

## Research Article

# Characterization of Various Honey Samples from Different Regions of Morocco Using Physicochemical Parameters, Minerals Content, Antioxidant Properties, and Honey-Specific Protein Pattern

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Honey is a bee product relatively expensive; therefore, it has been a target of adulteration by many sweeteners. In this work, we evaluated the good quality, authenticity, and content in bioactive molecules of twenty-two Moroccan honey from different botanical origins and geographical areas. For that, the following analyses were determined: the content in total protein and especially the major royal jelly protein (apalbumin 1), the analysis of total acidity, free acidity, lactic acidity, pH, ash, Pfund, electrical conductivity, and moisture. In addition, the content of sodium, potassium, calcium, and magnesium, the dosage of polyphenols, flavones, and flavonols, and the antioxidant activities were assessed. All analyzed samples had good antioxidant activities and present a source of antioxidant compounds, the predominant mineral in all honey samples was potassium, and the physicochemical parameters are in line with the standards' recommended limits. The content of honey samples in total protein and apalbumin 1 ranged between 212  $\mu\text{g/g}$  and 4121.2  $\mu\text{g/g}$  and between 27.4  $\mu\text{g/g}$  and 790.82  $\mu\text{g/g}$ , respectively. Overall, the detection of apalbumin 1 in all honey samples and the results of physicochemical parameters, minerals, bioactive compounds, and antioxidant activities confirm the authenticity and no adulteration of Moroccan honey.

## 1. Introduction

Honey is a sweet product produced by bees from the nectar of plants. It is nutritious and has traditionally been consumed by humans since the oldest times [1]. Honey is a complex mixture that comprises carbohydrates (60–85%) mainly glucose and fructose, water (12–23%), and other minor constituents such as proteins, enzymes, free amino acids, lipids, vitamins, phenolic acids, flavonoids, and mineral salts [2]. The biochemical composition of honey

mostly depends on its floral source, the honey bee species, weather conditions, and geographical origin [3].

There is a large volume of published studies describing the role of honey as functional food, it has been reported that honey has several pharmacological effects such as antioxidant, anticancer, antimicrobial, and inflammatory effects [4, 5]. It is widely used in wound healing and can counteract inflammation [6].

Honey is relatively expensive; thus, it has been a target of adulteration by many adulterants such as corn syrup, sugar,

and cane [7]. To verify the authenticity of honey, many tools are recommended such as physicochemical parameters, sensory analysis, microscopical examination, and the analysis of chemical composition [1, 8]. In addition to that, apalbumin 1 is among the main protein that exists in royal jelly and also in honey; it has been suggested to use it as a marker of authenticity and quality of honey since this protein is specific to bees and cannot be replaced by other ingredients [9]. Therefore, the objective of this work was to determine the authenticity and quality of honey samples of different botanical and geographical origins in Morocco by the analysis of physicochemical parameters (pH, free acidity, lactone acidity, total acidity, moisture, electrical conductivity, ash, and Pfund), minerals content (sodium, potassium, calcium, magnesium), antioxidant content and activities (polyphenols, flavones/flavonols, DPPH, ABTS, RP), and the analysis of total protein and more specifically apalbumin 1.

## 2. Material and Methods

**2.1. Honey Samples.** Twenty-two honey samples were obtained from beekeepers, who installed their hives in seven different regions of Morocco. Three samples were multifloral, and nineteen were monofloral (the pollen grains of the predominant plant are higher than 45%). The honey samples S1, S4, S8, S11, S12, S18, S20, and S21 were obtained from the Fez-Meknes region. The honey samples S2, S6, S13, S14, S17, and S22 were obtained from the Rabat-Salé-Kénitra region. The honey sample S3 was obtained from the Souss-Massa region. The honey samples S5 and S7 were presented from the Oriental region. The sample S9 was presented from the Drâa-Tafilalet region. The honey samples S10, S15, and S16 were obtained from the Beni Mellal-Khenefra region, and honey sample S19 was obtained from the Tangier-Tetouan-Al Hoceïma. The honey samples were produced by three different bee species: *Apis mellifera intermissa*, *Apis mellifera sahariensis*, and *Apis mellifera major* (Table 1 and Figure 1).

**2.2. Melissopalynological Analysis.** The botanical origin of honey samples was determined using the method described by Louveaux et al. [10]. A minimum of 1000 pollen grains were counted for each honey sample under a microscope. If the percentage of any type of pollen grains found in honey exceeds 45% of the total pollen grains content, it is classified as the predominant and the honey is classified as monofloral.

**2.3. Physicochemical Analysis.** Total acidity, free acidity, lactic acid, pH, ash, electrical conductivity, and moisture, were analyzed using the methods recommended by the International Honey Commission [11]. Pfund was determined as described previously by Laaroussi et al. [12].

**2.4. Minerals Content.** The analysis of minerals elements (Na, K, Ca, and Mg) of honey samples was carried out using ICP-AES after the calcination method as described by Laaroussi et al. [12]. Briefly, the honey ashes were mixed with

5 ml of nitric acid 0.1 M and stirred on a heating plate until the total evaporation of nitric acid. Then, 10 ml of nitric acid was added and the mixture was made up to 25 ml with ultrapure water. All samples were analyzed in triplicate.

**2.5. Polyphenols Content.** The polyphenols content was assessed using the Folin-Ciocalteu method [13]. Briefly, 100  $\mu$ L of aqueous extract of honey was mixed with 500  $\mu$ L of Folin-Ciocalteu reagent solution (10 g of sodium tungstate and 2.5 g of sodium molybdate (2.5 g) were dissolved in 70 ml of distilled water; then, 5 ml of phosphoric acid (85%) and 10 ml of concentrated hydrochloric acid were added. After 10 hours, 15 g of lithium sulfate and 5 ml of distilled water were added and brought to 100 ml with distilled water) for 6 min, and then 400  $\mu$ L of sodium carbonate (75 g/l) was added to the mixture. The absorbance was measured at 760 nm after 15 min of incubation. Gallic acid calibration was used as a standard for calibration. The results were expressed as milligrams of Gallic acid equivalents per gram (mg GAE/g).

**2.6. Flavones and Flavonols Content.** The content of flavones and flavonols was quantified by a colorimetric assay described previously by Bakour et al. [14]. Briefly, 500  $\mu$ L of honey sample or standard was added to 500  $\mu$ L of 20%  $\text{AlCl}_3$ . After 1 h at room temperature, the absorbance was measured at 420 nm. A quercetin calibration curve was prepared, and total content was expressed as mg of quercetin equivalents per 100 g of honey (mg QE/100 g).

**2.7. Radical Scavenging Activity (DPPH Assay).** The radical scavenging activity of the honey solution against DPPH free radical was measured using the method described by Bakour et al. [15]. Briefly, 100  $\mu$ L of the aqueous honey extract was mixed with 900  $\mu$ L of a 100  $\mu$ M solution of DPPH radical prepared in ethanol (96%). The absorbance of the solution was measured at 540 nm after 30 min of incubation in the dark. Several concentrations of samples were made, and the  $\text{IC}_{50}$  (concentration of sample able to scavenge 50% of DPPH free radical) was determined graphically using the curve plotted by the percentage of DPPH inhibition as a function of the sample concentration:

$$\% \text{ inhibition} = \frac{\text{Abs control} - \text{Abs sample}}{\text{Abs control}} \times 100. \quad (1)$$

**2.8. Azino-Bis (3-Ethylbenzothiazoline-6-Sulphonic Acid (ABTS)).** Azino-bis (3-ethylbenzothiazoline-6-sulphonic acid (ABTS) free radical scavenging activity was analyzed using the method described by Bakour et al. [15]. 75  $\mu$ L of aqueous extract of honey or standard control (BHT) was mixed with 825  $\mu$ L of ABTS solution, and the absorbance of the mixture was measured after 6 min at 734 nm. The tests were carried out in triplicate, and the  $\text{IC}_{50}$  (concentration of sample able to scavenge 50% of ABTS free radical) was determined graphically using the curve plotted by the

TABLE 1: Information on the harvested year, the botanical, bee species, and geographical origin of the honey samples.

Sample	Local name	Botanical origin (Predominant pollen grains)	Bee species	Location	Region	Year of harvest
S1	Azir	<i>Salvia rosmarinus</i> Spenn.	<i>Apis mellifera intermissa</i>	Tandit	Fez-Meknes	2015
S2	Bouchnikha	<i>Ammi visnaga</i> L.	<i>Apis mellifera intermissa</i>	Hedkourt	Rabat-Salé-Kénitra	2015
S3	Daghmous	<i>Euphorbia resinifera</i> Berg.	<i>Apis mellifera sahariensis</i>	Tiznit	Souss-Massa	2015
S4	Sadra	<i>Ziziphus lotus</i> L.	<i>Apis mellifera intermissa</i>	Marmoucha	Fez-Meknes	2015
S5	Latchin	<i>Citrus sinensis</i> L.	<i>Apis mellifera intermissa</i>	Nador	Oriental	2015
S6	Latchin	<i>Citrus sinensis</i> L.	<i>Apis mellifera intermissa</i>	Khnichat	Rabat-Salé-Kénitra	2015
S7	Multifloral	Multifloral	<i>Apis mellifera intermissa</i>	Nador	Oriental	2015
S8	Multifloral	Multifloral	<i>Apis mellifera intermissa</i>	Elmers	Fez-Meknes	2015
S9	Multifloral	Multifloral	<i>Apis mellifera sahariensis</i>	Skoura	Drâa-Tafilelet	2015
S10	Kharob	<i>Ceratonia siliqua</i> L.	<i>Apis mellifera intermissa</i>	Khenifra	BéniMellal-Khénifra	2015
S11	Kharob	<i>Ceratonia siliqua</i> L.	<i>Apis mellifera intermissa</i>	Taounate	Fez-Meknes	2015
S12	Kharob	<i>Ceratonia siliqua</i> L.	<i>Apis mellifera intermissa</i>	Taounate	Fez-Meknes	2015
S13	Khzama	<i>Lavandula angustifolia</i> L.	<i>Apis mellifera intermissa</i>	Oulmès	Rabat-Salé-Kénitra	2017
S14	Hamd	<i>Citrus limon</i> (L.) Burm. F.	<i>Apis mellifera intermissa</i>	Khnichat	Rabat-Salé-Kénitra	2017
S15	Sadra	<i>Ziziphus lotus</i> L.	<i>Apis mellifera intermissa</i>	Khenifra	BéniMellal-Khénifra	2017
S16	Chouk	<i>Silybum marianum</i> (L.) Gaertn.	<i>Apis mellifera intermissa</i>	Khenifra	BéniMellal-Khénifra	2017
S17	Bouchnikha	<i>Ammi visnaga</i> L.	<i>Apis mellifera intermissa</i>	Sidi Kacem	Rabat-Salé-Kénitra	2017
S18	Kebbar	<i>Capparis spinosa</i> L.	<i>Apis mellifera intermissa</i>	My yacoub	Fez-Meknes	2017
S19	Bakhenou	<i>Arbutus unedo</i> L.	<i>Apis mellifera major</i>	Tetouan	Tanger-Tétouan- AlHoceïma	2017
S20	Zandaz	<i>Bupleurum spinosum</i> Gouan	<i>Apis mellifera intermissa</i>	Boulemane	Fez-Meknes	2017
S21	Z'itra	<i>Thymus vulgaris</i> L.	<i>Apis mellifera intermissa</i>	Timhdit	Fez-Meknes	2017
S22	Zaatar	<i>Origanum vulgare</i> L.	<i>Apis mellifera intermissa</i>	Errachidia	Rabat-Salé-Kénitra	2017

percentage of ABTS inhibition as a function of the sample concentration (equation (1)).

**2.9. Ferric Reducing Power.** The reducing power of the aqueous honey extract was determined using the method described by Bakour et al. [15]. 50  $\mu$ l of aqueous honey extract (50% W/V) was mixed with 200  $\mu$ l of 0.2 M sodium phosphate buffer (pH 6.6) and 200  $\mu$ l of 1% potassium ferricyanide. The mixture was incubated at 50°C for 20 min. Then, 200  $\mu$ l of 10% trichloroacetic acid (TCA) was added, and the mixture was centrifuged at 3000 rpm for 10 min. 500  $\mu$ l of the above solution from each reaction was diluted with 500  $\mu$ l of distilled water, and 100  $\mu$ l of 0.1% ferric

chloride ( $\text{FeCl}_3$ ) was added. The absorbance was measured at 700 nm, and ascorbic acid was used as a reference standard. The results were represented in  $\text{EC}_{50}$  values, corresponding to the concentration providing 50% of the antioxidant activity or 0.5 of absorbance in the reducing power assay measured at 700 nm.

**2.10. Determination of Protein Concentration.** The total protein content of the honey samples was determined by microplate assay according to Bradford [16]. To 100  $\mu$ l of the sample or its dilution in physiological solution was added 100  $\mu$ l of QuickStart Bradford reagent (BioRad, Laboratories, Inc., USA). The absorbance was measured at 595 nm. Bovine

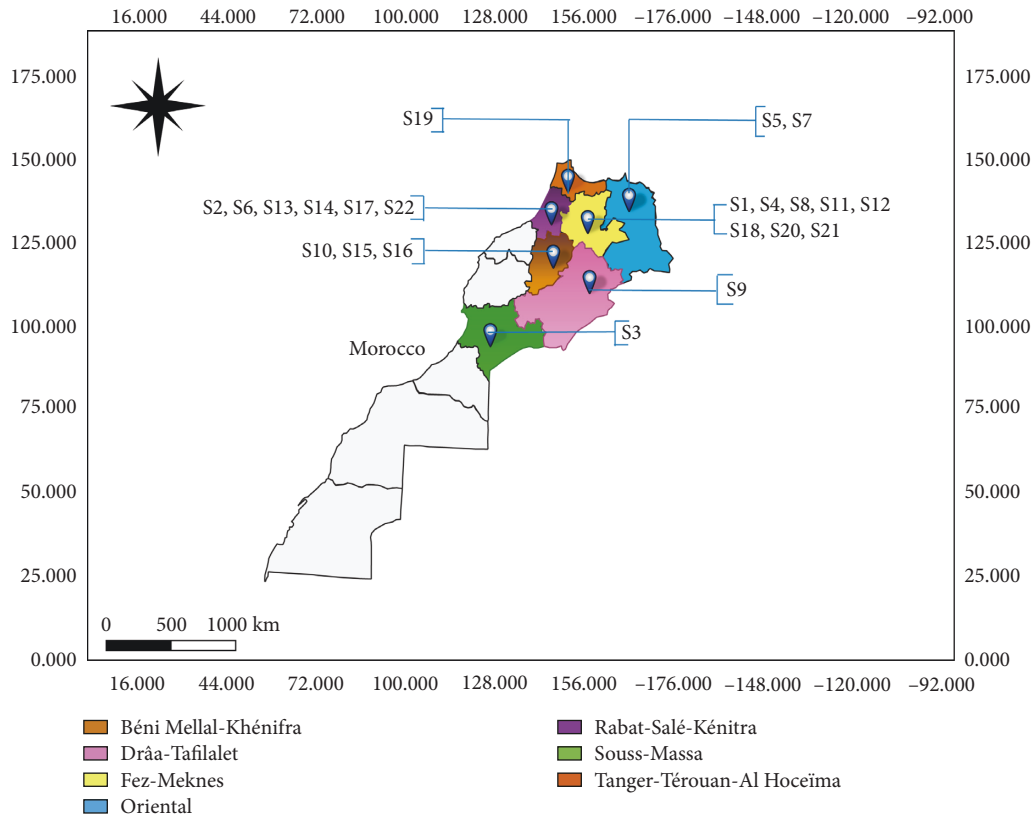


FIGURE 1: Map of Morocco showing honey sampling regions.

serum albumin (BSA, Sigma, USA) was used as standard. Each sample was analyzed at three dilutions and each dilution in three parallel analyses.

**2.11. Determination of Apalbumin 1 in Honey by ELISA.** Honey samples were analyzed using enzyme-linked immunosorbent assay (ELISA) for apalbumin 1 quantification as described previously in detail [17]. The 96 well/flat-bottom microtiter plates (Brand, Germany) were coated with antigen-diluted honey samples at dilution of 0.05% and/or 0.001% in Milli-Q water and/or standard solution of apalbumin 1 and incubated overnight at 4°C. After washing with TBS buffer (100 mmol/L Tris and 150 mmol/L NaCl, pH 7.5), the plates were incubated with polyclonal rabbit anti-apa1 antibody in milk buffer (2% nonfat milk in TBS) and then with peroxidase-conjugated anti-rabbit IgG in milk buffer for 1 h. Detection was done by adding 3% ABTS (2,2'-azino-bis-(3-benzthiazoline-6-sulfonic acid, Southern Biotech, USA), in 50 mmol/L citrate buffer pH 4.3; supplemented by hydrogen peroxide. The absorbance at 405 nm was read in a Microplate Spectrophotometer Power Wave TM XS (BioTek Instruments, INC, Winooski, Vermont, USA).

**2.12. Statistical Analysis.** All data are presented as mean  $\pm$  SD (standard deviation). Graphpad prism (version 5.0; GraphPad Software, Inc., San Diego, USA) was used to compare honey samples using a one-way analysis of variance

(ANOVA) followed by Tukey's test, and  $p < 0.05$  was considered significant. Correlations between the parameters studied were achieved by the Pearson correlation coefficient ( $r$ ). The principal component analysis (PCA) was accomplished using Past: paleontological statistics software package for education and data analysis, version 3.20.

### 3. Results and Discussion

**3.1. Physicochemical Parameters.** Twenty-two honey samples of different botanical and geographical origins of Morocco were analyzed for physicochemical parameters. The results presented in Table 2 showed that the pH values ranged between  $3.59 \pm 0.10$  in *Euphorbia resinifera* honey from Tiznit and  $4.30 \pm 0.20$  in *Ziziphus lotus* honey from Marmoucha; these values are within the range recommended by Council Directive 2001/110/EC [18]. Free acidity ranged between  $11.45 \pm 0.12$  mEq/kg in *Salvia rosmarinus* honey and  $30.81 \pm 1.20$  mEq/kg in *Thymus vulgaris* from Timhdit, lactone acidity ranged between  $5.09 \pm 0.96$  mEq/kg in *Ziziphus lotus* honey and  $17.50 \pm 0.22$  mEq/kg in *Bupleurum spinosum* honey, and total acidity ranged between  $18.30 \pm 0.30$  mEq/kg in *Citrus sinensis* honey and  $42.28 \pm 0.30$  mEq/kg in *Ceratonia siliqua* honey. These values are in line with those recommended by Codex Alimentarius Commission [19]. For moisture values, all honey samples are below the maximum limit (20%) except the *Arbutus unedo* honey sample from Tetouan which was slightly higher ( $20.90 \pm 0.15\%$ ). The

TABLE 2: Physicochemical parameters and minerals content in honey samples.

Sample	pH	Free acidity (mEq/kg)	Lactone acidity (mEq/kg)	Total acidity (mEq/kg)	Moisture (%)	Electrical			Ash%	Pfund (mm)	Na (mm/kg)	K (mm/kg)	Ca (mm/kg)	Mg (mm/kg)
						conductivity (mS/cm)	conductivity (mS/cm)	conductivity (mS/cm)						
S1	4.05 ± 0.03 <sup>b</sup>	11.45 ± 0.12 <sup>hi</sup>	9.51 ± 0.50 <sup>cd</sup>	20.96 ± 0.15 <sup>f</sup>	17.20 ± 0.22 <sup>bc</sup>	443.00 ± 20.00 <sup>h</sup>	0.28 ± 0.01 <sup>g</sup>	83.06 ± 0.01 <sup>j</sup>	41.64 ± 0.54 <sup>o</sup>	270.57 ± 0.85 <sup>s</sup>	28.49 ± 0.21 <sup>o</sup>	19.76 ± 1.25 <sup>n</sup>		
S2	3.71 ± 0.02 <sup>b</sup>	21.00 ± 1.00 <sup>d</sup>	11.10 ± 0.10 <sup>cd</sup>	32.10 ± 0.35 <sup>e</sup>	18.60 ± 0.41 <sup>b</sup>	472.33 ± 3.22 <sup>g</sup>	0.31 ± 0.01 <sup>ef</sup>	64.21 ± 0.10 <sup>m</sup>	128.16 ± 0.4 <sup>f</sup>	286.78 ± 0.42 <sup>r</sup>	25.98 ± 1.20 <sup>o</sup>	17.44 ± 0.56 <sup>o</sup>		
S3	3.59 ± 0.10 <sup>b</sup>	20.51 ± 0.50 <sup>d</sup>	8.10 ± 0.36 <sup>f</sup>	28.61 ± 0.52 <sup>g</sup>	17.50 ± 0.50 <sup>bc</sup>	444.00 ± 20.00 <sup>h</sup>	0.33 ± 0.02 <sup>ef</sup>	75.01 ± 0.12 <sup>j</sup>	117.89 ± 1.21 <sup>h</sup>	667.46 ± 0.78 <sup>h</sup>	77.27 ± 1.35 <sup>f</sup>	42.36 ± 1.25 <sup>e</sup>		
S4	4.30 ± 0.20 <sup>a</sup>	14.41 ± 0.90 <sup>g</sup>	7.51 ± 0.12 <sup>f</sup>	21.92 ± 0.45 <sup>f</sup>	17.70 ± 0.23 <sup>bc</sup>	620.83 ± 1.53 <sup>c</sup>	0.42 ± 0.05 <sup>d</sup>	63.43 ± 0.11 <sup>m</sup>	154.35 ± 0.54 <sup>d</sup>	1014.28 ± 2.36 <sup>c</sup>	67.57 ± 0.78 <sup>k</sup>	13.55 ± 0.56 <sup>p</sup>		
S5	4.07 ± 0.04 <sup>b</sup>	12.00 ± 0.15 <sup>hi</sup>	6.30 ± 0.22 <sup>f</sup>	18.30 ± 0.30 <sup>k</sup>	19.80 ± 0.12 <sup>a</sup>	242.00 ± 30.00 <sup>m</sup>	0.17 ± 0.01 <sup>gh</sup>	25.58 ± 0.23 <sup>q</sup>	114.74 ± 0.41 <sup>i</sup>	424.24 ± 1.25 <sup>o</sup>	79.35 ± 1.20 <sup>b</sup>	25.76 ± 0.87 <sup>j</sup>		
S6	4.20 ± 0.10 <sup>a</sup>	13.51 ± 0.60 <sup>h</sup>	6.00 ± 0.12 <sup>f</sup>	19.51 ± 0.40 <sup>k</sup>	19.50 ± 0.52 <sup>a</sup>	223.00 ± 40.00 <sup>n</sup>	0.20 ± 0.01 <sup>g</sup>	33.13 ± 0.13 <sup>p</sup>	71.48 ± 0.52 <sup>i</sup>	622.95 ± 2.32 <sup>j</sup>	86.36 ± 0.36 <sup>g</sup>	28.15 ± 0.47 <sup>h</sup>		
S7	3.94 ± 0.20 <sup>a</sup>	14.45 ± 0.80 <sup>g</sup>	7.41 ± 0.52 <sup>f</sup>	21.86 ± 0.40 <sup>j</sup>	17.10 ± 0.36 <sup>d</sup>	577.33 ± 4.22 <sup>d</sup>	0.35 ± 0.01 <sup>e</sup>	103.77 ± 0.62 <sup>e</sup>	114.63 ± 0.21 <sup>i</sup>	947.36 ± 3.25 <sup>f</sup>	93.21 ± 0.98 <sup>f</sup>	35.13 ± 0.36 <sup>f</sup>		
S8	3.94 ± 0.40 <sup>a</sup>	16.05 ± 0.80 <sup>g</sup>	10.11 ± 0.63 <sup>cd</sup>	26.16 ± 0.30 <sup>h</sup>	18.80 ± 0.20 <sup>b</sup>	510.67 ± 1.23 <sup>e</sup>	0.55 ± 0.02 <sup>c</sup>	78.74 ± 0.14 <sup>k</sup>	78.16 ± 1.21 <sup>k</sup>	847.21 ± 1.25 <sup>d</sup>	167.57 ± 1.25 <sup>d</sup>	218.72 ± 1.54 <sup>a</sup>		
S9	3.85 ± 0.00 <sup>b</sup>	21.51 ± 0.16 <sup>d</sup>	8.20 ± 0.10 <sup>f</sup>	29.71 ± 0.62 <sup>f</sup>	18.70 ± 0.12 <sup>b</sup>	762.33 ± 2.33 <sup>b</sup>	0.92 ± 0.02 <sup>a</sup>	76.19 ± 0.20 <sup>t</sup>	153.04 ± 1.54 <sup>d</sup>	987.89 ± 1.25 <sup>d</sup>	22.12 ± 0.21 <sup>r</sup>	34.35 ± 0.54 <sup>g</sup>		
S10	3.75 ± 0.10 <sup>b</sup>	11.45 ± 0.90 <sup>hi</sup>	9.81 ± 0.41 <sup>cd</sup>	21.26 ± 0.16 <sup>f</sup>	17.10 ± 0.32 <sup>bc</sup>	484.33 ± 4.55 <sup>f</sup>	0.31 ± 0.01 <sup>ef</sup>	102.92 ± 0.35 <sup>e</sup>	131.15 ± 0.65 <sup>e</sup>	1016.13 ± 4.23 <sup>c</sup>	26.00 ± 1.23 <sup>o</sup>	28.07 ± 0.88 <sup>h</sup>		
S11	3.79 ± 0.08 <sup>b</sup>	26.10 ± 0.60 <sup>bc</sup>	12.15 ± 0.10 <sup>c</sup>	38.25 ± 0.55 <sup>bc</sup>	18.80 ± 0.22 <sup>b</sup>	948.33 ± 1.63 <sup>a</sup>	0.74 ± 0.01 <sup>b</sup>	108.91 ± 0.35 <sup>e</sup>	174.24 ± 2.87 <sup>c</sup>	806.56 ± 1.23 <sup>g</sup>	252.43 ± 0.50 <sup>bb</sup>	60.97 ± 0.58 <sup>d</sup>		
S12	4.00 ± 0.01 <sup>a</sup>	28.21 ± 0.23 <sup>b</sup>	14.00 ± 0.36 <sup>b</sup>	42.28 ± 0.30 <sup>a</sup>	18.50 ± 0.52 <sup>b</sup>	700.00 ± 40.00 <sup>b</sup>	0.52 ± 0.04 <sup>c</sup>	128.74 ± 1.20 <sup>b</sup>	233.92 ± 1.32 <sup>b</sup>	1017.98 ± 3.24 <sup>c</sup>	80.3 ± 0.69 <sup>i</sup>	62.52 ± 0.41 <sup>c</sup>		
S13	4.18 ± 0.12 <sup>a</sup>	18.15 ± 0.90 <sup>f</sup>	8.03 ± 0.09 <sup>f</sup>	26.18 ± 0.22 <sup>hi</sup>	17.30 ± 0.23 <sup>bc</sup>	345.44 ± 3.12 <sup>k</sup>	0.31 ± 0.05 <sup>e</sup>	40.83 ± 0.52 <sup>q</sup>	104.77 ± 0.65 <sup>j</sup>	588.92 ± 1.25 <sup>i</sup>	59.44 ± 1.25 <sup>i</sup>	40.17 ± 0.78 <sup>e</sup>		
S14	3.80 ± 0.20 <sup>b</sup>	20.56 ± 0.12 <sup>d</sup>	6.70 ± 0.23 <sup>f</sup>	27.26 ± 0.30 <sup>h</sup>	18.74 ± 0.62 <sup>b</sup>	231.83 ± 2.21 <sup>n</sup>	0.13 ± 0.07 <sup>gh</sup>	19.67 ± 0.64 <sup>f</sup>	41.66 ± 0.36 <sup>o</sup>	331.89 ± 2.32 <sup>p</sup>	37.50 ± 1.80 <sup>n</sup>	19.44 ± 0.44 <sup>i</sup>		
S15	4.08 ± 0.02 <sup>a</sup>	21.47 ± 0.40 <sup>d</sup>	5.09 ± 0.96 <sup>g</sup>	26.56 ± 0.52 <sup>h</sup>	19.28 ± 0.20 <sup>a</sup>	521.60 ± 2.36 <sup>e</sup>	0.42 ± 0.09 <sup>e</sup>	56.20 ± 0.12 <sup>n</sup>	121.30 ± 0.21 <sup>g</sup>	616.38 ± 1.23 <sup>k</sup>	77.90 ± 0.36 <sup>i</sup>	24.02 ± 0.21 <sup>j</sup>		
S16	3.82 ± 0.01 <sup>b</sup>	18.05 ± 0.22 <sup>f</sup>	7.15 ± 0.52 <sup>f</sup>	25.20 ± 0.41 <sup>hi</sup>	19.15 ± 0.15 <sup>a</sup>	329.70 ± 5.21 <sup>f</sup>	0.20 ± 0.01 <sup>g</sup>	87.34 ± 0.69 <sup>i</sup>	43.30 ± 0.22 <sup>o</sup>	494.05 ± 0.23 <sup>n</sup>	42.05 ± 0.25 <sup>mm</sup>	20.24 ± 0.14 <sup>k</sup>		
S17	3.99 ± 0.01 <sup>a</sup>	28.43 ± 0.12 <sup>b</sup>	6.85 ± 0.23 <sup>f</sup>	35.28 ± 0.30 <sup>d</sup>	18.15 ± 0.52 <sup>b</sup>	489.99 ± 5.21 <sup>f</sup>	0.21 ± 0.06 <sup>g</sup>	97.24 ± 0.54 <sup>f</sup>	112.75 ± 0.25 <sup>i</sup>	303.67 ± 1.24 <sup>q</sup>	43.97 ± 0.87 <sup>mm</sup>	17.56 ± 0.25 <sup>mm</sup>		
S18	3.94 ± 0.02 <sup>a</sup>	20.04 ± 0.25 <sup>de</sup>	11.37 ± 0.63 <sup>c</sup>	31.41 ± 0.40 <sup>e</sup>	17.75 ± 0.63 <sup>bc</sup>	428.89 ± 6.12 <sup>i</sup>	0.29 ± 0.04 <sup>g</sup>	119.65 ± 0.41 <sup>b</sup>	78.42 ± 1.22 <sup>k</sup>	585.99 ± 1.23 <sup>i</sup>	172.42 ± 1.25 <sup>e</sup>	36.80 ± 0.87 <sup>f</sup>		
S19	4.14 ± 0.05 <sup>a</sup>	27.40 ± 0.63 <sup>b</sup>	7.70 ± 0.52 <sup>f</sup>	35.10 ± 0.23 <sup>d</sup>	20.90 ± 0.15 <sup>a</sup>	913.51 ± 6.21 <sup>b</sup>	0.49 ± 0.04 <sup>d</sup>	103.75 ± 0.25 <sup>e</sup>	264.56 ± 0.45 <sup>a</sup>	1299.56 ± 3.25 <sup>a</sup>	283.13 ± 2.36 <sup>a</sup>	77.23 ± 0.59 <sup>b</sup>		
S20	3.76 ± 0.06 <sup>b</sup>	22.05 ± 0.41 <sup>d</sup>	17.50 ± 0.22 <sup>a</sup>	39.55 ± 0.60 <sup>b</sup>	19.67 ± 0.34 <sup>a</sup>	466.67 ± 3.21 <sup>g</sup>	0.20 ± 0.05 <sup>g</sup>	89.13 ± 0.74 <sup>i</sup>	64.56 ± 0.98 <sup>m</sup>	521.26 ± 0.98 <sup>m</sup>	121.47 ± 1.54 <sup>e</sup>	26.42 ± 0.54 <sup>i</sup>		
S21	3.82 ± 0.02 <sup>b</sup>	30.81 ± 1.20 <sup>a</sup>	8.45 ± 0.40 <sup>e</sup>	39.26 ± 0.52 <sup>b</sup>	17.32 ± 0.42 <sup>bc</sup>	372.00 ± 2.35 <sup>j</sup>	0.48 ± 0.05 <sup>d</sup>	94.18 ± 0.98 <sup>g</sup>	103.29 ± 1.02 <sup>j</sup>	1105.58 ± 2.36 <sup>b</sup>	76.26 ± 0.65 <sup>i</sup>	30.10 ± 0.86 <sup>b</sup>		
S22	3.94 ± 0.04 <sup>a</sup>	18.70 ± 0.17 <sup>f</sup>	7.25 ± 0.54 <sup>f</sup>	25.95 ± 0.45 <sup>hi</sup>	19.00 ± 0.56 <sup>a</sup>	582.35 ± 4.21 <sup>d</sup>	0.32 ± 0.01 <sup>e</sup>	106.63 ± 1.21 <sup>d</sup>	51.60 ± 0.45 <sup>n</sup>	632.20 ± 3.20 <sup>i</sup>	71.85 ± 0.89 <sup>j</sup>	21.45 ± 0.32 <sup>i</sup>		
Mean	3.94	19.83	8.92	28.76	18.33	505.01	0.37	79.92	113.62	699.50	90.57	36.37		
SD	0.18	5.76	2.90	7.07	0.92	195.95	0.19	30.28	58.05	296.24	70.28	24.51		

All values are expressed as means of triplicate determinations ± SD. Values in the same column followed by the same letter are not significantly different by Tukey's multiple range test ( $p < 0.05$ ).

TABLE 3: Bioactive compounds and antioxidant activities (DPPH, ABTS, and RP) of honey samples.

Sample	Phenols (mg GAE/100 g)	Flavones and flavonols (mg QE/100 g)	DPPH (IC <sub>50</sub> = mg/ml)	ABTS (IC <sub>50</sub> = mg/ml)	RP (EC <sub>50</sub> = mg/ml)
S1	89.00 ± 1.40 <sup>de</sup>	10.10 ± 0.20 <sup>e</sup>	31.20 ± 1.60 <sup>e</sup>	11.17 ± 0.30 <sup>g</sup>	3.00 ± 0.02 <sup>hij</sup>
S2	63.20 ± 9.60 <sup>g</sup>	7.80 ± 1.30 <sup>ef</sup>	47.10 ± 2.20 <sup>d</sup>	8.76 ± 0.40 <sup>i</sup>	3.20 ± 0.01 <sup>hij</sup>
S3	89.10 ± 2.40 <sup>de</sup>	11.50 ± 0.30 <sup>e</sup>	36.60 ± 1.70 <sup>c</sup>	12.49 ± 0.10 <sup>f</sup>	5.40 ± 0.40 <sup>f</sup>
S4	171.70 ± 0.20 <sup>c</sup>	23.30 ± 0.00 <sup>c</sup>	9.30 ± 1.00 <sup>h</sup>	2.30 ± 0.10 <sup>l</sup>	1.60 ± 0.01 <sup>k</sup>
S5	13.70 ± 0.30 <sup>o</sup>	0.70 ± 0.10 <sup>h</sup>	93.40 ± 6.10 <sup>a</sup>	45.16 ± 0.70 <sup>b</sup>	20.10 ± 0.40 <sup>a</sup>
S6	15.60 ± 1.20 <sup>n</sup>	1.00 ± 0.10 <sup>h</sup>	62.00 ± 1.50 <sup>c</sup>	16.08 ± 0.30 <sup>c</sup>	14.20 ± 0.50 <sup>d</sup>
S7	168.40 ± 1.40 <sup>c</sup>	22.90 ± 0.20 <sup>c</sup>	36.70 ± 0.40 <sup>c</sup>	10.13 ± 0.20 <sup>g</sup>	2.10 ± 0.60 <sup>k</sup>
S8	39.40 ± 2.40 <sup>l</sup>	4.50 ± 1.10 <sup>g</sup>	56.10 ± 8.80 <sup>c</sup>	13.43 ± 0.30 <sup>e</sup>	12.80 ± 0.02 <sup>e</sup>
S9	104.10 ± 3.50 <sup>d</sup>	13.80 ± 0.60 <sup>d</sup>	36.60 ± 3.10 <sup>e</sup>	6.88 ± 0.20 <sup>j</sup>	3.80 ± 0.30 <sup>h</sup>
S10	96.30 ± 1.00 <sup>d</sup>	9.10 ± 1.00 <sup>ef</sup>	15.10 ± 0.70 <sup>f</sup>	2.66 ± 0.10 <sup>l</sup>	4.00 ± 0.01 <sup>h</sup>
S11	199.20 ± 8.70 <sup>b</sup>	25.90 ± 1.10 <sup>b</sup>	14.20 ± 0.20 <sup>f</sup>	5.06 ± 0.20 <sup>k</sup>	2.00 ± 0.01 <sup>k</sup>
S12	246.20 ± 10.40 <sup>a</sup>	31.70 ± 1.70 <sup>a</sup>	12.50 ± 0.50 <sup>f</sup>	2.50 ± 0.20 <sup>l</sup>	1.90 ± 0.01 <sup>k</sup>
S13	77.40 ± 0.16 <sup>def</sup>	8.80 ± 0.05 <sup>ef</sup>	16.29 ± 0.29 <sup>f</sup>	14.90 ± 0.13 <sup>d</sup>	7.95 ± 0.04 <sup>g</sup>
S14	17.28 ± 0.71 <sup>m</sup>	1.57 ± 0.04 <sup>h</sup>	77.65 ± 1.52 <sup>b</sup>	58.96 ± 0.92 <sup>a</sup>	17.58 ± 0.34 <sup>b</sup>
S15	73.72 ± 0.84 <sup>def</sup>	8.40 ± 0.08 <sup>ef</sup>	16.07 ± 0.16 <sup>f</sup>	9.39 ± 0.10 <sup>gh</sup>	5.54 ± 0.08 <sup>f</sup>
S16	57.43 ± 0.12 <sup>k</sup>	4.23 ± 0.04 <sup>g</sup>	30.92 ± 1.34 <sup>e</sup>	10.12 ± 0.21 <sup>g</sup>	14.62 ± 0.09 <sup>c</sup>
S17	75.31 ± 0.70 <sup>def</sup>	9.76 ± 0.12 <sup>ef</sup>	15.30 ± 0.20 <sup>f</sup>	3.27 ± 0.25 <sup>l</sup>	3.78 ± 0.01 <sup>h</sup>
S18	102.11 ± 1.03 <sup>d</sup>	12.70 ± 0.10 <sup>d</sup>	9.52 ± 0.13 <sup>fg</sup>	2.93 ± 0.10 <sup>l</sup>	3.46 ± 0.01 <sup>hi</sup>
S19	83.72 ± 1.43 <sup>def</sup>	10.11 ± 0.09 <sup>e</sup>	10.39 ± 0.11 <sup>fg</sup>	5.27 ± 0.32 <sup>k</sup>	4.25 ± 0.02 <sup>h</sup>
S20	98.01 ± 0.28 <sup>d</sup>	10.34 ± 0.23 <sup>e</sup>	13.85 ± 0.4 <sup>f</sup>	2.94 ± 0.16	3.78 ± 0.01 <sup>h</sup>
S21	97.56 ± 1.26 <sup>d</sup>	11.70 ± 0.22 <sup>e</sup>	15.27 ± 0.34 <sup>f</sup>	3.67 ± 0.20 <sup>l</sup>	4.31 ± 0.01 <sup>h</sup>
S22	78.20 ± 0.60 <sup>g</sup>	9.76 ± 0.08 <sup>e</sup>	18.59 ± 0.56 <sup>f</sup>	4.70 ± 0.61 <sup>k</sup>	7.34 ± 0.03 <sup>g</sup>

All values are expressed as means of triplicate determinations ± SD. Values in the same column followed by the same letter are not significantly different by Tukey's multiple range test ( $p < 0.05$ ).

recommended value of moisture in honey indicates its maturity and reflects the desirable density [20]. For electrical conductivity, all honey samples are bellowing the maximum limit allowed (800  $\mu$ S/cm) except *Ceratonia siliqua* honey from Taounate (948.33 ± 1.63  $\mu$ S/cm) and *Arbutus unedo* honey from Tetouan (913.51 ± 6.21  $\mu$ S/cm). Similarly, the ash contents in all honey samples are below the limits (0.6%) except for the multifloral honey sample from Skoura (0.92 ± 0.02%). The ash content reflects the mineral composition in honey. It is influenced by soil and botanical origins [12]. On the other hand, Pfund is a parameter that reflects honey color, the analysis of Pfund showed values ranging between 19.67 ± 0.64 mm in *Citrus limon* honey and 128.74 ± 1.20 mm in *Ceratonia siliqua* honey. According to the Pfund scale, the color of our honey samples ranged between white color and dark amber [21].

**3.2. Minerals Content.** The mineral contents in honey samples were summarized in Table 2; the predominant metal in the majority of honey samples was potassium followed by sodium, calcium, and then magnesium. The same results were obtained by Bouhlali et al. [22] for eleven Moroccan honey from various floral origins.

The *Arbutus unedo* honey sample presented the highest potassium content of 1299.56 ± 3.25 mg/kg, and *Salvia rosmarinus* honey presented the lowest content (270.57 ± 0.85 mg/kg). The contents in sodium ranged between 41.64 ± 0.54 mg/kg in *Salvia rosmarinus* honey and 264.56 ± 0.45 mg/kg in *Arbutus unedo* honey, and the contents in calcium ranged between 22.12 ± 0.21 in multifloral honey sample from Skoura and 283.13 ± 2.36 mg/kg in

*Arbutus unedo* honey, and the contents in magnesium ranged between 13.55 ± 0.56 mg/kg in *Ziziphus lotus* honey from Marmoucha and 218.72 ± 1.54 mg/kg in multifloral from Elmers. The mineral content in honey is influenced by soil composition, botanical origin, climatic conditions, and seasonal variations [23].

Honey is a good source of trace elements that are essential for the proper functioning of the body [24]. Many studies have shown the pharmacological effect of dietary minerals; for instance, it was proven that potassium plays a crucial role in endothelial and cardiovascular function and can reduce blood pressure [25]. Similarly, calcium dietary intake is very important for the good health of the skeleton, the function of skeletal muscles, and nerve conduction [26, 27]. In addition, it was proven in a study conducted by Kh et al. [28] that magnesium supplementation can prevent blood pressure elevation and significantly reduce platelet aggregation.

**3.3. Polyphenols, Flavones, Flavonols, and Antioxidant Activities.** Polyphenols are secondary metabolites widely present in the plant kingdom and known for their pharmacological properties, such as antioxidant, anti-inflammatory, immunomodulatory, and antidiabetic effects [29, 30]. The analysis of polyphenols in honey samples revealed a range between 13.70 ± 0.30 mg GAE/100 g found in the *Citrus sinensis* honey sample (S5) and 246.20 ± 10.40 mg GAE/100 g in *Ceratonia siliqua* honey (S12). Flavones and flavonols ranged between 0.70 ± 0.10 mg QE/100 g in *Citrus sinensis* honey sample (S5) and 31.70 ± 1.70 mg QE/100 g *Ceratonia siliqua* honey (S12)

TABLE 4: Total protein and apalbumin1 contents of the honey samples.

The botanical origin of honey	Code	Total protein		Apalbumin1		
		$\mu\text{g/g}$ of honey	% of honey	$\mu\text{g/g}$ of honey	%of total protein	% of honey
<i>Salvia rosmarinus</i>	S1	212.00 <sup>o</sup>	0.021 <sup>o</sup>	27.40 <sup>t</sup>	12.92 <sup>m</sup>	0.013 <sup>m</sup>
<i>Ammi visnaga</i>	S2	2215.40 <sup>d</sup>	0.222 <sup>d</sup>	242.60 <sup>n</sup>	10.95 <sup>n</sup>	0.011 <sup>mn</sup>
<i>Euphorbia resinifera</i>	S3	1245.00 <sup>i</sup>	0.125 <sup>i</sup>	185.70 <sup>o</sup>	14.92 <sup>k</sup>	0.015 <sup>l</sup>
<i>Ziziphus lotus</i>	S4	1245.00 <sup>i</sup>	0.125 <sup>i</sup>	338.90 <sup>i</sup>	27.22 <sup>fg</sup>	0.027 <sup>g</sup>
<i>Citrus sinensis</i>	S5	460.80 <sup>j</sup>	0.046 <sup>n</sup>	137.70 <sup>f</sup>	29.88 <sup>f</sup>	0.030 <sup>f</sup>
<i>Citrus sinensis</i>	S6	623.00 <sup>j</sup>	0.062 <sup>m</sup>	131.00 <sup>s</sup>	21.03 <sup>h</sup>	0.021 <sup>h</sup>
Multifloral	S7	2041.60 <sup>e</sup>	0.204 <sup>e</sup>	277.40 <sup>l</sup>	13.58 <sup>kl</sup>	0.014 <sup>m</sup>
Multifloral	S8	897.80 <sup>k</sup>	0.090 <sup>k</sup>	175.90 <sup>p</sup>	19.59 <sup>i</sup>	0.020 <sup>h</sup>
Multifloral	S9	1518.80 <sup>g</sup>	0.152 <sup>g</sup>	426.70 <sup>e</sup>	28.09 <sup>f</sup>	0.028 <sup>g</sup>
<i>Ceratonia siliqua</i>	S10	1659.90 <sup>f</sup>	0.166 <sup>f</sup>	302.20 <sup>j</sup>	18.21 <sup>j</sup>	0.018 <sup>i</sup>
<i>Ceratonia siliqua</i>	S11	2422.40 <sup>c</sup>	0.242 <sup>c</sup>	295.20 <sup>k</sup>	12.19 <sup>m</sup>	0.012 <sup>m</sup>
<i>Ceratonia siliqua</i>	S12	2052.40 <sup>e</sup>	0.205 <sup>e</sup>	258.10 <sup>h</sup>	12.57 <sup>m</sup>	0.013 <sup>i</sup>
<i>Lavandula angustifolia</i>	S13	249.60 <sup>n</sup>	0.025 <sup>o</sup>	157.44 <sup>d</sup>	63.08 <sup>a</sup>	0.016 <sup>j</sup>
<i>Citrus limon</i>	S14	446.00 <sup>m</sup>	0.045 <sup>n</sup>	260.05 <sup>m</sup>	58.31 <sup>b</sup>	0.026 <sup>hk</sup>
<i>Ziziphus lotus</i>	S15	840.80 <sup>j</sup>	0.084 <sup>l</sup>	404.06 <sup>f</sup>	48.06 <sup>d</sup>	0.040 <sup>d</sup>
<i>Silybum marianum</i>	S16	1630.50 <sup>f</sup>	0.163 <sup>f</sup>	357.89 <sup>h</sup>	21.95 <sup>h</sup>	0.036 <sup>e</sup>
<i>Ammi visnaga</i>	S17	2582.00 <sup>c</sup>	0.258 <sup>c</sup>	368.91 <sup>g</sup>	14.29 <sup>k</sup>	0.037 <sup>e</sup>
<i>Capparis spinosa</i>	S18	2010.90 <sup>e</sup>	0.201 <sup>e</sup>	688.34 <sup>b</sup>	34.23 <sup>e</sup>	0.069 <sup>b</sup>
<i>Arbutus unedo</i>	S19	1032.40 <sup>j</sup>	0.103 <sup>j</sup>	545.40 <sup>c</sup>	52.83 <sup>c</sup>	0.055 <sup>c</sup>
<i>Bupleurum spinosum</i>	S20	4121.20 <sup>a</sup>	0.412 <sup>a</sup>	787.73 <sup>a</sup>	19.11 <sup>i</sup>	0.079 <sup>a</sup>
<i>Thymus vulgaris</i>	S21	3326.20 <sup>b</sup>	0.333 <sup>b</sup>	422.87 <sup>d</sup>	12.71 <sup>m</sup>	0.042 <sup>d</sup>
<i>Origanum vulgare</i>	S22	1357.90 <sup>h</sup>	0.136 <sup>h</sup>	790.82 <sup>a</sup>	58.24 <sup>b</sup>	0.079 <sup>a</sup>

Values in the same column followed by the same letter are not significantly different by Tukey's multiple range test ( $p < 0.05$ ).

(Table 3). Our results are higher than those obtained by Petretto et al. [31] for seven commercial Moroccan honey samples from different floral origins and lower than those found in monofloral honey (*Bupleurum spinosum*) collected from Moroccan Middle Atlas [12]. Moreover, the antioxidant compounds' rate in honey is affected by several factors such as its floral source, geographical origin, honey maturation processing, handling, and storage [32]. In addition, we observed that honey samples from the same botanical origin (S2 and S17: *Ammi visnaga*), (S4 and S15: *Ziziphus lotus*), (S10, S11, S12: *Ceratonia siliqua*) have different content in antioxidant compounds; the same results were found by Laaroussi et al. [12]. This is explained by the presence of secondary pollen and nectar from other floral sources [33].

Concerning the antioxidant activity, the DPPH method is largely used to test the free radical scavenging ability of various samples [20, 34]. The DPPH  $\text{IC}_{50}$  values (the concentration with scavenging activity of 50%) of Moroccan honey samples showed significant differences among analyzed samples and ranged between  $9.3 \pm 1.0$  mg/ml in *Ziziphus lotus* honey sample and  $93.40 \pm 6.10$  mg/ml in *Citrus sinensis* honey sample.

The second method used to evaluate the antioxidant activity of honey samples was the ABTS cation radical assay (ABTS<sup>•+</sup>). It is based on the interaction between the nitrogen atom of ABTS and hydrogen donating antioxidant; this reaction produces the decolorization of the solution [34]. The  $\text{IC}_{50}$  of the ABTS test ranged between  $58.96 \pm 0.92$  mg/ml in the *Citrus limon* honey sample and  $2.30 \pm 0.10$  mg/ml in the *Ziziphus lotus* honey sample (Table 3). Furthermore, the reducing power on the ferric ion of honey samples was

analyzed; this method is based on the reduction of ferric ion Fe (III) to ferrous ion Fe (II) by the antioxidant compound. The reaction was visualized by the formation of Perl's Prussian blue complex with maximum absorption at 700 nm [35]. The results of reducing power showed a range of  $\text{EC}_{50}$  between  $1.60 \pm 0.01$  mg/ml obtained by *Ziziphus lotus* honey from Marmoucha and  $17.58 \pm 0.34$  mg/ml obtained by *Citrus limon* honey from Khnichat.

**3.4. Proteins and Apalbumin 1 Content.** Proteins are one of the minor compounds found in honey. Their percentages vary according to the honeybee origin. The honey produced by *Apis mellifera* contains a range between 0.6% and 1.6% of proteins while the honey produced by *Apis cerana* contains a range between 0.1% and 3.3% of proteins [36]. It was reported that the amount of protein from bee secretions in honey is higher than that of protein from plants [37]. Among these proteins, there are enzymes responsible for the transformation of nectar components into honey such as glucose-6-oxidase, invertase, and diastase [9]. Furthermore, the major royal jelly protein "apalbumin 1" is one of the most abundant proteins in honey originating from bee secretions, it is an important criterion for honey quality examination, and its detection in honey samples is an indicator of the authenticity and no adulteration [9]. In addition, this protein of 55 kDa had a wide range of biological properties and health-promoting functions such as the immunostimulation effect, the increase of TNF- $\alpha$  release by macrophages, and the antihypertensive activity [38–40].

The results of total protein and apalbumin-1 content in honey are summarized in Table 4 and Figure 2. According to

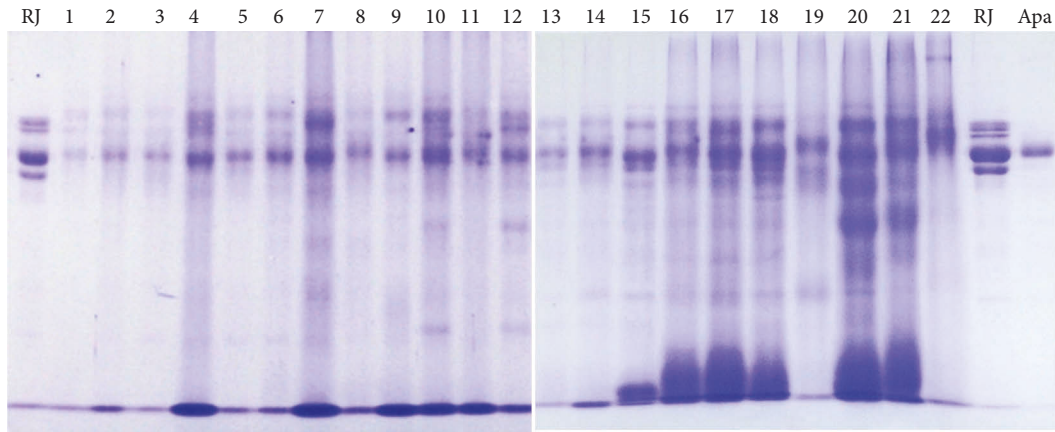


FIGURE 2: Electrophoretic profile of the proteins of honey samples, 12% SDS-PAGE, Coomassie Brilliant Blue staining. Lines RJ: royal jelly (1 mg/ml); Lines 1–22: honey samples (50%); Line Apa: apalbumin 1.

the results obtained, the range of protein content in honey samples was between 0.021% found in *Salvia rosmarinus* honey and 0.412% found in *Bupleurum spinosum* honey. These results are in line with those obtained in other studies [17, 36].

For apalbumin 1 analysis, the highest content was shown in *Origanum vulgare* honey (790.82  $\mu\text{g/g}$ ), it represents 58.24% of total protein presented in this honey, followed by *Bupleurum spinosum* honey (787.73  $\mu\text{g/g}$ ), and it represents 19.11% of total protein. Whereas the lowest content of apalbumin 1 was found in *Salvia rosmarinus* honey (212  $\mu\text{g/g}$ ), it represents 12.92% of total protein. As shown in Table 4, the percentage of apalbumin 1 in honey samples ranged between 0.011% and 0.079%; these results are higher than those obtained for honey samples from Italia and Slovakia ranging between 0.08% and 0.03% [17].

Overall, the obtained results showed that all analyzed samples contain an important amount of apalbumin 1, the detection of this protein in honey is a criterion of honey's good quality, and it is an indicator of authenticity and no adulteration [9].

**3.5. Correlation and Multivariate Analysis.** The correlation test between the studied parameters is presented in Table 5. The results revealed significant Pearson correlations ( $p \leq 0.05$ ) among the different parameters. The antioxidant compounds (polyphenols, flavones, and flavonols) correlate positively with total acidity, ash, Pfund, and the content of sodium and potassium. Polyphenols content correlates negatively with ABTS, DPPH, and RP tests ( $r = -0.420$ ,  $r = -0.595$ , and  $r = -0.746$ , respectively). On the other hand, flavones and flavonols correlate negatively with DPPH ( $r = -0.578$ ) and RP ( $r = -0.737$ ). RP test correlates positively with DPPH and ABTS ( $r = 0.553$  and  $r = 0.799$ , respectively). Total acidity correlates positively with free acidity ( $r = 0.917$ ) and lactonic acidity ( $r = 0.614$ ). Electrical conductivity correlates positively with total acidity ( $r = 0.459$ ), ash ( $r = 0.765$ ), Pfund ( $r = 0.611$ ), sodium ( $r = 0.738$ ), potassium ( $r = 0.738$ ), calcium ( $r = 0.562$ ), polyphenols ( $r = 0.678$ ), and flavones and flavonols ( $r = 0.685$ ). The content in apalbumin 1

correlates positively with total acidity ( $r = 0.431$ ), Pfund ( $r = 0.471$ ), and the content in protein ( $r = 0.554$ ) while the protein content correlates negatively with pH ( $r = -0.480$ ) and positively with free acidity ( $r = 0.554$ ), lactone acidity ( $r = 0.629$ ), total acidity ( $r = 0.710$ ), and Pfund ( $r = 0.570$ ). Furthermore, a principal component analysis (PCA) was applied to the obtained results (Figure 3). PCA is one of the techniques most used for performing multivariate analysis; it is characterized by low difficulty and rapid analysis [41]. Figure 3(a) represents the PCA for physicochemical analysis of the studied honey samples; the PC1 and PC2 explained a variance of 56%. The first component (PC1) explained 39.274% and represents in its positive part all parameters studied except pH that exists in the negative part. The second component (PC2) explained 16.726% and represents in its positive part pH, moisture, Mg, Ca, K, Na, ash, and electrical conductivity, while in the negative part we found Pfund, free acidity, total acidity, and lactonic acidity. The honey samples S8, S9, S7, S4, and S15 shared characteristics regarding pH, moisture, Mg, Ca, K, Na, ash, and electrical conductivity, whereas the honey samples S21 and S18 shared the characteristics for Pfund, free acidity, total acidity, and lactonic acidity.

Figure 3(b) represents the PCA for polyphenols, flavones/flavonols, the antioxidant activities (DPPH, ABTS, and RP) and the content in total protein and apalbumin 1. The two principal components (PC1 and PC2) explained a variance of 76.973%. The first component explained 55.936% and represents in its positive part apalbumin 1, proteins, polyphenols, and flavones/flavonols, and in its negative part, we found the antioxidant test (DPPH, ABTS, and RP), whereas the second component explained 21.037% and represents in its positive part proteins, apalbumin 1, DPPH, and ABTS and in its negative part polyphenols, flavones/flavonols, and ABTS. S4, S11, and S7 honey samples shared the characteristics for polyphenols and flavones/flavonols content while S15, S17, S18, S19, S20, S21, and S22 honey samples shared the characteristics for apalbumin 1 and proteins. It was suggested that the difference in the physicochemical parameters, minerals content, and bioactive molecules of



TABLE 5: Pearson correlation coefficients between the analyzed parameters.

Parameters	pH	FA	LA	TA	Moisture	EC	Ash	Pfund	Na	K	Ca	Mg	Polyphenols	Flavones and flavonols	ABTS	DPPH	RP	ApalbuminI	Protein
pH	1	0.313	0.083	0.124	0.467	0.013	-0.055	-0.245	0.183	0.139	0.120	0.010	0.017	0.037	0.030	-0.089	0.084	-0.145	-0.480*
FA	-0.225	1	0.249	0.917***	0.200	0.422	0.346	0.406	0.452*	0.243	0.331	0.039	0.333	0.333	-0.536*	-0.107	-0.357	0.378	0.554**
LA	0.083	0.249	1	0.614**	-0.012	0.281	0.153	0.493*	0.136	0.056	0.258	0.190	0.457*	0.415	-0.221	-0.365	-0.428*	0.302	0.629**
TA	0.124	0.917***	0.614**	1	0.158	0.459*	0.345	0.533*	0.425*	0.221	0.375	0.110	0.460*	0.442*	-0.527*	-0.238	-0.467*	0.431*	0.710**
Moisture	0.467	0.200	-0.012	0.158	1	0.178	0.013	-0.177	0.218	-0.001	0.446*	0.163	-0.313	-0.316	0.171	0.263	0.401	0.297	-0.051
EC	0.013	0.422	0.281	0.459*	0.178	1	0.765**	0.611**	0.738**	0.598**	0.562**	0.249	0.678**	0.685**	-0.338	-0.575**	-0.648**	0.283	0.236
Ash	-0.055	0.346	0.153	0.345	0.013	0.765**	1	0.347	0.561**	0.629**	0.318	0.383**	0.485*	0.527*	-0.076	-0.501**	-0.442*	0.045	0.122
Pfund	-0.245	0.406	0.493*	0.533*	-0.177	0.611**	0.347	1	0.342	0.432*	0.357	0.156	0.668**	0.626**	-0.509*	-0.624*	-0.683**	0.471*	0.570**
Na	0.183	0.452*	0.136	0.425*	0.218	0.738**	0.561**	0.342	1	0.670**	0.445*	0.156	0.572**	0.581**	-0.135	-0.455*	-0.453*	-0.002	0.098
K	0.139	0.243	0.056	0.221	-0.001	0.598**	0.629**	0.432*	0.670**	1	0.422	0.329	0.472*	0.501*	-0.222	-0.408	-0.377	0.180	0.159
Ca	0.120	0.331	0.258	0.375	0.446*	0.562**	0.318	0.357	0.445*	0.422	1	0.503*	0.212	0.230	-0.142	-0.213	-0.139	0.289	0.137
Mg	0.010	0.039	0.190	0.110	0.163	0.249	0.383**	0.156	0.156	0.329	0.503*	1	-0.025	-0.004	0.221	-0.154	0.129	-0.149	-0.117
Polyphenols	0.017	0.333	0.457*	0.460*	-0.313	0.678**	0.485*	0.668**	0.572**	0.472*	0.212	-0.025	1	0.990**	-0.420*	-0.595*	-0.746**	0.111	0.406
Flavones and flavonols	0.037	0.333	0.415	0.442*	-0.316	0.685**	0.527*	0.626**	0.581**	0.501*	0.230	-0.004	0.990**	1	-0.373	-0.578**	-0.737**	0.097	0.373
ABTS	0.030	-0.536*	-0.221	-0.527*	0.171	-0.338	-0.076	-0.509	-0.135	-0.222	-0.142	0.221	-0.420*	-0.373	1	0.218	0.553**	-0.581**	-0.398
DPPH	-0.089	-0.107	-0.365	-0.238	0.263	-0.575**	-0.501**	-0.624*	-0.455*	-0.408	-0.213	-0.154	-0.595*	-0.578**	0.218	1	0.799**	-0.127	-0.341
RP	0.084	-0.357	-0.428*	-0.467*	0.401	-0.648**	-0.442*	-0.683**	-0.453*	-0.377	-0.139	0.129	-0.746**	-0.737**	0.553**	0.799**	1	-0.288	-0.497*
ApalbuminI	-0.145	0.378	0.302	0.431*	0.297	0.283	0.045	0.471*	-0.002	0.180	0.289	-0.149	0.111	0.097	-0.581**	-0.127	-0.288	1	0.554**
Protein	-0.480*	0.554**	0.629**	0.710**	-0.051	0.236	0.122	0.570**	0.098	0.159	0.137	-0.117	0.406	0.373	-0.398	-0.341	-0.497*	0.554**	1

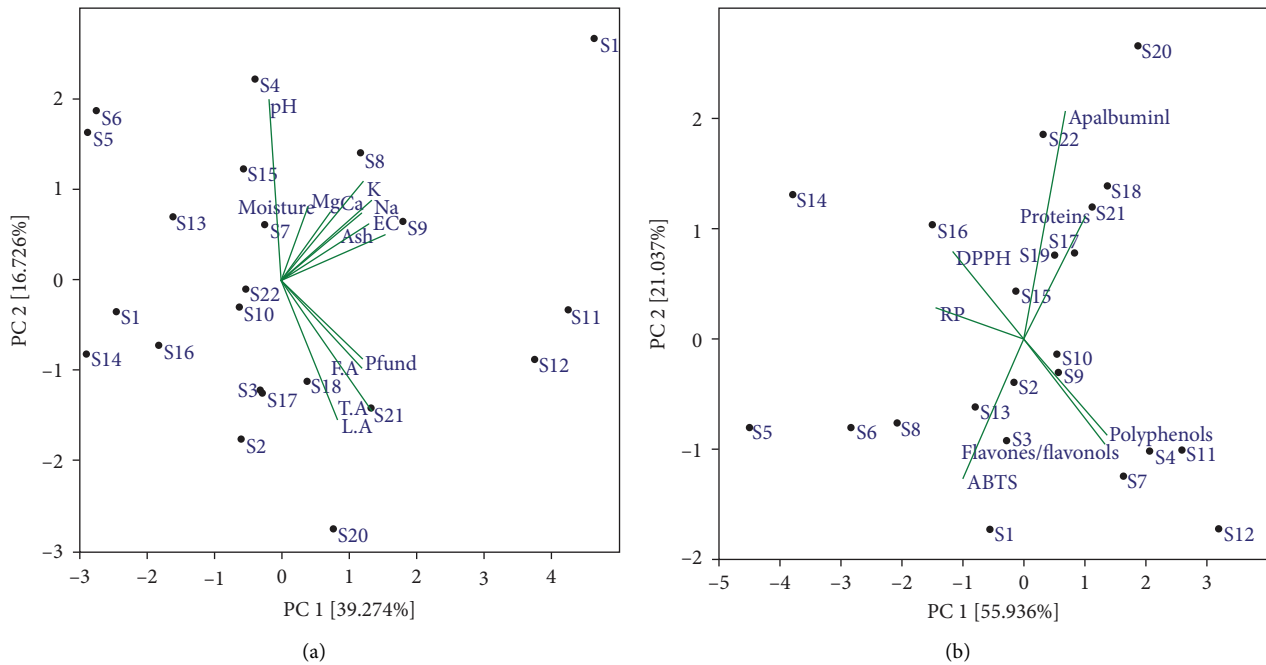


FIGURE 3: Principal component analysis (PCA). (a) Biplots for physicochemical analysis of honey samples; (b) biplots for antioxidant activities (ABTS, RP, and DPPH), polyphenols, flavones/flavonols, protein, and apalbumin I of honey samples. LA: lactic acid; TA: total acidity; FA: free acidity; EC: electrical conductivity.

honey is related to the soil composition, botanical origin, and climatic conditions [32]. In sum, this study supplied new information about the antioxidant activity, protein, and apalbumin contents in Moroccan honey obtained from different botanical origins.

#### 4. Conclusions

Our results assert that Moroccan honey samples are rich in bioactive compounds such as polyphenols, flavones/flavonols, and proteins and are endowed with great antioxidant activities. Nevertheless, the detection of the major royal jelly protein apalbumin I in all analyzed samples confirms the quality and no adulteration of Moroccan honey.

#### Data Availability

The data used to support the findings of this study are included within the article.

#### Conflicts of Interest

The authors declare that they have no conflicts of interest.

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