g) and MsaMT (total flavonoid: 0.3 ± 0.0 mg QE/g) samples. As observed by De-Melo (2015), bee pollen with a predominance of *Cocos nucifera* produced in Brazil seems to have a tendency toward low levels of total phenolic.

In previous studies, the total phenolic content varied from 5.4 to 132.4 mg GAE/g, and the total flavonoid content between 0.6 and 27.2 mg QE/g in bee pollen from Brazil (Menezes, Maciel, Miranda, & Druzian, 2010; Vecchia, Pegoraro, Carpes, & Pegoraro, 2009). In Portugal, the values fluctuated between 10.5 and 45.9 mg GAE/g (total phenolic) as well as between 4.5 and 7.1 mg QE/g (total flavonoid) (Féas, Vázquez-Tato, Estevinho, Seijas, & Iglesias, 2012; Morais et al., 2011). Mărghitas et al. (2009), who analyzed monofloral bee pollen samples produced in Romania, observed total phenolic values between 4.4 and 16.4 mg GAE/g as well as total flavonoid between 3.8 and 13.6 mg QE/g. The variation observed in these parameters could be due to the botanical origin of the samples and the processing conditions adopted by the beekeepers (Campos et al., 2008; De-Melo et al., 2016).

The Mya sample also presented a greater antioxidant capacity (ORAC: 542.0 ± 20.7 μmol TE/g and DPPH: 110.8 ± 1.3 μmol TE/g) and was one of the products with the greatest antimicrobial capacity against yeasts (MIC: 11.5 ± 0.3 to 13.3 ± 1.0 mg/mL). The antioxidant capacity of the eight samples, by the DPPH method, was below that observed by Mărghitas et al. (2009) in samples collected in Romania (135–2,814 μmol TE/g). By ORAC, the values were close to those

TABLE 4 Total phenolics, total flavonoids, antioxidant, and antimicrobial capacities (dry-matter basis) of monofloral bee pollen samples produced in seven Brazilian states

Parameters	Samples[1]							
	Ala	Ana	Cna	Mca	Mya	MsaSC	MsaRN	MsaMT
Total phenolics (mg GAE/g)	11.2 ± 1.0^{e}	16.1 ± 1.1^{b}	5.6 ± 0.0^{g}	12.7 ± 0.4^{c}	29.7 ± 0.3^{a}	$11.3 \pm 0.4^{d,e}$	7.4 ± 0.3^{f}	$12.5 \pm 0.3^{c,d}$
Total flavonoids (mg QE/g)	1.0 ± 0.1^{d}	1.3 ± 0.1^{c}	1.1 ± 0.0^{d}	$1.3 \pm 0.0^{c,d}$	19.0 ± 0.6^{a}	1.5 ± 0.0^{c}	4.1 ± 0.1^{b}	0.3 ± 0.0^{e}
Antioxidant capacity								
DPPH (μmol TE/g)	15.5 ± 0.3^{e}	26.8 ± 0.6^{d}	10.0 ± 0.3^{f}	25.6 ± 1.3^{d}	110.8 ± 1.3^{a}	71.6 ± 1.8^{b}	31.6 ± 0.0^{c}	15.9 ± 0.1^{e}
ORAC (μmol TE/g)	178.3 ± 8.6^{d}	299.6 ± 4.6^{b}	$156.9 \pm 4.4^{d,e}$	224.1 ± 7.4^{c}	542.0 ± 20.7^{a}	313.3 ± 8.9^{b}	232.9 ± 8.6^{c}	133.7 ± 7.3^{e}
Antimicrobial capacity[2] (MIC[3]: mg/mL)								
Sp ESA12	$4.6 \pm 0.5^{b,c}$	$5.2 \pm 0.5^{a,b}$	$5.3 \pm 0.7^{a,b}$	$4.3 \pm 1.0^{b,c}$	$4.7 \pm 0.5^{a,b}$	1.4 ± 0.5^{e}	2.8 ± 0.6^{d}	4.0 ± 0.6^{c}
Sp 12344	$2.6 \pm 0.7^{b,c}$	3.9 ± 0.7^{a}	4.3 ± 1.0^{a}	$2.4 \pm 0.7^{b,c}$	$3.0 \pm 0.6^{a,b}$	1.1 ± 0.3^{d}	2.3 ± 0.6^{c}	$3.1 \pm 0.5^{a,b}$
Sa ESA54	$5.0 \pm 0.4^{c,d}$	$6.4 \pm 0.7^{a,b}$	6.8 ± 1.0^{a}	$5.3 \pm 0.9^{b,c,d}$	$5.6 \pm 0.5^{b,c}$	2.2 ± 0.4^{e}	$5.6 \pm 0.8^{b,c,d}$	4.8 ± 0.5^{d}
Sa 25923	3.5 ± 0.2^{c}	$5.0 \pm 1.0^{a,b}$	5.7 ± 0.2^{a}	3.3 ± 0.7^{c}	$4.3 \pm 0.9^{a,b,c}$	1.6 ± 0.2^{d}	$4.2 \pm 0.1^{a,b,c}$	$3.6 \pm 0.4^{b,c}$
Ec ESA72	$5.4 \pm 0.4^{b,c}$	$6.8 \pm 0.2^{a,b}$	8.4 ± 0.7^{a}	$5.4 \pm 0.4^{b,c}$	$6.6 \pm 0.6^{b,c}$	2.9 ± 0.4^{d}	$6.7 \pm 1.1^{b,c}$	5.3 ± 0.3^{c}
Ec 25922	4.0 ± 0.4^{b}	5.6 ± 0.8^{a}	5.7 ± 0.2^{a}	$3.9 \pm 0.9^{b,c}$	$5.2 \pm 0.2^{a,b}$	2.2 ± 0.6^{c}	$5.0 \pm 0.8^{a,b}$	$4.3 \pm 0.4^{a,b}$
K ESA61	6.5 ± 0.5^{b}	7.3 ± 0.4^{b}	9.3 ± 0.7^{a}	6.3 ± 0.2^{b}	7.2 ± 0.9^{b}	4.0 ± 0.5^{c}	6.9 ± 0.2^{b}	6.1 ± 0.5^{b}
K 1705	$4.1 \pm 0.8^{d,e}$	$6.1 \pm 0.6^{a,b}$	7.4 ± 0.7^{a}	$4.6 \pm 0.2^{c,d}$	$5.8 \pm 0.5^{b,c}$	2.9 ± 0.4^{e}	$6.0 \pm 0.1^{a,b,c}$	$4.9 \pm 0.7^{b,c}$
Ca SA109	12.6 ± 1.7^{d}	18.9 ± 2.4^{b}	22.2 ± 1.4^{b}	28.1 ± 3.6^{a}	$13.3 \pm 1.0^{c,d}$	9.3 ± 0.8^{d}	$18.3 \pm 1.0^{b,c}$	12.8 ± 1.4^{d}
Ca 60193	9.0 ± 1.9^{e}	$13.4 \pm 1.4^{b,c,d}$	16.2 ± 0.8^{b}	20.6 ± 2.7^{a}	$11.5 \pm 0.3^{c,d,e}$	8.2 ± 0.7^{e}	$13.7 \pm 1.2^{b,c}$	$10.1 \pm 0.8^{d,e}$

[1]**Ala,** *Alternanthera*; **Ana,** *Anadenanthera*; **Cna,** *Cocos nucifera*; **Mca,** *Mimosa caesalpiniaefolia*; **Mya,** *Myrcia*; **MsaSC,** *Mimosa scabrella* from Santa Catarina; **MsaRN,** *Mimosa scabrella* from Rio Grande do Norte; **MsaMT,** *Mimosa scabrella* from Mato Grosso.
[2]*Sp: Streptococcus pyogenes; Sa: Staphylococcus aureus; Ec: Escherichia coli; K: Klebsiella; Ca: Candida albicans*.
[3]MIC, minimum inhibitory concentration (the lowest concentration that inhibited visible growth of microorganisms).
Values correspond to the average ± standard deviation of three replicates. Averages followed by the same letter are not significantly different ($p > .05$).

observed by Arruda (2013) (133–576 μmol TE/g) and De-Melo et al. (2016) (310.1–559.3 μmol TE/g) in samples from Brazil. All the results from using the ORAC method were above those for fruits in natura, such as strawberries (43.0 μmol TE/g), apples (30.5 μmol TE/g), blueberries (96.2 μmol TE/g), and cranberries (90.9 μmol TE/g) (USDA, 2010).

Although the Mya sample stood out due to its capacity to inhibit yeast, Msa and, especially, MsaSC bee pollen generally stood out for their capacity to inhibit bacterial growth. Arruda (2013), when evaluating Brazilian samples, observed MIC of 1.0–7.3 mg/mL for *Staphylococcus epidermides* and *S. aureus*, 1.9–8.0 mg/mL for *E. coli*, and 7.8–29.0 mg/mL for *C. albicans*. Pascoal, Rodrigues, Teixeira, Féas, and Estevinho (2014), who tested eight bee pollen samples produced in Portugal for the inhibition of *S. aureus*, *Pseudomonas aeruginosa*, *E. coli*, and *Candida glabrata*, found that *S. aureus* strains were more sensitive, with MIC values between 1.81 and 4.28 mg/mL. For the other microorganisms, the authors observed 3.71–6.96 mg/mL (*P. aeruginosa*), 4.08–9.42 mg/mL (*E. coli*), and 16.00–33.92 mg/mL (*C. glabrata*). The results of the present study corroborate these observations, since the Gram-positive bacteria have greater sensitivity than Gram-negative bacteria, which could be explained by the greater complexity of the cell wall of the latter.

The phenolic compounds are described as the main bioactive agents in bee pollen (Carpes et al., 2008), and as there is variation in the phenolic profile depending on botanical origin and processing conditions, it is to be expected that the antimicrobial capacity, as well as the antioxidant capacity, would undergo the same influences. It is necessary to consider that, though phenolic compounds are considered important, other substances present, such as spermidine derivatives, also have antimicrobial capacity (Bassard, Ullmann, Bernier, & Werck-Reichhart, 2010; Mihajlovic, Radosavljevic, Burazer, Smiljanic, & Velickovic, 2015).

In terms of *M. scabrella* monofloral bee pollen produced in Brazil, as was seen for the physicochemical parameters, it does not seem possible to establish a biological potential profile, given that there was significant variation in the parameters among the three samples, according to a harvesting site.

3.3 | Phenolic profile by HPLC-MS

The results obtained by HPLC-MS for the eight bee pollen samples from seven Brazilian states, as well as the proposed identification for the observed compounds, are presented in Table 5. Flavonoid glycosides predominated, mainly the 3-O-glycosides of quercetin, kaempferol, and isorhamnetin. Negri et al. (2011) also found flavonoid glycosides in bee pollen from Pindamonhagaba, Brazil. In plant extracts, there is a great variety of bioactive substances, and flavonoids are frequently found in the form of *O*- or *C*-glycosides, with sugar units connected directly to the hydroxyl or to one of the carbon atoms of the aglycone.

The pinobanksin derivative was found in the seven bee pollens, that is, it was identified not only in the Ana sample. It is a dihydroflavonol with antibacterial potential that had already been observed in honey and propolis (Falcão et al., 2013; Keckes et al., 2013). The 4-methylsulfonylbutyl glucosinolate (glucoraphanin), found in three samples (Ala, Mca, and MsaSC), is part of the group of glucosinolates

TABLE 5 Retention time (R_t), maximum absorbance wavelength, and main fragments of the polyphenols detected in the ethanolic extract of the monofloral bee pollen from seven Brazilian states, proposed identification, and references

Sample: Ala[1]							
				MS/MS fragments			
Peak	R_t (min)	λ/nm	MS $[M-H]^-$ (*m/z*)	MS^2 (*m/z*)	MS^3 (*m/z*)	Proposed identification	Reference
1	6.6	265	326	236, 278, 164	164	NI	
2	7.6	234, 264, 278	199	125, 153, 171	79	NI	
3	11.1	234, 265, 275	117	71		NI	
4	15.8	278	289	245, 205, 179	227, 203, 186, 161	Catechin	Standard
5	20.9	289	436	316	273	4-Methylsulfinylbutyl glucosinolate (glucoraphanin)	Phenol-Explorer (2015)
6	21.6	286	342	222, 164	147, 74, 164	Pinobanksin-3-O-butyrate (or isomer)	Keckes et al. (2013)
7	21.7	278	131	85	69	NI	
8	22.6	279	197	182, 166	153	Syringic acid	Standard
9	23.8	289	436	316	119, 273	4-Methylsulfinylbutyl glucosinolate (glucoraphanin)	Phenol-Explorer (2015)
10	30.6	308	163	119	93	p-Coumaric acid	Standard
11	34.9	275, 298, 307	639	519, 399, 315	399	Isorhamnetin-3-O-diglucoside or patuletin-3-O-rhamnosylglucoside	Mihajlovic et al. (2015); Negri et al. (2011)
12	38.1	264, 351	595	301	255	Quercetin-3-O-glucosyl-6-O-pentoside	Mihajlovic et al. (2015)
13	38.9	265, 330, 347	609	429, 285	339, 309, 297	Quercetin-3-O-rutinoside	Keckes et al. (2013)
14	40.4	265, 348	755	593, 285	285	Kaempferol-3-O-glucosyl-rutinoside	Bresciani et al. (2015)
15	40.9	301, 255, 354	623	459, 314, 299	299	Isorhamnetin-3-O-rhamnosylglucoside	Negri et al. (2011)
16	43.3	264, 300, 350	579	429, 284, 255	255	NI	
17	43.7	254, 352	609	301	151	Rutin	Standard
18	45.1	271, 289, 356	723	678	659 338, 225	1-Sinapoyl-2-feruloylgutiobiose	Phenol-Explorer (2015)
19	49.3	307	478	358	315, 145, 119	Petunidin-3-O-galactoside/or glucoside	Phenol-Explorer (2015)
20	50.2	321	630	494, 468	332, 296	NI	
21	51.6	292	950	904	886, 678, 565, 451	NI	
22	52.2	289	582	462, 342	342	Tri-*p*-coumaroyl spermidine (isomer)	Mihajlovic et al. (2015)
23	53.3	265,299,326,337	489	285	267, 257, 229, 213	Luteolin-7-O-6″-acetylglucoside	Lin and Harnly (2010)
24	53.8	371	301	179, 151	151	Quercetin	Standard

(Continues)

TABLE 5 (Continued)

Sample: Ala[1]							
				MS/MS fragments			
Peak	R_t (min)	λ/nm	MS $[M-H]^-$ (*m/z*)	MS^2 (*m/z*)	MS^3 (*m/z*)	Proposed identification	Reference
25	54.1	310	785	665, 545	545, 291	NI	
26	56.7	292, 308	582	462, 342	342	Tri-*p*-coumaroyl spermidine (isomer)	Mihajlovic et al. (2015)
Sample: Ana[1]							
				MS/MS fragments			
Peak	R_t (min)	λ/nm	MS $[M-H]^-$ (*m/z*)	MS^2 (*m/z*)	MS^3 (*m/z*)	Proposed identification	Reference
1	7.0	261	323	211, 280	97, 77, 193, 139	NI	
2	16.2	283	289	245, 205	203, 227, 187	Catechin	Standard
3	20.5		167	153, 123	108	Vanillic acid	Standard
4	22.1	290	319	193	165	Ampeloptin	Abu-Reidah, Ali-Shtayeh, Jamous, Arráez-Román, and Segura-Carretero (2015)
5	23.0	280	153	109	109, 65	β-Resorcylic acid	Standard
6	23.6	271, 280	440	253, 272, 422	179, 210, 171	NI	
7	27.8	286, 330	602	512, 440	422, 272, 254	NI	
8	32.6	288	303	285	241, 199, 175	NI	
9	32.2	316	193	149, 178, 134	134	Ferulic acid	Standard
10	34.5	256, 354	625	445, 301	265	Quercetin diglucoside	Mihajlovic et al. (2015)
11	35.2	255, 354	433	271	179, 151	Quercetin-3-O-arabinoside	Negri et al. (2011)
12	35.7	271	639	315, 299	476	Isorhamnetin-3-O-diglucoside or patuletin-3-O-rhamnosylglucoside	Mihajlovic et al. (2015); Negri et al. (2011)
13	37.0	351	609	300, 271	271, 255	Quercetin rutinoside (isomer)	Mihajlovic et al. (2015)
14	37.5	255, 301, 354	623	459, 314, 299	299	Isorhamnetin-3-O-rhamnosylglucoside	Negri et al. (2011)
15	38.9	266, 347	609	429, 285	151	Quercetin-3-O-rutinoside	Keckes et al. (2013)
16	40.3	291, 269, 328	287	269	125	Eriodictyol	Keckes et al. (2013)
17	40.9	265, 351	623	459, 314, 299	299	Isorhamnetin-3-O-rhamnosylglucoside (isomer)	Negri et al. (2011)
18	43.7	254, 352	609	301	151	Rutin	Standard
19	45.2	271	723	678	659, 338, 225	1-Sinapoyl-2-feruloylgutiobiose	Phenol-Explorer (2015)

(Continues)

TABLE 5 (Continued)

Sample: Ana[1]							
				MS/MS fragments			
Peak	R_t (min)	λ/nm	MS $[M-H]^-$ (*m/z*)	MS^2 (*m/z*)	MS^3 (*m/z*)	Proposed identification	Reference
20	47.2	328, 377, 389	433	271	151	Naringenin hexoside	Bystrom, Lewis, Brown, Rodriguez, and Obendorf (2008)
21	48.4	251	147			Cinnamic acid	Standard
22	49.1	268, 292, 315	837	791	773, 564, 451	NI	
23	49.6	289	582	462	342	Tri-*p*-coumaroyl spermidine	Mihajlovic et al. (2015)
24	50.2	297, 320	630	494, 468	332	NI	
25	51.6	265,393	301	255, 229	211, 185	Ellagic acid	
26	52.9	290	271	151, 177	107	Naringenin	Standard
27	53.8	371	301	179, 151	151	Quercetin	Standard
28	56.6	266, 284, 294	582	462, 342	342	Tri-*p*-coumaroyl spermidine (isomer)	Mihajlovic et al. (2015)
29	57.3	297, 309	672	536, 522	479, 372	N′,N′′,N′′′-tris-*p*-feruloyl spermidime	Negri et al. (2011)
30	60.6	294, 308	785	665, 545	545	NI	
31	62.3	290	596	476	433	NI	
Sample: Cna[1]							
				MS/MS fragments			
Peak	R_t (min)	λ/nm	MS $[M-H]^-$ (*m/z*)	MS^2 (*m/z*)	MS^3 (*m/z*)	Proposed identification	Reference
1	11.8	264, 353	606	403, 273, 323	272,97	NI	
2	19.3	323	353	191	172, 85, 93	Chlorogenic acid	Standard
3	32.1		193	149, 178, 134	133	Ferulic acid	Standard
4	32.9		223	208, 179, 164	164, 150	Synapic acid	Standard
5	36.4	259, 354	609	477, 315	300	Isorhamnetin rutinoside	Mihajlovic et al. (2015)
6	39.6	255, 354	623	314, 459, 299	299	Isorhamnetin-3-O-rhamnosylglucoside	Negri et al. (2011)
7	43.5	235, 289, 353	187	125	97	NI	
8	43.7	254, 352	609	301	151	Rutin	Standard
9	45.3	271, 356	723	678, 577	660, 225, 338, 451	1-Sinapoyl-2-feruloylgutiobiose	Phenol-Explorer (2015)
10	47.3	254, 355	709	665	501, 314, 299, 623	NI	
11	49.2	292, 316	837	791	773, 565, 452, 338	NI	

(Continues)

TABLE 5 (Continued)

Peak	R_t (min)	λ/nm	MS [M−H]$^-$ (*m/z*)	MS/MS fragments		Proposed identification	Reference
				MS^2 (*m/z*)	MS^3 (*m/z*)		
Sample: Cna[1]							
12	51.7	294, 320	950	904	886, 565, 451, 678	NI	
13	53.8	371	301	179, 151	150,88	Quercetin	Standard
14	54.4	299, 310	623	315, 300	300	Isorhamnetin-3-O-rhamnosylglucoside (isomer)	Negri et al. (2011)
15	56.6	295	582	462, 342	342	N′,N′′,N′′′-tris-*p*-coumaroyl spermidine	Mihajlovic et al. (2015)
16	57.1	296, 330	672	522, 536	479, 372	N′,N′′,N′′′-tris-*p*-feruloyl spermidime	Negri et al. (2011)
17	61.1	280	327	309, 291, 229, 211, 171	211, 209	Pinobanksin-5-methylether-3-O-acetate	Falcão et al. (2013)
18	61.9	282	695	677, 651, 569, 551	551, 525	NI	
19	62.5	282	785	623	615, 295	Isorhamnetin-3-O-rutinoside-7-O-glucoside	Truchado, Ferreres, and Tomas-Barberan (2009)
20	63.4	283	607	589, 481, 463, 437	463, 437	NI	
21	63.9	280	329	311, 229	211, 209	Bis-methylated quercetin	Keckes et al. (2013)
22	64.7	282	529	511, 297, 271	251, 185	NI	
Sample: Mca[1]							
1	15.8	278	289	245, 205, 179	227, 203, 187, 161	Catechin	Standard
2	18.8	326	353	191	93, 85	Chlorogenic acid	Standard
3	22.0	323	179	135	107	Caffeic acid	Standard
4	22.6	279	197	182, 166	153	Syringic acid	Standard
5	28.2	298, 309	436	316	273, 145	4-Methylsulfinylbutyl glucosinolate (glucoraphanin)	Phenol-Explorer (2015)
6	29.7	265, 347	593	285	155	Kaempferol-3-O-rhamnosyl-glucoside	Negri et al. (2011)
7	32.6	270, 344	639	476, 313	341, 298	Isorhamnetin-3-O-diglucoside or patuletin-3-O-rhamnosylglucoside	Mihajlovic et al. (2015); Negri et al. (2011)
8	36.5	267, 330, 346	785	639, 623, 605	315	Isorhamnetin-3-O-rutinoside-7-O-glucoside	Truchado et al. (2009)
9	39.1	255, 301, 354	623	459, 314, 299	299	Isorhamnetin-3-O-rhamnosylglucoside	Negri et al. (2011)
10	43.3	254, 352	609	301	151	Rutin	Standard
11	44.3	265, 366	447	285	151	Kaempferol-3-O-glucoside	Mihajlovic et al. (2015)
12	45.3	271, 289, 356	723	678	660, 451, 338, 225	1-Sinapoyl-2-feruloylgutiobiose	Phenol-Explorer (2015)

(Continues)

TABLE 5 (Continued)

Peak	R_t (min)	λ/nm	MS [M–H]$^-$ (*m/z*)	MS/MS fragments		Proposed identification	Reference
				MS^2 (*m/z*)	MS^3 (*m/z*)		
Sample: Mca[1]							
13	47.4	253, 355	709	665	314	NI	
14	49.3	308	477	357	314	Isorhamnetin-3-O-glucoside	Mihajlovic et al. (2015)
15	50.5	305, 320, 398	762	596, 582, 556, 402	567, 539, 402, 387	NI	
16	51.4		949	904	885, 677, 564, 451	NI	
17	53.9	371	301	179, 151	151	Quercetin	Standard
18	56.1	315, 337	762	596, 582, 556, 402	402, 387, 376, 205	NI	
19	58.2	319	489	442, 309	185	Kaempferol-3-O-acetylglucoside	Kajdzanoska, Gjamovski, and Stefova (2010)
20	59.2	265, 364	285	229, 151	185	Kaempferol	Standard
21	59.9	236, 319	315	300	271, 255, 151	Quercetin-3-methylether	Keckes et al. (2013)
22	61.2	289	327	239	183, 155, 125	Pinobanksin-5-methylether-3-O-acetate	Falcão et al. (2013)
23	64.0	285	329	311, 293	211, 209, 155, 130	Bis-methylated quercetin	Keckes et al. (2013)
24	66.2	284, 373	605	587, 461, 443	425, 399, 381	NI	
25	70.3	314	505	487, 469, 443	469, 443, 425, 373	Isorhamnetin 3-O-methyl hexuronide	Mihajlovic et al. (2015)
26	73.3	312	449	417	373, 163, 145	Dihydroquercetin-3-O-rhamnoside	Phenol-Explorer (2015)
Sample: Mya[1]							
1	20.5	260	167	153, 123	108	Vanillic acid	Standard
2	20.6	296	389	345, 209, 165, 121	183, 165, 143, 121	NI	
3	29.8	297	403	371, 223, 179	165, 121	NI	
4	30.6	308	163	119	119	*p*-Coumaric acid	Standard
5	32.8	320	223	208, 179, 164	164, 149	Synapic acid	Standard
6	34.6	265, 294	625	463, 445, 300, 505	271, 255	Quercetin diglucoside	Mihajlovic et al. (2015)
7	37.1	266, 294	609	300, 271	271, 255	Quercetin rutinoside (isomer)	Mihajlovic et al. (2015)
8	37.5	290	303	151	107	NI	
9	38.1	266, 297, 307	595	449	287	Cyanidin-3-rutinoside	Koolen, Silva, Gozzo, Souza, and Souza (2013)

(Continues)

TABLE 5 (Continued)

Sample: Mya[1]							
				MS/MS fragments			
Peak	R_t (min)	λ/nm	MS $[M-H]^-$ (m/z)	MS^2 (m/z)	MS^3 (m/z)	Proposed identification	Reference
10	38.8	255	610	564	547, 225	NI	
11	39.5	270, 297	639	519	476	Isorhamnetin-3-O-diglucoside or patuletin-3-O-rhamnosylglucoside	Mihajlovic et al. (2015); Negri et al. (2011)
12	41.2	256, 350, 400	463	316	287, 271	Myricetin-3-O-α-L-rhamnopyranoside	Saldanha et al. (2013)
13	41.9	284, 327	403	271	151	Pinobanksin-3-O-phenylpropionate	Falcão et al. (2013)
14	43.3	265, 347	579	429, 327	255	Orientin-2-O-xyloside	Sakalem, Negri, and Tabach (2012)
15	43.7	254, 352	609	301	151	Rutin	Standard
16	45.2	270	723	678	660, 451, 338, 225	1-Sinapoyl-2-feruloylgutiobiose	Phenol-Explorer (2015)
17	46.1	327	641	479	317	Myricetin-3,7-di-O-glucoside	Truchado et al. (2009)
18	47.8	264,306,326, 390, 399	317	179, 151	151	Myricetin	Bresciani et al. (2015)
19	48.2	253, 344	447	301, 285	283, 179, 151	Kaempferol-3-O-glucoside	
20	49.1	273, 294	837	791	772, 565, 451, 338	NI	
21	50.6	252,271,315, 377, 387	625	477, 315	151	Isorhamnetin rutinoside	Mihajlovic et al. (2015)
22	51.5	265, 345	301	255, 229	211, 185	Ellagic acid	
23	52.7	271, 319	609	463, 301	301	Quercetin rutinoside (isomer)	
24	52.3	265, 324	489	285	267, 257, 229, 213	Luteolin-7-O-6"-acetylglucoside	Lin and Harnly (2010)
25	53.8	371	301	179, 151	151	Quercetin	Standard
26	54.9	327	639	315, 301	300	Isorhamnetin-3-O-diglucoside (isomer)	Mihajlovic et al. (2015)
27	56.5	299, 308	593	447, 285	241, 217, 199, 175	Kaempferol-3-O-rhamnosyl-glucoside	Negri et al. (2011)
28	57.4	254, 360	623	323, 299, 285	241, 217, 199, 175	Isorhamnetin-3-O-rhamnosyl-glucoside	Negri et al. (2011)
29	58.5	266, 314	593	285	241, 217, 175, 151	Kaempferol-7-O-rutinoside	Bresciani et al. (2015)
30	59.7	271, 313	609	301	151	Quercetin rutinoside (isomer)	
31	60.3	267, 320, 347	269	225, 201, 149, 151	197, 181, 169	Apigenin	
32	60.7	299, 309	785	665, 545	545	NI	
33	61.3	238, 272, 314	593	285	241, 217, 199, 175	Kaempferol rutinoside (isomer)	Truchado et al. (2009)
34	61.7	289	615	597, 251, 277	231, 223, 209, 125, 169	NI	
35	62.6	252, 326, 364	583	301	179, 151	NI	

(Continues)

TABLE 5 (Continued)

Sample: Mya[1]							
Peak	R_t (min)	λ/nm	MS [M−H]$^-$ (*m/z*)	MS/MS fragments MS^2 (*m/z*)	MS/MS fragments MS^3 (*m/z*)	Proposed identification	Reference
36	63.5	265, 330, 339	567	285	241, 217, 199, 175	NI	
37	64.2	284	573	555, 297, 271	251	NI	
38	64.9	276	529	511, 297, 271	251	NI	
39	68.2	270	773	755, 737, 685	737, 667, 641	NI	
40	69.3	266	595	463, 445	301	Tricetin-7-O-(pentoside-glucoside)	Truchado et al. (2009)
Sample: MsaSC[1]							
Peak	R_t (min)	λ/nm	MS [M−H]$^-$ (*m/z*)	MS/MS fragments MS^2 (*m/z*)	MS/MS fragments MS^3 (*m/z*)	Proposed identification	Reference
1	7.6	288	434	419, 259, 275	157, 176, 224, 402	NI	
2	9.9	288	471	341, 425	341, 323	NI	
3	11.5	224, 260	153	109	109	Protocatechic acid	Standard
4	20.8	293	436	316	273	4-Methylsulfinylbutyl glucosinolate (glucoraphanin)	Phenol-Explorer (2015)
5	27.8	265, 357	639	477, 315	300	Isorhamnetin-diglucoside	Mihajlovic et al. (2015), Negri et al. (2011)
6	29.0	313	701	565, 535, 399, 555	399, 389	NI	
7	30.1	298, 307	639	519, 399	399	Isorhamnetin-diglucoside (isomer)	Mihajlovic et al. (2015), Negri et al. (2011)
8	32.4	316	193	149, 178, 134	134	Ferulic acid	Standard
9	33.0	311	715	569, 549, 399	506, 399	NI	
10	34.6	267, 310	625	463, 445, 301	151	Tricetin-7-O-sophoroside (glucosyl(1−2)glucoside)	Truchado et al. (2009)
11	35.0	271, 313	609	445, 285, 315	151	Quercetin rutinoside (isomer)	Mihajlovic et al. (2015)
12	35.3	310	715	549, 399	399, 506	NI	
13	35.7	297, 307	639	519, 399	399	Isorhamnetin-3-O-diglucoside or patuletin-3-O-rhamnosylglucoside	Mihajlovic et al. (2015), Negri et al. (2011)
14	38.7	265,295,330,347	609	429, 285, 255	339, 309	Luteolin-7-O-sophoroside (glucosyl(1−2)glucoside)	Truchado et al. (2009)
15	40.4	266, 343	755	593	285	Kaempferol-3-O-rhamnosyl-glucoside	Bresciani et al. (2015)

(Continues)

TABLE 5 (Continued)

Sample: MsaSC[1]							
				MS/MS fragments			
Peak	R_t (min)	λ/nm	MS [M−H]$^-$ (*m/z*)	MS^2 (*m/z*)	MS^3 (*m/z*)	Proposed identification	Reference
16	41.3	262, 282	593	429, 285	255	Kaempferol-3-O-rhamnosyl-glucoside	Negri et al. (2011)
17	41.8	282	403	271	253; 151	Pinobanksin-3-O-phenylpropionate	Falcão et al. (2013)
18	43.8	255, 354	609	301	179, 151	Rutin	Standard
19	45.2	271, 312	724	678	660, 451, 338, 225	NI	
20	46.1	271, 320	477	315, 300	300	Isorhamnetin-3-O-glucoside	Mihajlovic et al. (2015)
21	46.9	271, 324, 399	507	492, 345, 329	329	NI	
22	47.4	329, 366	433	313, 271	151	Quercetin-3-O-arabinoside	Negri et al. (2011)
23	47.9	301, 328, 354	317	179, 151	151	Myricetin	Bresciani et al. (2015)
24	49.6	292, 320	582	462, 342	342	Tri-*p*-coumaroyl spermidine (isomer)	Mihajlovic et al. (2015)
25	50.2	297, 321	630	494, 468, 358	332	NI	
26	51.0	371	301	179, 151	151	Quercetin	Standard
27	51.7	254, 366	301	257, 229	185	Ellagic acid	Kajdzanoska et al. (2010)
28	52.2	298, 310	582	462, 342	342	Tri-*p*-coumaroyl spermidine (isomer)	Mihajlovic et al. (2015)
29	52.6	324	403	271	151	Pinobanksin-3-O-phenylpropionate (isomer)	Falcão et al. (2013)
30	52.8	290	271	151, 177	107	Naringenin	Standard
31	53.5	310, 338	879	743, 717	581, 555, 551, 415	NI	
32	54.1	291	785	665, 545	545	NI	
33	55.3	319	863	727, 701, 671, 561	565, 535, 399	NI	
34	55.9	294	785	665, 545	545	NI	
35	57.3	317	672	536, 522	479, 372	N′,N″,N‴-tris-*p*-feruloyl spermidime	Negri et al. (2011)
36	58.8	297, 308	785	665, 545	545	NI	
37	59.3	265, 366	285	229, 151	185	Kaempferol	standard
38	63.9	280	329	311, 229, 211, 293	211, 209, 125	Bis-methylated quercetin	Keckes et al. (2013)
39	68.6	294, 308	575	557, 539, 415	539, 521, 441, 415	NI	
40	69.1	312	421	403, 377, 359, 333	359, 341, 317, 299	NI	

Sample: MsaRN[1]							
				MS/MS fragments			
Peak	R_t (min)	λ/nm	MS [M–H]$^-$ (*m/z*)	MS^2 (*m/z*)	MS^3 (*m/z*)	Proposed identification	Reference
1	16.2	267, 288	377	329, 287, 215	215	Caffeic acid derivative	Bystrom et al. (2008)
2	23.1	280	153	109	109, 65	β-Resorcylic acid	Standard
3	25.1	265, 345	609	447, 285	285	Orientin-7-glucoside	Sakalem et al. (2012)
4	26.6	265, 331, 345	755	593	285	Kaempferol-3-O-glucosylrutinoside (isomer)	Bresciani et al. (2015)
5	28.2	265, 331, 347	609	489, 447, 285	327, 255, 284	Kaempferol-3,7-di-O-glucoside	Bresciani et al. (2015)
6	32.8	320	223	208, 179, 164	164, 149	Synapic acid	Standard
7	38.5	265, 331, 350	609	429, 285	151	Quercetin-3-O-rutinoside	Keckes et al. (2013)
8	40.9	265, 349	593	429, 285, 255	255	Kaempferol-7-O-rutinoside	Truchado et al. (2009)
9	42.9	254, 352	609	301	151	Rutin	Standard
10	44.2	264, 367	447	285	151	Kaempferol-3-O-glucoside	Mihajlovic et al. (2015)
11	45.4	254, 370	723	678	660, 225, 338, 451	1-Sinapoyl-2-feruloylgutiobiose	Phenol-Explorer (2015)
12	47.4	303, 358	709	665	314, 299	NI	
13	48.4	266,327,345	447	284	255, 227	Kaempferol-3-O-glucoside (isomer)	Mihajlovic et al. (2015)
14	48.6	266,299,326,339	593	285	267, 257, 229, 213	Kaempferol-7-O-rutinoside	Truchado et al. (2009)
15	49.2	272,296,307	837	791	773, 565, 452, 338	NI	
16	50.8	361	331	316	287, 271	NI	
17	51.5	268, 345	301	255, 229	211, 185	Ellagic acid	
18	52.4	292	403	271	253	Pinobanksin-3-O-phenylpropionate	Falcão et al. (2013)
19	52.9	290	271	151	63, 83	Naringenin	Standard
20	53.9	297, 366	301	179, 151	151	Quercetin	Standard
21	54.2	297, 308	598	478	342	Di coumaroyl caffeoyl spermidine	Mihajlovic et al. (2015)
22	56.8	238, 319	635	617, 327, 309, 291	273, 247, 193, 165	NI	
23	58.1	297, 307	628	492, 466	449, 372	NI	
24	58.3	297	489	327, 309, 291	273, 247, 193, 165	Kaempferol-3-O-acetylglucoside	Kajdzanoska et al. (2010)
25	59.2	265, 366	285	229, 151	185	Kaempferol	Standard
26	60.1	256, 355	315	300	271, 255, 151	Quercetin-3-methyl ether	Falcão et al. (2013)
27	60.3	298, 307	612	492	449, 372	NI	

(Continues)

TABLE 5 (Continued)

Peak	R_t (min)	λ/nm	MS [M–H]$^-$ (*m/z*)	MS/MS fragments MS^2 (*m/z*)	MS^3 (*m/z*)	Proposed identification	Reference
Sample: MsaRN[1]							
28	61.1	290	327	291, 229, 211, 171	211, 125	Pinobanksin-5-methylether-3-O-acetate	Falcão et al. (2013)
29	61.6	284	327	309, 291, 229, 171	153, 127, 125	Pinobanksin-3-O-propionate	Falcão et al. (2013)
30	66.1	297	626	506	491	NI	
31	68.7	236, 280	577	559	443, 417	Procyanidin dimer B1	Sakalem et al. (2012)
32	69.5	313	489	442, 309	185	Kaempferol-3-O-acetylglucoside	Kajdzanoska et al. (2010)
Sample: MsaMT[1]							
Peak Peak	R_t (min) R_t (min)	λ/nm λ/nm	MS [M–H]$^-$ (*m/z*)	MS/MS fragments MS^2 (*m/z*)	MS^3 (*m/z*)	Proposed identification	Reference
1	12.5	293, 312	305	263, 149, 134	134	NI	
2	14.3	295	727	709, 623	303, 667, 521, 385	NI	
3	19.9	296	210	94	66	NI	
4	23.4	294	278	163, 132, 216, 234	119	NI	
5	30.7	266, 304	655	346, 331	331	NI	
6	33.9	270, 294, 314	625	300, 301, 445, 271	271, 255	Quercetin diglucoside	Mihajlovic et al. (2015)
7	37.0	275, 287	609	300, 271, 255	271, 255	Quercetin rutinoside (isomer)	Mihajlovic et al. (2015)
8	38.7	280	610	565	547, 225	NI	
9	39.1	282	403	271	253	Pinobanksin-3-O-phenylpropionate	Falcão et al. (2013)
10	41.3	266, 283, 314	593	429, 285, 255	255	Kaempferol rutinoside	Mihajlovic et al. (2015)
11	41.8	282	403	271	151	Pinobanksin-3-O-phenylpropionate (isomer)	Falcão et al. (2013)
12	45.1	271, 289, 356	723	678	659, 338, 225	1-Sinapoyl-2-feruloylgutiobiose	Phenol-Explorer (2015)
13	49.1	269	837	791	772, 565, 451, 338	NI	
14	49.6	292, 320	582	462, 342	342	Tri-p-coumaroyl spermidine	Mihajlovic et al. (2015)
15	50.6	304	762	582, 556, 596	567, 402, 539, 387	NI	
16	51.6	295	950	904	886, 678, 565, 451	NI	
17	52.2	296	672	536, 522	479, 372	N′,N″,N‴-tris-p-feruloyl spermidime	Negri et al. (2011)
18	52.7	292	403	271	253	Pinobanksin-3-O-phenylpropionate (isomer)	Falcão et al. (2013)
19	53.3	315	762	596, 582	568, 539, 402, 387	NI	

(Continues)

TABLE 5 (Continued)

Sample: MsaMT[1]						
Peak	R_t (min)	λ/nm	MS $[M-H]^-$ (m/z)	MS/MS fragments		Proposed identification	Reference
Peak	R_t (min)	λ/nm		MS^2 (m/z)	MS^3 (m/z)		
20	54.6	296, 316	672	536, 522	479, 372	N′,N″,N‴-tris-p-feruloyl spermidime (isomer)	Negri et al. (2011)
21	56.1	319	762	596, 582	567, 539, 402, 387	NI	
22	56.6	319	702	566, 552, 522	537, 509, 402, 372	NI	
23	57.2	300	672	536, 522	479, 372	N′,N″,N‴-tris-p-feruloyl spermidime (isomer)	Negri et al. (2011)
24	61.1	294	327	271	253	Pinobanksin-3-O-propionate	Falcão et al. (2013)
25	69.2	268, 329	537	443, 375	331	NI	

NI, not identified.
[1]**Ala**, *Alternanthera*; **Ana**, *Anadenanthera*; **Cna**, *Cocos nucifera*; **Mca**, *Mimosa caesalpiniaefolia*; **Mya**, *Myrcia*; **MsaSC**, *Mimosa scabrella* from Santa Catarina; **MsaRN**, *Mimosa scabrella* from Rio Grande do Norte; **MsaMT**, *Mimosa scabrella* from Mato Grosso do Sul.

whose compounds occur mainly in plants from the Brassicaceae but also in other angiosperms. Based on the literature reviewed, this was the first time that this substance was identified in bee pollen.

In the MS spectra of the Ala sample, 26 peaks were observed, of which 18 were identified: 5 confirmed using standards available in the laboratory (Table 2), and the remainder based on the literature. The compounds identified by comparison with the standards were catechin (0.73 ± 0.08 mg/100 g), syringic acid (4.81 ± 0.05 mg/100 g), *p*-coumaric acid (0.47 ± 0.02 mg/100 g), rutin (6.65 ± 0.05 mg/100 g), and quercetin (1.92 ± 0.02 mg/100 g).

In the Ala sample, of the compounds identified based on the literature, a glycosylated anthocyanin (petunidine-3-O-galactoside, retention time: 49.3 min) and a spermidine derivative (tri-*p*-coumaryl spermidine, retention time: 56.7 min) seem to be the most prominent. Petunidine-3-O-galactoside results from the substitution of a hydroxyl of the aglycone by a unit of galactose. Petunidin derivatives had been previously identified in red wine, but, from what we understand, the present study was the first to report the presence of these compounds in bee pollen. The tri-*p*-coumaryl-spermidine is a conjunction of polyamine with phenolic compounds, which are part of the group of bioactive substances with antimicrobial potential (Bassard et al., 2010). The occurrence of spermidine derivatives was reported in *Ambrosia artemisiifolia* L. pollen from Belgrade, Serbia (Mihajlovic et al., 2015).

In the analysis of the Ana sample, 31 peaks were observed, with 23 being identified based on the literature and from available information of the standards. The compounds identified based on the standards were also quantified: 0.69 ± 0.03 mg of catechin/100 g, 12.54 ± 0.12 mg of β-resorcylic acid/100 g, 1.04 ± 0.01 mg of ferulic acid/100 g, 3.66 ± 0.14 mg of rutin/100 g, 13.05 ± 0.08 mg of cinnamic acid/100 g, 7.42 ± 0.15 mg of quercetin/100 g, and 4.57 ± 0.17 mg of naringenin/100 g. Naringenin is a compound with antioxidant activity commonly found in grapefruit juice; however, Leblanc, Davis, Boue, Delucca, and Deeby (2009) also identified this substance in bee pollen from the Sonoran Desert, North of Tucson, AZ. In the present study, the compound was also observed in the MsaSC and MsaRN samples.

Considering the intensity of the peaks, the two spermidine derivatives stand out as the main compounds in the Ana bee pollen: tri-*p*-coumaryl-spermidine (retention time: 56.6 min) and N′,N″,N‴-tris-*p*-feruloyl-spermidine (retention time: 57.3 min). The longer retention time for the latter indicates a lower polarity in relation to the others, which coincides with the observations of Negri et al. (2011) in bee pollen samples. The authors attribute the lower polarity of this substance to the presence of a methoxyl group in the feruloyl moiety. The ampeloptin and eriodictyol compounds are only found in the Ana bee pollen. Ampeloptin (or dihydromyricetin) is used in Chinese medicine as an anti-inflammatory agent. Previously, Keckes et al. (2013) suggested that eriodictiol and quercetin can be used as markers for sunflower honey produced in Serbia.

Amongst the spectra generated, that of the Cna bee pollen had the lowest number of peaks (22), of which 14 were identified. This sample had the lowest antioxidant capacity and low antimicrobial capacity against bacteria, as previously mentioned, which could be related to the lower number and/or types of compounds present. An

isorhamnetin (isorhamnetin-3-O-rhamnosyl-glycoside) glycoside was the main compound, which had already been identified in bee pollen (Negri et al., 2011). Other authors also identified the presence of flavonoid-O-glycoside in honey, with the type and amount correlated with the botanical origin of the samples. Five compounds were confirmed by comparison with the standards, and the following amounts were found: chlorogenic acid (4.85 ± 0.12 mg/100 g), ferulic acid (0.71 ± 0.02 mg/100 g), synapic acid (0.29 ± 0.03 mg/100 g), rutin (4.78 ± 0.10 mg/100 g), and quercetin (1.86 ± 0.01 mg/100 g).

Of the 26 peaks observed in the Mca sample, 21 were identified. This was one of the two samples in which the main compound (retention time: 56.1 min), the compound that was also found in the MsaMT sample, was not identified. The second peak of highest intensity corresponds to the isorhamnetin-3-O-glycoside. Seven compounds were confirmed and quantified using the standard data: 0.75 ± 0.05 mg of catechin/100 g, 4.53 ± 0.19 mg of chlorogenic acid/100 g, 0.15 ± 0.01 mg of caffeic acid/100 g, 5.36 ± 0.24 mg of syringic acid/100 g, 8.39 ± 0.16 mg of rutin/100 g, 2.91 ± 0.06 mg of quercetin/100 g, and 7.40 ± 0.45 mg of kaempferol/100 g.

In the Mya bee pollen, 40 peaks were detected, with 28 being identified. The main compounds were suggested to be myricetin (retention time: 47.8 min) and ellagic acid (retention time: 51.5 min). Myricetin has antioxidant potential and, depending on the concentration and on the conditions of the medium, can act as a pro-oxidant. Ellagic acid is a dimeric derivative of gallic acid with anti-inflammatory (Bae et al. 2010) and antioxidant (Festa et al., 2001) activity. Saldanha, Vilegas, and Dokkedal (2013) analyzed extracts from *Mycia bella*, a species from the *Myrcia* genus common in areas of the Cerrado, and identified, as the present study, glycosides of myricetin, quercetin, and kaempferol. Of the 40 compounds detected, 4 corresponded to standards that were quantified in the Mya bee pollen for HPLC-PDA: vanillic acid (6.06 ± 0.08 mg/100 g), *p*-coumaric acid (6.68 ± 0.02 mg/100 g), rutin (3.98 ± 0.01 mg/100 g), and quercetin (67.91 ± 0.25 mg/100 g). This was the highest level of quercetin observed amongst the samples. The highest antioxidant capacity for both methods was also observed in the Mya bee pollen.

Cyanidin-3-rutinoside and apigenin compounds were only identified in the Mya bee pollen sample. The first is a polyphenol from the anthocyanin group with the potential to assist in the prevention of degenerative diseases and has already been identified in *Euterpe oleracea* (açaí) collected in the Pará state, Brazil (Gouvêa et al., 2012). Apigenin is a flavonoid from the flavonas class, found in fruits and vegetables, with anti-inflammatory, antioxidant, and antitumor activity (Shukla & Gupta, 2010).

Each of the three Msa samples presents a singular phenolic profile. This variability can be related to genotypic and agronomic differences; climatic conditions such as temperature, water stress, and light intensity; as well as soil conditions, which can influence the phenolic composition of these plants. In the MsaSC bee pollen, a larger number of peaks were observed (40), and, though the main compound was not identified (retention time: 53.6 min), the other 25 were. The determined phenolic content based on the standards using HPLC-PDA varied from 1.50 ± 0.01 to 46.80 ± 2.57 mg/100 g, with the quantified compounds being protocatechic acid (1.50 ± 0.01 mg/100 g), rutin (46.80 ± 2.57 mg/100 g), naringenin (18.36 ± 1.09 mg/100 g), quercetin (5.97 ± 0.29 mg/100 g), and kaempferol (5.50 ± 0.31 mg/100 g). It is worth noting that, in this monofloral bee pollen, from what we can tell, two compounds common to plants from the *Myrica* genus (myricetin and tricetin-7-O-sophoroside[glycosyl(1–2)glycoside]) were identified for the first time.

In the MsaRN sample, 32 peaks were observed in the MS spectra, of which 25 were identified. Kaempferol-3-O-glycoside was the main compound (retention time: 44.2 min). Additionally, seven other glycosides of this flavonol were identified. β-resorcylic acid, synaptic acid, rutin, naringenin, quercetin, and kaempferol were confirmed by comparison with the available standards, and their contents were 9.65 ± 0.12 mg/100 g, 0.33 ± 0.03 mg/100 g, 25.81 ± 0.31 mg/100 g, 10.41 ± 0.25 mg/100 g, 5.05 ± 0.21 mg/100 g, and 44.97 ± 2.88 mg/100 g, respectively. Procyanidin B1, which was only identified in MsaRN sample, is a substance with antioxidant activity (Amíc & Lucic, 2010).

In the MsaMT bee pollen, of the 25 observed peaks, 12 were identified, including the main one (N′,N″,N‴-tris-*p*-feruloyl-spermidine). Of the three *M. scabrella* samples, the lowest number of compounds was observed in MsaMT sample, together with the lowest antioxidant capacity for both methods (Table 4). LeBlanc et al. (2009) also identified the lowest antioxidant activity in bee pollen sample with the smallest number of polyphenols. The antimicrobial capacity of the MsaMT sample was intermediary, indicating that this parameter can experience a greater influence from the type of a phenolic compound present in the sample than from the total number of compounds.

4 | CONCLUSIONS

Monofloral bee pollen from Brazil has a variable composition and a singular profile. Though some researchers have suggested that there is a standard between monofloral samples from the same pollen type, in the present study, it can be seen that this is not the case for *M. scabrella* bee pollen. Monofloral bee pollen could be a protein source for consumers, especially for those who have a restrictive diet, such as some types of vegetarians. The content of minerals such as iron, manganese, and zinc were so relevant that the introduction of those monofloral bee pollens into diet could be an interesting alternative to avoid deficiencies. The antioxidant capacity of the products was greater than that reported for some red fruits, which are recognized as a dietary source of antioxidants. Monofloral bee pollen inhibited the growth of bacteria and yeast; however, the effect depends on the species tested. The phenolic profile of monofloral bee pollen is variable, and the products had 22–40 compounds, which were mostly identified. Flavonoid 3-O-glycosides glycosides predominated, but other compounds were also present, such as pinobanksin derivatives, glucosinolates, spermidine derivatives, a glycosylated anthocyanin, ampeloptin, eriodictyol, and ellagic acid. The type and the number of phenolic compounds can be due to the biological potential of monofloral bee pollen from Brazil.

ACKNOWLEDGMENTS

The authors are grateful to beekeepers who provided samples. MM Moreira is also grateful to FCT/MEC and FEDER, under the Partnership Agreement PT2020 through the project UID/QUI/50006/2013 - POCI/01/0145/FERDER/007265.

CONFLICT OF INTEREST

The authors declare that they have no conflict of interest.

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How to cite this article: De-Melo AAM, Estevinho LM, Moreira MM, et al. Phenolic profile by HPLC-MS, biological potential, and nutritional value of a promising food: Monofloral bee pollen. *J Food Biochem*. 2018;42:e12536. https://doi.org/10.1111/jfbc.12536