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Article

Authentication of *Ziziphus lotus* Honey from the Middle Atlas Mountains of Morocco: Physicochemical Properties, Mineral Content, Sugar, Polyphenol Profiles, and Antioxidant Capacity

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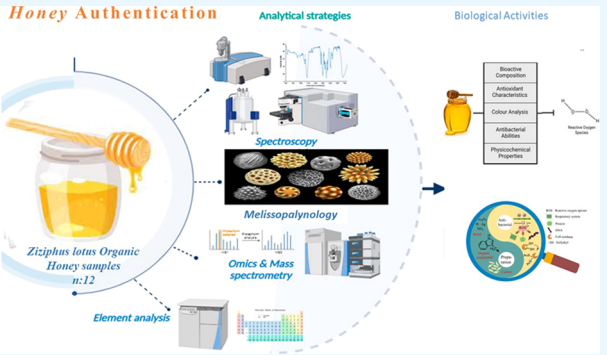
ABSTRACT: The jujube honey from the Moroccan Middle Atlas area is thoroughly described in this study, which takes into account melissopalynological, physicochemical, antioxidant, mineral, and phytochemical characteristics. Twelve samples of jujube honey underwent in-depth analyses between 2019 and 2021. The honey's unifloral origin was confirmed by pollen analysis, which revealed that *Ziziphus lotus* pollen predominated along with pollen from 21 other species. The honeys meet Codex Alimentarius criteria and displayed a variety of characteristics, including moisture content (13.7% to 18.6%), pH (3.9 to 6.4), electrical conductivity (406 to 713 μs/cm), ash content (0.31 to 1.21%), and the Invertase Index (7.1 to 26.4 U/kg). Hydroxymethylfurfural levels spanned from 1.1 to 40 mg/kg, indicating freshness. No significant differences were observed between honey groups for fructose and glucose profiles determined via GC-MS analysis. The honey samples, which varied in total phenolic content (TPC) from 48.3 mg of gallic acid equivalent (GAE)/100 g to 91.8 mg of GAE/100 g, showed strong antioxidant capacity, indicating possible health advantages. This study also revealed principal phenolic substances including gallic acid (1.18 to 6.36 mg/100 g), caffeic acid (0.07 to 3.25 mg/100 g), and *p*-coumaric acid (0.49 to 5.04 mg/100 g). Next, the bactericidal concentrations and minimum inhibitory concentrations (MBC and MIC) of each jujube honey were additionally examined and compared with two representative bacterial strains species *Listeria monocytogenes* and *Salmonella typhimurium* using broth microdilution, with MIC values ranging between 0.03 and 0.3 mg/mL for *Listeria monocytogenes* and 0.003 to 0.03 mg/mL for *Salmonella typhimurium*. There is a correlation between various parameters and the monofloral pollen content in honey, as determined by PCA analysis.



1. INTRODUCTION

The development of functional foods is currently a major trend in the field of food chemistry. This movement is driven by scientific findings indicating that diet plays a crucial role in preventing illnesses such as diabetes type 2, high blood pressure, and cancer.

One example of a functional food is honey. It contains bioactive compounds such as polyphenols, which have beneficial health properties. Honey is also known for being a complex mixture of various components since nectar and pollen are gathered by bees from different plants. In addition to sugars, honey is composed of moisture and valuable nutrients, such as vitamins, minerals, enzymes, free amino acids, and various volatile compounds. The bioactive compounds present in honey are largely determined by the plants that provided the nectar, with the most important bioactive fraction being the secondary metabolites found in nectar, such as phenolic acids and flavonoids. The composition of honey is influenced by its botanical and geographical source and can undergo substantial alterations based on the storage duration and circumstances.[1] Regarding its botanical origin, floral honey can be categorized as either polyfloral or monofloral. Polyfloral honey is derived from multiple botanical sources without any particular dominance, whereas monofloral honey is wholly or mainly produced from the nectar/honeydew of a single type of flower and the honey possesses the organoleptic, physico-chemical, and microscopic characteristics of that source.[2] Monofloral honeys have captured

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Table 1. Sampling Details: Honey Provenance and Extraction Periods

Reference	Geographical origin	Beekeepers declarations	Latin name	Local name	Latitude	Longitude	Altitude	Harvested period
JUB1	Ain Cheggag	jujubier + fleur	*Ziziphus lotus* + other flowers	Sdra + zher	33°51′32″ N	5°05′48″ W	864 m	June 2019
JUB2	Tandit	jujubier	*Ziziphus lotus*	Sdra	33°41′07″ N	3°40′17″ W	837 m	May 2019
JUB3	Amghasse	jujubier	*Ziziphus lotus*	Sdra	33°23′20″ N	5°32′53″ W	844 m	June 2019
JUB4	Oued Ifran	jujubier	*Ziziphus lotus*	Sdra	33°17′38″ N	5°28′55″ W	920 m	June 2019
JUB5	Outat Elhaj	jujubier	*Ziziphus lotus*	Sdra	33°24′07″ N	3°42′14″ W	772 m	May 2020
JUB6	Bouchbel	jujubier	*Ziziphus lotus*	Sdra	33°19′32″ N	5°34′58″ W	896 m	May 2020
JUB7	Sebt Ain Lahnech	jujubier	*Ziziphus lotus*	Sdra	33°47′58″ N	5°12′50″ W	834 m	June 2020
JUB8	El Ksabi	jujubier	*Ziziphus lotus*	Sdra	32°50′25″ N	4°24′22″ W	1048 m	June 2020
JUB9	Oued Amlil	jujubier	*Ziziphus lotus*	Sdra	34°14′46″ N	4°22′29″ W	600 m	May 2021
JUB10	Oued Ifran	jujuber	*Ziziphus lotus*	Sdra	33°18′25″ N	5°30′30″ W	906 m	June 2021
JUB11	Ain Chegag	jujubier + thym + buplèvre	*Ziziphus lotus* + *Thymus vulgaris* + *Bupleurum spinosum*	Sdra + Zaaitra + zandaz	33°51′06″ N	5°01′55″ W	708 m	June 2021
JUB12	Outat Elhaj	jujubier	*Ziziphus lotus*	Sdra	33°22′29″ N	3°39′50″ W	774 m	June 2021

more attention from consumers because they offer the potential to possess the properties of the plants from which they are derived. In fact, there is a suggestion that the medicinal properties of many plants can be conveyed through honey, making it a carrier of these properties.[3] Consequently, there has been a recent surge of interest in unconventional varieties of honey, such as monofloral honey, due to their distinct nutritional, sensory, and potential therapeutic characteristics. *Ziziphus lotus* honey serves as an example of this trend, as it has gained popularity among consumers for its fragrance and the medicinal properties associated with the *Ziziphus lotus*plant.[4]

The Moroccan wild jujube, also known as *Ziziphus lotus*, is a common plant found in various regions with arid and semi-arid climates, including Chaouia, Haouz, Zear, Rhamna, the Middle Atlas, Gharb, Errachidia, Souss, the coastal region of Safi in Sidi Ifni, Khenifra, eastern Morocco Sahara, and the region of Oujda. This is due to Morocco's diverse geography and distinct soil types, which contribute to its rich ecological and botanical diversity. Out of the 4500 species of vascular plants found in Morocco, 800 to 951 are endemic, with *Z. lotus* being a wild plant species from the Rhamnaceae family, which includes 135 to 179 species of *Ziziphus*.[5−7]

The presence of *Z. lotus* in various regions of Morocco makes it a significant home remedy for the local population. The leaves, fruits, and roots of plants are utilized in various forms such as infusions and decoctions to treat a wide range of ailments. Some of the health benefits associated with consuming *Z. lotus* include treatment for urinary tract infections, digestive and intestinal disorders, and properties that lower blood sugar, reduce diarrhea, lower blood pressure, and prevent ulcers.[7,8]

In addition to its medicinal properties, *Z. lotus* is also a valuable source of honey. The nectar from the flowers of this plant is collected by bees and used to produce honey that contains many of the same biologically active molecules found in the plant itself. These "fingerprint" molecules, such as polyphenols, triterpenes, anthraquinones, and alkaloids, give honey its health benefits and can be used to identify this specific type of honey. Previous studies have shown that honey made from *Z. lotus*'s nectar has strong antibacterial and antioxidant properties, making it a valuable addition to a healthy diet due to the presence of polyphenolics along with alkaloids.[9−11] In addition, these compounds can also be used to identify the source of honey and differentiate it from other types of honey.[12]

The data regarding *Z. lotus* honey are still limited, and the specifics of its chemical composition remain elusive, particularly for Moroccan *Z. lotus* honey. Our work aimed to characterize this type of honey by its physicochemical, pollinological, mineral, and sugar characteristics. Through LC-MS/MS analysis, the comprehensive composition of its phenolic compounds is here investigated. We next tested the antibacterial and antioxidant properties of this valuable product.

2. MATERIALS AND METHODS

2.1. Standards and Reagents. The following polyphenols were obtained from Sigma-Aldrich: these were 3,4,5-trimethoxycinnamic acid (T70408), 4-OH-phenyl acetic acid (H50004), avicularin (Quercetin 3-*O*-arabinoside) (44006), caffeic acid (C0625), chlorogenic acid (C3878), cyanidin (79457), daidzein (16587), dihydrocaffeic acid (3,4-dihydroxyhydrocinnamic acid) (102601), dihydroferullic acid (3-(4-hydroxy-3-methoxylphenyl)propionic acid) (17803), epicatechin (E1753), (trans)-ferulic acid (128708), gallic acid (91215), gentisic acid (149357), hesperetin (51864), hesperidin (50162), isoquercetin (0014-05-85), kaempferol (96353), naringenin (52186), *o*-coumaric acid (*trans*-2-hydroxycinnamic acid) (H22809), *p*-coumaric acid (*trans*-4-hydroxycinnamic acid) (C9008), phloretin (P7912), phloridzin (phloretin-*O*-2′-glucoside) (274313), pyrocatechol (C9510), quercetin (quercetin hydrate) (337951), quinic acid (138622), salicylic acid (S5922), sinapic acid (93878), and vanillic acid (68654). A selection of polyphenols, including apigenin (1102S), galangin (1114S), isorhamnetin (1120S), quercetrin (quercetin 3-*O*-rhamnoside) (1236S), and rutin (quercetin 3-*O*-rutinoside) (1139S), were purchased from Extrasynthese. Polyphenols from Phytolab include apigetrin (apigenin 7-*O*-glucoside) (89160), aromadendrin (dihydrokaempferol) (80430), catechin (D-catechin) (89172), cynaroside (luteolin 7-glucoside) (89724), luteolin (89245), miquelianin (quercetin 3-*O*-glucuronid) (90733), procyanidin B2 (89552), protocatechuic acid (3,4-dihydroxybenzoic acid) (89766), and taxifolin (89284). Sucrose, fructose, turanose, glucose, maltose, isomaltose, melibiose, trehalose, erlose, palatinose, melezitose, raffinose, maltotriose, and panose were also obtained from Sigma-Aldrich. Sodium nitroprusside dihydrate was purchased from Riedel-de Han (St. Louis, MO, USA). *N*-(1-Naphthyl)ethylenediamine dihydrochloride, potassium dihydrogen phosphate, and disodium hydrogen phosphate dihydrate were from purchased Merck (Darmstadt, Germany). Phosphoric acid, formic acid, methanol, and acetonitrile were purchased from Biosolve (Dieuze, France). Sulphanilamide, potassium hexacyanoferrate, zinc

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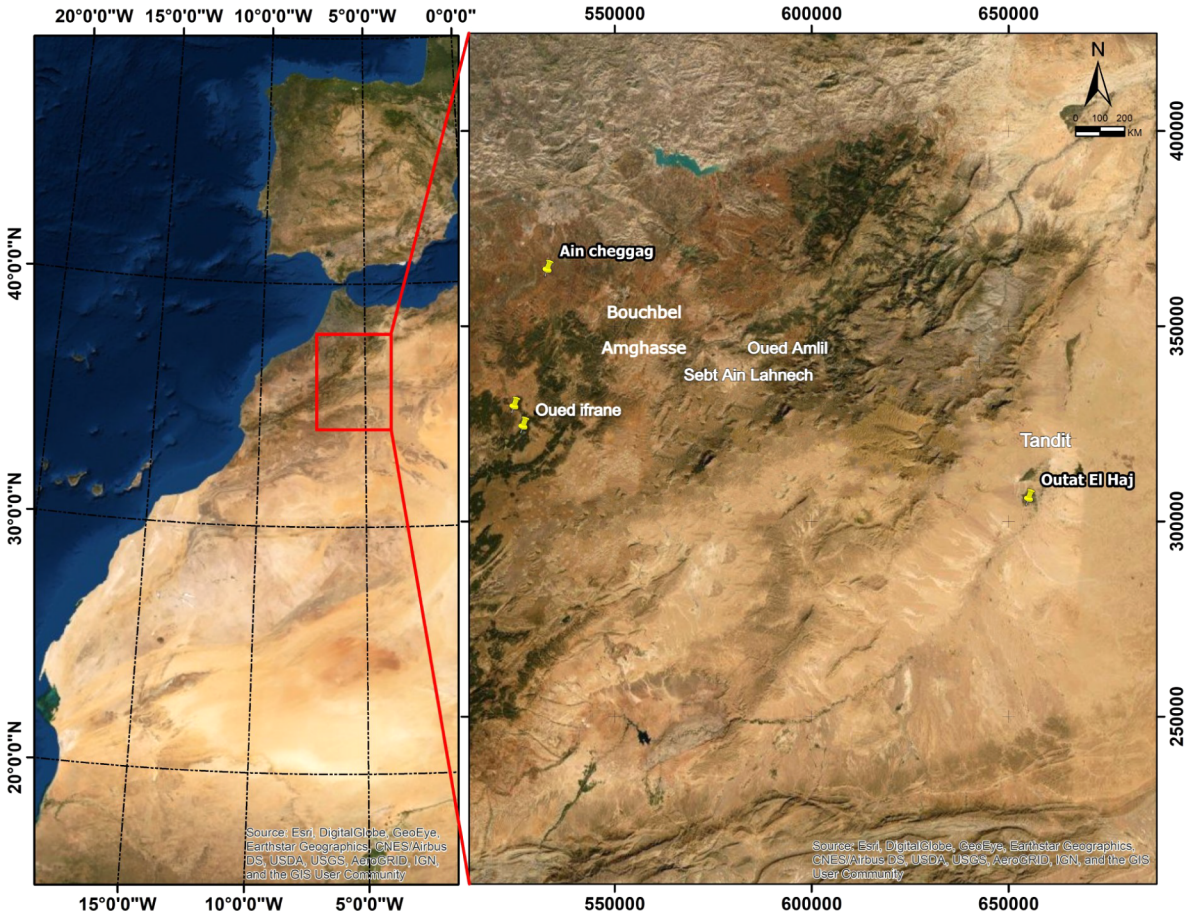

Figure 1. Map of Morocco showing honey sample regions in the Middle Atlas.

acetate dehydrate, 2,2′-azino-bis(3- ethylbenzothiazoline-6-sulfonic acid, 6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid, hexamethyldisilazane, and trifluoroacetic acid were also used. The water was treated in a Milli-Q water purification system, Millipore, Bedford, MA, USA.

2.1.1. Sampling. Between the middle of May and the end of June during a period of three years (2019−2021), 12 (n = 12) honey samples (500 g) were taken from healthy, contemporary hives situated in 12 distinct eco-geographical zones of Morocco (Table 1), across different zones of the Middle Atlas range in Morocco (Figure 1). Prior to analysis, all samples were centrifuged at the time of collection and kept at 4 ± 1 °C before analysis.

2.2. Melissopalynological Analysis. Pollen grains were acetolyzed by treating subsamples with a mixture of acetic anhydride and sulfuric acid (9:1 ratio) at 70 °C until they turned brown, and the melissopalynological examination was carried out qualitatively.[13] In order to filter out the contaminants, each 10 g sample of honey was diluted in 100 mL of distilled water before being centrifuged at 2500 rpm for 5 min. The pellet was suspended in 10 mL of glacial acetic acid and centrifuged once more, and the supernatant was discarded. A minimum of 400 pollen grains per sample were counted, recognized using the general pollen identification standard, and then compared to the pollen source catalogs of the studied area's flowers.[14,15] Various pollen atlases and bibliographic sources[16−18] were consulted for reference. The frequencies of pollen from different representative plant species or families in each sample were recorded and compiled. Frequency ranks were determined by dividing the percentage of pollen grains: predominance pollen: this refers to the primary or most abundant type of pollen present; secondary pollen (10−40%): this indicates pollen that is present in lesser quantities compared to the predominant pollen, typically comprising 10% to 40% of the total pollen count; and important minor pollen (<10%): this describes pollen types that are present in smaller quantities, constituting less than 10% of the total pollen count but are still noteworthy due to their significance.

2.3. GC-FID Determination of Sugars. GC-FID (gas chromatography with flame ionization detection) was employed to analyze sugar content, adhering to the Pierce−Portallier method.[19] The sugar analysis was carried out using two-step derivatization procedures (oximation and trimethylsilylation).

5 mL portion of mannitol and 3 g of each sample of honey, dissolved in distilled water, were put into a flask with a capacity of 500 mL. Then, distilled water was added until the line was reached in each flask. A conical-bottomed test tube was filled with 100 μL of each mixture after the samples had been well mixed for 10 min. It was allowed to dry at 50 °C while being sprayed with nitrogen. After that, the test tubes were securely closed with screw caps before 200 μL of oximation solution (0.06 g of hydroxylamine chloride diluted in 5 mL of pyridine) was added. Homogenized mixtures were heated to 65 °C for 30 min while being combined at 1400 rpm. The oximes produced at this stage were silylated for 30 min at 25 °C with hexamethyldisilazane (100 μL) and trifluoroacetic acid (10 μL). The trimethylsilyl derivatives were purified by centrifugation before being injected into a gas chromatograph (XL FID

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Autosystem, PerkinElmer, USA). Each sample was examined using a 0.6 μL injection under the following GC conditions: a 70 °C starting oven temperature, followed by 49 °C/min programming from 70 to 140 °C and 6 °C/min programming from 140 to 300 °C using helium as the carrier gas (Sabatini, 2001). Using the TurboChrom Navigator software, the chromatographic peak regions' data were collected. Retention times relative to those of mannitol were employed for both standards and sample peaks in qualitative analysis. Standards additions were used to honey samples in order to quantify each detected sugar after confirming the identity of each of them.

Duplicate injections were performed. The acquisition was performed using TurboChrom Navigator software operating in a Windows environment.

2.4. Mineral Content by ICP-AES. Inductively coupled plasma atomic emission spectroscopy (ICP-AES) was used to help determine minor and major mineral content in the digested powder of studied honey samples and was performed as described by Ben Mrid et al. (2019)[20] with some modifications. Briefly, this analysis was determined after a two-phase dry ashing. Around 5 g of honey was weighted in a ceramic crucible and put on a hot plate. The temperature of the hot plate was gradually increased, and when no fume was observed, the crucible was transferred into a muffle furnace, which was preheated at 550 °C. After 4 h of ashing, the crucible was removed from the furnace and left to cool. The ash from burned honey samples was digested by heating at 110 °C in 5 mL of concentrated nitric acid HNO_3 and 15 mL of hydrochloric acid. After complete digestion, the mixed samples were cooled at room temperature and made up a final volume of 100 mL with ultrapure water. Digestion was then analyzed in duplicate, and concentrations of trace metal elements were measured directly by using the ICP-AES Agilent 5110 ICP-OES Spectrometer (Agilent Technology Inc.). The plasma gas flow rate is maintained at a stable 12 L/min for ideal instrument performance. Auxiliary gas flows at 1 L/min, while the nebulizer gas is set to 0.7 L/min. Samples are introduced at 1.5 mL/min, and the system operates with 1200 W of radio frequency (RF) power.

2.5. Hydroxymethylfurfural (HMF) Determination. HMF content was determined using an HPLC Agilent 1200 system integrated with various Shimadzu components and controlled by Shimadzu Client/Server software (Version 7.3). This analytical setup, sourced from Shimadzu Corporation in Tokyo, Japan, was employed following the methodology described by Baloš et al.[21] Briefly, in a flask (50 mL), 3 g honey sample was dissolved in 25 mL of Milli-Q water. For solution purification, 0.5 mL each of Carrez I (potassium hexacyanoferrate) and Carrez II (zinc acetate dihydrate) solutions was sequentially added and mixed thoroughly. The flask was then filled to the mark with Milli-Q water, resulting in a milky solution that ranged from light yellow to brown depending on the honey type. This mixture was filtered through a 5 μm filter paper, discarding the initial 10 mL of filtrate. The remaining filtrate underwent a second filtration using a 0.45 μm PTFE filter before being transferred to glass vials for chromatographic analysis.

The chromatographic conditions were as follows: a ZORBAX Eclipse XDB-C18 reverse phase column (150 × 4.6 mm, 5 μm film thickness) from Supelco (Bellefonte, PA) was used. The injection volume was 25 μL with a mobile phase flow rate of 0.7 mL/min in isocratic mode. The mobile phase consisted of 90% water (with 1% formic acid) and 10% acetonitrile. The column was maintained at 30 °C, and detection was carried out with a DAD detector set to λ = 285 nm. The total run time was 15 min. Serial standard solutions of HMF, ranging from 1 to 50 mg/L, were prepared by using Milli-Q water.

2.6. Total Phenolic Content (TPC). The polyphenol content was determined using the Folin-Ciocalteau method as outlined by Yildiz.[22] A calibration curve was constructed using gallic acid (0−500 mg/L) with a correlation coefficient of R2 = 0.998. The results were expressed in milligrams of gallic acid equivalent (GAE) per 100 g of honey (mg GAE/100g)

2.7. Total Flavonoids Content (TFC). The total flavonoid content (TFC) was determined using the method described by Laaroussi et al.[23] A calibration curve was constructed using a range of quercetin standards (0.5−100 mg/L), which was analyzed in the same manner as for the honey samples. Two milliliters of 2% $AlCl_3$ reagent were added to 2 mL of honey solution (1 g/10 mL). After incubating for 30 min at room temperature, the absorbance was measured spectrophotometrically at 420 nm. The TFC of each honey sample was calculated using the regression equation ($y = 0.0304x + 0.0169$) from the quercetin standard curve (R^2 = 0.986). The results were expressed as milligrams of quercetin equivalent (QE) per 100 g of honey.

2.8. Free Radical Scavenging Activity (DDPH Assay). The DPPH radical scavenging activity was determined using the method described by Ak et al.[25] Honey samples at various concentrations (3.90−125 mg/mL) were added to a DPPH−methanol solution (150 μM, absorbance of 0.700 ± 0.01 at 515 nm). After incubating for 1 h at room temperature, the absorbance of the reaction mixtures was measured at 517 nm. The percentage inhibition was calculated using the provided equation. The honey sample concentration required for 50% inhibition (IC50) was determined using the linear regression algorithm of the plotted inhibition graph.

$$\%\text{Inhibition} = [(|\text{control}| - |\text{sample}|)/|\text{control}|] \times 100$$

The control solution consisted of ultra-pure water instead of honey, and a standard solution of 6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid (Trolox) was used as a positive control with a concentration range of 250−15 μM, R^2 = 0.98.

2.9. Radical Cation Decolorization (ABTS Assay). The ABTS assay for assessing various honey samples was performed by mixing 2 mL of an ABTS radical cation solution (2,2′-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid) diammonium salt) with 100 μL of honey solutions at different concentrations (from 0.97 to 125 mg/mL). These mixtures were then incubated for 30 min in the dark at room temperature. Following incubation, the intensity of the coloration produced was promptly measured at 734 nm using a UV−Vis spectrophotometer (Jasco V-730).[26]

Trolox (800−30 μM, R^2 = 0.998) was used as a positive control. The ABTS radical cation inhibition percent was determined using the equation of DPPH.

2.10. Polyphenol LC-MS/MS Analysis. The sample preparation method employed in our study was adapted from Zhu et al.,[27] and the LC-MS/MS analysis followed the method outlined by Kips et al.[28]

A total of 0.5 g of honey was combined with 50 μL of an internal standard (Daidzin) solution at a concentration of 100 ng/μL. Subsequently, 1 mL of acidified water, containing 10% NaCl with a pH adjusted to 2.0 using a 1 mol/L HCl solution, was added to the mixture and thoroughly mixed until the honey was dissolved. To this solution was added 1 mL of acetonitrile (ACN) followed by vortexing for 1 min. The resulting mixture

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Table 2. Melissopalynological Analysis of 12 Honey Samples

Samples	Predominant pollen	Secondary pollen (10−40%)	Important minor pollen (<10%)	
H1	*Ziziphus lotus* (40%)	Brassicaceae (13%), Rosaceae (15%)	Apiaceae, Astéraceae, Pin, Poaceae, Rutaceae, Chénopodiaceae, Pissenlit, Fruitiers, Fabaceae, Plantain, Ronces	monofloral
H2	*Ziziphus lotus* (64%)	Rosaceae (16%)	Chénopodiaceae, Cistaceae, Renonculaceae, Apiaceae, Pissenlit, Brassicaceae, plantain, Ronces, Fabaceae	monofloral
H3	*Ziziphus lotus* (68%)	Apiaceae (11%)	Acacia, Lamiaces, *Olea europaea*, plantain, Poaceae, Chénopodiaceae, Cistaceae, Pissenlit, Ronces, Asteraceae, Rosaceae, Trèfles, Brassicaceae	monofloral
H4	*Ziziphus lotus* (73%)	Rosaceae (14%)	Apiaceae, Myrthaceae, Plantain, Trèfles, Astearaceae, Cistaceae, Brassicaceae	monofloral
H5	*Ziziphus lotus* (63%)	Fabaceae (10%)	Multiporés, non identifié, Pissenlit, Poaceae, Renonculaceae, Tournesol, Apiaceae, Centaureae, Saule, Astéraceae, Cistaceae, Myrthaceae, Plantain, Ronces, Brassicaceae	monofloral
H6	*Ziziphus lotus* (61%)	Apiaceae (12%), Chénopodiaceae (20%)	Fabaceae, Rosaceae, Lamiaceae, Olea europaea, Pissenlit, Multiporés, Ronces, Trèfles, Asteraceae	monofloral
H7	*Ziziphus lotus* (64%)		Rosaceae (14%)	monofloral
H8	*Ziziphus lotus* (55%)	*Olea europaea* (14%)	Pissenlit, Poaceae, Rhamnaceae, Rutaceae, Vipérine, Brassicaceae, Plantain, Multiporés, Ronces, Cistaceae	monofloral
H9	*Ziziphus lotus* (68%)	Rosaceae (27%)	Chénopodiaceae, Cistaceae, Renonculaceae, Apiaceae, Pissenlit, Brassicaceae, Plantain, Ronces, Fabaceae	monofloral
H10	*Ziziphus lotus* (70%)	Fabaceae (12%)	Apiaceae, Astéraceae, Olea europaea, Pissenlit, Renonculaceae, Brassicaceae, Chénopodiaceae, Plantain, Anacardiaceae, Rosaceae	monofloral
H11	*Ziziphus lotus* (41%)	Apiaceae (10%), *Thymus vulgaris* (11%)	Astéraceae, Campanulaceae, Chénopodiaceae, Fabaceae, Oléaceae, Renonculaceae, Trèfles, Brassicaceae, Cistaceae, Plantain	monofloral
H12	*Ziziphus lotus* (91%)		Astéraceae, Aulne, Chénopodiaceae, Cistaceae, Fabaceae, Olea europaea, Pissenlit, Poaceae, Brassicaceae, non identifié, Rosaceae, Apiaceae	monofloral

underwent centrifugation at 4000 rpm for 1 min, and the upper organic layer was collected. This ACN extraction process was repeated two more times. The organic phases were then combined and dried at 40 °C under nitrogen. The dried residue was dissolved in 10 mL of a methanol/water solution (60/40; v/v). Finally, the solution was filtered through a 0.22 μm PVDF filter into a vial.

LC-MS/MS analysis was performed on an Acquity UPLC−XEVO TQ-XS (Waters, Milford, MA, USA) triple quadrupole mass spectrometer equipped with an electrospray source operated in negative ionization mode. The chromatographic separation was achieved on an Acquity UPLC BEH Shield RP18 (1.7 μm, 2.1 mm × 150 mm). The injection volume was 5 μL, and the column temperature was set at 40 °C. The mobile phase consisted of water (A) and acetonitrile (B), both containing 0.1% formic acid. The gradient elution program started with 1% B; it was increased to 26% B over 9.91 min. Next, it was further increased to 65% B over 18.51 min and finally increased to 99% B over 18.76 min (held 2 min). At 20.88 min, the column was re-equilibrated with 1% B during 2.12 min. The mobile phase flow rate was 196 μL min^{-1}. The total run time for each injection was 23 min. The selected reaction monitoring (SRM) acquisition mode was implemented monitoring 2 or 3 transitions per compound for an unequivocal identification and quantification of the target compounds. Quantification of the target compounds was obtained using calibration curves in solvent ranging from 0.02 to 80 mg/kg. The system was operated by MassLynx v4.2, and quantification was done using TargetLynx v4.2.

2.11. Antibacterial Activity. *2.11.1. Bacterial Strains and Growth Conditions.* The bacterial strains used in this study, *Salmonella typhimurium* (KX355308) and *Listeria monocytogenes* (ATCC 13932), were sourced from the Laboratory of Microbiology and Health at the Faculty of Sciences, Moulay Ismail University, Morocco. Bacterial strains stored at −80 °C were thawed and spread on Mueller Hinton agar (Merck Life Science, Merck KGaA, Darmstadt, Germany), followed by incubation at 37 °C for 24 h. Subsequently, bacterial suspensions were prepared in sterile distilled water and adjusted to a concentration equivalent to the 0.5 McFarland standard (10^8 cfu/mL).

2.11.2. Broth Microdilution Method. Minimum inhibitory concentration and minimum bactericidal concentration of samples of honey against *Salmonella typhimirium* and *Listeria monocytogenes* strains were determined by the broth microdilution method as described by Bouymajane et al. with some modifications.[29] Briefly, in a sterile flat-bottom 96-well microplate, 50 μL of Mueller Hinton Broth (MHB) was added to the well microplates. Afterward, 50 μL of honey samples (50 mg/mL of dH2O) were added to the first well microplate and mixed in order to determine cascade dilutions. Then, 50 μL of bacterial suspensions and 50 μL of MHB were added to each well. The well containing bacterial suspensions with MHB and the well containing honey samples and MHB were used as the control and blank, respectively. All microplates were incubated at 37 °C for 24 h. Afterward, 40 μL of TTC (2,3,5-triphenyl tetrazolium chloride) was added to each well microplate and reincubated at 37 °C for 30 min. The MIC was determined as the lowest concentration of honey samples that showed no visible bacterial growth whereas the MBC was determined as the lowest concentration of honey samples that did not produce any bacterial colony. The well microplate that showed no visible bacterial growth was streaked on Petri dishes containing MHA and incubated at 37 °C for 24 h. The MBC/MIC ratio is used to determine the bacteriostatic and bactericidal effects of honey samples. If MBC/MIC ≤ 4, the honey sample effect is bactericidal, and if MBC/MIC > 4, the honey sample effect is bacteriostatic. All of the experiments were carried out in triplicate.

2.12. Statistical Analysis. The data are presented as means ± SD. Statistical analyses, including analysis of variance (ANOVA) and principal component analysis (PCA), were

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Table 3. Physicochemical Parameters[ab]

	Moisture (%)	Conductivity (μs per cm)	pH	Free acidity (meq/kg)	HMF (mg per kg)	IS%	Ash content mg per kg
JUB1	15.7 ± 0.4^{cd}	0.427 ± 0.2^{a}	3.94 ± 0.2^{a}	18.1 ± 1.8^{d}	25.5 ± 2.3^{c}	13.6 ± 1.7^{b}	0.76 ± 0.05^{bc}
JUB2	16.3 ± 0.4^{de}	0.594 ± 0.2^{a}	4.21 ± 0.2^{ab}	16.6 ± 1.8^{cd}	16.3 ± 2.3^{b}	18.3 ± 1.7^{bcd}	0.89 ± 0.05^{cd}
JUB3	15.4 ± 0.4^{bcd}	0.507 ± 0.2^{a}	5.79 ± 0.2^{ef}	0^{a}	2.5 ± 0.3^{a}	20.7 ± 1.7^{cd}	1.00 ± 0.05^{d}
JUB4	15.1 ± 0.4^{bc}	0.467 ± 0.2^{a}	4.17 ± 0.2^{a}	15.1 ± 1.8^{cd}	29.2 ± 2.3^{c}	7.1 ± 1.7^{a}	0.78 ± 0.05^{bc}
JUB5	18.6 ± 0.4^{f}	0.607 ± 0.2^{a}	4.82 ± 0.2^{c}	7.2 ± 1.8^{b}	40.0 ± 2.3^{d}	18.5 ± 1.7^{bcd}	0.31 ± 0.05^{a}
JUB6	15.6 ± 0.4^{bcd}	0.649 ± 0.2^{a}	6.34 ± 0.2^{fg}	0^{a}	1.7 ± 0.3^{a}	21.0 ± 1.7^{d}	0.99 ± 0.05^{d}
JUB7	17.0 ± 0.4^{e}	0.527 ± 0.2^{a}	5.65 ± 0.2^{de}	0^{a}	1.2 ± 0.3^{a}	15.9 ± 1.7^{bc}	0.65 ± 0.05^{a}
JUB8	14.5 ± 0.4^{ab}	0.664 ± 0.2^{a}	4.78 ± 0.2^{bc}	7.8 ± 1.8^{b}	3.3 ± 0.3a	17.6 ± 1.7^{bcd}	1.22 ± 0.05^{e}
JUB9	13.7 ± 0.4^{a}	0.612 ± 0.2^{a}	5.94 ± 0.2^{efg}	0^{a}	1.1 ± 0.3^{a}	17.6 ± 1.7^{bcd}	1.16 ± 0.05^{e}
JUB10	15.2 ± 0.4^{bcd}	0.714 ± 0.2a	6.41 ± 0.2^{g}	0^{a}	1.7 ± 0.3^{a}	21.6 ± 1.7^{de}	0.43 ± 0.05^{a}
JUB11	15.2 ± 0.4^{bcd}	0.407 ± 0.2^{a}	4.10 ± 0.2^{a}	13.8 ± 1.8^{c}	4.2 ± 0.3^{a}	21.1 ± 1.7^{d}	0.33 ± 0.05^{a}
JUB12	15.6 ± 0.4^{bcd}	0.615 ± 0.2^{a}	5.08 ± 0.2^{cd}	8.4 ± 1.8^{b}	1.4 ± 0.3^{a}	26.4 ± 1.7^{e}	0.45 ± 0.05^{a}

[a]Different letters (a−g) in the same columns are significantly different at the 5% level ($p < 0.05$). [b]All values are expressed as means of triplicate determinations ± standard deviation (SD).

performed, and the least significant difference (LSD) was calculated according to the Student−Newman−Keuls method to compare and separate the means with significance accepted at the 5% level. The comparison of treatment means (LSD, 5% level) was conducted by using SPSS 22 statistical software.

3. RESULTS AND DISCUSSION

3.1. Pollen Analysis and Identification. Insights into the complex floral variability present in the Middle Atlas region are provided by a collection of 12 honey samples that reveal a remarkable spectrum of pollen variety (Table 2). This complex mix of pollen not only gives honey produced in this region unique qualities but also bears witness to the region's extensive floral variety. The gathered samples, which cover a wide geographic range, are all made up solely of monofloral honey varieties. Each of these samples reveals a monofloral composition, indicated by a pollen concentration over the 40% cutoff. The Middle Moroccan Atlas region plays a vital role in nectar production, as this study highlights by highlighting the wealth of aromatic and medicinal plant species that grow there.

In terms of origin, our findings are consistent with those of nearby beekeepers. The principal nectar-yielding plant in the area is *Ziziphus lotus*, which has a brief flowering period that lasts from May to June and lasts for around 25 days. This brief but vigorous blooming window occurs during an efflorescence of plant biodiversity, which has a significant impact on how frequently secondary pollen types are seen in pollen spectra. The gathered samples revealed a variety of pollen types, mostly originating from herbaceous species and supplemented by a small presence of woody and shrubby plants. Notably, a considerable presence is seen in plant families including Asteraceae, Fabaceae, Apiaceae, Rosaceae, Oleaceae, and Brassicaceae. These aforementioned plant groups make up the majority of the nectar supplies for honeybees in the area, together with *Z. lotus*.

Our results are consistent with prior studies by Otmani et al.,[30] Terrab et al.,[31] and Zerrouk et al.[32] The honey matrix consistently contains pollen grains from *Ziziphus lotus*, with their percentages varying from no less than 40% to as high as 91%. According to Zerrouk et al. (2020), these numbers are quite similar to those for related Algerian honey kinds, where the range is between 45.3% and 93.4%. Our results likewise show a striking similarity to the results described by Mekious et al., showing a range between 45.7% and 97.1%.

Sdra (*Z. lotus*) honeys show a strong presence of pollen sediment quantitatively, with 80% of samples falling into class III or higher. Although there has not been much scholarly discussion on the pollen content of this specific honey variant, it is universally accepted to be abundant (Zerrouk et al., 2018).[32] According to Pavlin et al. (2023),[33] there is a noticeable variation in pollen concentration depending on the kind of honey, and other variables including beekeeping practices, honey extraction methods, filtering systems, and hive variations all affect the variation.[34] Our findings, together with the identification of the honey's botanical ancestry, imply that management methods, such as bee bread during honeycomb harvests, may increase the pollen concentration.

3.2. Physicochemical Parameters. The moisture content and the presence of osmophilic yeasts in honey play a crucial role in its storage stability. These factors are influenced by the maturation process that occurs inside the hive and during harvesting. A 20% maximum moisture allowance is specified by laws like the EU Directive of 2002 and the Moroccan Directive in 2018.[2] The variance in moisture content across the samples in this study is a result of several factors, including environmental factors, harvest time, hive age, and extraction methods. All examined samples, ranging in moisture content from 13.7% to 18.6%, were below the 20% threshold. Additionally, the gathered samples were from humid and semi-arid locations throughout the summer, indicating the occurrence of high-quality honey in these places. The use of modern hives in modern beekeeping techniques is crucial for maintaining the correct moisture content and encouraging the desired honey development. Beekeepers using these hives help maintain the overall quality of the honey.

The pH value is another important parameter that is influenced by beekeeping practices and storage conditions. This parameter has an impact on the stability and shelf life of honey.[35] As shown in Table 3, all of the honeys tested were acidic. The pH varied between 4.2 and 6.41, giving our samples a unique character. Despite this, all our samples preserved their quality in terms of organoleptic parameters. Our finding is similar to those reported by Chakir et al. (2016)[36] and Bouddine et al.[24]

Free acidity, also known as titratable acidity, is an important parameter that is used to evaluate the quality of honey. It is a measure of the amount of acid present in the honey and is typically expressed in units of meq/kg. The Codex Alimentarius,[37] an international food safety organization, sets a maximum

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Table 4. Oligo Element (Minerals)

	K (mg/L)	Ca (mg/L)	Na (mg/L)	Mg (mg/L)	Fe (mg/L)	P (mg/L)	B (mg/L)	Zn (mg/L)	Mn (mg/L)	Cu (mg/L)
JUB1	457.03	80.81	111.79	23.52	38.53	34.04	6.41	1.04	0.82	0.37
JUB2	604.22	93.94	108.49	26.88	20.65	32.91	4.27	0.79	0.54	0.39
JUB3	394.81	344.40	101.66	101.74	34.42	10.75	2.31	3.74	0.43	0.45
JUB4	348.85	260.29	99.43	20.78	19.62	19.68	3.65	1.84	0.41	0.23
JUB5	123.94	57.45	88.05	12.59	17.90	7.34	2.07	0.41	0.25	0.18
JUB6	336.45	168.42	89.92	25.76	21.59	31.27	3.94	0.51	0.49	0.41
JUB7	350.50	63.59	75.50	18.14	81.82	25.57	2.33	2.24	0.81	0.81
JUB8	783.46	135.59	174.77	43.82	25.79	40.89	4.23	5.41	0.57	0.39
JUB9	671.85	224.93	130.36	85.32	17.88	19.69	2.71	0.82	0.46	0.22
JUB10	232.56	41.86	78.21	7.36	47.67	6.53	1.27	0.48	0.79	0.22
JUB11	1241.88	93.77	116.96	34.20	47.05	17.96	3.26	1.09	0.83	0.33
JUB12	274.11	42.67	62.39	12.28	30.66	16.33	2.31	0.36	0.55	0.20

limit of 50 meq/kg for free acidity in honey. In our study, we found that the free acidity values of all of the honeys we analyzed were between 0 and 16.55 meq/kg. These results are well below the limit set by the Codex Alimentarius, indicating that the honey samples are of good quality and free from fermentation. It is worth noting that free acidity is different from total acidity, which is a measure of all of the acidic compounds present in honey, including free acids and acids that are bound to other compounds. The free acidity values reported in our study refer specifically to the amount of free acids present in the honey samples. The low free acidity values found in our study suggest that the honey samples have undergone minimal fermentation and are of good quality. This is an important aspect to consider when assessing the shelf life and stability of honey during storage. Similar to Zerrouk et al.[32] results about the same plant origin, the values range between 10.1 and 14.8 meq/kg. Similar to pH, free acidity of honey is largely influenced by its origin, particularly the edafology (soil characteristics) and climate of the area, which determine the vegetation it is sourced from.

The ability of a substance to conduct electricity is measured by its electrical conductivity (EC). This parameter is closely related to the concentration of minerals, organic acids, and proteins. In this study, the EC values of the honey samples varied greatly depending on floristic diversity accompanied by the jujube plant of the honey. The EC ranged from 406.9 to 714 μS cm^{-1}, with the lowest value found in a sample collected from the Ain Cheggag area and the highest value found in a sample from the Oued Ifran station. These results demonstrate significant variability, even within the same floral origin. However, it is important to note that these values are below the maximum limit of 800 μS cm^{-1} for honey of floral origin as set by the Codex Alimentarius and EU Council. Honey with an EC of above 800 μS cm^{-1} is typically considered to be honeydew honey, with the exception of chestnut honey, similar to the values published by Chakir et al. (2016).[36] However, Haderbache et al. (2013)[38] and Zhou et al. (2013)[39] reported lower electrical conductivity (478 and 474 μS/cm) for Algerian and Chinese jujube honeys, respectively.

In honey analysis, the ash concentration is of utmost significance, since it represents the mineral constitution of the honey. Depending on the honey's regional and botanical sources, this content may change. A greater ash level can be a sign that the honey has contaminants or has been adulterated.[40] All of the samples we looked at met the permitted range defined by the Codex Alimentarius, which ranges from 0.31 to 1.22 ppm, according to our research. The honey sample from the Outat El Haj station (JUB5) included the lowest value, while the honey sample from the Elksabi station (JUB8) contained the highest value. A notable exception was discovered during our analysis involving the jujube honey sample (JUB3), which showed an abnormally high ash level of 2.4%. Similar findings from earlier investigations have shown that Sidr honey samples had a significantly high ash content.[32,41]

With the exception of sample JUB5, whose HMF concentration was exactly 40 mg/kg, all of the examined samples of honey were determined to have HMF levels below the established limit.[42] These results are quite similar to those found in honey samples from nearby countries like Libya (5.5 mg/kg)[43] and Algeria (1.1 mg/kg).[32] Additionally, they line up with the HMF levels reported for Indian *Ziziphus mauritiana* honey. Hydroxymethylfurfural (HMF) is a chemical formed during the heating and storage of honey. Increased HMF levels may be a sign of tainted honey quality or poor storage conditions. Although each nation has a different HMF tolerance level for honey, lower concentrations are typically desired. The fact that the analyzed honey samples have a low HMF concentration shows that the honey has not been heated or stored at a too high temperature. This is crucial to keeping honey's nutritive and sensory qualities.

The invertase index is recognized in scientific discussions as a key metric for evaluating the honey quality.

In our experimental investigation, the observed range of invertase index values among jujube honey samples was from 7.1% to 26.4%. This metric emerges as an important determinant in the quality assessment of honey, providing indispensable insights into its authenticity and botanical provenance.

3.3. Mineral Contents. A total of 19 trace elements and heavy metals were determined. It should be noted that the concentrations of 19 elements were variable, depending on the floral origin of honey. Among them, the most abundant elements were K, Ca and Na, and Mg with average concentrations ranging from 123.9 to 1241.9 mg/L, 41.86 to 344.4 mg/L, and 62.39 to 174.77 mg/L, respectively. Following our data, similar amounts of K in honey samples were previously reported from the Azilal and Beni Mellal provinces in Morocco with concentrations ranging from to 256 to 1023 mg/L[44] and in Tunisian honey (172.48 to 976.75 mg/L).[45] Overall, the concentrations of K in Moroccan Middle Atlas honey were also higher than those reported from the West Bank in Palestine 42.80 to 585.00 mg/L,[46] and Jableh and Tartous provinces in the western part of Syria, 38.2 to 174 mg/L,[47] but the levels were lower than those reported for Libya (1120.1 to 1980.6 mg/L).[48] A value of Ca was observed among the investigated Middle Atlas honey samples

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Table 5. Heavy Metals and Oligo Elements

	Cr (mg/L)	Ni (mg/L)	As (mg/L)	Pb (mg/L)	Se (mg/L)	Co (mg/L)	Mo (mg/L)	V (mg/L)	Cd (mg/L)
JUB1	5.77	2.80	0.001	0.22	0.04	0.11	0.12	0.01	0.001
JUB2	0.58	0.77	0.001	0.15	0.001	0.03	0.03	0.001	0.001
JUB3	5.48	3.12	0.001	0.30	0.13	0.12	0.11	0.03	0.001
JUB4	0.88	0.86	0.314	0.10	0.001	0.05	0.11	0.001	0.001
JUB5	0.52	0.79	0.097	0.18	0.001	0.03	0.04	0.001	0.001
JUB6	0.92	0.85	0.001	0.21	0.001	0.07	0.08	0.001	0.001
JUB7	18.44	9.5	0.001	0.23	0.10	0.26	0.22	0.03	0.001
JUB8	2.10	1.55	0.001	0.26	0.20	0.06	0.06	0.01	0.001
JUB9	0.68	0.79	0.145	0.07	0.18	0.06	0.07	0.19	0.001
JUB10	8.94	4.50	0.001	0.22	0.28	0.08	0.22	0.001	0.01
JUB11	8.34	4.18	0.001	0.19	0.001	0.06	0.2	0.01	0.001
JUB12	5.07	2.59	0.001	0.14	0.18	0.07	0.11	0.001	0.001

with the concentrations ranging from 41.86 to 344.4 mg/L, which were higher than those of honey samples reported for the Morocco Azilal province (19.71 to 200.1 mg/L),[44] Tunisia (113.85 to 221.07 mg/L),[45] Palestine (44.50 to 150.70 mg/L),[46] Syria (43.3 to 118 mg/L),[47] Greece (15.22 to 65.93 mg/L), and Egypt (44.79 to 112.10 mg/L)[49] (see Tables 4 and 5). Besides, honey samples collected from different locations in the west of Libya show the highest concentrations ranging within 923.92−1117.5 mg/L[48] of all the Mediterranean regions considered. It would be interesting, due to the presence of a large amount of this mineral, to propose it in a strategy for the prevention of osteoporosis. Interestingly, there are some similarities between the Ca contents of our samples and some types of west Algerian honey. For instance, the Ca concentration of *Ziziphus lotus* honey is similar to that of the same plant predominance honey from Algeria (502.00 to 33.10 mg/L[50]). The concentrations of Na in our region honey samples were higher than those reported for honey from Algeria (83.40 to 64.85 mg/L[50]) and Spain (11 to 84 mg/L[51]). The high content of both K and Na in honey makes it less dangerous when consumed by patients with hypertensive patients.

Magnesium was the fourth most abundant element in the present study, with contents ranging from 7.36 to 101.74 mg/L. These concentrations were lower than those of Algerian honey (20.80 to 162.00 mg/L) as reported by Bereksi-Reguig et al. (2016).[50] The concentrations are above the maximum limit set by the Codex Alimentarius: 25 mg kg^{-1} of Mg in honey and also higher than those reported by Karabagias et al. (2019) in multifloral honey from the Mediterranean area, and this can be explained by the presence of this element in the soils of the region.[52]

In the case of Fe, its concentrations showed levels spanning between 17,88 and 81.82 mg kg^{-1}; our finding is slightly higher than those reported by our Algerian neighbors (8.48 to 59.60 mg/kg)[50] similar to those reported by a study of Yaiche Achour (2014).[53] The values were well below the provisional tolerable weekly intake (PTWI) by body weight (5.6 mg/kg b.w.) recommended by the Joint FAO/WHO Expert Committee on Food Additives (JECFA).[54]

The levels of zinc and copper are below the maximum limit of 5 mg kg^{-1} of honey (Codex Alimentarius 2001) except for the honey coming from El Ksabi that exceeds the limit for zinc. The Mn values range between 0.83 ± 0.8 and 0.25 ± 0.08 mg kg^{-1} much lower than those reported by Algerian honey, which was between 1.36 and 13.90 mg/kg.[32]

In the present study, the Zn concentrations ranged from 0.41 to 3.74 mg/kg. The highest Zn level was 3.74 mg/kg of honey from JUB3, while the lowest one was 0.41 mg/kg in honey from JUB5. The levels of Zn in our honey samples are lower than those from Palestine (0.13−25.20 and 1.00−19.90 mg/kg),[41,42] respectively, but higher than those from beni mellal province Morocco (<0.1−0.69 mg/kg),[39] Tunisia (0.42−2.06 mg/kg),[40] Egypt (0.55−1.68 mg/kg),[26] and Syria (0.206−2.76 mg/kg).[43] In the case of the concentrations of Zn, all honey samples are in the maximum tolerable weekly intake range (2.1−7 mg/kg b.w.).[54]

Under the optimized parameters, average copper concentrations in our samples ranged from 0.45 to 0.18 mg/kg. The highest concentrations of Cu were in JUB1, and the lowest value is reported in JUB5 0.18 mg/L. The levels in all honey samples are in line with the PTWI (3.5 mg/kg b.w.) for Cu established by Joint FAO/WHO.[54] The copper levels in the study area range from comparable to somewhat higher than those found in other regions of Morocco (<0.1 mg/kg)[44] and Tunisia (0.12−0.34 mg/kg).[45]

Our samples are rich in essential microelements, making them valuable food products. These elements, such as Mg, Fe, Mn, Zn, Cr, Mo, and Cu, are crucial for activating enzymes and facilitating metabolic processes in the body. Moreover, elements such as K, Ca, and Na contribute to bone and teeth strength, muscle function, nerve transmission, heart rhythm regulation, and cellular fluid balance.[55] Their deficiencies play critical roles in many disorders such as hypertension and osteoporosis.[56] To verify the quality of Moroccan Middle Atlas honey, in addition to Mn, Fe, Zn, and Cu, it is very important to assess and monitor the concentrations of other heavy metals and metalloids, which are potentially toxic. The levels of nickel and selenium in honey samples were investigated. The nickel levels ranged from 0.21 to 0.94 mg per kg, while the selenium levels ranged from 0.28 to 0.001 mg per kg. These levels are comparable to the levels found in other studies.[36,5057] The maximum tolerable daily intake (TDI) of nickel set by the joint FAO/WHO Expert Committee on Food Additives (JEFCA) is 5 mg per kg of body weight.[54] The selenium levels in our samples are much higher than the levels found in Argentine honey samples. The lead content in our honey samples ranged from 0.3 to 0.04 mg per kg. This is higher than the average level of 0.22 mg/kg found in other studies. The lead levels in our samples are all except one above the limit set at 0.100 mg/kg in Regulation 2021/1317 set by the FAO/WHO.[54]

The concentrations of cadmium (Cd) in the honey samples analyzed in our research ranged from 0.001 mg/kg to 0.01 mg per kg. These concentrations are lower than the limits set by the European legislation and the Codex Alimentarius, which is 0.05

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mg per kg.[58] Cadmium is a toxic heavy metal that can cause health problems if consumed in high amounts. The European Food Safety Authority (EFSA) has set a tolerable weekly intake (TWI) of 2.5 μg per kg of body weight for cadmium.[59] This means that an adult weighing 70 kg should not consume more than 175 μg of cadmium per week. The cadmium levels in the honey samples analyzed in our study were significantly lower than the EFSA's TWI. Nevertheless, it is worth noting that cadmium levels in honey can vary depending on its source.

The arsenic levels obtained in this work are similar to those levels found in honey from the same country, Morocco (Chakir et al. 2011).[60] Arsenic is a naturally occurring element that can be found in the environment. It can also be released into the environment from industrial activities, such as mining and smelting. Honey can become contaminated with arsenic through the nectar that bees collect from flowers.

3.4. Sugars. Based on Table 6, the sugar composition of honey is intricate and varies based on the floral sources and the ecogeographical features of the area. A study of honey samples from different regions found that the most common sugars were glucose and fructose, both of which were present in all samples. Fructose was the predominant sugar with an average concentration of 34.63 g/100 g. Glucose was the second most common sugar with an average concentration of 27.03 g/100 g. Other sugars found in honey include maltose, turanose, melibiose, isomaltose, raffinose, and sucrose. These sugars were detected in all investigated samples, but their concentrations varied. Minor sugars such as panose, melezitose, maltotriose, erlose, palatinose, and trehalose were also found in some honey samples. However, they were not detected in all of the honey samples that were analyzed. Honey from flowers that bloom early in the season tends to have a higher concentration of fructose, while honey from flowers that bloom later in the season tends to have a higher concentration of glucose. The sugar composition of honey can also be affected by the processing methods used.[36]

The total sugar content of honey samples from the Oued Amlil and Bouchbel regions varied between 65.2% and 76.8%. The honey sample from Oued Amlil (H9) had the highest level of total sugars, while the honey sample from Bouchbel (H6) had the lowest. The average total sugar content of 76.8% for organic Atlas honeys is consistent with the findings of Mouhoubi-Tafinine et al. (2019) for Algerian honeys.[61] Fructose was the most abundant sugar, ranging from 31.4% to 40.7%, followed by glucose (22.3−30.3%). The monosaccharide sugar content (glucose and fructose) was within the limits authorized by the Council of the European Union (2002) (>60%).[62] The fructose/glucose ratio varied considerably between samples, from 1.16 for sample H1 to 1.51 for sample 10. The fructose/glucose ratio is a measure of honey's tendency to crystallize. Honey is more likely to remain liquid when fructose is high and glucose is low. In addition to its impact on the sensory characteristics and physical state of honey, the fructose/glucose ratio is also an important criterion for its use in certain critical physiological conditions, such as lipid and glucose metabolic disorders. Pasupuleti et al. (2020)[63] found that fructose in honey improved hyperglycemia in experimental diabetic animals and diabetic patients. Dietary fructose has also been shown to improve the glycemic status by enhancing glucokinase activity and catalyzing the conversion of glucose to glucose-6-phosphate, which is then stored as glycogen in the liver. Therefore, fructose-rich honey may be beneficial for human

Table 6. Sugar Values (g/100 g) of Honey Samples[ab]

	Fructose	Glucose	Maltose+	Turanose +	Melibiose and isomaltose	Sucrose	Trehalose	Raffinose	Erlose	Melezitose	Maltotriose	Panose
JUB1	35.34 ± 3.32^{a}	30.30 ± 2.14^{b}	4.34 ± 1.32^{a}	1.10 ± 0.64^{a}	0.33 ± 0.10^{abc}	0.17 ± 0.01^{d}	ND	0.08 ± 0.12^{a}	1.43 ± 0.16^{e}	0.04 ± 0.01^{a}	0.08 ± 0.02^{c}	ND
JUB2	36.35 ± 3.32^{a}	26.03 ± 2.14^{ab}	2.86 ± 1.32^{a}	0.89 ± 0.64^{a}	0.32 ± 0.10^{abc}	0.08 ± 0.01^{b}	0.10 ± 0.01^{b}	0.10 ± 0.12^{a}	0.54 ± 0.16^{bc}	0.05 ± 0.01^{a}	ND	ND
JUB3	34.07 ± 3.32^{a}	25.66 ± 2.14^{ab}	5.36 ± 1.32^{a}	1.33 ± 0.64^{a}	1.01 ± 0.10^{e}	0.20 ± 0.01^{e}	ND	0.10 ± 0.12^{a}	1.29 ± 0.16^{e}	0.11 ± 0.01^{b}	0.39 ± 0.02^{g}	0.24 ± 0.01^{g}
JUB4	33.37 ± 3.32^{a}	24.72 ± 2.14^{ab}	4.56 ± 1.32^{a}	1.17 ± 0.64^{a}	0.63 ± 0.38^{bcde}	1.49 ± 0.01^{f}	ND	0.27 ± 0.12^{a}	2.77 ± 0.16^{f}	0.15 ± 0.01^{c}	0.15 ± 0.02^{d}	0.23 ± 0.01^{g}
JUB5	33.38 ± 3.32^{a}	28.17 ± 2.14^{b}	4.45 ± 1.32a	0.91 ± 0.64^{a}	0.77 ± 0.10^{cde}	0.04 ± 0.01^{a}	ND	0.08 ± 0.12^{a}	0.14 ± 0.16^{a}	0.04 ± 0.01^{a}	0.05 ± 0.01^{bc}	0.13 ± 0.01^{e}
JUB6	31.37 ± 3.32^{a}	24.59 ± 2.14^{ab}	4.47 ± 1.32^{a}	1.19 ± 0.64^{a}	0.92 ± 0.10^{de}	2.07 ± 0.01^{g}	ND	0.07 ± 0.12^{a}	2.65 ± 0.16^{f}	0.14 ± 0.01^{c}	0.31 ± 0.02^{f}	0.15 ± 0.01^{f}
JUB7	32.44 ± 3.32^{a}	25.56 ± 2.14^{ab}	4.51 ± 1.32^{a}	1.13 ± 0.64^{a}	0.55 ± 0.10^{bcd}	0.11 ± 0.01^{c}	0.02^{a}	0.07 ± 0.12^{a}	0.52 ± 0.02^{bc}	0.06 ± 0.01^{a}	ND	0.06 ± 0.01^{c}
JUB8	34.41 ± 3.32^{a}	28.55 ± 2.14^{b}	4.83 ± 1.32^{a}	0.63 ± 0.64^{a}	0.06 ± 0.10^{a}	0.11 ± 0.01^{c}	0.02^{a}	0.08 ± 0.12^{a}	1.01 ± 0.02^{d}	0.68 ± 0.01^{e}	0.03 ± 0.02^{ab}	0.09 ± 0.01^{d}
JUB9	40.66 ± 3.32^{a}	29.58 ± 2.14^{b}	3.74 ± 1.32^{a}	1.11 ± 0.64^{a}	0.27 ± 0.10^{ab}	0.22 ± 0.01^{e}	ND	0.09 ± 0.12^{a}	0.7 ± 0.16^{c}	0.22 ± 0.01^{d}	ND	ND
JUB10	33.80 ± 3.32^{a}	22.31 ± 2.14^{a}	5.36 ± 1.32^{a}	1.33 ± 0.64^{a}	1.49 ± 0.38^{f}	0.22 ± 0.01^{e}	ND	0.35 ± 0.12^{ab}	1.5 ± 0.16^{e}	0.21 ± 0.01^{d}	0.18 ± 0.01^{e}	0.28 ± 0.01^{h}
JUB11	35.05 ± 3.32^{a}	29.63 ± 2.14^{b}	3.96 ± 1.32^{a}	0.95 ± 0.64^{a}	0.23 ± 0.10^{ab}	0.05 ± 0.01^{a}	0.06 ± 0.01^{ab}	0.54 ± 0.12^{b}	0.26 ± 0.16^{ab}	0.12 ± 0.01^{b}	0.06 ± 0.02^{bc}	0.02 ± 0.01^{b}
JUB12	35.39 ± 3.32^{a}	29.36 ± 2.14^{b}	4.00 ± 1.32^{a}	0.96 ± 0.64^{a}	0.61 ± 0.10^{bcde}	0.08 ± 0.01^{b}	ND	0.40 ± 0.12^{ab}	0.49 ± 0.16^{bc}	0.04 ± 0.01^{a}	0.04 ± 0.02^{ab}	0.09 ± 0.01^{d}

^{a}Different letters (a−g) in the same columns are significantly different at the 5% level ($p < 0.05$). ^{b}All values are expressed as means of triplicate determinations ± standard deviation (SD).

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Table 7. Retention Time and Related MS Data of Compounds Detected in the UPLC-QqQ MS Analysis

Compound name	Retention time	Precursor ion	Product ion	Collision energy (CE)	Ionisation mode
quinic acid	2.33	191	85	40 V	negative
pyrocatechol	5.47	109.2	108.896	40 V	negative
protocatechuic acid	6.25	153	109	40 V	negative
4-hydroxyphenylacetic acid	7.34	151	151	40 V	negative
catechin	8.78	289	245	40 V	negative
vanillic acid	8.92	167	125	40 V	negative
caffeic acid	9.59	179	135	40 V	negative
epicatechin	9.81	289	245	40 V	negative
hydroferulic acid	10.10	195	121	40 V	negative
p-coumaric acid	11.49	163	119	40 V	negative
ferulic acid	11.96	193	134	40 V	negative
sinapinic acid	12.00	223	208	40 V	negative
salicylic acid	12.45	137	93	40 V	negative
taxifolin	12.46	303	285	40 V	negative
rutin	12.78	609	300	40 V	negative
hesperidin	13.05	609	301	40 V	negative
isoquercetin	13.12	463	300	40 V	negative
aromadendrin	14.15	287	259	40 V	negative
quercetrin	14.22	447	300	40 V	negative
luteolin	16.48	285	133	40 V	negative
quercetin	16.53	301	164	40 V	negative
naringenin	16.74	271	151	40 V	negative
hesperetin	16.88	301	164	40 V	negative
apigenin	17.75	269	117	40 V	negative
isorhamnetin	17.92	315	300	40 V	negative
kaempferol	18.01	285	93	40 V	negative
galangin	19.81	269	171	40 V	negative

health, including preventing metabolic disorders, such as diabetes.

When we compare honeys, in the context of the fructose contents in Middle Atlas and honey from our Algerian neighbor having the same origin, they are nearly the same.[32] However, due to seasonal climatic variation as well as differing geographic origins, the composition of monosaccharides from the same floral source may change.

The major disaccharides detected in the honey samples were maltose, turanose, sucrose, and trehalose. All four disaccharides were present in all samples, and maltose was the most abundant with a mean value of 1.05 g/100 g. Turanose was the second most abundant disaccharide, followed by sucrose and trehalose.

A high content of apparent sucrose indicates that the honey was harvested early as the sucrose has not been fully converted into glucose and fructose by the action of invertase. Authentic honey samples typically have a sucrose content of less than 5%. All of the samples in this study had sucrose levels below 5%, which is the maximum limit set by the Codex Alimentarius Commission. It has been reported that sucrose levels can decrease during honey storage due to the presence of invertase. Santos et al. (2014)[64] suggest that the variable levels of sucrose in honey may be due to a transglycosylation reaction, in which the α-D-glucopyranosyl unit from sucrose is transferred to an acceptor molecule. Additionally, the melibiose and isomaltose contents are detected in all samples with a mean value of 1.05%. However, this sugar has been found at a low level (mean value of 0.09%) in Moroccan Eucalyptus honey analyzed by Terrab et al.[65]

The monofloral honey H4 has the highest erlose content, followed by honeys H6 and H10. Trisaccharides are non-reducing saccharides except panose. Erlose, raffinose, melezitose, maltotriose, and panose are present in all honey samples, but erlose is present in only 12 samples. The erlose content ranges from 0.146% to 2.776%, which is higher than the values reported by Tedesco et al. (2022).[66] Raffinose is present in all samples, but its content does not exceed 0.55%. Melezitose is present in 9 samples and ranges from 0.04% to 0.7%. This sugar is usually indicative of honeydew honey, but the low percentage in these samples suggests that they are nectar honeys.

3.5. Phenolic Compound Quantification from Honey Samples. Phenolic compounds are naturally occurring substances that are synthesized in response to conditions such as infection, injury, and exposure to UV light. These compounds are the most widely distributed secondary metabolites in plants, contributing to the color, taste, bitterness, and hardness of foods. More than 8000 distinct structures of phenolic compounds are known.[67] 46 phenolic substances were targeted for detection and quantification including benzoic acids exemplified by (1) gentisic acid; trimethoxycinnamic acid; quinic acid; 4-OH-phenylacetic acid, gallic acid and its ester ethyl gallate, and *p*-hydroxybenzoic, vanillic, syringic acid; and protocatechuic acid and salicylic acid as well as cinnamic acids; (2) exemplified by chicoric acid, caffeic, sinapinic acid, hydrocaffeic, ferulic, hydroferulic, and o-, *p*-, and *m*-coumaric acids and chlorogenic acid); and (3) flavonoids: these were flavonols (quercetin, kaempferol, rutin, astragalin, avicularin, galangin, isoquercetin, and isorhamnetin), flavone (apigenin, luteolin, cynaroside, and apigetrin); flavanone (taxifolin, hesperidin, naringin, and aromadendrin), a flavanol (procyanidin B2), and finally an anthocyanidine (cyanidin).

Interestingly, the profiles of jujube honeys from the Midlle Atlas region of Morocco differ from those we have previously studied from the Southeastern region of Morocco. In our

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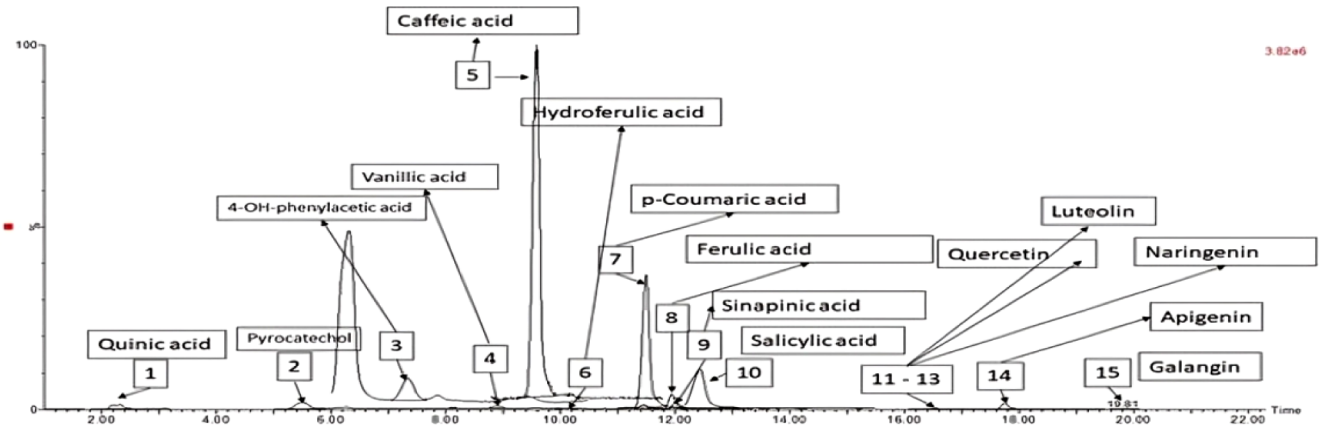

Figure 2. LC-MS/MS chromatogram of honey sample. 1: Quinic acid; 2: pyrocatechol; 3: 4-OH-phenylacetic acid; 4: vanillic acid; 5: caffeic acid; 6: hydroferulic acid; 7: *p*-coumaric acid; 8: ferulic acid; 9: sinapic acid; 10: salicylic acid; 11: quercetin; 12: luteolin; 13: naringenin; 14: apigenin; and 15: galangin.

previous study, we identified only a few phenolic compounds, including caffeic acid, methylsyringate, and alkaloids.

These differences are probably due to the plant flowering stage, climate, season, beekeeping practices, cross contamination, and methodology used. As a matter of fact, LC-MS/MS has provided more selective identification using specific fragmentation transitions of known phenolics present in honeys. Moreover, in our previous study, the honeys were injected directly without taking into account the notable matrix effect or interferences that may render the method less sensitive than the present LC-MS/MS.[10] The transitions used of each component are recapitulated in Table 7 below.

There are some observable patterns among the phenolic compounds that have been found. Catechin was detected in four samples (JUB3, JUB7, JUB9, and JUB10) with high concentrations of 8.891, 1.049, 20.971, and 1627.621 mg/kg, respectively Similarly, epicatechin was found in the same samples with concentrations of 14 .038, 7.958, 124.652, and 36.861 mg/kg. The chemical family of catechins is well-known for its various health-related benefits, including anticancer, antiobesity, antidiabetic, anticardiovascular, anti-infectious, hepatoprotective, and neuroprotective effects. Catechins exhibit biochemical properties, antioxidant activities, and mechanisms of action that contribute to preventing diseases caused by oxidative stress, such as cancer, cardiovascular diseases, and neurodegenerative diseases. A large body of literature has shown that catechins as inhibitors of oxidative stress enhance immune responses, induce epigenetic modifications, and inhibit receptor tyrosine kinase activity. Alzheimer's disease (AD) is a major global neurodegenerative condition linked to oxidative stress, which can lead to neuroinflammation due to an imbalance between reactive oxygen species (ROS) and antioxidants. Ide et al. summarized catechins' effects on AD, highlighting antioxidative, anti-inflammatory, protein kinase C-related, and neurotransmission-related properties. Some compounds in tea, such as epigallocatechin gallate in green tea, methylated epigallocatechin gallate in oolong tea, theaflavins in black tea, and polyphenol metabolites in dark tea, have shown promise in weight loss. Rothenberg et al.'s "Short Chain Fatty Acid (SCFA) hypothesis" explains how different teas can promote weight loss. Achieving effective concentrations of epigallocatechin gallate and other catechins often requires chemical modification and specific delivery systems, as seen with peracetylated epigallocatechin gallate suppressing colon tumorigenesis. Additionally, epigallocatechin gallate-fatty acid derivatives exhibit improved antiviral activities against viruses. A liposomal mixture of curcumin, epigallocatechin gallate, and resveratrol emerges as a potential onco-immunotherapeutic agent against glioblastoma.[68] Encapsulation further enhances epigallocatechin gallate's stability, bioavailability, and function, with encapsulated epigallocatechin gallate showing improved stability, sustained release, and direct absorption. Naringenin is a member of the flavanone group (ranging from 0.115 to 0.0025 mg/kg). Naringenin primarily exists in its aglycone form, although glycosylated and neohesperidoside forms are also present. Commonly sourced from grapefruits, lemons, oranges, and tomatoes, naringenin has garnered significant attention due to its pharmacological properties. Numerous studies have underscored the diverse pharmacological attributes of naringenin and its derivatives. These include estrogen-like activity and potent anti-cancer effects achieved through carcinogen inactivation and cell cycle arrest, as succinctly.[69] Another compound of interest, kaempferol (3.975−0.024 mg/kg), is a widely encountered aglycone flavonoid, often found in glycoside form. It has a tetrahydroxyflavone structure with hydroxy groups at positions 3, 5, 7, and 4′, imparting a yellow color.[70] Kaempferol is present in various plant parts, including seeds, leaves, fruits, flowers, and vegetables, explaining its presence in honey, propolis, and pollen. This compound, along with its glycosylated derivatives, has demonstrated cardioprotective, neuroprotective, anti-inflammatory, antidiabetic, antioxidant, antimicrobial, and antitumor activities.[71] Quercetin (0.744−0.036 mg/kg), a major flavonol representative, is found in numerous fruits and vegetables. Onions, asparagus, and red leaf lettuce are particularly rich sources of quercetin.[72] In foods, quercetin is present in glycoside form rather than as an aglycone.[73] Upon ingestion, quercetin glycosides are hydrolyzed to release the aglycone, which is then metabolized into glucuronidated, methylated, and sulfated derivatives. Quercetrin (3.596 mg/kg) was present in 4 honey samples (JUB2, JUB4, and JUB7) (Figure 2).

3.6. Antioxidant Properties. Our data underscore extensive diversity in phenolic compounds. Such variability was evident in the recorded values for JUB5 and JUB4, spanning from 48.3 to 91.8 milligrams of gallic acid equivalent (GAE) per 100 grams, respectively. It is noteworthy that although informative, these results were observed to be slightly lower than those documented by Zerrouk et al. in their 2018

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Table 8. Antioxidant Activities of *Ziziphus Lotus* Honey Samples[abc]

Samples	Phenols (mg GAE/100 g)	Flanovoids (mg QE/100 g)	DPPH IC50 (mg/mL)	ABTS IC50 (mg/mL)
JUB1	62.3 ± 0.03^{e}	14.72 ± 0.03^{h}	17.51 ± 0.81^{c}	0.98 ± 0.25^{a}
JUB2	87.6 ± 0.03^{i}	15.90 ± 0.03^{i}	13.54 ± 0.32^{a}	1.55 ± 0.11^{a}
JUB3	66.5 ± 0.03^{f}	5.17 ± 0.03^{a}	22.58 ± 0.33^{e}	2.84 ± 0.21^{b}
JUB4	91.8 ± 0.03^{l}	17.85 ± 0.03^{l}	37.06 ± 0.32^{h}	3.64 ± 0.07^{bcd}
JUB5	48.3 ± 0.03^{a}	16.73 ± 0.03^{k}	45.34 ± 0.57^{h}	4.23 ± 0.43^{cd}
JUB6	90.7 ± 0.03^{k}	14.38 ± 0.03^{f}	14.61 ± 0.18^{ab}	1.73 ± 0.11^{a}
JUB7	51.1 ± 0.03^{b}	11.85 ± 0.03^{d}	35.14 ± 0.75^{f}	0.87 ± 0.31^{a}
JUB8	76.5 ± 0.03^{h}	12.98 ± 0.03^{e}	35.68 ± 0.43^{f}	4.60 ± 1.06^{d}
JUB9	71.3 ± 0.03^{g}	6.95 ± 0.03^{b}	13.89 ± 0.33^{ab}	3.29 ± 0.07^{bc}
JUB10	88.6 ± 0.03^{j}	14.52 ± 0.03^{g}	15.05 ± 0.20^{b}	1.04 ± 0.02^{a}
JUB11	51.7 ± 0.03^{c}	16.02 ± 0.03^{j}	23.10 ± 0.45^{e}	3.77 ± 0.08^{bcd}
JUB12	58.3 ± 0.03^{d}	11.69 ± 0.03^{c}	18.99 ± 0.26^{d}	2.91 ± 0.11^{b}
Trolox (μg/mL)			10.81 ± 0.1	23.15 ± 4.0

[a]Notes: GAE: galic acid equivelent, QE: quercitin equivalent, DPPH IC 50: inhibitory concentration that reduces 50% of free radicals, ABTS IC 50: inhibitory concentration that reduces 50% of free radicals. [b]Different letters (a–l) in the same columns are significantly different at the 5% level ($p < 0.05$). [c]All data were expressed as the mean ± standard deviation (SD).

Table 9. Determination of the Minimum Inhibitory Concentration and Minimum Bactericidal Concentration of Jujube Honey against Pathogenic Bacteria (mg/mL)

	Listeria monocytogenes				*Salmonella typhimirium*			
Sample of honey	MIC	MBC	MBC/MIC	effect	MIC	MBC	MBC/MIC	effect
JUB1	0.30	2.77	9	bacteriostatic	0.03	0.10	3	bactericidal
JUB2	0.03	0.10	3	bactericidal	0.01	0.03	3	bactericidal
JUB3	0.03	0.10	3	bactericidal	0.01	0.03	3	bactericidal
JUB4	0.03	-	-	bactericidal	0.01	0.03	3	bactericidal
JUB5	0.10	-	-	bacteriostatic	0.003	0.01	3	bactericidal
JUB7	0.10	0.92	9	bacteriostatic	0.01	0.03	3	bactericidal
JUB8	0.30	2.77	9	bacteriostatic	0.01	0.03	3	bactericidal
JUB9	0.10	0.92	9	bacteriostatic	0.03	0.10	3	bactericidal
JUB10	0.10	0.92	9	bacteriostatic	0.03	0.10	3	bactericidal
JUB11	0.10	0.92	9	bacteriostatic	0.01	0.03	3	bactericidal
JUB12	0.03	-	-	bactericidal	0.01	0.03	3	bactericidal

investigation, focusing on phenolic content in monofloral honey sourced from the same plant origin.[32]

The analysis of flavonoids unveiled disparities among various jujube samples. Unlike the minimal flavonoid content, which was ascertained in JUB3 and quantified at about 5.17 mg of quercetin equivalent (QE) per 100 g of sample, JUB4 in contrast demonstrated a remarkable flavonoid concentration of about 17.85 mg QE/100 g. These diverse findings spotlight the spectrum of flavonoid presence in the scrutinized honey samples. As regards the antioxidant activity evaluation, two distinct and complementary assays, namely, the DPPH and ABTS tests, were employed to evaluate different antioxidant mechanisms. As a result, the significant variations in the chemical composition of phenolic and non-phenolic antioxidant molecules, along with their concentrations, coupled with the intricate chemistry of the utilized tests, contribute to disparities in the outcomes of antioxidant assays.[74] As illustrated in Table 8, the evaluated samples (JUB1–JUB12) manifest distinct antioxidant activities, regardless of the methodology employed.

The scavenging of the 2,2-diphenyl-1-picrylhydrazyl (DPPH) radical is one of the most used assays that help provide a first approach.[75] As indicated in Table 8, the quantity of honey needed to inhibit 50% of the DPPH• radical displayed a significant variation among the samples studied. *Ziziphus lotus* honey (JUB2) sourced from the TANDIT area, exhibiting the highest polyphenol content (87.6 ± 0.03 mg GAE/100 g), exhibited the most robust antiradical activity (IC50 = 13.54 mg/mL). However, *Ziziphus lotus* honey (JUB5) harvested from OUTAT ELHAJ, with the lowest polyphenol content, displayed the weakest DPPH scavenging capacity with an IC50 of 45.35 mg/mL. Despite both samples being categorized as monofloral *Ziziphus lotus* honeys, they showed different aspects related to their antiradical activity; such behavior underscores the impact of the secondary nectar flora origin and the pedo-climatic characteristics of each harvesting site. The observed variations might also be attributed to the distinct composition of phenolic and non-phenolic antioxidant components in each tested honey.[76] These findings align with our previous data, where 23 honey samples collected from diverse regions of the Moroccan Middle Atlas exhibited substantial variations in phenol content, total flavonoids, ascorbic acid, total antioxidant capacity, and antiradical activity.[24]

The obtained values surpass those observed for Trolox (10.81 ± 0.1 μg/mL), indicating lower antiradical activities.

In terms of ABTS scavenging capacities, the honey sample sourced from the Sebt Ain Lahnech region (JUB7) and characterized by the highest flavonoid content (11.85 mg QE/100 g) displayed the most effective ABTS radical scavenging activity with an IC50 value of 0.87 mg/mL. Conversely, the sample harvested from the El Ksabi area (JUB8) exhibited the lowest inhibition percentage of the ABTS free radical (IC50 = 4.60 mg/mL). These reported activities fall below those

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Table 10. Eigenvalues and Percentages of Explained Variability

	Initial eigenvalues			Sums extracted from the load square		
Component	Total	% variance	Cumulative %	Total	% variance	Cumulative %
1	12.27	37.19	37.19	12.27	37.19	37.19
2	4.80	14.55	51.75	4.80	14.55	51.75

recorded for Trolox (IC50 = 23.15 μg/mL), and they lie within the range of values reported for 23 mono- and polyfloral honeys collected from diverse eco-geographical regions of the Moroccan Middle Atlas. Specifically, the IC_{50} values were ranged from 4.49 to 31.00 mg/mL for *Origanum* and jujube honeys, respectively.[24]

The observed variation in the antioxidant activity across the analyzed honey samples aligns with data reported for 17 honey samples from Algeria and 28 monofloral honey samples harvested from China, as reported by Zerrouk[32] and Zhao in 2016.[77] The examined honey samples showcased pronounced and extensive discrepancies in their antioxidant activities. These variations can likely be attributed to their distinct phytochemical compositions, notably, the presence of diverse antioxidant constituents.[78] These findings emphasize the potential significance of integrating organic honey into the daily human diet as a bioactive functional food, yielding favorable implications.

3.7. Antibacterial Activity. The activity of honey as an antibacterial agent has been used for ages. Recent studies have demonstrated that honey possesses antibacterial properties against Gram-positive, Gram-negative, aerobic, and anaerobic bacteria. However, honey may also include spores that were introduced during the beekeeping process.

The antibacterial activity of honey was evaluated against *Listeria monocytogenes* (Gram-positive), and *Salmonella typhimirium* (Gram-negative) through a broth microdilution method. As seen in Table 9, the MIC values for honey against *Listeria monocytogenes* and *Salmonella typhimurium* ranged from 0.03 to 0.30 mg/mL and from 0.003 to 0.03 mg/mL, respectively. The honey samples (JUB2 and JUB3) displayed a bactericidal activity against *Listeria monocytogenes* with a MBC/MIC value of 3. While the samples (JUB1, JUB5, JUB8, JUB9, JUB10, and JUB11) exhibited a bacteriostatic activity against *Listeria monocytogenes* with an MBC/MIC value of 9. Furthermore, all honey samples presented a bactericidal activity against *Salmonella typhimurium* with an MBC/MIC value of 3. It is well established that the antibacterial activity of honey depends on its chemical composition such as polyphenols content, hydrogen peroxide (H_2O_2), high sugar content, gluconic acid, methylglyoxal, and bee peptide defensin-1.[10]

These results confirm findings already documented in the literature, exemplified by the antimicrobial study of honeys from the Greece Island of Lemnos and Manuka honey from New Zealand against clinically important bacteria, which demonstrated significant potential for developing natural antimicrobial systems for use in food and medicine.[79]

3.8. Principal Component Analysis (PCA). Principal component analysis (PCA) was performed using the software version statistical software. PCA serves as a versatile instrument to help minimize the dimensionality of large datasets, offering a holistic perspective on the topic under study. Indeed, one of the paramount strengths of PCA is its unique capability to provide us with a visual representation of data and allow us to distill complex systems down to comprehensible two- or three-dimensional representations, thus making it possible for the human eye to discern patterns and structures that would otherwise be obscured in the murky depths of higher-dimensional spaces and consequently retain the most essential information while removing redundancy and noise, resulting in a more efficient and effective analytical process.[80]

The rotated component matrix for 12 honey samples is summarized in Table 10. This table includes the PCA loadings for the factor plane, where the variables were projected, and showed that the first and second axes of the PCs explained 37% and 14.5% of the variation in total. Principal component 1 (PC1) best explains the variability of groups F (naringenin, luteolin, and rutin), G (quercetin, hesperetin, taxifolin, etc.), and H (pyrocatechol and sinapinic acid); these values rise with increasing PC1, as shown by Figure 3, which showed the

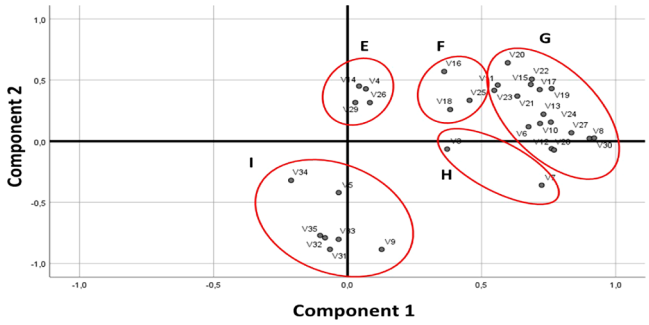

Figure 3. Component plot in rotated space.

projection of the fore variables on the first and second factor planes. Additionally, there is a considerable correlation between the final variables; this is expected given that the most frequently stated species and botanical groups are those that are well known to informants and have a high degree of popularity. In contrast, the principal component 2 (PC2) best explains the variability of E (salicylic acid, galangin, astragalin, and gallic acid) and this value increases with the increasing PC2; indeed, these variables are uncorrelated with I (4-OH-phenylacetic acid, caffeic acid, epicatechin, catechin, etc.).

The extrapolation of the data from Figure 4 to Figure 3 provides valuable insights into the relationship between jujube honey samples and their respective phytochemical compositions. Notably, it reveals distinct patterns of positive correlation between different groups of jujube honey samples (labeled as groups B, C, and D) and specific sets of phytochemical compounds (grouped as E, F, G, H, and I). In the case of group B, represented by JUB1 and JUB4, these samples exhibit a pronounced positive correlation with phytochemicals from groups E and F. This observation suggests that JUB4 is enriched with an array of phytochemicals, including but not limited to salicylic acid, galangin, astragalin, naringenin, luteolin, rutin, and gallic acid. Conversely, the jujube honey samples in group C, consisting of JUB2 and JUB7, showcase a different correlation pattern. They are positively correlated with phytochemicals from groups G and H, indicating that these samples are notably rich in compounds like quercetin, hesperetin, taxifolin, pyrocatechol, and sinapinic acid. Group D, which includes

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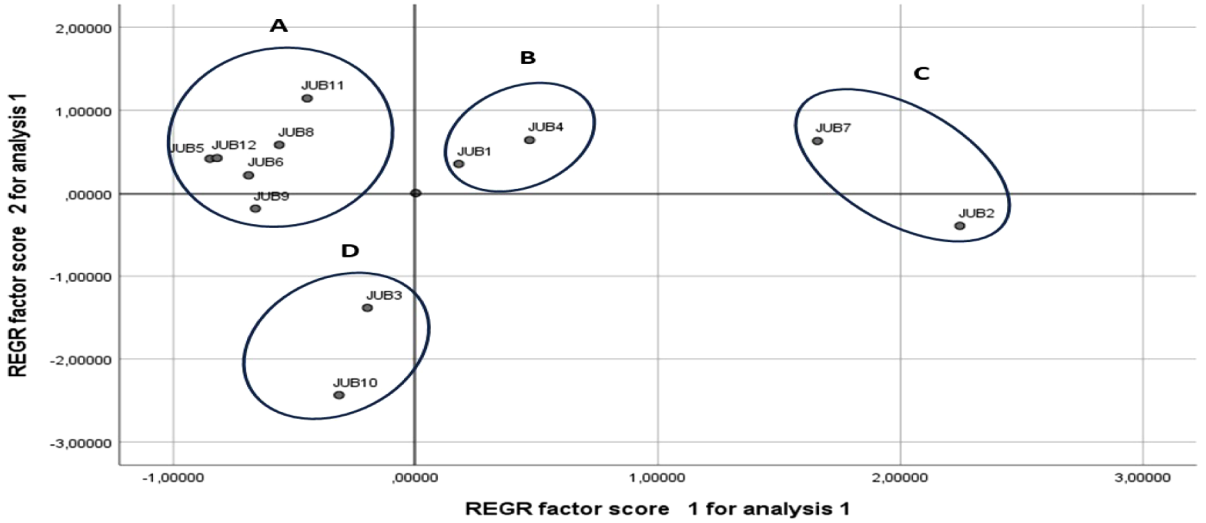

Figure 4. Scatterplot of bivar = Fact1 with Fact2 by different Jujube honey samples.

Table 11. Matrix of Component Coefficients

Compounds	Components		Compounds	Components	
	1	2		1	2
pyrocatechol	0.05	−0.03	hesperetin	0.05	0.03
salicylic acid	−0.02	0.07	taxifolin	0.05	0.05
4-OH-phenylacetic acid	0.02	−0.07	isorhamnetin	0.04	0.04
protocatechuic acid	0.07	−0.02	hesperidin	0.08	−0.02
sinapinic acid	0.10	−0.10	rutin	0.03	0.03
p-coumaric acid	0.10	−0.05	astragalin	−0.01	0.05
caffeic acid	0.06	−0.16	isoquercetin	0.09	−0.03
vanillic acid	0.07	−0.01	quercetrin	0.09	−0.06
quinic acid	0.04	0.05	gallic acid	−0.01	0.05
ferulic acid	0.09	−0.05	gentisic	0.10	−0.05
hydroferulic acid	0.07	−0.01	resveratrol	0.04	−0.15
galangin	−0.02	0.08	3,4,5 trimethoxycinnamic acid	0.03	−0.13
apigenin	0.05	0.04	phloretin	0.041	−0.14
naringenin	0.01	0.08	epicatechin	−0.01	−0.04
kaempferol	0.06	0.03	catechin	0.03	−0.13
luteolin	0.03	0.02	aromadendrin	0.06	0.03
			quercetin	0.03	0.08

JUB3 and JUB10, stands out with a distinct profile. These samples are found to be particularly abundant in phytochemicals belonging to group I. Consequently, they boast a heightened concentration of compounds such as 4-OH-phenylacetic acid, caffeic acid, epicatechin, and catechin.

This analytical approach not only elucidates the unique phytochemical profiles of different jujube honey groups but also provides a foundation for further investigation of the factors contributing to these variations. Understanding the specific phytochemical compositions of different jujube honey samples can be invaluable for various applications, including quality assessment and nutritional labeling and potentially even highlighting the potential health benefits associated with these distinct compositions.

The scatterplot (Figure 4) and PCA biplot (Figure 3) reveal distinct groupings among the honey samples based on their phenolic compound profiles. This analysis aimed to classify the honey samples according to the types of phenolic compounds identified in this study.

Table 10 further strengthens these findings. By examining the coefficients of the linear discriminant function, we can assess the effectiveness of specific phenolic compounds in differentiating honey types. Gentisic acid, *p*-sinapinic acid, and coumaric acid emerged as the most significant contributors to the classification, highlighting their discriminatory power (see Table 11 for specific values). This translates to a high degree of precision in segregating the honey samples into four distinct classes (A, B, C, and D) based on their phenolic profiles.

Interestingly, the scatterplot (Figure 4) suggests a negative correlation between groups A (containing samples JUB5, JUB6, JUB8, JUB9, JUB11, and JUB12) and C (containing samples JUB2 and JUB7) along the first principal component axis. Similarly, groups B (JUB1 and JUB4) and D (JUB3 and JUB10) appear negatively correlated on the second principal component axis. This suggests that the specific phenolic compound profiles of these groups differ substantially. This analysis demonstrates the effectiveness of PCA and the identified key phenolic compounds in classifying honey samples based on their unique chemical compositions.

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4. CONCLUSIONS

In summary, our analysis of the Moroccan Middle Atlas jujube honey highlights its quality and potential health benefits. The honey samples met the Codex Alimentarius standards and have favorable physicochemical attributes such as moisture content, pH, electrical conductivity, and ash content. Additionally, the low concentration of HMF in these samples confirmed their high quality and proper storage conditions. The invertase index indicated the glucose and fructose content and confirms the purity and botanical source. Our melissopalynological analysis supported its unifloral origin, mainly dominated by *Ziziphus lotus* pollen. The mineral content analysis showed high levels of potassium and calcium, although the levels vary depending on the floral source. The elevated Pb content observed in our findings exceeds the EU Maximum Limits (EU-ML). This suggests a potential correlation with divergences in cultural practices such as farming and cattle rearing as well as variances in soil composition. Additionally, these honey samples contained essential micronutrients. The bioactive potential of honey and its potential to promote health and well-being are augmented by the presence of diverse phenolic compounds, such as catechin, epicatechin, galangin, quercetin, kaempferol, and luteolin. The PCA analysis confirms that environmental conditions clearly influence the composition of the phenolic compounds. Moreover, the monofloral pollen content affects various parameters and activities, such as conductivity, antioxidant potential, and antimicrobial potential, in different jujube honeys. In conclusion, our study highlights the high quality, diverse floral composition, and bioactive properties of the Moroccan Middle Atlas jujube honey. This underscores its importance as a valuable food product with potential health benefits. This research represents the first comprehensive exploration of phytochemical compounds in this honey variety conducted by LC-MS/MS analysis and provides valuable insights into its composition and its potential applications in various industries and health contexts.

■ ASSOCIATED CONTENT

Data Availability Statement

The data supporting this study are available upon request.

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Notes

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