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Bioactive signatures of native forest honeys from Northeastern Brazil: linking botanical origin, chemical composition, and *in vitro* biological effects

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ABSTRACT

Honey is a natural product valued for its nutritional and therapeutic properties, which are largely attributed to its chemical compounds. This study evaluated the pollen analysis, the color, the minerals, the volatile compounds, the phenolic composition and antioxidant-related phytochemicals of four honey samples collected from a preserved native forest region in Brazil, an area characterized by minimal anthropogenic impact and significant potential for sustainable beekeeping. Total phenolic content (TPC), total flavonoid content (TFC), spectrophotometric methods, and volatile compounds were conducted by gas chromatography mass spectrometry (GC–MS) and individual polyphenols were determined using high-performance liquid chromatography coupled with mass spectrometry (HPLC-MS), respectively. The results revealed considerable variation among the honey samples. The predominance of phenolic acids and flavonoids confirms the rich phytochemical composition of these honeys and demonstrates that their chemical profiles are strongly influenced by botanical origin. The findings highlight the high quality of honey produced in this preserved Brazilian ecosystem and support its potential as a valuable natural product with promising antioxidant properties. Furthermore, the results emphasize the importance of conserving native forests to promote sustainable apiculture and the production of authentic, contamination-free honey.

1. Introduction

Honey is one of the oldest natural foods known to humans and has been used for centuries not only as a sweetener but also for nutritional, medicinal, and cultural purposes. Today, honey remains the most important primary product of beekeeping, both economically and nutritionally, with a global production more than 1.8 million tons annually and representing a significant share of the international food

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market (FAO, 2025). According to the definition established by the *Codex Alimentarius*, honey is a natural sweet substance produced by honeybees from the nectar of flowers, plant secretions, or excretions of plant-sucking insects. The bees collect materials from nectar sources, transform them by combining them with enzymes, and store them in honeycombs where the product undergoes dehydration and maturation. Honey can be classified according to its botanical origin into blossom honey and honeydew honey. Blossom honey is produced mainly from floral nectar, whereas honeydew honey originates from secretions of plant-sucking insects such as aphids that feed on plant sap. Within blossom honeys, products can be either multifloral—derived from multiple plant sources—or monofloral, where the nectar of a single plant species predominates and gives the honey its characteristic properties. Chemically, honey is a complex natural matrix composed of carbohydrates and water, together with minor components including organic acids, proteins, enzymes, amino acids, vitamins, minerals, phenolic compounds, and volatile aromatic substances. These constituents are responsible for the nutritional value, sensory characteristics, and biological properties of honey. Moreover, the composition of honey can vary considerably depending on factors such as botanical origin, geographical location, environmental conditions, and processing or storage practices (Santos-Buelga & González-Paramás, 2017). Despite its natural stability and suitability for long-term storage, honey may undergo compositional changes over time due to chemical and biochemical processes such as fermentation, oxidation, or sugar dehydration. Consequently, understanding the chemical composition of honey and the factors influencing it is essential for evaluating its quality, authenticity, and nutritional value (Solayman et al., 2016a).

Honey production is increasing worldwide. Bee are bioindicators of environmental pollution, and it is very important to verify its authenticity and purity, which contribute to the wide price variation in the market (Kadry et al., 2025; Mărgăoan et al., 2021a). It is crucial to verify the botanical and geographical origins of honey to confirm its provenance, to provide consumer safety, and promote production and sales within the beekeeping chain (Margaoan et al., 2025; Zhang & Abdulla, 2022). Finding products free from contamination and originating from pollution-free regions is crucial. The honeys analyzed in this study come from a new region where beekeeping is practiced, a native forest far from polluting sources, and with a great potential for increased production, because this location is free from deforestation and ecosystem

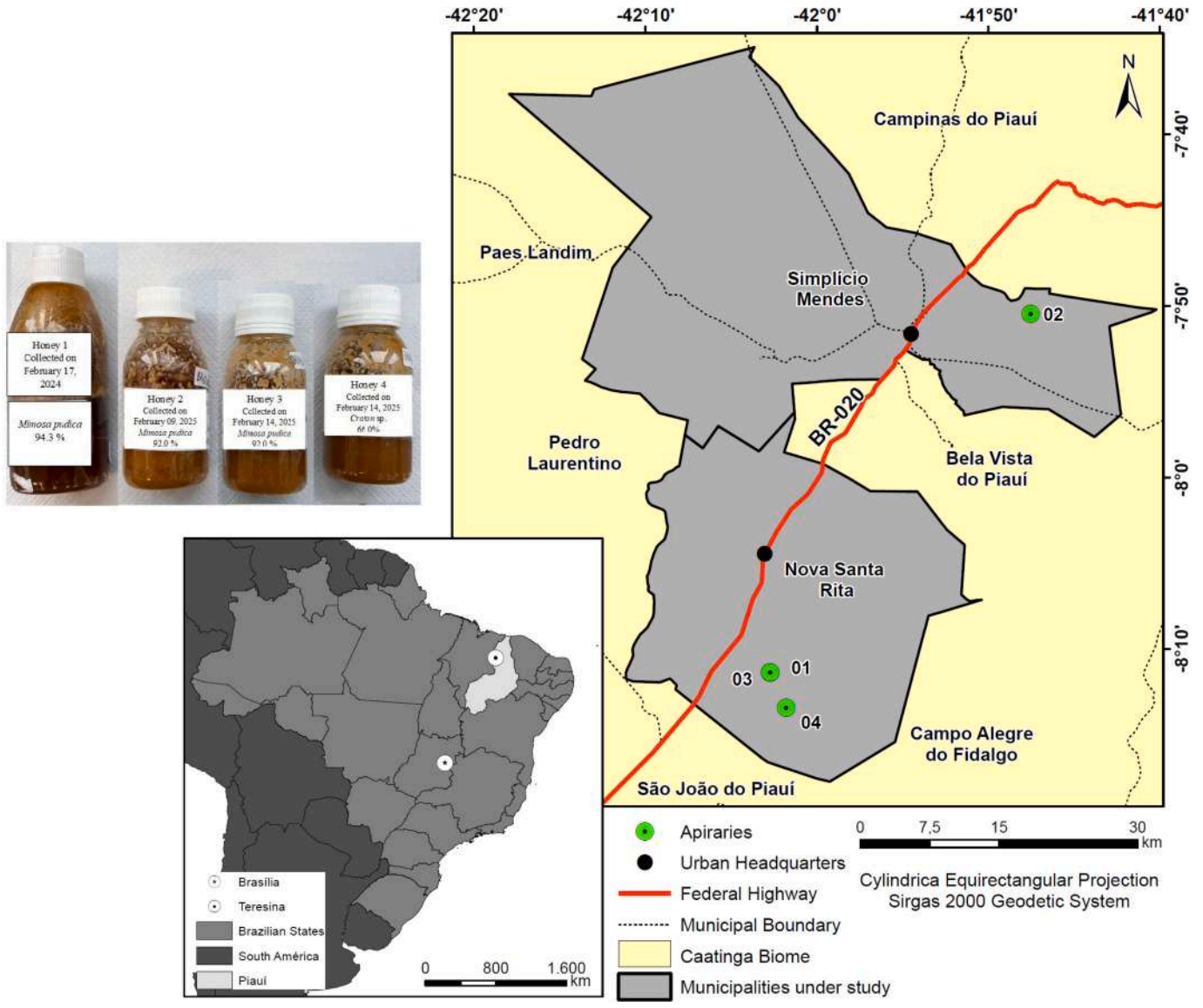


Fig. 1. Image of the honey samples and the geographic location of each apiary where honey samples were collected. Geographic location: honeys **1** and **3**, https://maps.app.goo.gl/xWDZavSmEz9zZpmd9?g_st=aw, honey **2**, https://maps.app.goo.gl/EYuQmWrjBPqzyznP7, honey **4**, https://maps.app.goo.gl/xWDZavSmEz9zZpmd9?g_st=aw.

destruction. Beekeepers are native to the region and are aware of the need to preserve the environment where they harvest honey.

2. Materials and methods

2.1. Honey samples

The honey samples from *Apis mellifera* were collected in Nova Santa Rita, Piauí State, Brazil. The location is native semi-arid forest. The first sample (honey **1**) collected in February 17, 2024, the second (honey **2**) collected in February 09, 2025, honey **3** (the same location of honey **1**), collected in February 14, 2025, honey **4** collected in the same date of honey **3** (Fig. 1 and Fig. S1).

2.2. Pollen analysis

The botanical origin of honey was determined by melissopalynological analysis (Louveaux et al., 1978). Fresh honey samples were prepared for microscopic analysis of pollen profiles, including qualitative identification of pollen types and quantitative determination of relative pollen grain frequency (Da Luz et al., 2021). The pollen frequency was classified as predominant pollen (>45% of total pollen), secondary pollen (16–45%), important minor pollen (3–15%), and minor pollen (<3%) (Barth, 1989; Da Luz et al., 2021; Louveaux et al., 1978).

2.3. Chemical characterization of honeys

2.3.1. Determination of color by Bianchi

The determination of color of honey is followed by the developed method by Bianchi. 5 g of honey are weighed and transfer to 25 mL beaker, adding 3 mL of distilled water. The volume is then made up to 10 mL with distilled water in a calibrated volumetric flask. The prepared solution is placed in a test tube and allowed to stand for 10 to 15 min before reading. Honey with impurities due to improper handling is filtered with filter paper immediately after the 10 or 15 min of standing and before taking the reading. The absorbance is measured using a spectrophotometer at 635 nm, the zero value is made with distilled water. The color of the honey is determined using the scale of Pfund (Archilia et al., 2023; Bianchi, 1990). To obtain the color (mm Pfund) the following formula is used: mmPFund = (−38,7 + 371,39) X A, where "A" is the absorbance read at 635 nm and the numbers are factors of the linear regression (Table S1). Perform the procedure in triplicate and measure the absorbance of each aliquot eight times.

2.3.2. Determination of TPC and TFC

Total phenolic content (TPC) was determined according to Mustafa et al. (Mustafa et al., 2016), with modifications. Methanolic extract (0.5 mL) was mixed with 2.5 mL of diluted Folin-Ciocalteu reagent and 7 mL of 7.5% Na_2CO_3 solution and incubated in the dark for 2 h at 25 °C. Absorbance was measured at 714 nm using an Agilent Cary 8454 UV–vis spectrophotometer (Agilent Technologies, Woburn, MA, USA). TPC was determined from a gallic acid calibration curve and expressed as mg gallic acid equivalents (GAE) per kilogram of dry extract (DE). Measurements were performed in quadruplicate. Total flavonoid content (TFC) was determined by mixing 0.5 mL of extract with 0.15 mL of 0.5 M $NaNO_2$, 3.2 mL of 30% methanol (*v*/v), and 0.15 mL of 0.3 M $AlCl_3$. After 5 min, 1 mL of 1 M NaOH was added, and absorbance was measured at 506 nm against a reagent blank. TFC was determined using a rutin calibration curve and expressed as mg rutin equivalents per 100 g of dry extract (mg RE/100 g). Analyses were performed in triplicate, with RSD values below 20%.

2.3.3. Analysis of polyphenols

The extraction of phenolic compounds from honey was performed by purification with solid phase extraction (SPE). Firstly, 2.5 g of honey was mixed with 2.5 mL of 37% HCl solution (pH = 2) and this solution was purified on cartridges C_{18}. The cartridges were preconditioned with 3 mL of methanol followed by 3 mL of 37% HCl solution (pH 2.0). 5 mL of sample was purified through the cartridges, then washed with 4 mL of deionized water and finally eluted with 2 mL of 90% *v*/v methanolic solution. Samples were then dried by nitrogen and redissolved with 1 mL of methanol. Phenolic compounds in honey extracts have been analyzed using an Agilent 1290 Infinity series coupled with Triple Quadrupole 6420 from Agilent Technology (Santa Clara, CA) equipped with an electrospray ionization (ESI) source operating in negative and positive ionization modes.

Phenolic compounds were separated on a Synergi Polar-RP C18 analytical column (250 mm × 4,6 mm, 4 μm) by Phenomenex (Chesire, UK), coupled with a pre-column Polar RP security guard cartridge (4 mm × 3 mm ID). The mobile phase was a mixture of (A) water and (B) methanol, both with formic acid 0,1%, at a flow rate of 0.8 mL/min in gradient elution mode. The elution program used for the separation was: 0–1 min, isocratic condition, 20% B; 1–25 min, 20–85% B; 25–26 min, isocratic condition, 85% B; 26–32 min, 85–20% B. All solvents and solutions were filtered through a 0.2 μm polyamide filter from Sartorius Stedim (Goettingen, Germany). The injection volume was 2 μL. The temperature of the column was 30 °C, and the temperature of the drying gas in the ionization source was 350 °C. The gas flow was 12 L/min, the nebulizer pressure was 55 psi, and the capillary voltage was 4000 V. Detection was performed in the dynamic-multiple reaction monitoring (dynamic-MRM) mode, and the dynamic-MRM peak areas were integrated for quantification. The most abundant product ion was used for quantitation, and the others for qualification. The specific time window for each compound (Δ retention time) was set at 2 min. The selected ion transitions and the mass spectrometer parameters for the analyzed compounds are reported in Table S3 and Fig. S2. Analysis was performed in triplicate (Mustafa et al., 2022a).

2.3.4. Analysis of volatiles

Volatile organic compounds (VOCs) were extracted using a headspace solid-phase microextraction (HS-SPME) procedure based on Rivelino et al. (Rivellino et al., 2013), with minor modifications. A 1-cm SPME fiber coated with 65 μm polydimethylsiloxane/divinylbenzene (PDMS/DVB) (Supelco - USA) was used for analyte extraction. Extractions were carried out in 20 mL glass headspace vials sealed with magnetic stainless-steel screw caps fitted with PTFE/Silicone septa. Prior to use, the SPME fiber was conditioned according to the manufacturer's instructions. HS-SPME extractions were performed using a TriPlus RSH autosampler (ThermoFisher Scientific, USA). The honey blend was preheated at 40 °C for 30 s and manually stirred to ensure homogeneity. Subsequently, 1.0 g of honey blend was weighed into a 20 mL headspace vial containing 1 mL of a saturated aqueous sodium chloride solution. The extraction was performed at 250 rpm agitation. The vials were pre-equilibrated at 45 °C for 5 min. The SPME extraction was then performed at 45 °C for 30 min. After extraction, the analytes were thermally desorbed from the fiber coating in the GC injector at 260 °C for 1 min. For compound identification, a standard solution of *n*-alkanes (C_8-C_{20}) was also analyzed to determine the first-dimension linear temperature programmed retention index (^{1}D-LTPRI).

2.3.4.1. GC × GC–MS. Analyses were performed using a TRACE 1300 gas chromatograph coupled to an ISQ single-quadrupole mass spectrometer (QMS) (Thermo Fisher Scientific). The GC system was equipped with a split/splitless injector operated in splitless mode for 1 min at 260 °C. Helium was used as the carrier gas at a constant flow rate of 0.6 mL/min. The GC oven temperature program was as follows: initial temperature of 60 °C, ramped to 250 °C at 3 °C/min, followed by a final isotherm hold of 5 min. Thermal modulation was performed using a consumable-free solid-state modulator, SSM1800 (J&X Technologies, China). The column configuration consisted of a SUPELCOWAX 10

column (30 m × 0.25 mm × 0.25 μm) (Merck Millipore) in the first dimension, connected to an HP-5 column (1.75 m × 0.25 mm × 0.25 μm) (Agilent Technologies, USA) in the second dimension. Siltite micro-unions (Trajan Scientific and Medical, Australia) were used for connecting these two capillary columns with the SV modulation capillary. The modulation period was set to 7 s. The cold trap temperature was maintained at −51 °C, while the modulator entry and exit zones were held at 250 °C and 280 °C, respectively. The MS transfer line and ion source temperatures were set at 250 °C and 275 °C, respectively. Electron ionization (EI) at 70 eV was employed. Mass spectra were acquired over a mass range of 35–500 *m/z*. Instrument control and data acquisition were performed using Chromeleon software v7.3.2 (ThermoFisher Scientific). Peak detection was carried out using GC IMAGE software (GC Image, LLC, USA). Feature annotation was performed using a minimum mass spectral similarity match of 75% and a retention index tolerance of ±30 units. Compound identification was achieved by comparison of experimental mass spectra with those in the NIST20 mass spectral library (National Institute of Standards and Technology, USA), using standard polar column reference values for ^{1}D-LTPRI filtering. Analysis was performed in duplicates (Rivellino et al., 2013).

2.3.5. Analysis of minerals

Honey samples (~10 g) were collected in previously acid-cleaned polyethylene containers, transported to the laboratory under refrigerated conditions, and stored at −20 °C in the dark until analysis. All sample handling and preparation steps were performed in an ISO 5 clean room using ultrapure reagents (Carlo Erba, Milan, Italy) and high-purity water obtained from Elix and Milli-Q systems (Millipore, Bedford, MA, USA); all labware was decontaminated by soaking in 10% HCl and rinsed with ultrapure water. For elemental analysis, approximately 1.00 ± 0.01 g of each honey sample was weighed into PTFE microwave digestion vessels, followed by the addition of 3 mL HNO_3 and 3 mL H_2O_2. Samples were digested using a MARS-5 microwave system (CEM Corporation, Matthews, NC, USA), including one temperature-control vessel per batch (Table S4), then cooled, diluted with 4 mL of ultrapure water, and stored at +4 °C in acid-cleaned polyethylene containers protected from light until measurement. The concentrations of Ca, Cu, Fe, K, Mg, Mn, Na and Zn were determined by inductively coupled plasma optical emission spectrometry (ICP-OES, Agilent 5800, Agilent Technologies, Santa Clara, CA, USA) after 50% or 1% (*v*/v) dilution of digested samples, using high-purity argon (Ar 5.0, 99.999%) as plasma and carrier gas (Table S5).

All analyses were performed in triplicate; for each element, the optimal emission line was selected based on minimal spectral interference and recovery performance. Method validation parameters, including linearity (R^2), limits of detection (LOD), and limits of quantification (LOQ), were established according to European Medicines Agency guidelines (2022), with procedural blanks processed alongside samples and subtracted when above the LOD (Table S6). Analytical accuracy was assessed using certified reference materials DORM-2 (Dogfish Muscle, NRC Canada) and SRM 1567b (Wheat Flour, NIST, USA), yielding recoveries between 91% and 108%, confirming the suitability of the method for honey matrices. Analysis was performed in triplicate (Girolametti et al., 2026).

2.4. Antioxidant activity

The antioxidant activity was determined spectrophotometrically using the DPPH method, described by Mustafa et al. (Mustafa et al., 2022b). Briefly, 0.5 mL of extract solution was mixed with 4.5 mL of ethanolic solution of DPPH (0.1 mM). After 30 min of incubation in the dark at room temperature, the DPPH disappearance was measured spectrophotometrically at 517 nm using Agilent Cary 8454 UV–vis spectrophotometer. Trolox was used as the reference antioxidant and the results were expressed as mg Trolox equivalent (TE)/kg fresh weight (FW). Analysis was performed in triplicate.

2.5. Biological assays

2.5.1. Antimicrobial tests

The antimicrobial activity of fresh honey samples was evaluated by the broth microdilution method, with adaptations according to the recommendations of the Clinical and Laboratory Standards Institute (CLSI) for bacteria and yeasts (Humphries et al., 2018; Pfaller et al., 2022). The tested microorganisms included *Cryptococcus neoformans* (ATCC 32045), *Candida glabrata* (ATCC 9030), *Candida albicans* (ATCC 64550), and *Staphylococcus aureus* (ATCC 6538). Microbial cultures were stored at −80 °C in nonfat milk and reactivated before the assays. *Staphylococcus aureus* was reactivated in Mueller-Hinton broth, whereas yeasts were reactivated in Sabouraud Dextrose broth (5%, pH 5.6), at 37 °C for 24 h. After incubation, cultures were harvested and suspended in sterile saline solution. The bacterial suspension was adjusted to the 0.5 McFarland turbidity standard and subsequently diluted to obtain the final inoculum concentration used in the assay. Yeast suspensions were adjusted to obtain a final concentration of 0.5 to 2.5 × 10^3 CFU/mL. Inoculum density was confirmed by spectrophotometric reading at 450 nm using an ELISA microplate reader (MR-96 A, Mindray, Brazil).

Fresh honey samples were diluted in the appropriate culture medium and distributed into sterile 96-well microplates. Each well received the microbial suspension and the respective honey dilution, and the assays were performed in duplicate. The positive controls were amphotericin for yeasts and erythromycin for *S. aureus*, both tested at an initial concentration of 20 μg/mL and subsequently subjected to serial two-fold dilutions ranging from 0.04 to 20 μg/mL. The plates were incubated at 37 °C, and microbial growth was determined by optical density reading at 450 nm using an ELISA microplate reader (Mindray-R96A, São Paulo, Brazil). The antimicrobial activity of honey was expressed as the percentage of microbial inhibition at the tested concentration. The percentage of inhibition was calculated by comparing the absorbance of treated wells with the growth control, consisting of culture medium plus microbial inoculum without honey. Culture medium sterility control, microbial growth control, honey sterility control, and honey blank were included to minimize possible interference from the natural color and turbidity of honey in the spectrophotometric readings.

2.5.2. Antitumor potential

2.5.2.1. Cell culture. Human melanoma A375 cell LINE ($BRAF^{V600E}$) was authenticated by short tandem repeat (STR) DNA profiling and routinely tested to ensure the absence of mycoplasma contamination. For assays, cells were used until 6 passages after thawing. Cells were cultured in Dulbecco's Modified Eagle's Medium (DMEM) high glucose (Sigma-Aldrich, St. Louis, MO, USA), pH 7.2, supplemented with 10% fetal bovine serum (FBS) (Gibco, Invitrogen, Waltham, MA, USA), 100 U/mL penicillin, and 100 μg/mL streptomycin, and maintained at 37 °C in a humidified atmosphere with 5% CO_2. For the experiments, cells were detached by trypsin/EDTA (Sigma-Aldrich), centrifuged at 160 ×*g* for 5 min, and resuspended in supplemented DMEM prior to seeding.

2.5.2.2. Cell viability assay. The antitumor potential was evaluated in vitro estimating the cytotoxicity of honey samples by the MTT (3-(4,5-dimethyl-2-thiazolyl)-2,5-diphenyl-2H-tetrazolium bromide, Sigma-Aldrich, St. Louis, MO, USA) reduction assay. For cell adhesion, A375 cells (9600 cells/well) were incubated in 96-well plates with 200 μL of DMEM high glucose supplemented with 10% SFB for 24 h in an incubator with 5% CO_2 at 37 °C. After the incubation period, honey samples at increasing concentrations (mg/mL) were added to each well followed by additional 24 h incubation. After, 10 μL of MTT solution (0.25 mg/mL) was added to each well and the plates were incubated for more 4 h.

Formazan crystals were solubilized by adding 100 μL of a solution of 10% (*w/v*) SDS (sodium dodecyl sulfate) prepared in 0.01 mol/L HCl and incubated for 12 h in 5% CO_2 atmosphere at 37 °C. After that, absorbance was measured in a multiplate reader, model 800 TS (Biotek, Winooski, VT, USA), at 570 nm, subtracted by the background measured at 620 nm (Pichichero et al., 2010a).

2.6. Chemometric analysis

Chemometric analysis was carried out by the MetaboAnalyst 6.0 open-source program. The data applied to the Statistical Analysis [one factor]. Chemometric analyses were performed by Principal Component Analysis (PCA) and therefore, cluster analyses were performed by Hierarchical Clustering Heatmaps and Hierarchical Clustering Dendrogram.

2.7. Graphical and statistical analysis

All values were obtained from at least three independent experiments, each one run in triplicate. Data were expressed as mean ± standard error of the mean (SEM), and statistical analyses were performed by one way ANOVA followed by Tukey's post hoc test for multiple comparisons. Significance was defined as $*p < 0.05$, $**p < 0.01$, *** $p < 0.001$, and **** $p < 0.0001$.

3. Results

3.1. Melissopalynological analysis

The predominant botanical species in honey were *Mimosa pudica* L (honey **1, 2** and **3**) and *Croton* sp. (honey **4**) (Table 1 and Table 2, Fig. 2). Honey **3** presented secondary pollen of *Mimosa pudica*, common to other honey samples, at a frequency of 18%. The pollens with a frequency of less than 15% were *Anadenanthera colubrina* (Vell.) and *Croton* sp., *Croton jacobinensis* Baill., *Miconia* sp., *Mimosa caesalpiniifolia* Benth., *Pityrocarpa moniliformis* (Benth.) Luckow & R.W.Jobson, *Senna* sp. and *Serjania* sp. (Table 2, Fig. 3). The pollen grains identified as *Croton* sp. (Fig. 2f) and *Croton jacobinensis* (Fig. 2g) showed the characteristic Croton-type exine ornamentation but differed in size. The pollen grain assigned to Croton sp. was medium-sized (25–50 μm), whereas that of *C. jacobinensis* was large (50–100 μm). Because the morphological characteristics of the specimen shown in Fig. 2f did not allow reliable identification at the species level, it was retained as *Croton* sp. The classification of monofloral honeys according to Brazilian law (Barth, 1989; Brazil, 2000) is showed on Table 2.

3.2. Chemical characterization of honeys

3.2.1. Determination of color by Bianchi

Color values of each honey sample are presented in Pfund values (mm) and Pfund scale. To classify colors, the scale starts from water white, extra white, white, extra light amber, light amber, amber and end

Table 1
Frequency of dominant pollen in studied honey.

Predominant specie (Frequency) (%)	Popular name	Secondary specie	Number of isolated species
Honey **1** *Mimosa pudica* (94.3%)	Dormideira	Absent	05
Honey **2** *Mimosa pudica* (92.0%)	Dormideira	Absent	03
Honey **3** *Mimosa* sp. (92.0%)		Absent	03
Honey **4** *Croton* sp. (66.0%)		*Mimosa pudica* (18%)	02

Table 2
Classification of honey according to Brazilian law* and other information.

Honey #	Classification	Observation	Information described by the beekeeper
1	Monofloral because it originates primarily from flowers. The nomenclature is *Mimosa* honey.	The plant species described by the beekeeper were found as isolated, due to a frequency of less than 15%, such as *Croton* sp., with a frequency of 2.90%. The pollen of *Miconia* sp. showed a frequency of 0.71%. *Mimosa caesalpiniifolia* showed a frequency of 0.97% (common name sansão do campo, sabiá). *Anadenanthera colubrina* (0.80%) (common name angico). *Serjania* sp. (0.27%).	Isolated, due to a frequency of less than 15%, such as *Croton jacobinensis* (common name marmeleiro do brejo), with a frequency of 1.8%. The pollen of *Miconia* sp. showed a frequency of 1.2%. *Miconia albicans* (common name canela de velho). *Croton heliotropiifolius* (common name velame).
2	Monofloral because it originates primarily from flowers. The nomenclature is *Mimosa* honey.	Isolated species found in the sample with a frequency of less than 15% were: *Croton* sp. (6.90%), *Anadenanthera colubrina* (0.90%) and *Croton jacobinensis* (0.13%) (common name marmeleiro do brejo).	*Sarcomphalus joazeiro* (Mart.) Hauenschild (common name juazeiro), *Cydonia oblonga* Mill. (common name marmeleiro) not occur in Northeast Brazil, *Croton jacobinensis* Baill. (common name marmeleiro-do-mato or marmeleiro do brejo), *Croton heliotropiifolius* (common name velame), *Tanacetum vulgare* L. (common name mulatinha) and *Miconia albicans* (Sw.) Steud. (common name canela de velho).
3	Monofloral because it originates primarily from flowers. The nomenclature is *Mimosa* honey.	Isolated species found in the sample with a frequency of less than 15% were: *Croton* sp. (5.30%), *Mimosa caesalpiniifolia* Benth. (2.30%) and *Pityrocarpa moniliformis* (0.77%).	*Croton heliotropiifolius* (common name velame) *Pityrocarpa moniliformis* (Benth.) Luckow & R.W. Jobson (common name angico de bezerro) *Cydonia oblonga* Mill. (common name marmeleiro) not found in Northeast Brazil *Croton jacobinensis* Baill. (common name marmeleiro-do-mato) *Tanacetum vulgare* L. (common name mulatinha or catinga de mulata)
4	Monofloral because it originates primarily from flowers. The nomenclature is *Croton* honey.	Secundary specie found in the sample *Mimosa pudica* (18%). Isolated species found in the sample with a frequency of less than 15% were: *Senna* sp. (14.30%) and *Miconia* sp. (2.10%).	*Sarcomphalus joazeiro* (Mart.) Hauenschild (common name juazeiro) *Cydonia oblonga* Mill. (common name marmeleiro) not found in Northeast Brazil *Croton jacobinensis* Baill. (common name

(*continued on next page*)

Table 2 (*continued*)

Honey #	Classification	Observation	Information described by the beekeeper
			marmeleiro-do-mato) *Tanacetum vulgare* L. (common name mulatinha or catinga de mulata).

* (Barth, 1989; Brazil, 2000).

up on dark amber, where the water white is the brightest, and dark amber is the darkest. In the present study, the color of honey varied from light amber to amber, that means the intensity of color values ranged from 76.9 to 90.2 mm. The results in mm Pfund, are: honey **1**, 85.9 ± 0.4; honey **2**, 76.9 ± 1.0, honey **3**, 77.5 ± 1.0 and honey **4**, 90.2 ± 1.3. They are classified as amber (**1** and **4**), light amber (**2** and **3**) according to the color in Pfund scale (See Tables S1 and S2).

3.2.2. Determination of TPC and TFC

The total phenolic content (TPC) and total flavonoid content (TFC) varied considerably among the four honey samples (Table 3). TPC values ranged from 133.7 ± 1.1 to 1696.8 ± 1.3 mg GAE/kg DE. Honey 1 was clearly distinguished from the other samples, exhibiting the highest TPC (1696.8 ± 1.3 mg GAE/kg DE), whereas Honeys 2, 3, and 4 showed substantially lower and more comparable values, ranging from 133.7 ± 1.1 to 152.9 ± 0.8 mg GAE/kg DE. Within this group, Honey 2 showed the highest TPC (152.9 ± 0.8 mg GAE/kg DE), while Honey 3 exhibited the lowest value (133.7 ± 1.1 mg GAE/kg DE). A similar pattern was observed for TFC, which ranged from 73.25 ± 0.25 to 325.61 ± 2.93 mg RE/100 g DE. Honey 1 again showed the highest value (325.61 ± 2.93 mg RE/100 g DE), approximately three-fold higher than that observed for Honey 2 (108.96 ± 0.08 mg RE/100 g DE). Honeys 3 and 4 exhibited the lowest flavonoid contents, with values of 78.39 ± 1.43 and 73.25 ± 0.25 mg RE/100 g DE, respectively. Overall, Honey 1 showed markedly higher levels of both phenolic and flavonoid compounds than the other analyzed samples.

3.2.3. Analysis of polyphenols by HPLC-MS/MS

The HPLC-MS/MS analysis targeted 35 polyphenolic compounds and revealed distinct qualitative and quantitative profiles among the four honey samples (Table 4; Fig. S3). The total concentration of quantified polyphenols was highest in Honey 1 (4.06 mg/kg), followed by Honey 2 (2.12 mg/kg), Honey 4 (1.80 mg/kg), and Honey 3 (1.79 mg/kg). Ellagic acid was the most abundant individual polyphenol in all samples, reaching 2.29 ± 0.23 mg/kg in Honey 1, compared with 0.801 ± 0.02, 0.530 ± 0.08, and 0.472 ± 0.01 mg/kg in Honeys 2, 3, and 4, respectively. Honey 1 also showed comparatively high concentrations of 4-hydroxybenzoic acid (0.63 ± 0.05 mg/kg), ferulic acid (0.280 ± 0.05 mg/kg), and p-coumaric acid (0.230 ± 0.01 mg/kg). In contrast, Honeys 2–4 exhibited similar concentrations of several compounds, including vanillic acid, syringic acid, procyanidin B2, 3-hydroxybenzoic acid, and procyanidin A2.

Some compounds were detected selectively among the samples. Hyperoside, hesperidin, phloretin, and kaempferol were detected only in Honey 1, whereas procyanidin B2, 3-hydroxybenzoic acid, procyanidin A2, myricetin, and naringin were detected in Honeys 2–4 but not in Honey 1. Overall, the HPLC-MS/MS profiles showed that Honey 1 differed most markedly from the other samples, while Honeys 3 and 4 displayed highly similar total polyphenol contents and broadly comparable profiles.

3.2.4. Analysis of volatiles by GC × GC–MS

GC × GC–MS analysis identified a total of 198 volatile organic compounds (VOCs) across the four honey samples, although no individual sample contained the complete set of compounds detected in the study (Table 5; Fig. S4). The relative abundance profiles differed substantially among the samples, indicating marked variation in volatile composition. Among the most abundant compounds, trans-linalool oxide (furanoid) was detected in all samples and increased from 8.172 ± 0.130% of total area in Honey 1 to 17.927 ± 2.460% in Honey 2, 21.667 ± 1.297% in Honey 3, and 28.447 ± 2.899% in Honey 4. The compound 2-furanmethanol, 5-ethenyltetrahydro-α,α,5-trimethyl-, cis- was also a major constituent, particularly in Honey 2 (24.656 ± 3.565%) and Honey 3 (22.400 ± 0.294%), whereas lower proportions were

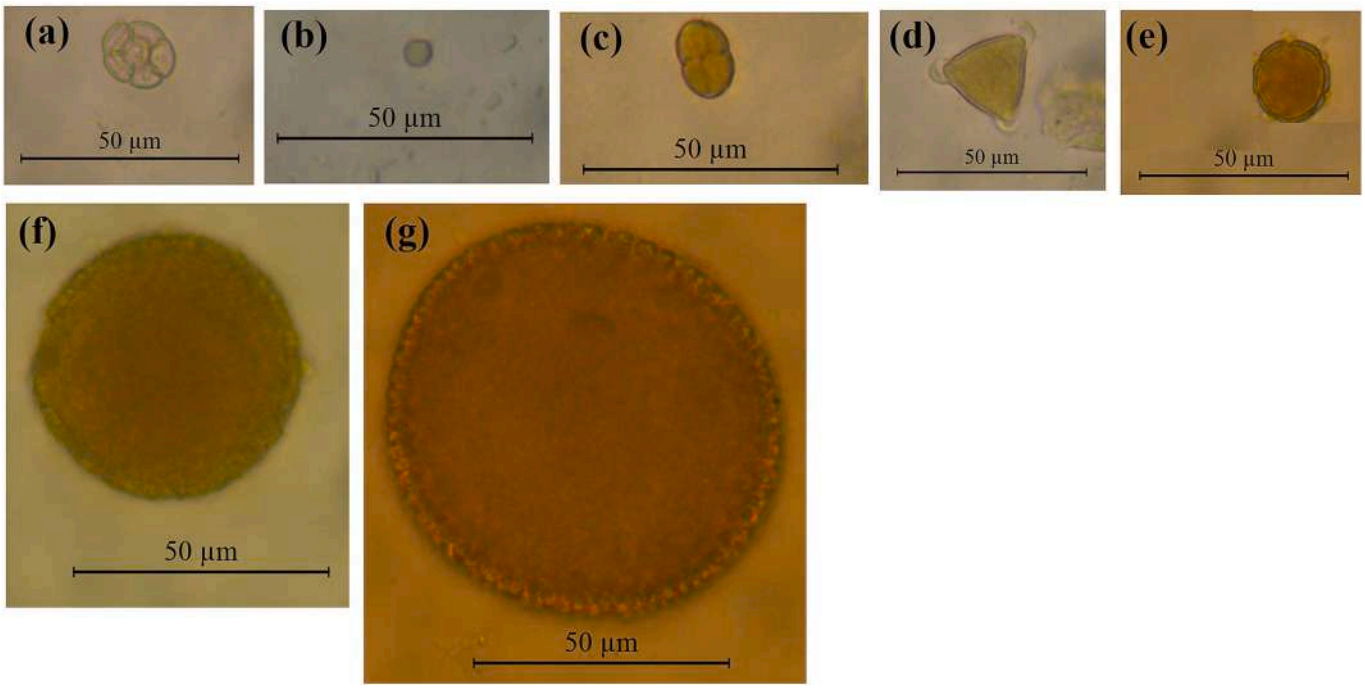
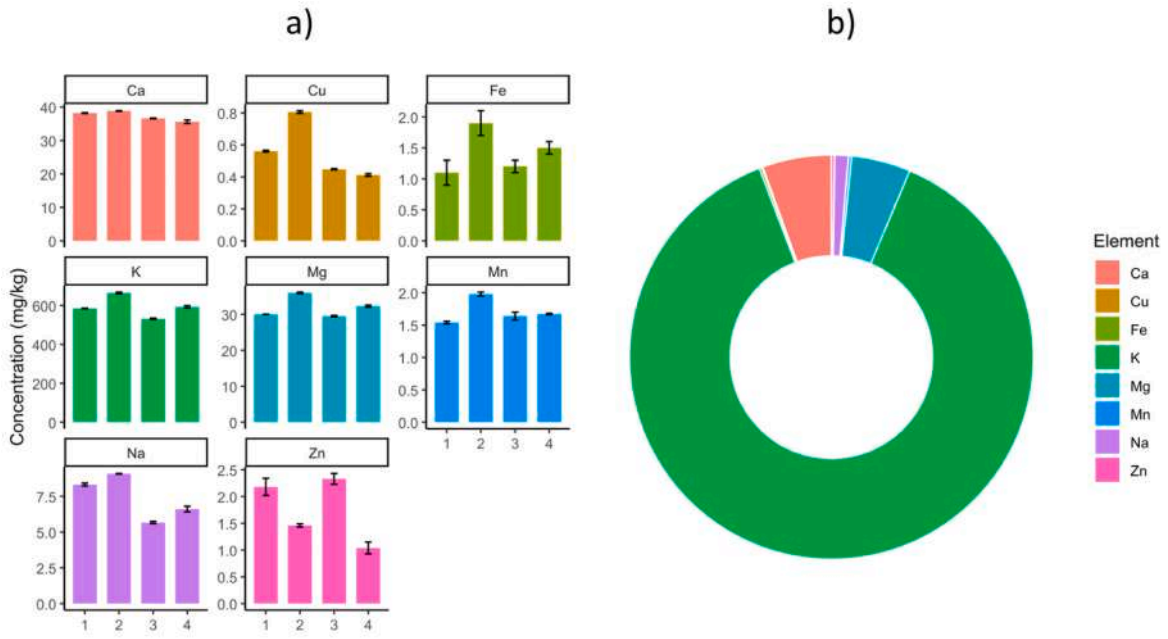


Fig. 2. Optical microscopy images of pollen of the *Anadenathera colubrina* (a); *Mimosa pudica* (b); *Mimosa caesalpiniifolia* (c); *Senna* sp. (d); *Miconia* sp. (e), *Serjania* sp.; *Croton* sp. (f) and *Croton jacobinensis* (g). 400 x magnification.



C

Honey	Ca	Cu	Fe	K	Mg	Mn	Na	Zn
1	38.2 ± 0.1	0.561 ± 0.005	1.1 ± 0.2	585 ± 1	30.01 ± 0.02	1.54 ± 0.02	8.3 ± 0.1	2.18 ± 0.16
2	38.8 ± 0.1	0.806 ± 0.008	1.9 ± 0.2	665 ± 4	36.0 ± 0.2	1.98 ± 0.03	9.08 ± 0.02	1.46 ± 0.03
3	36.6 ± 0.1	0.448 ± 0.003	1.2 ± 0.1	532 ± 3	29.5 ± 0.2	1.64 ± 0.06	5.67 ± 0.08	2.33 ± 0.10
4	35.6 ± 0.5	0.412 ± 0.008	1.5 ± 0.1	593 ± 6	32.3 ± 0.3	1.67 ± 0.01	6.61 ± 0.20	1.04 ± 0.11

Fig. 3. Elemental composition of honey samples. (A) Barplot of individual samples showing concentrations of Ca, Cu, Fe, K, Mg, Mn, Na, and Zn (mg/kg) with standard deviation from triplicate measurements. (B) Donut chart representing the percentage contribution of each element to the global mean composition across all samples. (C) Elemental composition in mg/kg, wet weight.

Table 3
TPC and TFC values in honey.

Honey sample	TPC (GAE/kg DE)	TFC (mg RE/100 g DE)
1	1696.8 ± 1.3	325.61 ± 2.93
2	152.9 ± 0.8	108.96 ± 0.08
3	133.7 ± 1.1	78.39 ± 1.43
4	147.2 ± 0.9	73.25 ± 0.25

DE is dry extract. GAE/kg is the number of equivalents of gallic acid per kg of dry extract. RE/100 g DE is the number of equivalents rutin per 100 g of dry extract. Analysis was performed in triplicate and RSD was maintained below 20%.

observed in Honey 1 (8.550 ± 0.136%) and Honey 4 (7.833 ± 0.310%).

Other abundant VOCs also showed sample-dependent variation. Ethanol ranged from 2.028 ± 0.032% in Honey 1 to 11.167 ± 0.099% in Honey 4, while 1-butanol, 3-methyl- increased from 4.175 ± 0.066% in Honey 1 to 7.313 ± 0.309% in Honey 4. In contrast, Honey 1 showed the highest relative abundances of D-limonene (5.094 ± 0.081%), phenylethyl alcohol (6.178 ± 0.098%), and 2,5-furandicarboxaldehyde (8.850 ± 0.141%). Several compounds were detected only in specific samples, further supporting distinct volatile signatures among the four honeys.

3.2.5. *Analysis of minerals*

K was the dominant element in all honey samples, with concentrations ranging from 532 ± 3 mg/kg in Honey **3** to 665 ± 4 mg/kg in Honey **2**, followed by Ca, which varied between 35.6 ± 0.5 and 38.8 ± 0.1 mg/kg (Fig. 3A, B and C). Mg was the third most abundant element, with concentrations ranging from 29.5 ± 0.2 to 36.0 ± 0.2 mg/kg, whereas Na was detected at lower concentrations, between 5.67 ± 0.08 and 9.08 ± 0.02 mg/kg. Trace elements were consistently detected in all samples, with Zn ranging from 1.04 ± 0.11 to 2.33 ± 0.10 mg/kg, Mn from 1.54 ± 0.02 to 1.98 ± 0.03 mg/kg, Fe from 1.1 ± 0.2 to 1.9 ± 0.2 mg/kg, and Cu from 0.412 ± 0.008 to 0.806 ± 0.008 mg/kg. Honey **2** exhibited the highest concentrations of K, Ca, Mg, Na, Cu, Fe, and Mn, whereas Honey **3** showed the highest Zn concentration. Conversely, the lowest concentrations of K, Mg, and Na were observed in Honey **3**, while Honey **4** presented the lowest Ca, Cu, and Zn contents. Overall, the elemental abundance followed the order K > Ca > Mg > Na > Zn > Mn > Fe > Cu across all analyzed samples.

3.3. *Antioxidant activity*

2,2-diphenyl-1-picrylhydrazyl (DPPH) analysis is used to evaluate the scavenging ability of tested substances. In this analysis the purple color solution of DPPH reagent reacts with given substances and changes color light yellow to colorless. These changes are due to the scavenging activity of compounds in substances to the DPPH reagent (Tuksitha et al., 2018). In addition, the antioxidant potential in substances involves several factors such as botanical origins, climates, surrounding of hives, methods of harvesting and honey pigments (Bakar et al., 2017). Honey samples showed the following antioxidant activities (TE/kg): 343.08 ± 0.07 (**1**), 64.29 ± 0.08 (**2**), 75.52 ± 0.06 (**3**) and 70.42 ± 0.11 (**4**). Honey **1** showed the highest content while honey **2** had the lowest phenolic content, its DPPH value was slightly higher than honey **3** and **4**, suggesting the presence of specific high-potency non-phenolic antioxidants.

Table 4
Concentration (mg/kg) of 35 polyphenols in honey.

Compound	Honey 1	Honey 2	Honey 3	Honey 4
	mg/kg			
Gallic acid	0.030 ± 0.01	0.009 ± 0.01	0.011 ± 0.01	0.014 ± 0.01
Neochlorogenic acid	n.d.	0.013 ± 0.01	0.012 ± 0.01	0.012 ± 0.01
Delphindin3-galactoside	n.d.	n.d	n.d	n.d
Catechin	n.d.	n.d	n.d	n.d
Procyanidin B2	n.d.	0.167 ± 0.01	0.166 ± 0.01	0.167 ± 0.02
Chlorogenic acid	n.d.	0.06 ± 0.01	0.029 ± 0.01	0.05 ± 0.01
4-Hydroxy benzoic acid	0.63 ± 0.05	0.083 ± 0.01	0.082 ± 0.01	0.088 ± 0.03
Epicatechin	n.d.	n.d	n.d	n.d
Cyanidin-3-glucoside	n.d.	n.d	n.d	n.d
Petunidin-3-glucoside	n.d.	n.d	n.d	n.d
3-Hydroxy benzoic acid	n.d.	0.183 ± 0.01	0.182 ± 0.02	0.186 ± 0.01
Caffeic acid	0.041 ± 0.01	0.045 ± 0.01	0.044 ± 0.01	0.046 ± 0.01
Vanillic acid	0.173 ± 0.03	0.221 ± 0.01	0.220 ± 0.03	0.222 ± 0.02
Pelargonidin-3-glucoside	n.d.	n.d		n.d
Pelargonidin-3-rutinoside	n.d.	n.d		n.d
Malvidin-3-galactoside	n.d.	n.d		n.d
Syringic acid	0.084 ± 0.01	0.110 ± 0.01	0.108 ± 0.02	0.110 ± 0.01
Procyanidin A2	n.d.	0.088 ± 0.01	0.087 ± 0.01	0.089 ± 0.01
p-Coumaric acid	0.230 ± 0.01	0.046 ± 0.01	0.023 ± 0.01	0.047 ± 0.01
Ferulic acid	0.280 ± 0.05	0.089 ± 0.01	0.085 ± 0.05	0.094 ± 0.01
Rutin	0.061 ± 0.01	0.032 ± 0.01	0.032 ± 0.02	0.044 ± 0.01
Hyperoside	$5.09*10^{-3}$ ± 0.01	n.d	n.d	n.d
Isoquercitrin	$5.10*10^{-3}$ ± 0.01	0.003 ± 0.01	0.003 ± 0.01	0.005 ± 0.01
Delphindin 3,5 diglucoside	0.011 ± 0.00	0.005 ± 0.01	0.005 ± 0.01	0.008 ± 0.01
Phloridzin	n.d.	0.001 ± 0.0.01	0.001 ± 0.01	0.001 ± 0.01
Quercitrin	n.d.	0.001 ± 0.01	0.001 ± 0.01	0.001 ± 0.01
Myricetin	n.d.	0.080 ± 0.01	0.079 ± 0.02	0.080 ± 0.01
Naringin	n.d.	0.048 ± 0.01	0.047 ± 0.01	0.047 ± 0.02
Kaempferol-3-glucoside	0.010 ± 0.01	0.007 ± 0.01	0.003 ± 0.01	0.004 ± 0.01
Hesperidin	0.03 ± 0.01	n.d	n.d	n.d
Ellagic acid	2.29 ± 0.23	0.801 ± 0.02	0.530 ± 0.08	0.472 ± 0.01
Quercetin	0.050 ± 0.01	0.022 ± 0.001	0.012 ± 0.01	0.023 ± 0.02
Phloretin	0.15 $*10^{-3}$ ± 0.01	n.d	n.d	n.d
Kaempferol	0.07 ± 0.01	n.d	n.d	n.d
Isorhamnetin	0.060 ± 0.01	0.057 ± 0.001	0.028 ± 0.01	0.028 ± 0.02
TOTAL	**4.06**	**2.12**	**1.79**	**1.80**

3.4. Biological activities

Biological activities of honey samples were evaluated through antimicrobial and antitumor screening assays. The antimicrobial activity of fresh honey samples is shown in Table 6. At a concentration of 2 mg/mL, the honey samples showed microbial inhibition ranging from approximately 56% to 79%, depending on the sample and microorganism tested. The highest inhibitory effect was observed against *Candida albicans*, with inhibition values ranging from 71.6% for honey **2** to 78.78% for honey **1**. Honey **1** showed the broadest antimicrobial activity among the evaluated samples. This sample inhibited *Cryptococcus neoformans* by 60.96%, *Candida glabrata* by 55.95%, *Candida albicans* by 78.78%, and *Staphylococcus aureus* by 72.01%. In contrast, honey **2**, **3**, and **4** were tested only against *Candida albicans* and *Staphylococcus aureus*, showing inhibition values of 71.6%, 74.8%, and 72.1% against *C. albicans*, and 57.5%, 59.2%, and 57.6% against *S. aureus*, respectively.

Further, to evaluate the cytotoxic effects and antitumor potential of the different honey samples, human melanoma cells (A375) were incubated with increasing concentrations (from 1 to 500 mg/mL) of four different honey samples for 24 h and cell viability was assessed using the MTT assay. As observed in Fig. 4, all honey samples exhibited a concentration-dependent cytotoxicity at the higher concentrations (250 and 500 mg/mL). Only one sample (honey 3) presented statistically significant cytotoxicity starting at 100 mg/mg (Fig. 4C). To analyze the cytotoxic potency among samples, a fixed concentration (250 mg/mL) was selected and their effects on cell viability was compared (Fig. 4E) and the most cytotoxicity was observed by honey **4**. The following cytotoxic potency order was achieved: **4** > **3** > **2** > **1**, indicating that, despite the similar cytotoxicity profile, there are distinct potencies among the samples. Collectively, these data show that while all honey samples decreased the viability of melanoma cells in vitro at higher concentrations, their cytotoxic potency varies significantly according to sample origin.

These findings support the notion that the chemical composition and botanical/geographical origin of honey may influence its biological activity, reinforcing the relevance of sample-specific characterization when investigating its potential cytotoxic effects against melanoma cells.

3.5. Chemometric analysis

The chemometric analysis Fig. 5 visualized through the hierarchical clustering dendrogram shows clear patterns of similarity and differentiation among the honey samples across harvest years and groups. It can be noted that samples cluster primarily according to their labeled groups **H1** through **H4** (honey **1** through honey **4**), in triplicate), indicating strong intra-group consistency and suggesting that each group shares a distinct chemical profile. Replicates within each group **H3** (e.g., honey **3**, honey **3.1** and honey **3.2**) cluster very tightly, demonstrating high analytical reproducibility and minimal within-sample variability. A notable separation is observed between honey **1** and the honey **2,** honey **3** and honey **4**, indicating a significant shift in chemical composition that may be attributed to seasonal variation, environmental factors, or differences in floral origin between years (Khamitova et al., 2026; Pourmoradian et al., 2025). Therefore, honey **2** and honey **4** form subclusters that are more closely related to each other than to honey **1** or honey **3**, suggesting partial overlap in their compositional characteristics. The larger linkage distances separating major clusters reflect substantial dissimilarity, reinforcing the presence of distinct chemometric fingerprints among the sample groups.

Table 5
Volatile organic compounds (VOCs) in honey samples analyzed by GC × GC–MS expressed in % of total area.

Compound Name	Honey 1	Honey 2	Honey 3	Honey 4
	% Total area			
(2*R*,5*S*)-2-Methyl-5-(prop-1-en-2-yl)-2-vinyltetrahydrofuran	0.096 ± 0.002	0.182 ± 0.007	0.333 ± 0.035	0.414 ± 0.082
(3*R*,6*S*)-2,2,6-Trimethyl-6-vinyltetrahydro-2H-pyran-3-ol	4.925 ± 0.078	3.067 ± 0.002	3.378 ± 0.273	4.197 ± 0.338
(*E*)-1-(2,3,6-trimethylphenyl)buta-1,3-diene (TPB, 1)	–	–	–	0.094 ± 0.004
(E)-1,3-Butadien-1-ol	–	0.177 ± 0.007	–	–
1-(2,4-Dimethyl-furan-3-yl)-ethanone	–	–	0.249 ± 0.003	–
1-(4-*tert*-Butylphenyl)propan-2-one	–	–	–	0.219 ± 0.009
1, 1, 5-Trimethyl-1, 2-dihydronaphthalene	–	–	0.054 ± 0.001	–
1,3-Cyclohexadiene, 1-methyl-4-(1-methylethyl)-		0.046 ± 0.002	0.049 ± 0.001	0.061 ± 0.002
1,3-Cyclohexadiene-1-carboxaldehyde, 2,6,6-trimethyl-	0.449 ± 0.007	–	–	1.222 ± 0.048
1,3-Dioxolane, 2,4,5-trimethyl-	1.265 ± 0.020	–	–	–
1,5,7-Octatrien-3-ol, 3,7-dimethyl-	3.149 ± 0.050	5.300 ± 0.045	4.570 ± 0.049	7.419 ± 0.310
1,5-Hexadiene, 2,5-dimethyl-	0.218 ± 0.003	–	–	–
1,7-Octadiene-3,6-diol, 2,6-dimethyl-	–	0.264 ± 0.011	–	0.254 ± 0.010
1-Butanol, 2-methyl-, acetate	0.473 ± 0.008	–	–	–
1-Butanol, 3-methyl-	4.175 ± 0.066	4.972 ± 0.200	5.695 ± 0.223	7.313 ± 0.309
1-Butanol, 4-(1-methylethoxy)-	–	0.292 ± 0.031	0.266 ± 0.003	0.104 ± 0.004
1-Cyclohexene-1-carboxaldehyde, 2,6,6-trimethyl-	–	0.576 ± 0.022	0.597 ± 0.032	0.880 ± 0.043
1H-Indene, 2,3-dihydro-1,1,5,6-tetramethyl-	0.247 ± 0.004	0.041 ± 0.001	0.047 ± 0.005	0.070 ± 0.018
1-Methoxydecane	–	–	–	0.777 ± 0.031
1-Nonanol	–	0.415 ± 0.017	0.348 ± 0.005	0.746 ± 0.293
1-Propanol, 2-methyl-	1.436 ± 0.023	1.269 ± 0.053	1.135 ± 0.329	1.764 ± 0.250
1-Undecanol	–	0.112 ± 0.004	–	0.238 ± 0.009
2,2-Dimethyl-3-morpholin-4-ylpropan-1-amine	–	–	–	0.129 ± 0.005
2,4-Cycloheptadien-1-one, 2,6,6-trimethyl-	–	0.758 ± 0.050	0.731 ± 0.001	1.164 ± 0.124
2,4-Di-tert-butylphenol	–	–	1.512 ± 0.080	–
2,4-Hexadienoic acid, ethyl ester	–	–	0.037 ± 0.005	0.052 ± 0.002
2,4-Hexadienoic acid, ethyl ester, (2E,4E)-	–	0.050 ± 0.002	–	–
2,5,8,11,14,17-Hexaoxanonadecan-19-ol	0.294 ± 0.005	–	–	–
2,5,8,11,14-Pentaoxahexadecan-16-ol	0.135 ± 0.002	–	–	–
2,5-Furandicarboxaldehyde	8.850 ± 0.141	0.179 ± 0.007	0.863 ± 0.011	–
2,6,6-Trimethyl-2-cyclohexene-1,4-dione	0.956 ± 0.015	0.322 ± 0.009	0.357 ± 0.008	0.419 ± 0.024
2-[2-[2-[2-[2-[2-[2-[2-(2-Methoxyethoxy)ethoxy]ethoxy]ethoxy]ethoxy]ethoxy]ethoxy]ethoxy] ethanol	–	–	0.053 ± 0.001	0.156 ± 0.006
2-[2-[2-[2-[2-[2-[2-[2-[2-(2-Hydroxyethoxy)ethoxy]ethoxy]ethoxy]ethoxy]ethoxy]ethoxy]ethoxy] ethoxy]etanol	–	0.305 ± 0.012	0.375 ± 0.005	0.353 ± 0.014
21-Crown-7	–	–	0.052 ± 0.003	0.101 ± 0.004
2-Butanol, 4-(2,2-dimethyl-6-methylenecyclohexylidene)-	0.216 ± 0.003	–	–	–
2-Buten-1-one, 1-(2,6,6-trimethyl-1,3-cyclohexadien-1-yl)-, (E)-	–	0.184 ± 0.014	0.203 ± 0.013	0.286 ± 0.048
2-Dodecanone	–	0.089 ± 0.009	–	–
2-Ethylhexyl acrylate	0.332 ± 0.005	–	–	–
2-Ethylhexyl salicylate	–	0.955 ± 0.075	–	–
2-Furancarboxaldehyde, 5-methyl-	0.547 ± 0.009	–	–	–
2-Furancarboxylic acid, ethyl ester	0.317 ± 0.005	–	–	–
2-Furanmethanol, 5-ethenyltetrahydro-α,α,5-trimethyl-, cis-	8.550 ± 0.136	24.656 ± 3.565	22.400 ± 0.294	7.833 ± 0.310
2-Heptanol, 4-methyl-	–	–	0.217 ± 0.022	–
2-Hexadecanone	–	0.203 ± 0.185	0.067 ± 0.001	0.135 ± 0.005
2-Hexen-1-ol, (*Z*)-	–	–	0.126 ± 0.002	–
2H-Pyran-3-ol, 6-ethenyltetrahydro-2,2,6-trimethyl-	–	–	–	0.113 ± 0.004
2H-Pyranmethanol, tetrahydro-2,5-dimethyl-	1.118 ± 0.018	–	–	–
2-Isopropylidene-5-methylhex-4-enal	–	0.048 ± 0.015	–	–
2-Nonen-1-ol	–	–	0.239 ± 0.003	–
2-Pentadecanone	–	0.097 ± 0.007	0.055 ± 0.009	0.219 ± 0.009
2-Propanol, 1-(2-butoxy-1-methylethoxy)-	–	–	–	0.076 ± 0.003
2-Tetradecanone	–	0.389 ± 0.016	0.141 ± 0.002	0.341 ± 0.014
2-Tridecanone	–	0.095 ± 0.004	–	0.187 ± 0.007
3,5-di-tert-Butyl-4-hydroxybenzaldehyde	–	0.203 ± 0.008	0.071 ± 0.001	0.220 ± 0.009
3,6,9,12-Tetraoxatetradecan-1-ol	0.308 ± 0.005	–	–	–
3,7-Octadiene-2,6-diol, 2,6-dimethyl-	–	0.090 ± 0.004	0.113 ± 0.025	0.148 ± 0.006
3-Buten-2-ol, 4-(2,6,6-trimethyl-1-cyclohexen-1-yl)-	–	0.434 ± 0.017	0.481 ± 0.006	0.641 ± 0.056
3-Cyclohexene-1-methanol, α,α,4-trimethyl-, (*R*)-	–	0.660 ± 0.026	–	–
3-Furaldehyde	–	0.127 ± 0.005	0.099 ± 0.001	0.160 ± 0.006
3-Furanmethanol	–	–	–	0.296 ± 0.012
3-Phenyl-1-propanol, acetate	–	–	0.116 ± 0.002	0.134 ± 0.005
4-Hydroxybutyl acrylate	–	–	–	0.345 ± 0.014
4-Methyleneisophorone	0.804 ± 0.013	–	–	–
4-Tetradecylmorpholine	–	–	0.093 ± 0.005	0.154 ± 0.006
5-Hepten-2-ol, 6-methyl-	–	0.118 ± 0.005	0.164 ± 0.001	0.417 ± 0.141
7-Acetyl-6-ethyl-1,1,4,4-tetramethyltetralin	–	0.141 ± 0.006	–	0.354 ± 0.014
Acetic acid, (1,2,3,4,5,6,7,8-octahydro-3,8,8-trimethylnaphth-2-yl)methyl ester	–	–	0.107 ± 0.001	0.355 ± 0.014
Acetic acid, 2-phenylethyl ester	–	1.519 ± 0.167	2.170 ± 0.011	2.841 ± 0.187
Acetic acid, chloro-, 3-phenylpropyl ester	–	0.100 ± 0.004	–	0.159 ± 0.006
Aromandendrene	–	0.171 ± 0.007	–	–
Benzaldehyde	1.497 ± 0.024	–	–	–

(*continued on next page*)

Table 5 (*continued*)

Compound Name	Honey 1	Honey 2	Honey 3	Honey 4
	% Total area			
Benzaldehyde, 2-methyl-	4.187 ± 0.067	–	–	–
Benzaldehyde, 5-(1,1-dimethylethyl)-2-hydroxy-	–	0.115 ± 0.005	–	–
Benzene, 1-(1,1-dimethylethyl)-4-ethoxy-	–	0.105 ± 0.004	–	0.297 ± 0.012
Benzene, 1,2,3-trimethyl-	–	–	–	0.045 ± 0.002
Benzene, 1,2,4,5-tetramethyl-	–	–	0.048 ± 0.001	–
Benzene, 1,2,4-trimethyl-Bicyclo[3.1.1]hept-3-en-2-one, 4,6,6-trimethyl-, (1*S*)-trans-Verbenol	–	–	–	0.046 ± 0.002
Benzene, 1,3-bis(1,1-dimethylethyl)-	0.313 ± 0.005	–	0.252 ± 0.027	–
Benzene, 1-ethenyl-4-methoxy-	–	0.500 ± 0.001	0.457 ± 0.014	0.686 ± 0.022
Benzene, 1-ethyl-2,3-dimethyl-	–	–	–	0.061 ± 0.002
Benzene, 1-methyl-3,5-bis(1-methylethyl)-	–	0.040 ± 0.002	0.038 ± 0.001	–
Benzene, 2-methyl-1,4-bis(1-methylethyl)-	–	0.034 ± 0.001	0.055 ± 0.001	0.068 ± 0.001
Benzeneacetaldehyde	1.350 ± 0.021	–	–	–
Benzeneacetaldehyde, α-methyl-	–	0.092 ± 0.004	–	–
Benzeneacetic acid, ethyl ester	1.972 ± 0.031	1.539 ± 0.189	1.754 ± 0.050	2.112 ± 0.079
Benzenemethanol, α,α,4-trimethyl-	–	0.042 ± 0.002	0.042 ± 0.001	–
Benzoic acid, 2-ethylhexyl ester	–	0.064 ± 0.011	0.043 ± 0.001	0.114 ± 0.005
Benzoic acid, ethyl ester	0.724 ± 0.012	1.029 ± 0.013	1.067 ± 0.014	1.279 ± 0.051
Bicyclo[2.2.1]hept-2-ene, 1,7,7-trimethyl-	–	0.174 ± 0.006	0.212 ± 0.008	0.246 ± 0.003
Bicyclo[3.1.0]hex-2-ene, 2-methyl-5-(1-methylethyl)-	–	–	0.067 ± 0.001	–
Bicyclo[3.1.1]hept-2-ene, 3,6,6-trimethyl-	–	–	0.083 ± 0.001	–
Bicyclo[3.1.1]hept-2-ene, 3,6,6-trimethyl-	–	–	–	0.083 ± 0.003
Butane, 1,1′-[oxybis(2,1-ethanediyloxy)]bis-	–	–	–	0.069 ± 0.003
Butanedioic acid, diethyl ester	1.979 ± 0.031	–	–	–
Butanoic acid, 2-methyl-, ethyl ester	0.716 ± 0.011	0.398 ± 0.008	0.439 ± 0.049	0.557 ± 0.024
Butanoic acid, 3-methyl-, 2-methylbutyl ester	–	0.056 ± 0.002	0.066 ± 0.001	0.062 ± 0.002
Butanoic acid, 3-methyl-, 3-methylbutyl ester	–	0.064 ± 0.003	–	–
Butanoic acid, 3-methyl-, ethyl ester	1.026 ± 0.016	1.371 ± 0.004	1.492 ± 0.087	1.883 ± 0.137
Butyrolactone	–	0.228 ± 0.009	0.234 ± 0.020	0.324 ± 0.013
Cadina-1(10),6,8-triene	–	–	0.057 ± 0.014	–
Camphene	–	0.033 ± 0.001	–	–
Caryophyllene	–	0.055 ± 0.002	0.065 ± 0.004	0.077 ± 0.001
cis-Arbusculone	0.572 ± 0.009	0.705 ± 0.019	0.724 ± 0.033	1.074 ± 0.034
Cyclododecane	–	–	–	1.050 ± 0.042
Cyclohexane, 1,2,4-trimethyl-, (1α,2β,4β)-	–	0.298 ± 0.009	0.319 ± 0.014	0.429 ± 0.017
Cyclohexane, 1,3,5-trimethyl-	–	–	–	0.396 ± 0.016
Cyclohexanol	–	–	0.094 ± 0.001	–
Cyclohexanol, 1-methyl-4-(1-methylethenyl)-, acetate	–	0.081 ± 0.003	–	–
Cyclohexanol, 2,6-dimethyl-	0.200 ± 0.003	–	–	–
Cyclohexanone, 2,2,6-trimethyl-	–	0.036 ± 0.001	–	0.039 ± 0.004
Cyclohexanone, 5-methyl-2-(1-methylethylidene)-	–	–	–	0.197 ± 0.008
Cyclohexene, 1,5,5-trimethyl-3-methylene-	1.233 ± 0.020	0.692 ± 0.029	0.804 ± 0.010	1.150 ± 0.054
Cyclohexene, 1-methyl-4-(1-methylethylidene)-	–	–	0.051 ± 0.001	0.100 ± 0.004
Cyclopenta[g]-2-benzopyran, 1,3,4,6,7,8-hexahydro-4,6,6,7,8,8-hexamethyl-	–	0.277 ± 0.011	0.327 ± 0.004	0.311 ± 0.012
Cyclopentadecanone, 2-hydroxy-	–	–	–	0.417 ± 0.016
Cyclopentasiloxane, decamethyl-	0.686 ± 0.011	–	–	–
Cycloprop[*a*]indene, 1,1a,6,6a-tetrahydro-	–	–	–	0.454 ± 0.018
Cyclopropane, octyl-	–	0.251 ± 0.010	–	–
Decanal	0.807 ± 0.013	–	–	–
Decanoic acid, ethyl ester	0.592 ± 0.009	0.342 ± 0.039	0.219 ± 0.112	0.533 ± 0.218
Diethyl phthalate	–	–	–	2.670 ± 0.106
Dill ether	–	0.049 ± 0.002	–	0.086 ± 0.005
dl-Alanyl-l-alanine	–	0.014 ± 0.001	–	–
D-Limonene	5.094 ± 0.081	1.873 ± 1.034	1.069 ± 0.034	1.622 ± 0.122
Dodecanoic acid, ethyl ester	–	0.312 ± 0.051	0.310 ± 0.001	0.354 ± 0.043
Dodecyl acrylate	–	0.177 ± 0.047	0.044 ± 0.001	0.118 ± 0.005
Ethanol	2.028 ± 0.032	8.684 ± 0.255	8.172 ± 0.391	11.167 ± 0.099
Ethanol, 2-[2-(ethenyloxy)ethoxy]-	1.014 ± 0.016	–	–	–
Ethyl 2-(5-methyl-5-vinyltetrahydrofuran-2-yl)propan-2-yl carbonate	–	–	–	0.705 ± 0.028
Ethyl 2,6-dimethylbenzoate	–	0.276 ± 0.006	–	–
Ethyl 2-hydroxy-3-phenylpropanoate	0.319 ± 0.005	–	0.041 ± 0.002	–
Ethyl 4-methylbenzoate	1.660 ± 0.026	–	–	–
Ethyl Acetate	2.183 ± 0.035	2.644 ± 0.108	2.881 ± 0.292	3.762 ± 0.178
Eugenol	0.354 ± 0.006	0.259 ± 0.037	0.254 ± 0.008	0.344 ± 0.063
Formic acid, octyl ester	–	–	–	0.471 ± 0.019
Formic acid, TMS derivative	–	–	0.052 ± 0.002	–
Heptanoic acid, ethyl ester	0.257 ± 0.004	0.074 ± 0.003	0.125 ± 0.016	0.195 ± 0.092
Hexadecanoic acid, ethyl ester	–	–	–	0.279 ± 0.011
Hexadecanoic acid, methyl ester	–	0.035 ± 0.009	–	0.028 ± 0.001
Hexanoic acid, ethyl ester	0.417 ± 0.007	0.244 ± 0.004	0.256 ± 0.024	0.317 ± 0.034
Hydroperoxide, 1-methylbutyl	2.266 ± 0.036	–	–	–
Hydroxyurea	–	–	0.018 ± 0.001	–
Isopropyl myristate		0.183 ± 0.007	–	–
Levomenthol	0.852 ± 0.014	–	–	–
Lilac alcohol B	0.614 ± 0.010	–	0.239 ± 0.003	0.371 ± 0.015

(*continued on next page*)

Table 5 (*continued*)

Compound Name	Honey 1	Honey 2	Honey 3	Honey 4
	% Total area			
Lilac alcohol C	–	–	–	0.445 ± 0.018
Lilac alcohol D	–	0.270 ± 0.020	0.240 ± 0.003	–
Lilac aldehyde A	–	0.093 ± 0.004	–	0.071 ± 0.003
Lilac aldehyde B	0.410 ± 0.007	0.083 ± 0.003	0.057 ± 0.011	–
Lilac aldehyde C	0.073 ± 0.001	–	–	–
Lilac aldehyde D	0.353 ± 0.006	0.097 ± 0.004	–	–
Megastigma-4,6(E),8(E)-triene	–	0.271 ± 0.011	0.209 ± 0.090	0.288 ± 0.127
Megastigma-4,6(E),8(Z)-triene	–	0.249 ± 0.010	–	0.430 ± 0.017
Megastigma-4,6(Z),8(E)-triene	–	0.123 ± 0.005	0.283 ± 0.004	0.046 ± 0.003
Mesitylene	–	0.032 ± 0.001	–	–
Methacrolein	–	0.156 ± 0.006	0.161 ± 0.004	–
Methyl n-hexadecyl ketone	–	0.087 ± 0.003	–	–
Methylene chloride	–	0.036 ± 0.019	0.025 ± 0.015	0.077 ± 0.053
Methyleugenol	–	0.142 ± 0.015	0.160 ± 0.020	0.207 ± 0.024
Naphthalene, 1,2,3,4,4a,5,6,8a-octahydro-4a,8-dimethyl-2-(1-methylethenyl)-, [2R-(2α,4aα,8aβ)]-	–	0.054 ± 0.002	–	0.146 ± 0.006
Naphthalene, 1,2,3,4-tetrahydro-1,1,6-trimethyl-	–	0.145 ± 0.008	0.222 ± 0.037	0.234 ± 0.035
Naphthalene, 1,2,3,5,6,7,8,8a-octahydro-1,8a-dimethyl-7-(1-methylethenyl)-, [1R-(1α,7β,8aα)]-	–	0.037 ± 0.001	–	0.136 ± 0.005
Naphthalene, 1,2-dihydro-	–	0.335 ± 0.013	–	0.421 ± 0.017
Naphthalene, 1,2-dihydro-1,1,6-trimethyl-	–	–	–	0.291 ± 0.012
Naphthalene, 1,6-dimethyl-4-(1-methylethyl)-	–	0.286 ± 0.081	–	–
Naphthalene, decahydro-4a-methyl-1-methylene-7-(1-methylethenyl)-, [4aR-(4aα,7α,8aβ)]-	–	0.116 ± 0.005	0.118 ± 0.017	–
n-Hexyl salicylate	–	0.053 ± 0.019	–	0.236 ± 0.009
Nonadecane	–	0.640 ± 0.025	–	–
Nonadecane, 2-methyl-	–	0.224 ± 0.009	–	–
Nonanal	0.824 ± 0.013	–	–	–
Nonanoic acid	–	–	–	2.505 ± 0.099
Nonanoic acid, ethyl ester	1.200 ± 0.019	0.521 ± 0.036	0.508 ± 0.039	1.145 ± 0.641
Octanal, 2-(phenylmethylene)-	–	0.209 ± 0.029	0.204 ± 0.003	0.350 ± 0.014
Octanoic acid	–	–	–	1.743 ± 0.069
Octanoic acid, ethyl ester	1.074 ± 0.017	0.587 ± 0.002	0.579 ± 0.018	0.894 ± 0.271
Orthoformic acid, triisobutyl ester	0.440 ± 0.017	0.371 ± 0.015	0.173 ± 0.002	–
Pentadecane	–	0.215 ± 0.009	0.355 ± 0.005	–
Pentanoic acid, 3-methyl-, ethyl ester	–	0.024 ± 0.001	–	–
Phenol, 2,5-bis(1,1-dimethylethyl)-	4.798 ± 0.076	–	–	–
Phenol, 2-methoxy-4-(1-propenyl)-, (Z)-	–	–	–	0.069 ± 0.003
Phenylethyl Alcohol	6.178 ± 0.098	2.446 ± 0.299	2.948 ± 0.216	3.683 ± 0.313
Phthalic acid, butyl 2-pentyl ester	–	–	–	1.678 ± 0.066
Phthalic acid, isobutyl 3-methylbutyl ester	–	–	–	0.790 ± 0.031
Propanoic acid, 2-hydroxy-, ethyl ester	–	0.228 ± 0.008	0.228 ± 0.003	–
Propanoic acid, 2-hydroxy-, ethyl ester, (L)-	0.724 ± 0.012	–	0.239 ± 0.003	0.335 ± 0.013
p-Xylene	–	0.029 ± 0.001	–	0.113 ± 0.004
Styrene	–	–	0.015 ± 0.001	0.031 ± 0.001
Tetracyclo[5.3.0.0 < 2,6 > 0.0 ⟨3,10⟩]deca-4,8-diene	–	0.519 ± 0.021	0.276 ± 0.010	–
Tetradecanal	–	0.428 ± 0.116	0.217 ± 0.003	0.114 ± 0.005
trans-Arbusculone	–	–	–	1.220 ± 0.048
trans-Ascaridol glycol	0.460 ± 0.007	–	–	–
trans-Linalool oxide (furanoid)	8.172 ± 0.130	17.927 ± 2.460	21.667 ± 1.297	28.447 ± 2.899
trans-β-Ocimene	–	0.083 ± 0.003	–	–
α-Corocalene	–	–	–	0.065 ± 0.003
α-Farnesene	–	0.037 ± 0.001	–	–
α-Methyl-α-[4-methyl-3-pentenyl]oxiranemethanol	–	0.045 ± 0.002	0.037 ± 0.001	0.033 ± 0.001
α-Pinene	–	0.058 ± 0.002	–	0.100 ± 0.004
α-Terpineol	0.876 ± 0.014	0.697 ± 0.028	0.647 ± 0.046	1.030 ± 0.117
TOTAL (% Area)	**99.200**	**100.20**	**99.75**	**99.77**

- Not detected. 0.000 ± 0.000% of area.

Table 6
Average percentage of inhibition (%) of fresh honey (2 mg/mL) for microorganisms in the microdilution test.

Samples	Microorganisms*			
	CN	CG	CA	SA
Honey **1**	60.96 ± 1.68	55.95 ± 4.05	78.78 ± 3.20	72.01 ± 1.13
Positive control (amphotericin 2.5 μg/ mL)	100 ± 0.0	100 ± 0.0	100 ± 0.0	na
Positive control (erithromycin 2.5 μg/ mL)	na	na	na	98.04 ± 0.79
Honey **2**	nt	nt	71.6 ± 6.3	57.5 ± 7.9
Honey **3**	nt	nt	74.8 ± 2.6	59.2 ± 5.1
Honey **4**	nt	nt	72.1 ± 2.1	57.6 ± 4.1
Positive control (amphotericin 2.5 μg/ mL)	nt	nt	100 ± 0.0	na
Positive control (erythromycin 2.5 μg/ mL)	nt	nt	na	99.98 ± 1.24

na = not applicable, nt = not tested.

* **CN**: *Cryptococcus neoformans* (ATCC 32045), **CG**: *Candida glabr*ata (ATCC 9030), **CA**: *Candida albicans* (ATCC 64550), and **SA**: *Staphylococcus aureus* ATCC 6538).

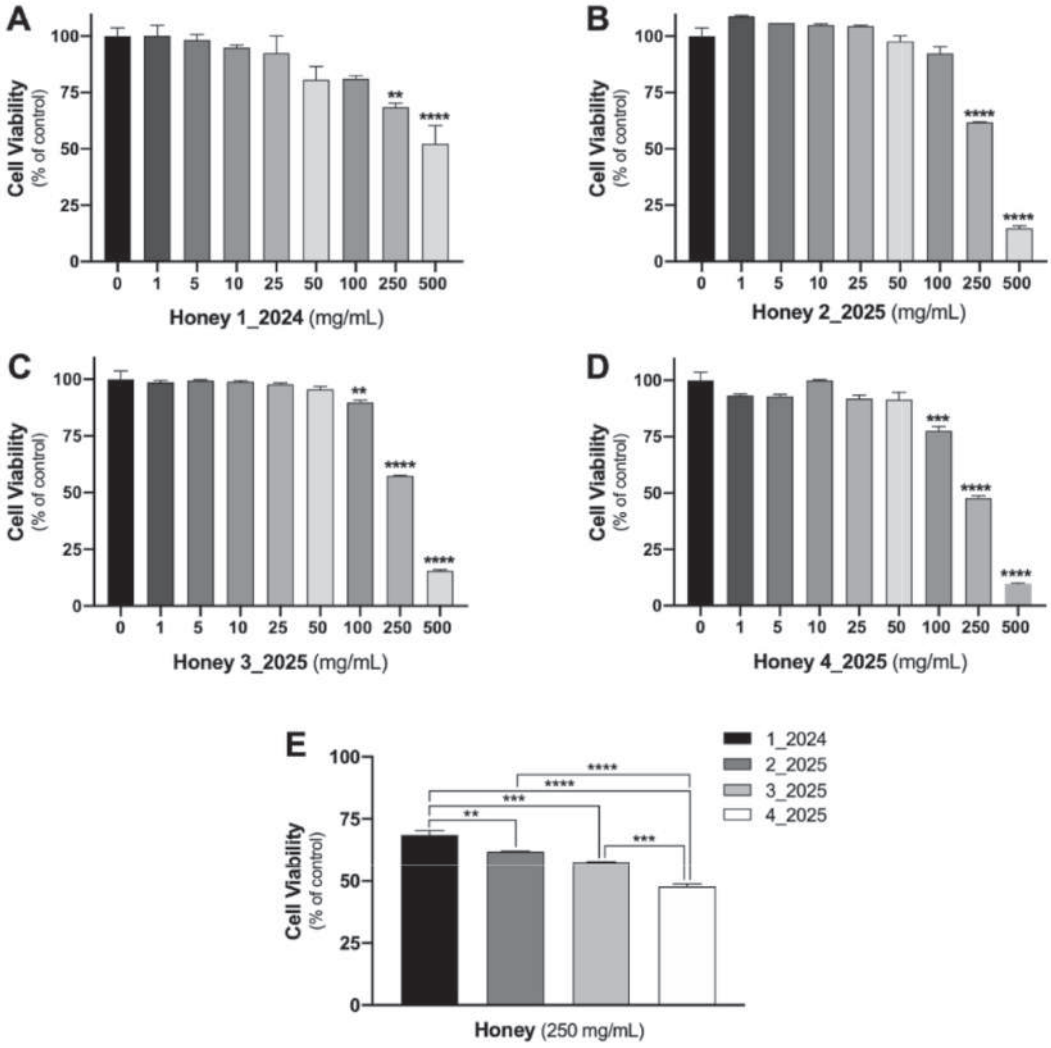


Fig. 4. Effects of different honey samples on the viability of melanoma cells. Cytotoxicity was evaluated by MTT assay in human melanoma A375 cells incubated with increasing concentrations of different honey samples (from 1 to 500 mg/mL) after 24 h of incubation. (A) Honey_1 2024; (B) Honey 2_2025; (C) Honey 3_2025; (D) Honey 4_2025. (E) Comparison of cytotoxic potency among the four honey samples at 250 mg/mL concentration. Cell viability was expressed as a percentage relative to the negative control (untreated cells). Data are presented as mean ± SEM from three independent experiments performed in triplicate. Statistical analysis was conducted using one-way analysis of variance (one-way ANOVA), followed by Tukey's post hoc test for multiple comparisons. Statistically significant differences are indicated by asterisks (**$p < 0.01$; ***$p < 0.001$; ****$p < 0.0001$).

The heatmap (Fig. 6) coupled with hierarchical clustering provides the chemometric differentiation among the honey samples, highlighting both sample grouping and compound-level variation. The clustering pattern clearly separates the samples into four primary classes (H**1**–H**4**), consistent with the dendrogram results Fig. 5, confirming that each honey group possesses a distinct chemical signature. At the metabolite level, distinct blocks of variables exhibit class-specific abundance patterns, as indicated by the contrasting color gradients (ranging from low in blue to high in red). For instance, **H1** samples show elevated intensities for a specific subset of compounds, while these same variables are comparatively suppressed in **H3** and **H4**, suggesting potential marker compounds responsible for discrimination. On the other hand, another group of metabolites is highly expressed in **H4** but remains low across the other classes, further reinforcing compositional uniqueness. The clustering of variables also reveals co-regulated or chemically related compound groups that may be linked to shared botanical or geographical origins. The combined sample and variable clustering demonstrate that the applied chemometric approach effectively captures both inter-class differences and intra-class consistency, supporting its utility for classification, authentication, and identification of characteristic chemical markers in honey samples (Jandrić et al., 2015; Zhou et al., 2014).

4. Discussion

Mimosa pudica honey is common in Brazil, particularly in the central-west and Northeast regions (Da Luz et al., 2021). Plants of the genus *Croton* (common name velane) have been described by beekeepers in Northeast Brazil as a resource for *Apis mellifera* (Bandeira & Novais, 2021). *Croton* sp. was recorded in melissopalynological analysis as a dominant pollen at low frequency in honey samples from Piauí; it is most commonly reported as a pollen of minor importance (Campos et al., 2025). In the honey **4** sample, *Croton* sp. was the predominant pollen species; this species flowers from January to March in Northeast Brazil, a period during which *A. mellifera* also flowers (Bendini et al., 2021; da Silva Moraes et al., 2020). *Anadenanthera colubrina* and *Anadenanthera brasiliensis* are present in several honeys from Brazil, classified as pollen of minor importance due to their low frequency in the total number of species in honey, and were present in two of the four honey samples (Campos et al., 2025; Da Luz et al., 2021; Dos Reis et al., 2023).

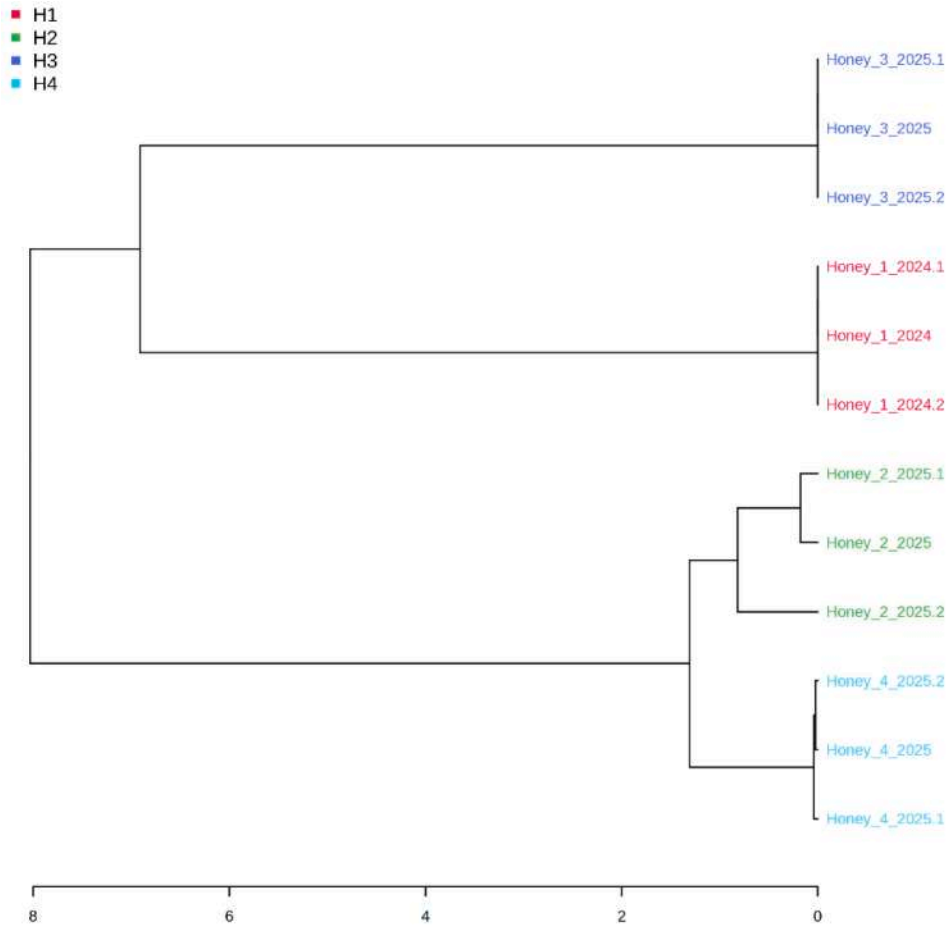


Fig. 5. Hierarchical clustering dendrogram of chemometric profiles of honey samples (1–4), discrimination and compositional variability across harvest years.

Honey color is an important quality attribute that influences consumer acceptance and is often associated with its botanical origin, mineral content, phenolic composition, and processing history (Tahir et al., 2016a). In the present study, the color of the analyzed honey samples ranged from light amber to amber, with Pfund values between 76.9 and 90.2 mm. According to the Pfund classification scale (Bodor et al., 2021), honey **1** and **4** were categorized as amber, whereas honey **2** and **3** were classified as light amber. The relatively narrow range of color values suggests a similar overall color intensity among the samples, although honey **4** exhibited the darkest appearance (90.2 ± 1.3 mm), while honey 2 showed the lowest color intensity (76.9 ± 1.0 mm).

Variations in honey color are generally attributed to differences in floral origin and the concentration of pigments, minerals, phenolic compounds, and Maillard reaction products (Kumar et al., 2018; Yan et al., 2023). Darker honeys are frequently reported to contain higher levels of phenolic compounds and antioxidant activity compared with lighter honeys, although such relationships depend strongly on botanical source (Becerril-Sánchez et al., 2021; Escuredo et al., 2025; Girolametti et al., 2026; Machado et al., 2020). In addition, storage conditions and thermal treatment may contribute to color development through non-enzymatic browning reactions (Gonzales et al., 1999; Vaikousi et al., 2009). Therefore, the darker color observed in samples **1** and **4** may reflect differences in their chemical composition or post-harvest history (Albu et al., 2025; Visquert et al., 2014). However, because the botanical origin and processing conditions were not specifically evaluated in this study, the observed color differences should be interpreted as indicative of compositional variability rather than being attributed to a single factor (Santos-Buelga & González-Paramás, 2025; Szanto et al., 2025).

Polyphenol compounds are important compounds that have a crucial contribution to the functional properties and appearance of honey (Bakar et al., 2017). The total phenolic content (TPC) of the honey 1, 2024 was of 1696.8 mg GAE/kg, which showed a substantially higher phenolic properties than the other three samples. The other samples remained in a lower range between 133.7 and 152.9 mg GAE/kg. Therefore, following the TPC trend, Honey 1 recorded the highest flavonoid concentration (325.61 mg RE/100 g). Honey 3 and 4 showed the lowest flavonoid content at 78.39 and 73.25 mg RE/100 g, respectively. These TPC values were compared to the TPC value reported for several type of honey from different countries. It was found that Black locust honey (*Robinia pseudoacacia*) honey from Italy and Poland had similar TPC value (112.99 mg GAE/kg and 142.8 mg GAE/kg, respectively) and Thyme honey (*Thymus vulgare*) from Italy (126.55 mg GAE/100 g) with Honey 3 (Mărgăoan et al., 2021a). In contrast Buckwheat honey (*Fagopyrum esculentum*) from Poland showed (1113.0 mg GAE/100 g) high result that can be closer to Honey 1 (Mărgăoan et al., 2021a). Additionally, stingless honey from Brazil such as *Melipona q. anthidioides* (161.8 ± 3.4 mg GAE/kg DE) also demonstrated nearly similar result to Honey sample 2 whereas *Melipona subnitida* was higher TPC (854.62 ± 3.8 mg GAE/kg DE) as Honey sample 1 (Oliveira et al., 2017).

A similar trend of pronounced variation was observed for the total flavonoid content (TFC). The TFC value varied considerably among the samples, as an instance, Honey 1 again stood out with the highest TFC, measured at 325.61 ± 2.93 mg RE/100 g DE. This was approximately three times greater than the next highest value, which was found in Honey 2 (108.96 ± 0.08 mg RE/100 g DE). The remaining two samples,

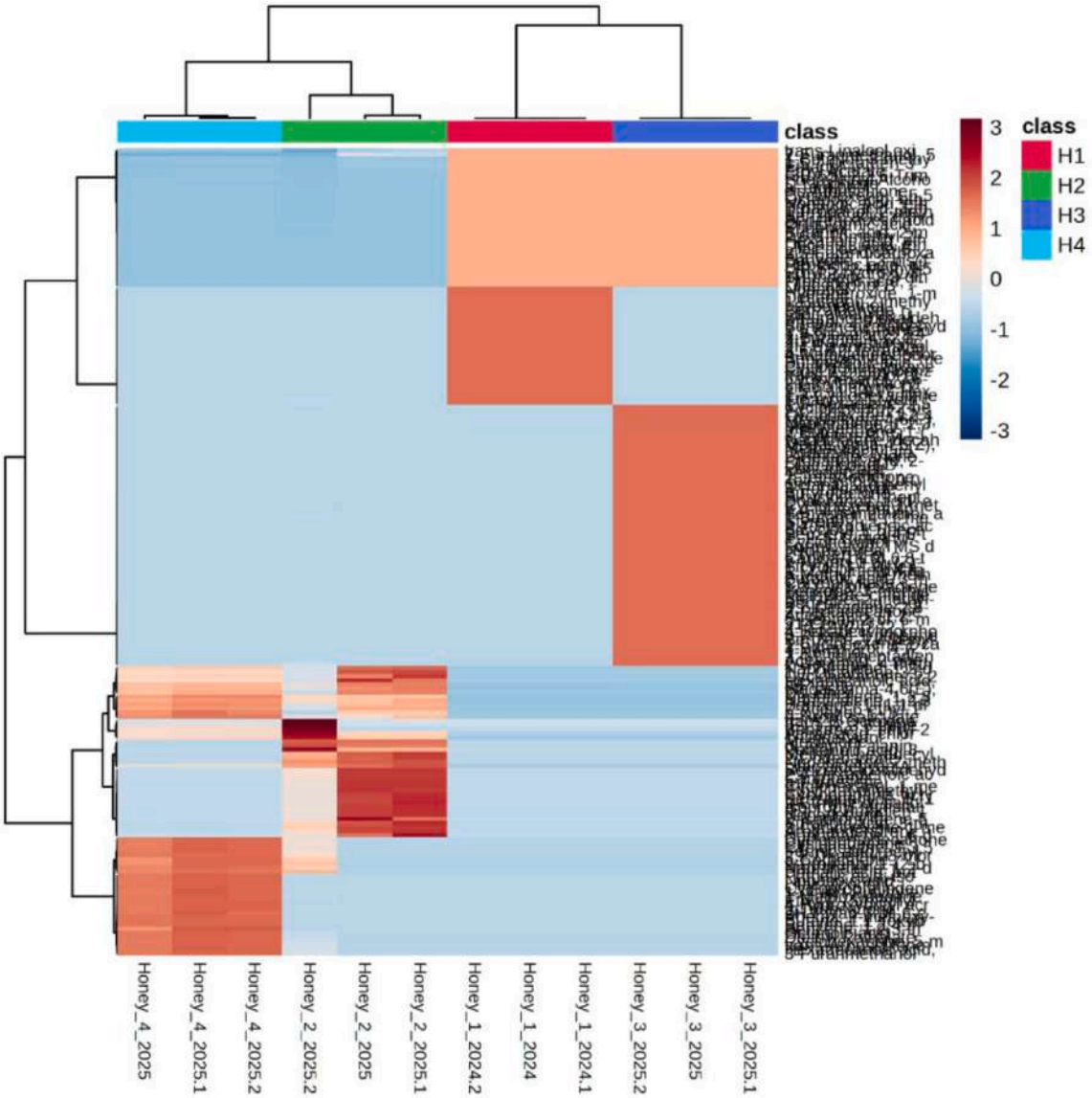


Fig. 6. Hierarchical clustering heatmap of chemometric profiles of honey samples (1–4), discrimination and compositional variability across harvest years.

Honeys 3 and 4, had the lowest flavonoid contents, with values of 78.39 ± 1.43 and 73.25 ± 0.25 mg RE/100 g DE, respectively. The TFC values of analyzed four honey samples were compared to the TFC value reported for other type of honey from different countries. Orange honey (*Citrus sinensis*) from Italy (69.8 mg RE/100 g) and Oak Honey (*Quercus* sp.*)* from Turkey (77.36 mg RE/100 g) demonstrated TFC value similar with Honey 3 and 4. Therefore, the TFC value of Tree of heaven honey (*Ailanthus altissima*) from Italy (91.55 mg RE/100 g) and Manuka honey (*Leptospermum scoparium*) from New Zealand (97.62 RE mg/kg) shows similar results with Honey 1 and 2 (Mărgăoan et al., 2021a). In general, total flavonoid content (TFC) in honey contribute the aroma and antioxidant potentiality. TFC dependent in the floral origin of honey, including bee species and foraging area of the bees (Oliveira et al., 2017).

The polyphenolic profiling of the four Brazilian honey samples revealed distinct differences in both the diversity and concentration of phenolic compounds, reflecting variations in botanical origin (Kumar et al., 2018; Pourmoradian et al., 2026) or environmental factors influencing nectar composition (Abouelenein et al., 2025; Mulheron et al., 2024). Honey 1 exhibited the highest total quantified phenolic content (4.06 mg/kg), nearly twice that of Honey 2 (2.12 mg/kg) and substantially higher than Honey 3 (1.79 mg/kg) and Honey 4 (1.80 mg/kg), suggesting a richer antioxidant phytochemical composition (Table 4). Among the identified compounds, ellagic acid was the predominant phenolic constituent in all samples, with the highest concentration observed in Honey 1 (2.29 mg/kg). The marked reduction in ellagic acid content in Honeys 2–4 indicates considerable variability in floral source (Kumar et al., 2018; Mustafa et al., 2022b). Hydroxybenzoic acids were also important contributors to the phenolic profile. Notably, 4-hydroxybenzoic acid was present at a substantially higher concentration in Honey 1 (0.63 mg/kg) than in the remaining samples (approximately 0.08 mg/kg), while vanillic acid was consistently detected in all samples, with slightly higher levels in Honeys 2–4. Similarly, syringic acid and caffeic acid were found across all samples, indicating that these compounds may represent common constituents of these Brazilian honey (Bakar et al., 2017; Mărgăoan et al., 2021a; Mustafa et al., 2022b). Although Honey 2 contained a greater diversity of detectable compounds than Honey 1, its overall phenolic concentration was lower because the major phenolic constituents occurred at much lower levels. Likewise, Honeys 3 and 4 showed very similar qualitative and quantitative phenolic profiles, suggesting comparable botanical origins or environmental conditions during nectar collection

(Bakar et al., 2017; Kumar et al., 2018).

One hundred and ninety-eight volatile organic compounds (VOCs) were identified in the honey samples. Several of them were not present in one or another sample. The percentage of area (% area), calculated relative to the total area, ranged from 0 to 2% for many compounds. However, we highlight the following compounds (incidence in % area): (3*R*,6*S*)-2,2,6-trimethyl-6-vinyltetrahydro-2H-pyran-3-ol, varying in honey between 3.067 (**2**) to 4.925 (**4**), 1,5,7-octatrien-3-ol, 3,7-dimethyl- from 3.149 (**1**) to 7.419 (**4**), 1-butanol, 3-methyl- from 4.175 (**1**) to 7.313 (**4**), 2,5-furandicarboxaldehyde from 0.000 (**4**) to 8.850 (**1**) with large variation in the percentage of area. 2-furanmethanol, 5-ethenyltetrahydro-α,α,5-trimethyl-, cis- 7833 (**4**) to 24,656 (**2**), in high incidence, benzaldehyde, 2-methyl- was only found in honey **1**, 4187%, D-limonene, from 1069 (**3**) to 5094 (**1**). Phenol, 2,5-bis(1,1-dimethylethyl)- was only identified in sample **1**, 4.798%, phenylethyl alcohol from 2446 (**2**) to 6178 (**1**). *trans*-Linalool oxide was found in significant quantities, from 8172 (**1**) to 28,447 (**4**). Some of these compounds were reported by different authors: 1,5,7-octatrien-3-ol, 3,7-dimethyl- from 2.01 to 2.26% (Nhu & Nguyen, 2026) in honey from Vietnam, 20.40 to 37.90% in Italian honey (Abouelenein et al., 2025), 1-butanol, 3-methyl- from 0.13 to 0.19% (Nhu et al. 2026) in honey from Vietnam and 20.40 to 40.00% in honey from Italy (Abouelenein et al., 2025), 2,5-furandicarboxaldehyde 0.60% in honey from Algeria (Sonia et al., 2026) and 0.68% in honey from Italy (Abouelenein et al., 2025), 2-furanmethanol 4.1% in honey from Algeria (Sonia et al., 2026), D-limonene from 0.19 to 0.22% in honey from Vietnam (Nhu & Nguyen, 2026) and 0.63 to 1.88% in honey from Tenerife, Canary Islands, Spain (Zalewski et al., 2025), phenylethyl alcohol from 6.24 to 6.47% in Vietnamese honey (Nhu & Nguyen, 2026), between 0.5 and 1.0% in honey from Greece (Pateraki et al., 2026) and from 1.64 to 13.87% in Italian honey (Abouelenein et al., 2025), *trans*-linalool oxide from 1.27 to 6.08% in Spanish honey (Pourmoradian et al., 2026), 5.2% in honey from Algeria (Sonia et al., 2026), (E) 0.89 to 5.69%, (Z) 0.36 to 4.45% in honey from Spain (Zalewski et al., 2025) and 2.68 to 16.70% in Italian honey (Abouelenein et al., 2025).

In our results, 1,5,7-octatrien-3-ol, 3,7-dimethyl- showed a higher percentage than that described (Nhu & Nguyen, 2026) and lower than Abouelenein et al. (Abouelenein et al., 2025), 1-butanol, 3-methyl- higher % than (Nhu & Nguyen, 2026) and lower than reported (Abouelenein et al., 2025), 2,5-furandicarboxaldehyde higher than (Sonia et al., 2026) and higher than (Abouelenein et al., 2025), 2-furanmethanol 4.1% higher than (Sonia et al., 2026), D-limonene higher than those reported (Nhu & Nguyen, 2026; Zalewski et al., 2025), phenylethyl alcohol higher than that mentioned by Nhu (Nhu & Nguyen, 2026) and Pateraki (Pateraki et al., 2026) and in the range found by Abouelenein et al. 2025 (Abouelenein et al., 2025), trans-linalool oxide, on average, always higher than all (Abouelenein et al., 2025; Sonia et al., 2026). Many of these identified compounds demonstrate their importance as markers of the floral and geographical origin, as well as the authenticity of honey (Sotiropoulou et al., 2021).

Mineral elements naturally occur in honey as a consequence of nectar composition, botanical origin, soil characteristics, and plant physiological metabolism. Essential macroelements and trace elements are transferred from soil to plants and subsequently incorporated into nectar and pollen, making the elemental profile of honey closely associated with the environmental and botanical conditions of the production area. At the same time, because bees forage over wide territories, honey composition may also reflect environmental exposure and has therefore been proposed as a useful indicator of ecosystem quality and contamination status (Mărgăoan et al., 2021b). In the analyzed samples, K was the dominant element, followed by Ca and Mg, in agreement with mineral profiles commonly reported for honeys from natural and forest ecosystems. K is generally the most abundant mineral in honey and is considered strongly dependent on botanical origin and soil mineral availability (Girolametti et al., 2026). The relatively narrow concentration ranges observed among samples suggest a substantial geochemical homogeneity of the collection area despite differences in pollen composition. Na occurred at lower concentrations, whereas Cu, Fe, Mn, and Zn were detected at trace levels, all within concentration ranges compatible with naturally occurring essential elements in honey matrices. These elements play important physiological roles in both plant and animal metabolism and contribute to the nutritional value of honey, although their abundance is generally much lower than that of major macroelements. Similar distributions have been reported in several monofloral and multifloral honeys from preserved environments (Solayman et al., 2016b). Overall, the elemental composition observed in the present study reflects the natural mineral signature of native Brazilian forest honeys and further supports the environmental quality of the production area.

The DPPH assay revealed substantial differences in the antioxidant capacity of the analyzed honey samples, with values ranging from 64.29 ± 0.08 to 343.08 ± 0.07 TE/kg. Honey **1** exhibited markedly higher radical scavenging activity than the other samples, indicating a greater capacity to neutralize free radicals. Such variation in antioxidant activity is commonly associated with differences in botanical origin, environmental conditions, nectar composition, and beekeeping practices (Dzugan et al., 2018; Poggi et al., 2026). In honey **2** exhibited the lowest total phenolic content, its DPPH value was slightly higher than those of honey **3** and **4**. This observation suggests that antioxidant activity cannot be explained solely by the concentration of total phenolics and may also be influenced by the presence of highly active non-phenolic antioxidants, such as enzymes, organic acids, peptides, vitamins, or specific phenolic compounds with greater radical-scavenging efficiency (Ajayi et al., 2025; Gajger et al., 2025). Furthermore, synergistic interactions among different antioxidant constituents may contribute to the overall antioxidant potential of honey (Ajayi et al., 2025). Therefore, the observed differences in DPPH activity likely reflect qualitative as well as quantitative variations in the antioxidant composition of the analyzed samples (Dimitriu et al., 2020).

Honey showed antimicrobial activity at 2 mg/mL (inhibition between 56% and 79%), with greater inhibition against *Candida albicans* (72% inhibition of honey **1**), a yeast-like fungus with greater sensitivity to the antimicrobial components of honey under the evaluated conditions (Stefanis et al., 2023). Honey from Piauí had antibacterial potential against *Staphylococcus aureus* (72% inhibition at a concentration of 0.2%), as observed for Manuka honey (from New Zealand and Australia), New Caledonia honey (from France), and Western Serbian honey, at concentrations greater than 5% for total inhibition (MIC) (Bucekova et al., 2023; Nedić et al., 2022). The antimicrobial activity of honey **1**, the only honey sample that inhibited *Cryptococcus neoformans* and *Candida glabrata*, can be attributed to the higher content of phenolic/flavonoid compounds, which modulate antimicrobial activity (Alcivar-Saldaña et al., 2024; Ogwu & Izah, 2025). Honey **1** contained hyperoside as its major compound (5.09 mg/kg), present only in this honey sample; this flavonoid compound was responsible for reducing *Candida albicans* biofilm and is present in plants such as *Nephrolepis exaltata* (L.) Schott and *Limonium* species (Baysal et al., 2021; Elhawary et al., 2025). The antimicrobial activity of honey **1** may be related to its multifactorial action, highlighting the presence of bioactive compounds such as flavonoids, in addition to its low pH, high sugar concentration, low water activity, and hydrogen peroxide production (Kunat-Budzyńska et al., 2023; Ogwu & Izah, 2025; Steffan-Dewenter & Kuhn, 2003).

Our findings are consistent with the growing body of evidence demonstrating that honey can exert cytotoxic effects against tumor cells in vitro, although its biological activity appears to depend strongly on chemical composition. Here, all honey samples decreased A375 melanoma cell viability in a concentration-dependent manner, but their cytotoxic potency differed markedly despite similar overall response profiles. These observations reinforce the concept that honey should not be regarded as a uniform biological product, since variations in botanical and geographical origin directly influence its chemical composition

and, consequently, its biological properties.

Previous studies have shown that acacia honey inhibits the proliferation of A375 and B16-F1 melanoma cells in concentration- and time-dependent manners (Pichichero et al., 2010b). Mechanistically, this effect was associated with G0/G1 cell-cycle arrest and accumulation of hyperploid cells, suggesting that growth inhibition resulted primarily from cytostatic rather than strictly cytotoxic mechanisms. In addition, the authors proposed that chrysin, a flavonoid present in acacia honey, substantially contributed to the observed activity in melanoma cells (Pichichero et al., 2010b). Supporting this hypothesis, isolated chrysin was also shown to suppress tumor growth in a B16-F10 melanoma in vivo model (Sassi et al., 2018). In contrast, Manuka honey suppressed B16-F1 melanoma cell growth through activation of the intrinsic apoptotic pathway, characterized by caspase-9 and caspase-3 activation, reduced Bcl-2 expression, and DNA fragmentation (Fernandez-Cabezudo et al., 2013). Similar effects have been reported in other tumor cell models, including prostate, hepatoma, and colon cancer cells, in which honey has been associated with reduced viability, apoptosis induction, cell-cycle arrest, oxidative stress modulation, and changes in metastatic behavior (Afrin et al., 2018; Erejuwa et al., 2014; Samarghandian et al., 2014). A plausible interpretation is that the biological activity of honey is driven mainly by bioactive low-molecular-weight compounds, particularly phenolic constituents, rather than by honey as a uniform matrix. This interpretation may help explain why different honey samples exhibit distinct cytotoxic potencies (Jaganathan et al., 2010; Mohd Hatta et al., 2026). Together, these studies indicate that honey-induced inhibition of melanoma cells may arise through different biological mechanisms depending on the specific composition of each honey sample.

Overall, our results expand the still limited literature regarding the cytotoxic effects of honey against melanoma cells by demonstrating that honey samples differing in origin exhibit distinct cytotoxic potencies. These findings reinforce the concept that chemical composition is a critical determinant of the biological activity of honey. Future studies integrating detailed phytochemical characterization with mechanistic analyses will be essential to identify the compounds responsible for these effects and to clarify their therapeutic potential against melanoma.

The hierarchical clustering dendrogram and heatmap consistently demonstrated clear differentiation among the four honey groups, indicating distinct chemical profiles associated with each sample type. The tight clustering of replicate samples within each group confirms the high reproducibility of the analytical method and the low variability among replicates. Notably, honey **1** formed a separate cluster from the other honey groups, suggesting substantial compositional differences that may be related to variations in floral source, environmental conditions, or harvest year (Mulheron et al., 2024; Pattamayutanon et al., 2017; Popa & Ustunol, 2011; Sonia et al., 2026). In contrast, honey **2** and honey **4** exhibited greater similarity, indicating partial overlap in their chemical composition. The heatmap further revealed class-specific abundance patterns, with several compounds showing marked differences among honey groups, highlighting their potential contribution to sample discrimination (Khamitova et al., 2026; Louppis et al., 2017; Tahir et al., 2016b). Overall, the chemometric results demonstrate the effectiveness of multivariate analysis in distinguishing honey samples based on their chemical fingerprints and provide valuable insights into the factors influencing honey composition (Kivima et al., 2021; Rahman et al., 2017; Seraglio et al., 2021).

5. Conclusion

This study provides a comprehensive characterization of honey produced in a preserved native forest region of Brazil by integrating melissopalynological, physicochemical, mineral, volatile, and phenolic analyses. The results demonstrated that the honey samples possess distinct chemical profiles, reflecting differences in their botanical origin while confirming their authenticity and high quality. The identification of diverse phenolic acids, flavonoids, volatile compounds, and mineral constituents, together with the observed variations in total phenolic and flavonoid contents, highlights the complexity of these honeys and their potential as natural sources of bioactive compounds with antioxidant properties. These results support the importance of conserving native forests and promoting sustainable beekeeping practices, which not only protect biodiversity but also enhance the production of high-quality honey with added nutritional and commercial value. Furthermore, the chemical fingerprint established in this study provides useful information for the authentication of Brazilian honeys according to their geographical and botanical origins and may contribute to future quality control and traceability studies.

CRediT authorship contribution statement

Maria Cristina Marcucci: Writing – review & editing, Writing – original draft, Visualization, Supervision, Resources, Project administration, Methodology, Investigation, Funding acquisition, Data curation, Conceptualization. **Gulzhan Khamitova:** Writing – review & editing, Writing – original draft, Visualization, Supervision, Resources, Project administration, Methodology, Investigation, Funding acquisition, Data curation, Conceptualization. **Laura Acquaticci:** Investigation, Conceptualization. **Lazzat Karasholakova:** Resources, Investigation, Funding acquisition, Conceptualization. **Giovanni Caprioli:** Writing – review & editing, Resources, Investigation, Conceptualization. **Carla Galbiati:** Writing – review & editing, Writing – original draft, Visualization, Supervision, Resources, Methodology, Investigation, Data curation, Conceptualization. **Mariana Lenina Menezes Aleixo:** Investigation, Conceptualization. **Joelma Nascimento da Silva:** Investigation, Conceptualization. **Anna Clara de Freitas Couto:** Methodology, Investigation, Conceptualization. **Leandro Wang Hantao:** Writing – original draft, Visualization, Methodology, Investigation, Conceptualization. **Cristina Truzzi:** Writing – review & editing, Supervision, Methodology, Investigation, Conceptualization. **Federico Girolametti:** Methodology, Investigation, Conceptualization. **Pablo Augusto Barreto de Morais:** Methodology, Investigation, Conceptualization. **Laura Francisca Leite do Prado-Souza:** Methodology, Investigation, Conceptualization. **Letícia Silva Ferraz:** Methodology, Investigation, Conceptualization. **Tiago Rodrigues:** Writing – review & editing, Writing – original draft, Visualization, Supervision, Resources, Methodology, Investigation, Funding acquisition, Data curation, Conceptualization.

Informed consent statement

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Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.foodres.2026.120999.

Data availability

The original contributions presented in this study are included in the Article and in supplementary material. Further inquiries can be directed towards the corresponding author.

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