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Comprehensive Characterization of Moroccan Honey Varieties (cedar, euphorbia, eucalyptus, carob, and thyme): Insights into Phytochemical and Physico-chemical Properties

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ABSTRACT

We analyzed five Moroccan honey varieties: cedar, euphorbia, eucalyptus, carob, and thyme, focusing on their physicochemical and phytochemical properties. Using gas chromatography-mass spectrometry, high-performance liquid chromatography, and X-ray fluorescence, we measured key parameters like pH, refractive index, moisture content, and Brix degree, comparing them to Codex Alimentarius standards. The chemical composition varied significantly between samples. We also evaluated antioxidant capacity through DPPH and total antioxidant assays. Methyl hepta-2,4-dienoate was predominant in euphorbia and eucalyptus honey, while 3-methoxy-2-methyl-cyclohex-2-enone was unique to carob honey. All samples contained polyphenols like caffeic acid and naringenin. Carob honey has high fructose and glucose levels and exceptional antioxidant capacity. These findings highlight the health-promoting potential of Moroccan kinds of honey for food and nutraceutical applications.

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1. INTRODUCTION

Honey, a complex biological substance (Tomczyk *et al*., 2020, Cohen and Weihs, 2010), serves as a natural sweetener rich in energy Stihi *et al*. (2016) and has been used for millennia (Kamal & Klein, 2011). It is made from nectar that honeybees, Apis mellifera, gather from different flowers (Trisha *et al*., 2023, Pereira *et al*., 2023). Recent scientific interest has grown due to its health benefits and identifying over 200 bioactive components (Hossain *et al*., 2022). Ancient societies harnessed honey's medicinal properties for wound healing, sunburn treatment, blood pressure reduction, and soothing throat inflammations (Duru & Duru, 2021).

Honey offers diverse nutrients, including water. (Cianciosi *et al*., 2018), sugars, amino acid compounds, different vitamins, active enzymes, organic acids, phenols, pigments, volatile oils, and a wide range of aromatic compounds (Terzo *et al*., 2020, Alghamdi *et al*., 2020).

The sensory, chemical, and physical significantly impact honey quality (Finola *et al*., 2007). The primary constituents of honey largely consist of sugars, with approximately 95 to 97% of its dry weight composed of monosaccharides (Gela *et al*., 2021). The dominant sugars in honey are fructose (38%) and glucose (31%), contributing to its nutritional qualities (Bastos & Alves, 2003, Alvarez-Suarez *et al*., 2010, Hussein *et al*., 2014, Doner, 1977, Duru & Duru, 2021).

It is well established that honey possesses various beneficial properties such as anti-inflammatory effects. Markelov & Trushin (2006), antioxidant activity (Chua *et al*., 2013, Aljadi & Kamaruddin, 2004), and even antibiotic potential (Rikohe *et al*., 2023). The varying concentrations of phenolic compounds in different honey types influence their antioxidant levels (Moniruzzaman *et al*., 2012, Gheldof *et al*., 2002). Consequently, honey stands out as a natural source of vital antioxidants for human health (Yamani & Ali, 2023). A particularly accurate and effective technique for identifying these phenolic compounds is high-performance liquid chromatograph y with diode array detection (HPLC-DAD). This method allows precise identification and quantification of phenolic compounds. (Zhang *et al*., 2023, Chen *et al*., 2011, Marini *et al*., 2011, Regos & Treutter, 2010, Zhang *et al*., 2013).

To ensure quality and authenticity, authorities establish guidelines based on botanical and geographical origins. These guidelines are intended to protect honey's competitiveness in the market and guarantee its quality (Castro-Vázquez *et al*., 2010, Ballabio *et al*., 2018). While melissopalynological analysis identifies floral sources, its limitations necessitate supplementing or replacing them with other techniques (Patrignani *et al*., 2018, Stanimirova *et al*., 2010). Unfortunately, this analytical procedure alone cannot reliably ensure the characterization of honey's floral source due to its cost and time-consuming nature. Additionally, its dependability may be restricted by the analyzer's capabilities, which significantly impact its effectiveness (Castro-Vázquez *et al*., 2014, Costa *et al*., 2018). Therefore, it is imperative to either supplement or completely replace this analysis with other techniques, such as physicochemical, organoleptic, and chromatographic methods.

In recent decades, gas chromatography-mass spectrometry (GC-MS) has been widely used to determine the volatile profile of honey (Castro-Vázquez *et al*., 2010, Devi *et al*., 2018, Escriche *et al*., 2009, Castro-Vázquez *et al*., 2014, Soria *et al*., 2008, Juan-Borrás *et al*., 2014). Another valuable technique is the energy dispersive X-ray fluorescence (ED-XRF) technique, which accurately determines the concentrations of elements (K, Ca, Ti, Cr, Mn, Fe, Ni, Cu, Zn, Rb, and Sr) in honey samples. ED-XRF, utilizing a range of tubes and radionuclide sources, demonstrated adequate precision for identifying specific elements in biological samples like honey (Khuder *et al*., 2010).

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Based on our previous studies (Errich *et al*., 2021; Sabbani *et al*., 2021; Nasri *et al*., 2021; El Ati *et al*., 2022; Maarouf *et al*., 2022; Hamdaoui *et al*., 2021; Boutebib *et al*., 2023; Laita *et al*., 2024; Ech-Chihbi *et al*., 2024; Sellam *et al*., 2024), this work aimed to define specific physicochemical characteristics, including water content, pH, refractive index, acidity index, saponification index, and Brix degree. Additionally, it focused on analyzing the phytochemical profiles of Moroccan honey using analytical techniques such as HPLC-DAD, GC-MS, and EDXRF. These analyses were conducted based on the floral richness of the honey samples. Furthermore, the study evaluated the antioxidant capacity of the honey varieties. Ultimately, this research contributes to the valorization of different honey samples.

2. METHODS

2.1. Chemical Reagents

The following compounds were purchased from Sigma-Aldrich: ascorbic acid, gallic acid, chlorogenic acid, caffeic acid, cinnamic acid, p-coumaric acid, benzoic acid, naringenin, rutin, kaempferol, and 2,2-diphenyl-1-picrylhydrazyl (DPPH). Methanol, sulfuric acid, hydrochloric acid, ethanol, acetonitrile, and sodium hydroxide (NaOH) were also used. All chemicals used in this study were analytical grade.

2.2. Honey Samples

Five distinct honey samples were collected between 1 April and 30 September 2022 from modern and healthy hives strategically placed across diverse eco-geographical regions in Morocco. These regions included Oulad Barhil, Ait Ba Amran, Agadir, Tiznit, and Errachidia as shown in **Figure 1** and detailed in **Table 1**. Each sample was weighed 250 g and was carefully transported to our laboratory in February 2023. Then, they were stored at room temperature. To ensure accuracy and consistency, we conducted all tests within three months of obtaining the samples.

Table 1. Honey samples, origins, and floral sources.

Sample code	Sample name	Location	Latitude	Longitude	Altitude (m)
M 1	Cedar	Oulad Barhil	30°38'47.029" N	8°28' 47.737" W	491.00
M 2	Euphorbia	Ait Ba Amran	31°22'48" N	8°33'0" W	665.23
M 3	Eucalyptus	Agadir	30°25'39.9180'' N	9°35'53.1852'' W	74.00
M 4	Carob	Tiznit	29°41'48.8436'' N	9°43'59.5128'' W	1200.00
M 5	Thyme	Erachidia	31°55'38.05" N	4°25'42.593" W	1009.00

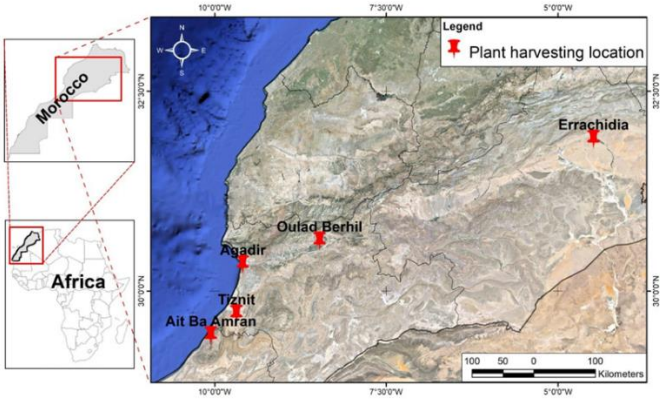

Figure 1. Geographical location of honey samples.

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2.3. Evaluation of Physicochemical Characteristics

We did measurements of parameters, such as humidity, pH, saponification index, acidity index, Brix degree, and refractive index, which are part of the physicochemical studies. These analyses were carried out three times to guarantee the accuracy and reliability of the results.

2.3.1. Moisture Content

Two methods were used to evaluate the moisture content of honey samples. First, 1 g of homogenized honey was placed in a Petri dish and heated in an oven at 105°C for at least 3 h to ensure complete evaporation of water. After cooling, the dried honey was weighed, and the difference between the initial and final masses provided the moisture content.

2.3.2. Refractive index

In the second method, a refractometer was employed to determine the refractive indices of the honey samples at room temperature. The Wedmore table was used to calculate the moisture percentage. This approach allowed us to get an accurate assessment of the honey's water content (Hussein *et al.*, 2014).

2.3.3. Determination of pH

The pH of the honey samples was measured using a pH meter (Bante, instruments, 920). Using buffer solutions, a calibrated pH meter with pH values of 4.00, 7.00, and 9.00 was used to measure the pH directly. In a 250 mL beaker, 10 g of honey was dissolved in 75 mL of distilled water and stirred using a magnetic stirrer. This approach aligned with the methodology described by other reports (Damto *et al.*, 2023), and adhered to guidelines for sample preparation.

2.3.4. Acidity value

To prepare the titrant solution, first make a standardized alcoholic mixture of KOH with a concentration of 0.01 M KOH, according to Aloune *et al.* (2012). Next, get the samples of honey ready for analysis. To dissolve 0.5 g of honey, add 5 mL of ethanol to it. For titration, the sample material is added to phenolphthalein, a colored indicator. The KOH titrant solution is then added gradually until the indicator's color changes, indicating that the acid has been neutralized.

Following other reports (Alloune *et al.*, 2012), the acidity value was calculated using the equation (1):

$$Acidity\ value\ \left(\frac{mgKOH}{g}\right) = IA = \frac{(VKOH\ x\ NKOH\ x\ 56{,}1)}{me} \quad (1)$$

where *V* is the KOH titrant solution volume (measured in mL), *N* is the KOH titrant solution's normalcy, 56.1 is the KOH's molar mass denoted by M of KOH, and *Me* is the weight (g) of the honey sample under test.

2.3.5. Saponification value

We gave 2 g of honey for analysis. Next, we made a standard solution of potassium hydroxide (KOH) with a concentration of 0.5 M. We combined the honey sample and 25 mL of the KOH solution for the saponification reaction. The esters in the honey were converted into soap and alcohol by this saponification reaction. The mixture contained an excess of

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potassium hydroxide after the saponification reaction. The excess KOH must then be titrated with hydrochloric acid (HCl) (0.5 M).

Following Aloune *et al*. (2012), the saponification value is calculated using the following formula (Equestion 2):

Saponification value (mg KOH/g) = IS = ((V0 – V1) x NHCl x 56,1)/me (2)

where V0 is the volume of 0.5 N of HCl used in the blank test (measured in mL), V1 is the volume of the test with 0.5 N of HCl (measured in mL), NHCl is the potassium hydroxide titrant solution's normalcy, and 56.1 is the potassium hydroxide equivalent mass, Me is the mass of the honey sample under test (measured in g).

2.3.6. Total soluble (Brix)

Refractometric analysis was used to measure the total soluble solids, which were expressed as degrees Brix on a scale from 0 to 90 degrees.

2.4. Phytochemical studies

2.4.1. Evaluation of phenolic compounds by HPLC-DAD

The honey samples were prepared in the physical measurement room of the Faculty of Sciences of University Mohamed IV in Oujda, using high-performance liquid chromatography coupled with a UV-visible detector (Shimadzu Corp., Kyoto, Japan) connected to a UV PDA Waters 2996 detector (210–400 nm), somewhat modified from the method developed by (Amarowicz *et al*., 2005). An SPD-M 10 A photodiode array detector, an SCTL 10 A system controller, and an LC-10AD pump made up the system. The reverse-phase column Alliance ew2695, C18 (250*4.0 mm, 5 µm) was injected with 20 µl of each sample. The mobile phase composition was found to be 4% aqueous acetic acid (A) and methanol (B) (v/v) from 0 to 10 min, 20-40% B at 10-15 min, 40-60% B at 15-20 min, and 60% B at 20-25 min. The HPLC analysis was carried out using a linear gradient elution. There was a 1.0 mL/min flow rate. The wavelengths that the diode detector used were 288 and 320 nm. The HPLC's Empower software was utilized to examine the peak's areas and heights. Many chemicals, including gallic acid, caffeic acid, rutin, naringenin, kaempferol, and benzoic acid, were used for the HPLC profile. After preparing analytical standards in DMSO (1 mg/ml), 10 µl were injected into the system using the previously mentioned protocol (Dalli *et al*., 2021).

2.4.2. Measurement of sugars by HPLC-DAD

The quantification of fructose, glucose, and sucrose was carried out following the standardized procedures established by the International Honey Commission (Bogdanov *et al*., 2002), with a few minor adjustments.

1 g of honey was combined with 4 mL of distilled water, and the mixture was shaken for 30 minutes at room temperature. For 10 minutes, the samples were centrifuged at 4000Xg. A 0.45 µm filter was used to filter the supernatants, which were then put in a vial for analysis. Using high-performance liquid chromatography (HPLC) (Agilent Technologies 1200 series) with an ELSD detector (Agilent Technologies 1200 Series ELSD) operating at 40 °C and 3.5 bar of pressure, the contents of glucose, fructose, and sucrose were ascertained. At a temperature of 30°C, separation was accomplished using an HPLC column Spherisorb NH2 (5 µm, L × I.D. 25 cm × 3.2 mm). Acetonitrile/Water (85/15) (v/v) was used in an isocratic elution system with a flow rate of 1 mL/min and an analysis time of 20 minutes. The results were expressed in mg per 100 g of dry matter and were computed using an external calibration curve.

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2.4.3. Identification of Chemical Compounds by (GC-MS)

The NF T60-233 standard protocol was followed in the preparation of the methyl esters of honey fatty acids (Loukili *et al*., 2021). A Shimadzu GC program (Kyoto, Japan) with an MS QP2010 coupled with a BPX25 capillary column containing a 5% diphenyl and 95% dimethylpolysiloxane phase (30 m × 0.25 mm inner diameter × 0.25 µm film thickness) was used to perform their separation and identification. The carrier gas was selected to be pure helium (99.99%) at a steady flow rate of 3 mL/min. The temperatures of the injection, ion source, and interface were all set to 250°C. The temperature program for the column oven went from 50°C (held for 1 minute) to 250°C (10 °C/min), with another 1 minute for holding time. Sample components were ionized at an energy of 70 eV in the EI mode (70%) of ionization. 40 to 300 m/z was the mass range that was scanned. Splitless mode (90:1) was used to inject 1 µl of each prepared extract diluted with the appropriate solvent. The sample was analyzed in a single run. Ultimately, the compounds were identified through the comparison of retention times with standards and mass spectra fragmentation with information from the NIST compounds 147, and 198 databases. Data processing and collection were done using LabSolutions (version 2.5) (Loukili *et al*., 2021). Detailed information regarding how to read GC-MS is reported elsewhere (Subagyono *et al*., 021; Diass *et al*., 2023; Nugraha & Nandiyanto, 2021).

2.4.4. Elemental Composition Determination (EDXRF)

The experiment was carried out using the Shimadzu X-ray Fluorescence Energy Dispersion Spectroscopy (EDX-7000) instrument to interpret the analysis of illicit liqueur samples. The X-ray source has been set at 50 kV, 144 uA for Al-U, and 15 kV, 999 uA for NaSc. The collimator was set to 3 mm for each sample. Fluorescence allows for the simultaneous detection of many elements to be analyzed. This apparatus analyzed the samples in less than 1 minute.

2.5. Antioxidant Activities

The antioxidant activity was studied by the DPPH (2,2-diphenyl-1-picrylhydrazyl) test and the measurement of total antioxidant capacity (TAC).

2.5.1. Study of Antioxidant Activity Using the DPPH Test

The DPPH radical scavenging potential was explored based on previous studies (Ferreira *et al*., 2009). However, a modified version of the previously approved DPPH free radical scavenging assay was used to evaluate the antioxidant activity of the honey sample (Damto *et al*., 2023). To do this, 0.5 mg of DPPH was dissolved in 25 mL of methanol to create a DPPH solution. By calculating their antioxidant capacity relative to ascorbic acid (AAEAC), the antioxidant compounds found in the honey samples were evaluated (Trisha *et al*., 2023). Butylated hydroxytoluene (BHT) was frequently used as a standard reference in earlier research. However, for our investigation, we decided to use ascorbic acid or vitamin C.

For comparison's sake, an ascorbic acid reference solution at a concentration of 10 mg/ml was employed. 30 mg of honey and 1 mL of methanol were combined to create the honey solution. 0.75 mL of this methanolic honey solution was then added to 1.5 mL of the DPPH solution. Using a UV-Vis spectrophotometer, the decrease in the DPPH solution's absorption following the addition of an antioxidant was calculated at 517 nm (Flieger & Flieger, 2020).

The equation used to evaluate the radical scavenging activity (%RSA) is as follows Equation (3):

$$\%RSA = ((ADPPH - AS)\ 100)/ADPPH \quad (3)$$

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Where ADPPH is the DPPH solution's initial absorbance. The absorbance of the solution after a specific quantity of the test sample has been added is known as AS.

2.5.2. Measurement of Total Antioxidant Capacity (TAC)

The ability of the honey samples to reduce ammonium molybdate was used to assess their overall antioxidant capacity. Their total antioxidant potential is estimated using this method (El Menyiy *et al*., 2020). The assay method is based on the conversion of molybdenum (VI) to molybdenum (V) by the sample, resulting in the formation of a subsequent coating of molybdenum (V) (Al-Hindi and Shehata, 2014). To achieve this, 100 µL of the honey sample was combined with 1 mL of a reactive solution that contained 0.6 M sulfuric acid, 28 mM sodium phosphate, and 4 mM ammonium molybdate. After shaking the reaction tubes, they were placed in a water bath and heated to boiling (95 °C for 90 min). The absorbance at 695 nm was measured after the sample cooled to room temperature. The values obtained were analyzed concerning a calibration curve (1- 200 µg/mL) established for ascorbic acid. The calibration curves were linear ($y = 0.0091x + 0.0188$; $R^2 = 0.9875$).

3. RESULTS AND DISCUSSION

3.1. Examination of Physicochemical Properties

The physicochemical parameters of the five Moroccan honeys, namely, cedar, euphorbia, eucalyptus, carob, and thyme, are presented in Table 2. The variables that can also affect the moisture content of honey include temperature, the relative humidity of the climate in a particular area, the conditions inside the beehives, and the time of harvest (Gomes *et al*., 2010). The outcomes obtained for the underwater content using the two methods closely resemble each other. Accordingly, the water content of the samples made using the traditional method ranged from 15.45% (carob honey) to 18.7% (eucalyptus honey). According to the second method, it ranged from 17.2% (carob honey) to 19.8% (euphorbia honey). These findings confirm that carob honey has the lowest humidity rate among the other samples, which contradicts the findings of Belhaj *et al*. (2015) Who discovered that the moisture content of carob honey is typically high (20.58%). Additionally, we noticed that, out of all the samples examined, samples M2 (euphorbia honey) and M3 (eucalyptus honey) had the highest moisture content. Eucalyptus honey has a moisture content that is quite similar to what the recommended norms suggest (Belhaj *et al*., 2015). It is important to remember that this value is lower than the 20% maximum tolerated limit for honey set by the Codex Alimentarius (Ciric *et al*., 2018). We have been able to comprehend the storage conditions, ascertain the possibility of honey fermentation, and assess the influence of climate and honey extraction and preservation methods thanks to this analysis (Amri *et al*., 2008).

Most honey has an acidic pH, typically falling between 3.42 and 6.10 (Islam *et al*., 2022). All of the samples (M1 through M5) that were tested showed acidity as well, the pH values of honey samples M3 (eucalyptus honey), M4 (carob honey), and M5 (thyme honey) are almost identical to the findings of the pH ranges from 3.90 (carob honey) to 4.56 (thyme honey) (Belhaj *et al*., 2015), but with slight differences. On the other hand (Mohamed *et al*., 1981), the average pH of eucalyptus honey is 5.36, and that of thyme honey is 6.2, according to reports, which is different from the values we measured. According to reports that have been published, the ideal pH range is 3.2 to 4.5 (Meda *et al*., 2005). No sample examined went over the upper limit permitted by Belhaj *et al*. (2015), this might be regarded as a freshness indicator.

The acidity index values of the examined honey samples ranged from 31.70 to 39.60 mg KOH/kg of honey. The measurements of free acidity varied between 20.3 ± 0.4 and 60.8 ± 0.4

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meq/kg. Regarding the 2001 Codex guidelines (Meda *et al*., 2005), the five honey samples that were analyzed met the established criteria for acidity index values. These results were also lower than the permitted 50 meq/kg limits (Chakir *et al*., 2016). High levels of free acidity were detected in some honeydew kinds of honey from Morocco. Díez *et al*. (2004), suggesting a tendency toward fermentation.

The results of the saponification index point to a comparatively low-fat content in the different honey samples. These indices ranged from 5.64 mg KOH/g (for carob honey) to 8.54 mg KOH/g (for thyme honey).

Sugars make up most of the honey's dry weight more than 90% of it. Additionally, honey contains at least 25 different types of sugars, all of which are closely related to the product's maturation stage and botanical source (Ng *et al*., 2023). The total sugar content of honey is directly correlated with the total amount of soluble matter because of its high sugar saturation. Thus, there is a strong correlation between the total amount of soluble solids in honey and its overall sugar concentration (Ng *et al*., 2021, Manikis & Thrasivoulou, 2001, Smanalieva & Senge, 2009). The concentration of soluble matter varies among the different honey samples (M1-M5), with Brix degrees varying from 59.75 to 76.25. When comparing Sample M4 (carob honey) to the other samples (M1 to M4), the Brix degree indicates a higher concentration of soluble solids in Sample M4. This indicates that sample M4's honey contains more sugars or other soluble compounds than the other samples under examination.

Our results showed that each honey sample possesses unique properties depending on the region of origin. For example, carob honey from Tiznit has the lowest moisture content (15.45% ± 0.21) and the highest Brix degree (76.25 ± 1.02), while euphorbia honey from Ait Ba Amran has the highest moisture content (18.13% ± 0.14). Similarly, the levels of acidity and saponification indices vary significantly between samples, highlighting the influence of local geographical conditions on the chemical composition and properties of the honey.

These differences underscore the importance of conducting regional studies to better understand and value the specific characteristics of Moroccan honey.

Overall, the composition of honey exhibits significant variability due to a combination of local and environmental factors. Key contributors include the bee species responsible for honey production, the extraction techniques employed, and the specific floral sources from which bees collect nectar (Young & Blundell, 2023). Several critical parameters, such as moisture content, pH, and acidity, directly impact honey quality (Zapata-Vahos *et al*., 2023). Additionally, climatic conditions and the types of flowers available to bees play a crucial role in shaping the composition of honey. In the context of Moroccan honey production, diverse floral origins contribute to the rich tapestry of honey varieties (Abu-Tarboush *et al*., 1993). Moroccan honey production involves various types of floral origins (Devillers *et al*., 2004). Researchers have extensively explored these parameters, particularly in the northwest region of Morocco (Malika *et al*., 2005, Terrab *et al*., 2003).

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Moroccan honey production involves various types of floral origins (Devillers *et al*., 2004). Researchers have extensively explored these parameters, particularly in the northwest region of Morocco (Malika *et al*., 2005, Terrab *et al*., 2003).

Table 2. Physicochemical characteristics of honey samples.

Sample	M1	M2	M3	M4	M5	Norm*
Moisture content (%)	17.07 ± 0.53 [a]	18.13 ± 0.14 [b]	17.00 ± 0.01 [a]	15.45 ± 0.21 [c]	16.32 ± 0.28 [d]	≤ 20 (Young and Blundell, 2023)
pH	4.03 ± 0.13 [a]	4.55 ± 0.17 [b]	4.05 ± 0.28 [a]	3.90 ± 0.24 [a]	4.56 ± 0.17 [b]	3.3 - 4.6 (Benaziza-Bouchema & Schweitzer, 2010)
Refractive index	1.4910	1.4870	1.4900	1.4935	1.4925	1.4865 (Bogdanov *et al*., 2002)
Acidity (mgKOH/g)	31.70 ± 0.56 [a]	35.41 ± 0.36 [b]	33.07 ± 0.63 [a]	39.65 ± 0.17 [c]	37.21 ± 0.62 [c]	≤ 50 (Young & Blundell, 2023)
Saponification (mgKOH/g)	7.55 ± 2.34 [c]	6.81± 2.97 [b]	6.68 ± 1.03 [b]	5.64 ± 2.32 [a]	8.54 ± 2.56 [d]	-
Degree Brix°	60.75 ±0.92 [a]	59.75 ± 0.87 [a]	60.90 ± 0.97 [a]	76.25 ± 1.02 [c]	69.50 ± 0.59 [b]	≤ 83 (Conti *et al*., 2014)

*M1: cedar honey, M2: euphorbia honey, M3: eucalyptus honey, M4: carob honey, and M5: thyme honey. * The composition criteria of honey according to the Codex Alimentarius. Each value is the mean ± SD of three repetitions. The means in the same row, followed by the same letter(s) are not statistically different (p > 0.05) using the LSD test.*

3.2. Phytochemical Study Results

3.2.1. Phenolic Compound Analysis

To examine the phenolic compounds, present in the honey samples, the High-Performance Liquid Chromatography with Diode Array Detector (HPLC-DAD) method was used. The results obtained are documented in **Table 3** and were compared against standards based on retention time and ultraviolet spectra.

The results indicated the presence of various phenolic compounds. The most prevalent ones were found in all analyzed samples and ranged from 4.47 to 37.08% for chlorogenic acid and 19.43 to 59.52% for caffeic acid. Benzoic acid, which is only measured in M2, M3, and M4 and ranges from 17.23 to 27.49%, comes after these two acids. While M1 (euphorbia honey) and M5 (thyme honey) have higher percentages of caffeic acid (34.45 to 59.52%), the M4 (carob honey) and M3 (eucalyptus honey) samples have higher percentages of chlorogenic acid (36.12 to 37.08%).

Honey contains polyphenols primarily in the form of flavonoids, phenolic acids, and their derivatives. It is well known that these substances give honey its antimicrobial and antioxidant qualities [76]. These compounds can serve as chemical attractants for pollinators, like bees, in the kingdom of plants, and they also affect the flavor and color of honey in humans [77]. Numerous studies have emphasized the existence of different phenolic compounds, like gallic acid, caffeic acid, chlorogenic acid, and p-coumaric acid, that possess antioxidant qualities [65]. These substances have been linked to consequences like cytoplasmic and nucleotide leakage, increased permeability of the cell membrane, harm to the integrity of the cell membrane, and disruption of bacterial DNA binding and the cell membrane [78-80].

Contrasting our findings with the polyphenol content of Tuscany honey samples by Mattonai *et al*., [81], we have observed that similar results have been obtained for the p-coumaric and benzoic acids. Contrary to what we discovered in our Moroccan samples, the

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Tuscany samples showed significantly lower concentrations of coffee acid. Be aware that our Moroccan sample lacks rutin, except for sample M1 (cedar honey); rutin is found in multiple Tuscany samples, though. Different phenolic compounds, such as ferulic acid, sinapic acid, hesperidin, and quercetin, have been found in Tuscan honey samples, but have not been found in Moroccan samples.

Our findings indicate the distinct phenolic profiles of each honey sample, further illustrating the unique properties influenced by their geographical origins. For instance, caffeic acid is most abundant in thyme honey (M5) from Errachidia (59.52%), while chlorogenic acid is predominantly found in carob honey (M4) from Tiznit (37.08%). These differences highlight the significant variation in phenolic composition among the different types of honey, emphasizing the importance of regional studies to fully understand and utilize the unique characteristics of Moroccan honey.

The study by Tafere *et al*., [82] mentions that the composition of honey varies not only according to the floral source but also due to seasonal and environmental factors, which significantly affect its chemical composition.

Furthermore, the variation in the availability of specific flowers during different seasons can alter the levels of phenolic compounds and other antioxidants in honey, affecting its antioxidant capacity Tafere *et al*., [82].

We conclude that the floral, geographic, and climatic characteristics of a given place have a significant impact on the polyphenol content of bee products, including honey, royal jelly, and bee bread, as well as the chemical composition and floral origin of the bees Tafere *et al*., [83].

Table 2. Phenolic compounds in honey samples determined by high-performance liquid chromatography with diode array detection.

N°	Compound	TR (min)	Phenolic compounds (%)				
			M1	M2	M3	M4	M5
1	Gallic acid	2.91	10.17	12.53	17.33	1.56	1.1
2	Chlorogenic acid	3.83	9.96	27.94	37.08	36.12	4.47
3	Caffeic acid	4.84	34.45	29.55	19.43	37.19	59.52
4	Cinnamic acid	12.53	nd	nd	0.14	nd	4.00
5	p-Coumaric acid	21.81	nd	2.49	1.34	1.95	6.69
6	Naringenin	23.23	29.18	nd	7.25	nd	1.82
7	Rutin	25.27	16.24	nd	nd	nd	nd
8	Benzoic acid	26.51	nd	27.49	17.23	23.19	nd
9	Kaempferol	29.02	nd	nd	0.21	nd	nd

TR: retention time, nd: not detected, M1: cedar honey, M2: euphorbia honey, M3: eucalyptus honey, M4: carob honey, and M5: thyme honey.

3.2.2. Sugar content quantification

Fructose and glucose are the main sugars in each sample, as the table illustrates. In all the examined samples, trace amounts of sucrose in low concentrations were measured, ranging from 0.56 to 7.86 g/100g of honey. The primary sugars found in honey's sweet composition are fructose and glucose (Aljohar *et al*., 2018). The European regulations and the Codex Alimentarius set standards that these results meet. They concur with (Zammit Young & Blundell, 2023). According to **Table 2**, sample M2 (euphorbia honey) had the lowest fructose concentration (29.22% ± 0.81), while sample M4 (carob honey) had the highest fructose concentration (42.64% ± 4.15). The main sugar that gives honey its sweet flavor is fructose (Gomes *et al*., 2010). There are minor differences between these and Joshi *et al*.'s results

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(45.93%) (Joshi *et al*., 2000), Erjuwa *et al*. (2012) (21-43.5%), Kucuk *et al*. (2007) (7.7-43,9%), and the monofloral Ethiopian honey studied by Belay *et al*. (2017) (43.1%). This implies that the predominant sugar in naturally high-grade honey is fructose. **Table 4** shows the glucose levels in our study, which varied from (22.77% ± 0.20) to (32.96% ± 1.34). The glucose average that was highest in M4, carob honey, was noted. This finding may have multiple explanations, such as the production of honey from particular plant sources, bee feeding with syrup, or possible contamination of honey by artificial ingredients high in glucose (Damto *et al*., 2023).

Table 3. Sugar content in various honey samples (g/100g).

	M1	M2	M3	M4	M5	Norm*
Fructose	32.02±0.52 [a]	29.22±0.81 [b]	31.75±0.58 [a]	42.64±4.15 [c]	37.72±0.19 [d]	≥ 60 (Bogdanov *et al*., 1999)
Glucose	27.17± 0.08 [a]	22.77±0.20 [c]	24.80±0.17 [d]	32.96±1.34 [b]	30.53±0.47 [a]	≥ 60 (Bogdanov *et al*., 1999)
Sucrose	1.09±0.01 [a]	7.86±0.15 [d]	4.31±0.05 [b]	0.56±0.08 [c]	1.25±0.02 [a]	≥ 5 (Bogdanov *et al*., 1999)

**Composition criteria of honey according to the Codex Alimentarius; Each value is the mean ± SD of three repetitions. The means in the same row, followed by the same letter(s) are not statistically different (p > 0.05) using the LSD test.*

In comparison to other studies, Kucuk *et al.* (2007) and Amri *et al*. (2008), reported glucose concentrations ranging from 20.3 to 40.2% and from 34.9 to 40.2%, respectively. Our findings fall within these ranges, suggesting modest consistency. Notably, all the honey samples examined had glucose levels within the Codex's tolerance (Nombré *et al*., 2010). When glucose content exceeds 40%, honey tends to crystallize more quickly than when fructose content is higher (Damto *et al*., 2023).

3.2.3. Volatile compound analysis

The primary volatile compounds found in the different samples from GC-MS chromatography are presented in **Table 5**. Numerous volatile compounds were found to be present, with 2-Propene-1,1-diol diacetate being the most prevalent. It was quantified in all analyzed samples, with a range of 3.8 to 17.06%. Other compounds that were found to be present included hepta-2,4-dienoic acid, methyl ester and 2,2,4-Trimethyl-5-oxo-2,5-dihydro-3-furancarboxylic acid. Measuring 3,4-dihydro-2,5-dimethyl-2H-Pyran-2-carboxaldehyde, the range of 25.1 to 38.84% is limited to samples M4 and M5. Stearic acid, or octadecanoic acid methyl ester, is only measured in M5 at a 14.28% percentage. As a result, it is evident that every honey sample has a unique composition. Only the n-hexadecanoic acid, methyl ester, which is detected only in M1 with a very low percentage, and the octa decanoic acid methyl ester, which is detected in M1 with a low percentage but in M5 with a percentage of 14.28%, are saturated fatty acids.

Our findings indicate the distinct volatile profiles of each honey sample, further illustrating the unique properties influenced by their geographical origins. For instance, 3,4-dihydro-2,5-dimethyl-2H-Pyran-2-carboxaldehyde is most abundant in thyme honey (M5) from Errachidia (38.84%), while hepta-2,4-dienoic acid, methyl ester, is predominantly found in euphorbia honey (M2) from Ait Ba Amran (56.83%). These differences highlight the significant variation in volatile composition among the different types of honey, emphasizing the importance of

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regional studies to fully understand and utilize the unique characteristics of Moroccan honeys.

Table 4. Chemical volatile compound profiles analyzed by gas chromatography-mass spectrometry.

N°	Compound	TR (min)	Percentage of volatile compound				
			M1	M2	M3	M4	M5
1	N, N-Dimethyl-4-nitroso-3- (trimethylsilyl) aniline	4.40	nd	nd	nd	3.58	nd
2	Dimethyl 2-oxomalonate	7.47	nd	nd	nd	20.22	1.60
3	2-Propene -1,1-diol, diacetate	9.28	3.80	16.04	17.06	15.65	15.20
4	4-Octanol, 7-methyl-, acetate	9.46	nd	nd	nd	3.74	2.71
5	Butanedioic acid, dimethyl ester	10.27	nd	nd	nd	2.25	nd
6	n-Undecane	11.51	nd	nd	nd	6.04	nd
7	3,4-dihydro-2,5-dimethyl-2H-Pyran-2-carboxaldehyde	12.68	nd	nd	nd	25.10	38.84
8	Hepta-2,4 -dienoic acid, methyl ester	12.78	24.13	56.83	53.30	23.42	nd
9	2,2,4-Trimethyl-5-oxo-2,5-dihydro-3-furancarboxylic acid	14.43	14.11	21.10	26.85	nd	18.49
10	2-Propanol, 1,1-dimethoxy-, acetate	15.53	5.65	2.41	1.44	nd	0.50
11	alpha,-Methylene butyrolactone	15.75	3.49	0.38	nd	nd	nd
12	E-14-Hexadecenal	16.3	1.99	0.47	nd	nd	nd
13	Trimethyl citrate	16.41	1.54	1.27	0.93	nd	0.44
14	trimethyl -Silanol	16.97	1.18	0.64	nd	nd	0.22
15	Hexanedioic acid, 3-methyl-, bis (1-methylpropyl) ester	17.48	1.26	nd	nd	nd	nd
16	7-Dimethyl (prop-2-enyl) silyloxytridecane	17.64	1.19	nd	nd	nd	nd
17	Allyl (2-tetrahydrofurylmethoxy) dimethylsilane	18.04	1.43	nd	nd	nd	nd
18	1-Hydroxy-2,4-di-tert-butylbenzene	18.19	2.82	nd	nd	nd	nd
19	alpha,-Octadecene	18.98	2.72	nd	nd	nd	nd
20	1-Octadecanol	21.37	2.60	nd	nd	nd	nd
21	n-Hexadecanoic acid, methyl ester (palmitic acid)	22.86	2.21	nd	nd	nd	nd
22	Dibutyl phthalate	22.89	2.85	nd	nd	nd	nd
23	Octyl butyl phthalate	23.40	3.03	nd	nd	nd	nd
24	n-Eicosanol	23.53	2.44	nd	nd	nd	nd
25	1,2-Benzenedicarboxylic acid, bis (2-methylpropyl) ester	23.60	2.17	nd	nd	nd	nd
26	Pyruvic aldehyde dimethyl acetal	24.53	nd	0.58	0.40	nd	7.72
27	3-methyl -4-Hexen-2-one	24.65	10.47	0.28	nd	nd	nd
28	Octadecanoic acid, methyl ester (stearic acid)	24.89	2.38	nd	nd	nd	14.28
29	1-Docosene	25.52	4.82	nd	nd	nd	nd

Honey's aroma, flavor, taste, and texture are all known to be significantly influenced by the organic compounds found in honey (Manickavasagam *et al*., 2024). The entomological, geographic, and botanical origins of volatile organic compounds affect their type and composition; as a result, these compounds may serve as chemical markers (Manickavasagam *et al*., 2024). The development and growth of the human body depend heavily on fatty acids. Their roles include acting as structural elements of the lipids that make up cell membranes and taking an active part in processes like gene expression and intercellular communication (Jarukas *et al*., 2020).

3.2.4. Elemental Composition (EDXRF)

Five samples of natural honey were analyzed for their EDXRF spectra, and **Table 6** contains the elemental concentration results.

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Table 5. Mineral composition of honey samples determined by energy dispersive X-ray fluorescence.

Sample	Mineral (%)									
	K	S	Si	Ca	Cu	Pd	P	Hf	Ag	Fe
M1	25.60 ± 0.35 [d]	24.60 ± 0.76 [d]	20.76 ± 0.78 [c]	11.91 ± 0.61 b	8.72 ± 0.32 [a]	8.38 ± 0.56 [a]	-	-	-	-
M2	18.09 ± 0.45 [b]	18.23 ± 0.67 [b]	-	35.88 ± 0.87 [c]	-	-	13.07 ± 0.54 [a]	-	-	-
M3	19.78 ± 0.27 [c]	-	-	33.95 ± 0.53 [d]	10.79 ± 0.62 [a]	-	17.29 ± 0.23 [b]	18.17 ± 0.48 [b]	-	-
M4	18.90 ± 0.67 [b]	18.45 ± 0.56 [b]	28.40 ± 0.42 [d]	24.44 ± 0.47 [c]	9.79 ± 0.85 [a]	-	-	-	-	-
M5	17.64 ± 0.87 [e]	11.87 ± 0.42 [d]	13.50 ± 0.64 [d]	34.90 ± 0.48 [f]	5.90 ± 0.59 [b]	-	4.87 ± 0.21 [b]	-	9.36 ± 0.31 [c]	2.36 ± 0.04 [a]

K: potassium, S: sulfur, Si: silicon, Ca: calcium, Cu: cupper, Pd palladium, P: phosphorus, Hf: hafnium, Ag: silver, and Fe: iron; Each value is the mean ± SD of three repetitions. The means in the same row, followed by the same letter(s) are not statistically different ($p > 0.05$) using the LSD test.

Numerous minerals are present, according to the results, with calcium (Ca) ranging from 11.91 to 35.88% and potassium (K) ranging from 18.09 to 25.60% being the two most common ones. All samples had sulfur (S), except for M3 (eucalyptus honey). All samples showed copper, except for M2 (euphorbia honey). The sample with the highest potassium content, M1 (cedar honey), has a content of 25.60%, while M3 (eucalyptus honey) has a content of 19.78%. The quantification of calcium (Ca) is based on M2 (euphorbia honey), which has a high amount of Ca (35.88%), and M5 (34.90%).

Our results demonstrate the unique mineral composition of each honey sample, further emphasizing the influence of geographical origin. For example, cedar honey (M1) from Oulad Berhil has high levels of potassium (25.60%) and sulfur (24.60%), whereas thyme honey (M5) from Errachidia has significant amounts of calcium (34.90%) and silver (9.36%). These differences in mineral content highlight the importance of regional studies to better understand and utilize the unique characteristics of Moroccan honeys.

Ismail *et al*. (2021) conducted similar studies on Saudi Arabian honey. They found potassium (K) percentages ranging from 9 to 48.9%. The results we found fall within this range. However, they found low percentages of calcium (Ca) (between 1.6 and 5.57%), while we found higher percentages.

3.2.5. Antioxidant Activity

Antioxidants are of interest to researchers because of their potential to treat oxidative stress-related diseases and preserve food quality (Ouahabi *et al*., 2023). One of the quickest ways to evaluate the total activity of hydrogen and electron release from specific antioxidants, as well as health-promoting antioxidant dietary supplements, is the DPPH radical scavenging test (Beretta *et al*., 2005).

The variation of inhibition percentage with honey concentration (0- 40 mg/mL) is shown in **Figure 2**. The IC50 DPPH method values and the TAC method results are presented in **Table 7**. Ascorbic acid, a common antioxidant compound, is used for comparison.

The smallest IC50 value indicates a stronger antioxidant activity. Thus, based on the calculation of the inhibitory concentration at 50% and by comparison with the IC50 value of ascorbic acid, which is presented as a standard, it can be concluded that the analyzed samples exhibit notable antioxidant activity. Carob honey (M4) shows the highest antioxidant activity

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with an IC50 value of 5.20 mg/ml, followed by sample M1 (cedar honey) and M5 (euphorbia honey). These results are confirmed by the second TAC method, which also shows that carob honey (M4) has the highest antioxidant activity.

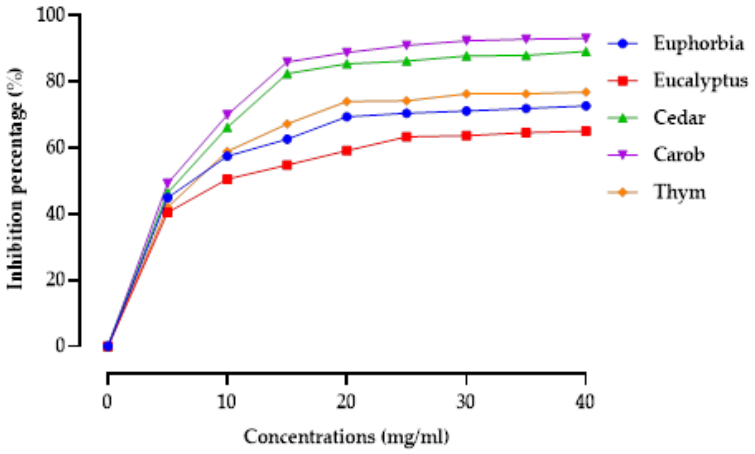

Figure 2. Percentage inhibition of DPPH at various honey sample concentrations.

Table 6. Antioxidant activity of the honey samples.

Sample	DPPH scavenging capacity IC_{50} (mg/mL)	Total antioxidant capacity*
M1	6.21 ± 0.74 ^{c}	183.16 ± 0.43 ^{d}
M2	7.89 ± 0.27 ^{d}	117.89 ± 0.95 ^{b}
M3	8.59 ± 0.82 ^{e}	104.51 ± 0.24 ^{a}
M4	5.20 ± 0.83 ^{b}	194.94 ± 0.82 ^{d}
M5	6.71 ± 0.62 ^{c}	164.50 ± 0.21 ^{c}
IC_{50} (AA)	0.34 ± 0.13 ^{a}	253.82 ± 0.36 ^{e}

** Total antioxidant capacity expressed in mM ascorbic acid equivalents/mg honey; Each value is the mean ± SD of three repetitions. The means in the same row, followed by the same letter(s) are not statistically different ($p > 0.05$) using the LSD test.*

Our results highlight the antioxidant capacities of different honey samples. Carob honey (M4) from Tiznit exhibited the lowest IC_{50} value (5.20 ± 0.83 mg/mL), indicating the highest DPPH scavenging activity, while it also showed the highest total antioxidant capacity (194.94 ± 0.82 mM ascorbic acid equivalents/mg honey). In contrast, eucalyptus honey (M3) from Agadir had the highest IC_{50} value (8.59 ± 0.82 mg/mL) and the lowest total antioxidant capacity (104.51 ± 0.24 mM ascorbic acid equivalents/mg honey). These variations underscore the significant differences in antioxidant properties among the various types of honey, influenced by their geographical and floral origins.

The results obtained for the identification of polyphenols by HPLC-DAD liquid chromatography indicate a slightly higher polyphenol content in carob honey compared to the other samples: chlorogenic acid (36.12%) and caffeic acid (37.19%). Similarly, for cedar honey (M1), this could be justified by the presence of rutin (16.24%) and caffeic acid (34.45%). This could explain why these samples show the highest antioxidant activity. Ascorbic acid, proteins, enzymes, flavonoids, phenolic acids, organic acids, and other microbiological components are among the substances in honey that have antioxidant properties. In human nutrition, it serves as a natural food source and antioxidant reservoir (Bouyahya *et al.*, 2018). Because of this quality, honey may be regarded as a nutraceutical that can be used as a food or medication (Socha *et al.*, 2009).

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Because it contains bioactive compounds, honey has strong antioxidant properties that are essential for scavenging free radicals and shielding the body from oxidative stress (Hameed *et al*., 2024). Aging, cellular damage, and several chronic diseases are linked to oxidative stress. Analyzing honey's antioxidant content sheds light on the substance's potential to lessen oxidative damage and lower risk factors for diseases like cancer, heart disease, and neurological disorders (Hameed *et al*., 2024).

4. CONCLUSION

In this study, we meticulously analyzed five distinct Moroccan honey samples, delving into their physicochemical characteristics, including water content, pH, acidity, saponifica-tion index, and Brix degree. Our findings not only allow us to evaluate the quality of these honeys but also establish rigorous standards that validate their superiority. The geographic and botanical origins of the honey significantly influence its properties. Our investigation revealed diverse phytochemical profiles in the samples, including volatile compounds and polyphenols. These compounds are important for human health due to their potent antiox-idant properties. Notably, carob honey stood out with exceptional antioxidant qualities. We assessed its antioxidant activity using both the TAC assay and the DPPH radical scavenging method. In summary, honey emerges as a natural treasure, brimming with remarkable therapeutic potential.

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6. AUTHORS' NOTE

The authors declare that there is no conflict of interest regarding the publication of this article. The authors confirmed that the paper was free of plagiarism.

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