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Bioactive compounds and pollen profile of honeys from northern Minas Gerais, Brazil

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Abstract

The composition of honey is influenced by its botanical origin and geographical area. Monofloral honeys, such as those from Aroeira (*Astronium urundeuva*), Betônica (*Hyptis* sp.), Coffee (*Coffea arabica*), Cipó-uva (*Serjania lethalis*), Pequi (*Caryocar brasilense*), and Velame (*Croton urucurana*), have distinct chemical profiles that impact their sensory properties and bioactive potential. There is limited information on the chemical composition of specific monofloral honeys, and this study provides valuable data for their characterization. This study analyzed seven honey samples collected in the northern region of Minas Gerais and commercialized by Cooperativa dos Apicultores e Agricultores Familiares do Norte de Minas in 2022. The aim was to classify the honeys based on melissopalynological analysis and chemical characterization using liquid chromatography coupled with high-resolution mass spectrometry. Melissopalynological analysis identified three monofloral honeys, three with dominant flowering sources, and one multifloral honey. Chemical characterization by liquid chromatography coupled with high-resolution mass spectrometry revealed 26 bioactive compounds, including plant hormones, alkaloids, flavonoids, and coumarins. Among the identified compounds, flavonoids and coumarins are noteworthy due to their potential antioxidant and antimicrobial properties. This study expands knowledge on the chemical composition of monofloral honeys from Brazilian Cerrado species, highlighting their bioactive potential and possible pharmaceutical, nutritional, and medicinal applications.

Keywords: honey composition; melissopalynology; secondary metabolites; dereplication; spectrometry

Practical Application: Natural substance produced by bees with bioactive compounds and a pollen profile defined by botanical origin and geographic area.

1 INTRODUCTION

Honey is a complex mixture of substances in a viscous and aromatic solution, produced by bees from floral nectar, secretions of living plant parts, or sap-sucking insects (Pătruică et al., 2022). It is predominantly composed of carbohydrates, with smaller concentrations of organic acids, vitamins, minerals, enzymes, proteins, pigments, and other plant-derived substances (Almeida-Muradian et al., 2020). Honey is considered a high-value product, both biologically and economically, with versatile applications in the food, cosmetics, and medical sectors (Šedík et al., 2019).

Floral nectar is collected by bees and hydrolyzed in their hypopharyngeal glands, which cleave disaccharides into monosaccharides. This class of substances constitutes approximately 80% of honey's volume (Wu et al., 2020). The nectar is then deposited in honeycomb cells and concentrated sufficiently to prevent bacterial deterioration during maturation and storage in the hive (Pătruică et al., 2022).

According to international regulations, monofloral honey is defined as honey derived from a single botanical origin, with a pollen frequency exceeding 45% (Louveaux et al., 1978). In addition, it must have a distinct composition, as well as specific organoleptic, physicochemical, and microscopic properties (Food and Agriculture Organization of the United Nations [FAO] & World Health Organization [WHO], 2019). Multifloral honeys, on the other hand, are obtained from various nectariferous floras without the dominance of pollen from a single plant species (Ikegbunam et al., 2023).

Honeys are classified as monofloral or polyfloral based on their pollen content. Quantitatively, classification can be based on the relative frequency of pollen in honey, where a honey is classified as pure if it contains over 90% of a particular pollen type, as having a dominant flowering source if between 45 and 89%, as frequent pollen between 15 and 44%, as isolated pollen between 4 and 15%, and as rare pollen if below 3% (Barth, 1989). When classification is based on pollen size, monofloral honeys are defined as containing at least 96% pollen for grains smaller

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than 20 μm and a minimum of 7% for those larger than 85 μm. However, quantitative classifications do not apply universally to all nectariferous plants, as certain species are classified as representative even with low pollen counts (Soares et al., 2017). *Citrus* spp., *Lavandula* spp., *Rosmarinus officinalis*, and others are considered monofloral honeys despite having low pollen proportions, typically ranging between 10% and 20% (Lopes et al., 2023).

The differences between honeys are strongly influenced by floral diversity, geographical origin, and environmental conditions (Escuredo & Seijo, 2022). Some honey constituents exhibit biological activity, including antibacterial, antifungal, antiviral, antioxidant, antidiabetic, antitumor, anti-inflammatory, anticancer, immunomodulatory, wound-healing, and hepatoprotective properties (Al-Kafaween et al., 2023). Among bioactive compounds, phenolic substances, which are derived from plant secondary metabolism, play a significant role in inhibiting oxidative reactions and contributing to antimicrobial and medicinal activities (Hailu & Belay, 2020). These bioactivities make honey a prophylactic food with preventive health benefits (Olas, 2020).

Studies on honey constituents have employed analytical techniques such as liquid chromatography coupled with mass spectrometry (LC-MS) to detect bioactive substances like sulforaphane and methylglyoxal (Ares et al., 2015). Other frequently identified compounds in honey include abscisic acid (ABA), 4-hydroxyquinoline, 3,4-dihydroxybenzoic acid, 4-hydroxybenzoic acid, ellagic acid, 2,5-dihydroxybenzoic acid, 3,4-dimethoxycinnamic acid, rutin, naringenin, kaempferol, and chrysin, among others (Yi et al., 2023). Chromatographic profile analysis is useful for assessing honey quality and investigating the presence of compounds that may contribute to health and nutrition (Pita-Calvo et al., 2017).

Organic acids are known to be present in honey at low concentrations, necessitating the use of highly sensitive and selective analytical techniques for their detection (Suto et al., 2020). Techniques such as LC-HRMS (liquid chromatography coupled with high-resolution mass spectrometry) are essential for identifying potential compounds based on acquired data, in accordance with established methodologies (Vazquez et al., 2021). LC-HRMS is considered one of the most promising metabolic profiling tools, offering high resolution, speed, and sensitivity, providing accurate analyses in foodomics and natural product research (Chen et al., 2021).

Due to honey's complex matrix, appropriate analytical methods and techniques are required for its analysis. LC-HRMS is applied to obtain chromatographic profiles, potential molecular formulas, and fragmentation patterns of detected compounds (Berton et al., 2020).

This study aims to conduct a melissopalynological analysis of seven honey samples produced in the northern region of Minas Gerais, Brazil, and to characterize them using LC-HRMS.

1.1 Relevance of the work

This article presents information on the pollen profile, composition, and bioactive potential of honeys produced in northern Minas Gerais. The results reveal a pollen profile influenced by the botanical origin and geographic area. The diversity of 26 metabolites in the composition of honeys belongs to different classes of natural products, including plant hormones, alkaloids, flavonoids, and coumarins. Substances with antioxidant, antimicrobial, and pharmacological potential.

2 MATERIALS AND METHODS

2.1 Chemicals

Deionized water (Merck Millipore, EUA), glycerin (Dinâmica, Brazil), sodium chloride, ethyl acetate, sodium sulfate, methanol (Sigma-Aldrich, EUA), formic acid (Supelco, USA), and high performance liquid chromatography (HPLC)-grade acetonitrile (Merck, Germany).

2.2 Melissopalynological analysis

To prepare the slides, 10 g of honey was dissolved in 20 mL of deionized water. The solution was centrifuged (Eppendorf, Germany), and the supernatant was discarded (Barth, 1989). The precipitate was immersed in uncolored glycerinated gelatin, and the slides were sealed with paraffin and observed under a light microscope (Zeiss, Germany). Pollen counting was performed as described by Barth (1989) and Louveaux et al. (1978), with the reference sheet being PROBEE Ltd. The results were expressed as a percentage of pollen type dominance.

2.3 LC-MS/MS analysis method

The methodology followed the procedures and parameters established by Acacio et al. (2023) in positive ionization mode.

2.4 Preparation of crude extracts

Due to the high viscosity of honey, the samples were preheated in a water bath at 45°C. A 2% sodium chloride solution was then prepared. Subsequently, 5 mL of the saline solution was added to 5 g of honey. The mixture was vortexed, and then three 5 mL extractions were performed with ethyl acetate. After liquid–liquid extraction, sodium sulfate (Na_2SO_4) was added to the organic phase as a drying agent, and the mixture was stirred and filtered through cotton. The final solution was subjected to SpeedVac equipment (Thermo Fisher Scientific, Waltham, Massachusetts, USA) to obtain a concentrated dry sample.

2.5 LC-HRMS analysis method and annotations

For LC-HRMS, the extract (5 mg) was prepared in safe-lock microtubes and solubilized in a 3:2 ratio of methanol and water. The polypropylene vials were conditioned with a final concentration of 5.0 mg mL^{-1} (50 μL) on the sampler rack (Soares-Bezerra et al., 2019). LC-HRMS analyses were performed using a Nexera UHPLC system (Shimadzu, Japan) coupled to a mass spectrometer with electrospray ionization and a quadrupole/time-of-flight detection (ESI-Q-TOF) of high-resolution MaXis ETD (Bruker, USA), controlled by Compass 1.5 software package (Bruker, USA).

Aliquots of 2 μL were injected into a Shimpack XR-ODSIII column (C18, 150 × 2.0 mm, 2.2 μm) (Shimadzu, Japan) at

40°C, with a flow rate of 400 µL min^{-1}. Mobile phases A and B (0.1% formic acid in water and HPLC-grade acetonitrile, respectively) were used in gradient elution, starting with 5% B for 5 min, followed by a linear ramp to 100% B over 45 min, and holding at 100% B for 5 min. Mass spectra were acquired in positive ionization mode at a spectral rate of 5.0 Hz. The ion source parameters were set to −500 V end plate offset, capillary voltage of 4,500 V, nebulizer pressure of 3.0 bar, and drying gas flow and temperature at 8.0 L min^{-1} and 200°C, respectively. Fragmentation spectra were obtained in data-dependent mode (automated MS/MS) using a collision energy ramp from 12 to 60 eV. Ion cooler settings were optimized for a mass range of 100–1,500 *m/z* using a sodium formate calibration solution (Sigma-Aldrich, USA) at 1 mM in 50% 2-propanol (Sigma--Aldrich, USA). Mass calibration was performed via initial infusion of the calibration solution (20 µL) into the ion source and post-acquisition recalibration of raw data. The compound detection was carried out by chromatographic peak dissection, followed by the determination of the formula based on exact mass and isotopic pattern (MS1). Putative identification was based on the comparison of fragmentation spectra (MS2) with reference spectra from standard substances available in the in-house public MassBank database (Horai et al., 2010) at the René Rachou Research Centre—CPqRR, as well as searches in public libraries, available literature, and annotation using the Sirius software (Lehrstuhl Bioinformatik Jena, version 5.7.2) with reliability scores above 90%.

3 RESULTS AND DISCUSSION

3.1 Melissopalynological analyses

From the data obtained, it can be observed that of the seven samples analyzed, three were classified as monofloral, three exhibited dominant flowering, and one was considered multifloral. According to the classification criteria established, honeys with more than 90% pollen from a single species are monofloral, between 45 and 89% are classified as having dominant flowering, and below 45% are considered multifloral (ref). The monofloral honeys identified in this study were *Caryocar brasiliense* (99%), *Astronium urundeuva* (94.34%), and *Coffea arabica* (90.09%). The samples classified as dominant flowering were *Serjania lethalis* (83.33%), *Croton urucurana* (83.34%), and *Hyptis* sp. (69.38%). The multifloral sample contained pollen from various species, with two considered accessory pollen: *Baccharis calvescens* (25.42%) and *C. urucurana* (16.95%) (Šedík et al., 2019) (Table 1).

Table 1. Mesopalynological analysis.

Honey	Type of pollen	Pollen count	Index %
Aroeira (Monofloral)	*Astronium urundeuva*	500	94.34
	Eucalyptus robusta	30	5.66
Betônica (Dominant flowering)	*Hyptis* sp.	213	69.38
	Croton urucurana	30	9.77
	Eucalyptus robusta	28	9.12
	Baccharis calvescens	11	3.58
	Astronium urundeuva	10	3.26
	Mimosa scabrella	4	1.30
	Protium sp.	3	0.97
	Sida sp.	2	0.65
	Serjania lethalis	2	0.65
	Cecropia glazioui	2	0.65
	Anadenanthera colubrina	2	0.65
Café (Monofloral)	*Coffea arabica*	100	90.09
	Baccharis calvescens	5	4.50
	Serjania lethalis	2	1.80
	Citrus sinensis	2	1.80
	Eucalyptus robusta	1	0.90
	Vernonia scorpioides	1	0.90
Cipó-uva (Dominant flowering)	*Serjania lethalis*	150	83.33
	Astronium urundeuva	30	16.66
Pequi (Monofloral)	*Caryocar brasiliense*	200	99.00
	Piptadenia communis	1	0.50
	Eucalyptus robusta	1	0.50
Silvestre (Multifloral)	*Baccharis calvescens*	30	25.42
	Hyptis sp.	10	8.47
	Myracrodruon urundeuva	15	12.71
	Croton urucurana	20	16.95
	Ipomoea sp.	16	13.56
	Richardia sp.	17	14.41
	Serjania sp.	5	4.24
	Mimosa caesalpiniaefolia	5	4.24
Velame (Dominant flowering)	*Croton urucurana*	150	83.34
	Eucalyptus robusta	20	11.11
	Anadenanthera colubrina	10	5.55

The diversity of pollen found in the analyzed honey samples is indicative of their botanical authenticity, confirming that they originate from specific geographic regions with distinct floral compositions. In Brazil, the country's remarkable biodiversity and well-defined biomes allow for clear distinctions in the botanical origin of honeys. The samples in this study were produced in the Cerrado *sensu stricto*, known as the Brazilian savannah, a biome with a rich variety of plant species that significantly influences the composition of local honeys.

3.2 LC-HRMS analyses

The seven samples of honey (Aroeira, Betônica, Coffee, Cipó-uva, Pequi, Velame, and Polyfloral) present a total of 26 substances identified, varying from 8 to 13 for the sample. Screening of data obtained through the system for detecting chemical substances present in honey revealed different chemical content among the analyzed materials, as well as common metabolites among the honeys. The data obtained can be observed in Table 2.

Substances 1–4, annotated as ABA, when fragmented in positive ionization mode, form fragments corresponding to two dehydrations, the first with *m/z* 247 [M+H-H_2O], the second with *m/z* 229 [M+H-2H_2O], and the fragment with *m/z* 201 corresponding to two dehydrations and the loss of a CO group [M+H-2H_2O-CO], as described in the literature (Zhao et al., 2013). Substance 5 annotated as indole-3-carboxaldehyde (3-IAld) or the isomers 2-hydroxyquinoline (2-OHQ) or 4-hydroxyquinoline (4-OHQ), the substance with *m/z* 146, protonated and with the molecular formula C_9H_7NO, possesses the characteristic fragments *m/z*118 and *m/z*117.

Substance 6 annotated as indole-3-acetic acid, the substance with *m/z* 176 and molecular formula $C_{10}H_9NO_2$, possesses the characteristic fragment *m/z* 130, relative to the loss of carboxyl [M+H-COOH] already reported in the literature (Matsuda et al., 2005). In addition, other fragments were detected in the analysis, namely *m/z* 158 relative to the loss of [M+H-H_2O] and *m/z* 118 relative to the loss of [M+H-$C_2H_3O_2$], annotated with the Sirius software. Substance 7 annotated as tuberonic acid, with *m/z* 227 and molecular formula $C_{12}H_{18}O_4$, possesses characteristic fragments of *m/z* 209 relative to a dehydration [M+H-H_2O], *m/z* 191 to the loss of two water molecules [M+H-2H2O], *m/z* 163 to the loss of two water molecules and one CO group [M+H-2H_2O-CO], fragments already reported in the literature for this substance (Sung et al., 2021). The *m/z* 149, possibly related to the loss of [M+H-2H_2O-C_2H_2O] and *m/z* 131 [M+H-3H_2O-C_2H_2O] were also detected and annotated based on the Sirius software. Substance 8 annotated as jasmonoyl-L-isoleucine with *m/z* 324 and molecular formula $C_{18}H_{29}NO_4$ possesses the characteristic fragment of *m/z* 151 already reported in the literature (Widemann et al., 2015). The *m/z* 278 fragment may be related to the loss of the carboxyl group [M+H-CO_2H], and the *m/z* 306 fragment may be related to a dehydration [M+H-H_2O] as suggested by Sirius. The fragment with *m/z* 132 has the characteristic mass of the protonated amino acid isoleucine. Substance 9 annotated as vomifoliol, with *m/z* 225 and molecular formula $C_{13}H_{20}O_3$, showed peaks at *m/z* 207 corresponding to the first dehydration [M+H-H_2O] and *m/z* 189 [M+H-2H_2O] corresponding to the second dehydration, *m/z* 149 to the loss of [M+H-$C_3H_8O_2$] and *m/z* 123 to the loss of [M+H-$C_4H_6O_3$] annotation made with data obtained from Sirius. Substance 10, with *m/z* 205 fragments resulting from dehydration [M+H-H_2O] and *m/z* 121 related to the loss of [M+H-$C_5H_{10}O_2$] are characteristic fragments reported for dehydrovomifoliol (Mannima et al., 2015). Annotated substance 11, caffeine with *m/z* 195 produces a characteristic main product ion of *m/z* 138, corresponding to the neutral loss of methyl isocyanate [M+H-O=C=NCH_3] *m/z* 57 due to a retro--Diels-Alder rearrangement (Bianco et al., 2009). Substance 12 annotated as theobromine, with the fragments *m/z* 181 corresponding to the addition of a hydrogen atom to the structure, *m/z* 138 representing the loss of [M+H-OCNH], and *m/z* 135 representing the loss of [M+H-H_2O-CO], both fragments previously reported in the literature (Vonaparti et al., 2009). Substance 13 annotated as flazin, an alkaloid already described in honey, exhibits characteristic peaks in the protonated form at *m/z* 281 after the loss of [M+H-CO], *m/z* 263 relative to the loss of carboxyl [M+H-COOH], *m/z* 235 after the loss [M+H-$C_2H_2O_3$], *m/z* 206 [M+H-CO_2-$C_2H_3O_2$], and *m/z* 180 relative to the loss [M+H-$C_2H_2O_2$], followed by a retro-Diels-Alder rearrangement (Guan et al., 2022). EIt is speculated that substance 14 annotated as 2′,3′,6-Trimethoxyflavone, with *m/z* 313, protonated, with molecular formula $C_{18}H_{16}O_5$, was detected with characteristic fragments *m/z* 295 [M+H-H_2O] (Zhang et al., 2022). Other fragments were also detected, *m/z* 267 possibly related to the loss of a methoxy and a methyl group [M+H-CH_3O-CH_3], *m/z* 239 possibly related to the loss of two methoxy groups and a methyl group [M+H-2CH_3O-CH_3], *m/z* 224 possibly related to the loss of the three methoxy groups [M+H-3CH_3O], and *m/z* 135 possibly formed from the fragmentation of the heterocyclic ring [M+H-$C_{10}H_{10}O_3$]. Substance 15 annotated as kaempferol, with *m/z* 287 and molecular formula $C_{15}H_{10}O_6$, has characteristic fragmentations, *m/z* 258 related to the loss of [M+H-CHO], and *m/z* 121 [M+H-$C_8H_5O_4$] and *m/z* 165 [M+H-$C_7H_5O_2$] corresponding to retro-Diels-Alder fragmentation (Satheeshkumar et al., 2014) and fragments *m/z* 153 [M+H-C_7H_5O] and *m/z* 133 [M+H-$C_8H_5O_2$] related to retro-Diels-Alder reaction (March & Miao, 2004). Substance 16 annotated as sakuranetin, with molecular formula $C_{16}H_{14}O_5$ and *m/z* 287 in its protonated form, has fragments *m/z* 270 related to demethylation [M+H-CH_3], *m/z* 242 to the loss of [M+H-C_2H_5O], *m/z* 167 [M+H-C_8H_9O], and *m/z* 119 [M+H-$C_8H_6O_4$] possibly resulting from retro-Diels-Alder reaction, *m/z* 153 [M+H-$C_9H_{10}O$] a possible fragment from retro-Diels-Alder-type reactions, and *m/z* 147 related to the loss of [M+H-$C_7H_8O_3$]. Annotated as santin, substance 17 with *m/z* 345 has peaks, *m/z* 330, resulting from demethylation [M+H-CH_3], *m/z* 329 the loss of [M+H-CH_4], *m/z* 313 the loss of [M+H-CH_4O], and *m/z* 287 relative to the loss of two methyls and a CO group [M+H-2CH_3-CO] annotated with support from the sirius software. Substance 18 annotated as suberic acid annotated with *m/z* 175 undergoes dehydration by the loss of a water molecule [M+H-H_2O] corresponding to the peak *m/z* 157 (Kasiotis et al., 2023). Annotated as monomethyl sebacate, substance 19, with *m/z* 217 and molecular formula $C_{11}H_{20}O_4$ has the fragment *m/z* 139 according to the literature (Grossert et al., 2005). Fragments *m/z* 171 possibly related to the loss of [M+H-COOH], and *m/z* 121 to the loss of [M+H-COOH-CH_4O-H_2O] were detected. Substance 20 annotated as isofraxidin with *m/z* 223 has characteristic peaks

Table 2. LC-HRMS annotations of the substances detected in the honeys.

	Compound name	Rt*	Massa $[M+H]^+$	Ms/Ms** *(m/z)*	Molecular Formula	Honey
1	Abscisic acid and or isomers	34.2 35.9 35.7 35.8 36	265.1424	247.1323 229.1218 201.1270	$C_{15}H_{20}O_4$	Aroeira Coffee Cipó-Uva Pequi Polyfloral
2		33.8 35.7	265.1424	247.1322 229.1220 209.0803 205.1216 201.1270	$C_{15}H_{20}O_4$	Aroeira Polyfloral
3		35,3	265.1423	247.1320 229.1216 209.0808 153.0897	$C_{15}H_{20}O_4$	Betônica
4		35.7 36 36.2 36.4 36.1	265.1423	247.1320 229.1216 201.1268 187.1111	$C_{15}H_{20}O_4$	Betônica Cipó-Uva Pequi Velame Polyfloral
5	Indole-3-carboxaldehyde or 2-hydroxyquinoline or 4-hydroxyquinoline	32.9	146.0596	118.0647 117.0568	C_9H_7NO	Aroeira
		32.9	146.0596	118.0647 117.0568	C_9H_7NO	Aroeira
		32.9	146.0596	118.0647 117.0568	C_9H_7NO	Aroeira
6	Indole-3-acetic acid	32.3	176.0703	158.0596 131.0680 130.0647 118.0647	$C_{10}H_9NO_2$	Aroeira
7	Tuberonic acid	33.6 34 33.8	227.1272	209.1172 191.1066 163.1115 149.0958 131.0852	$C_{12}H_{18}O_4$	Coffee Velame Polyfloral
8	Jasmonoyl-L-isoleucine	38.5 38.7	324.2167	306.2061 278.2111 151.1113 132.1016	$C_{18}H_{29}NO_4$	Pequi Cipó-Uva
9	Vomifoliol	33.5 32.2 33.5 33.9 31.4	225.1489	207.1374 189.1270 149.0958 123.1163	$C_{13}H_{20}O_3$	Betônica Aroeira Cipó-uva Pequi Polyfloral
10	Dehydrovomifoliol	34.3 32.9 34.6 34.7 34.9 35 34.8	223.1322	205.1218 121.0644	$C_{13}H_{18}O_3$	Betônica Aroeira Coffee Cipó-uva Pequi Velame Polyfloral
11	Caffeine	32.6 32.8 32.9 33.1	195.0871	138.0654 110.0720	$C_8H_{10}N_4O_2$	Betônica Coffee Cipó-uva Pequi
12	Theobromine	26.7	181.0719	138.0661 135.0662	$C_7H_8N_4O_2$	Coffee
13	Flazin	35.9 34.4 36.3 36.7	309.0863	281.0919 263.0812 235.0856 206.0832 180.0804	$C_{17}H_{12}O_4N_2$	Betônica Aroeira Coffee Velame

Continue...

Table 2. Continuation.

	Compound name	Rt*	Massa $[M+H]^+$	Ms/Ms** *(m/z)*	Molecular Formula	Honey
14	2′,3′,6-Trimethoxyflavone	36.8	313.1061	295.2248 268.1166 267.1006 252.0773 239.1058 224.0825 209.0592 181.0643 177.0546 135.0434	$C_{18}H_{16}O_5$	Aroeira
15	Kaempferol	37.5 37	287.0547	258.0514 165.0185 153.0183 121.0283	$C_{15}H_{10}O_6$	Velame Coffee
16	Sakuranetin	39 39.3	287.0909	167.0336 147.0437 119.0486	$C_{16}H_{14}O_5$	Cipó-uva Velame
17	Santin	40	345.0960	330.0723 329.0645 312.0621 287.0539 253.0481	$C_{18}H_{16}O_7$	Velame
18	Suberic acid	33.6	175.0954	157.0852	$C_8H_{14}O_4$	Betônica
19	Monomethyl sebacate	36.3 37.8 38.2 38.5	217.1429	171.1374 139.1111 121.1005	$C_{11}H_{20}O_4$	Aroeira Betônica Coffee Pequi
20	Isofraxidin	34.9	223.0600	208.0362 190.0259 163.0375 162.0310	$C_{11}H_{10}O_5$	Velame
21	Scopoletin	33.9 34.3 34.7	193.0490	178.0259 165.0541 133.0283	$C_{10}H_8O_4$	Betônica Coffee Velame
22	Phenyllactic acid	33.7	167.0699	121.0644	$C_9H_{10}O_3$	Aroeira
23	Lumichrome	34.5 34.8 34.9 35.2	243.8650	216.0765 200.0813 198.0660 172.0868	$C_{12}H_{10}N_4O_2$	Betônica Coffee Cipo-Uva Velame
24	Plastoquinol-1	32.1 33.4 36.7 33.9 34.1 34 34.3	207.1373	189.1268 149.0963 135.1166 123.1161	$C_{13}H_{18}O_2$	Aroeira Betonica Coffee Cipó-uva Pequi Polyfloral Velame
25	Roseoside	32.9	387.2003	225.1478 207.1372 189.1268 163.1096 149.0953	$C_{19}H_{30}O_8$	Betônica
26	Dihydroconiferin	32.5 33.4 33.8 34 34.5 34.5 34.3	345.1539	183.1011 165.0906 137.0956	$C_{16}H_{24}O_8$	Aroeira Betônica Coffee Cipó-Uva Pequi Polyfloral Velame

*Retention in minutes; **UHPLC-ESI-Q-TOF-MS: ultra-high-performance liquid chromatography coupled with electrospray ionization quadrupole time-of-flight mass spectrometry. MS: tandem mass spectrometry MS/MS is usually used, which indicates mass analysis at levels 1 and 2.

of *m/z* 208 relative to the loss of [M+H-CH_3], *m/z* 190 [M+H--CH_3-OH], and *m/z* 162 [M+H-CO-OH-CH_3] as described in the literature for the respective substance (Sun et al., 2007). The substance annotated as phenylacetic acid, with *m/z* 167 and molecular formula $C_9H_{10}O_3$, has the characteristic fragment of *m/z* 121 relative to the loss of the carboxyl group present in the structure [M+H-COOH] (Tuberoso et al., 2010). Substance 21 annotated as scopoletin, the substance with *m/z* 193 undergoes demethylation [M+H-CH_3] corresponding to the peak *m/z* 178, [M+H-CO] corresponding to the peak *m/z* 165, and the loss of [M+H-CO_2-H_2O] corresponding to the peak *m/z* 132 (Singh et al., 2021). Substance 22 annotated as phenyllactic acid with *m/z* 167 and molecular formula $C_9H_{10}O_3$, exhibits a characteristic peak of *m/z* 121 characterized by the loss [M+H-COOH] annotated with support from the sirius software.

Substance 23, annotated as lumichrome with *m/z* 243 and molecular formula $C_{12}H_{10}N_4O_2$, exhibits characteristic peaks of *m/z* 216, *m/z* 198, *m/z* 200, and a fragment of *m/z* 172 characterized by the loss [M+H-C_2HNO_2] (Stanojević et al., 2015). Substance 24, annotated as plastoquinol-1, protonated at *m/z* 207, exhibits the characteristic peaks already described in the literature at *m/z* 189, *m/z* 149, *m/z* 135, and *m/z* 123 (Tortosa et al. 2018). Substance 25, annotated as roseoside with *m/z* 387, exhibits the characteristic peak at *m/z* 225 resulting from the loss of the hexose sugar (*m/z* 163) [M+H-$C_6H_{11}O_5$]. The second aglycone fragment with *m/z* 207 [M+H-$C_6H_{12}O_6$] can be seen as the loss of the C1 bond of the hexose sugar, and *m/z* 189 [M+H--$C_6H_{12}O_6$-H_2O] (Xiao et al., 2018). Substance 26, annotated as dihydroconiferyl alcohol, with *m/z* 345 and molecular formula $C_{16}H_{24}O_8$, exhibits the characteristic fragment at *m/z* 165 [M+H--$C_9H_{11}O_6$] as previously reported in the literature (Shan et al., 2018). The proposed peaks for *m/z* 183 correspond to the loss of the glucoside [M+H-$C_6H_{11}O_5$], and *m/z* 137 corresponds to the loss of [M+H-$C_7H_{17}O_7$].

4 CONCLUSION

The LC-HRMS scan in positive ionization mode, combined with melissopalynological analyses, provided detailed insights into the analyzed honeys, classified as three monofloral, three with dominant flowering, and one multifloral. Chemical analysis revealed the presence of bioactive metabolites, including plant hormones, quinones, alkaloids, flavonoids, fatty acids, coumarins, and glycosides. These findings expand knowledge on the pollen composition and chemical profile of honeys produced in northern Minas Gerais, enhancing their value and potential application in food and pharmaceutical products. Moreover, they guide future research and improvements in regional beekeeping practices, promoting the quality and authenticity of local honeys.

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