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Antioxidant and anti-glycation activity of eight Moroccan honeys from different botanical origins

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Abstract

Eight monofloral honey samples from different botanical and geographical origins in the central and eastern parts of Morocco were examined for their phenolic composition, capacity to scavenge free radicals, and ability to inhibit advanced glycation end–products. According to the melissopalynological analysis, the examined honey samples were considered to be natural honeys, and were classified as monofloral with very frequent pollen grains characteristic of each types of the selected Moroccan honey samples: Zantaz (*Bupleurum* sp.), fennel, thistle, eucalyptus, spurge, thyme, jujube, and carob. Our results showed that the average moisture content varied from $16.3 \pm 0.1\%$ to $19.9 \pm 0.1\%$, and the mean color intensity ranged from 30.94 ± 7.42 to 166.9 ± 6.43 mm Pfund. The highest total phenolic content recorded was 163.83 ± 1.84 mg of gallic acid equivalent per kg, while the highest flavonoid content was 84.44 ± 1.20 mg of catechin equivalent per kg. Zantaz honey exhibited potent antioxidant capacity, as demonstrated by ABTS, DPPH, β-carotene bleaching, FRAP, and ORAC assays. The most significant results were obtained for the ORAC test, with the highest ORAC value of 4.65 mM Trolox equivalent per gram was recorded for Zantaz honey extract. HPLC–DAD analysis revealed different polyphenolic profiles, which can be attributed to differences in floral origin. Furthermore, our study revealed that the extracts of thistle honey, fennel honey, and Zantaz honey were the most effective glycation inhibitors, with the highest inhibition percentage of 96% obtained for the thistle honey extract.

Abbreviations

AAPH	2.20-Azobis (2-amidinopropane) dihydrochloride
ABTS	2,2'-Azino-bis(3-ethylbenzothiazoline-6-sulfonic acid)
AGEs	Advanced glycation end products
AUC	Area under the fluorescence curve
BSA	Bovine serum albumin
CE	Catechin equivalent
DNA	Deoxyribonucleic acid
DPPH	2,2-Diphenyl-1-picrylhydrazyl
FRAP	Ferric reducing antioxidant power

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GAE	Gallic acid equivalent
HPLC–DAD	High performance liquid chromatography with diode array detection
IC50	Concentration required to inhibit 50%
IHC	International honey commission
MG	Methylglyoxal
ORAC	Oxygen radical absorbance capacity
PBS	Phosphate buffer
RNA	Ribonucleic acid
TE	Trolox equivalent
TFC	Total flavonoid content
TPC	Total phenolic content
λ_{em}	Emission wavelength
λ_{exc}	Excitation wavelength

1 Introduction

Honey is a natural food produced by *Apis mellifera* bees from the nectar of flowers or from the honeydew that bees collect, process, and store in hives [1]. Its composition varies depending on its floral origin and climatic conditions[2]. Typically, carbohydrates, namely, glucose and fructose, constitute the main fraction and account for almost 60% to 70% of the total mass of honey [3]. In addition, honey contains minor fractions of other components, such as organic acids, vitamins, minerals, free amino acids, proteins, enzymes, volatile compounds and phenolic compounds derived from plants. Some of these compounds (e.g. organic acids, proline, enzymes) are used to assess honey quality according to standards specified by international commissions [4].

Morocco has significant and unique potential for beekeeping due to the diversity of its flora, fauna, and landscape, resulting in an estimated annual honey production of 7 960 tons [5]. However, in the last 5 years, there has been a stagnation or even a decrease in production, which contrasts with the increasing trend observed worldwide. This decline in Moroccan honey production has been attributed to several factors, including climate change and successive droughts over the past few decades, which have disrupted the flowering calendar and honey flow [6]. Moroccan honey production offers nine distinct flavors, including thyme, eucalyptus, euphorbia, jujube, and carob tree honey, as well as other types of honeys [6].

Scientific investigations on Moroccan honey have focused mainly on physicochemical and palynological characterization, color determination, and sugar composition, particularly in samples from northwestern Morocco [7–9]. Only a few studies have investigated the biological activities of Moroccan honey [10, 11], which have documented antibacterial and antimycotic effects as well as the ability to scavenge free radicals. These functional properties are closely related to the bioactive constituents, which may vary depending on their minor constituents. It is well known that honey may transfer many of the health benefits of plants [12–14]. Therefore, honey is considered to be a source of bioactive compounds, particularly carotenoids, phenolic compounds (flavonoids and phenolic acids), and other compounds [15–17], which are known to provide significant health benefits. For instance, honey samples from the Qinling Mountains (China) rich in caffeic acid was found to alleviate acute alcohol-induced liver damage in mice [18]. Furthermore, a significant and positive correlations were found between carotenoids content and antioxidant activity in 24 honey samples from the state of Rio Grande do Sul (Brazil) [19].

Oxidative stress caused by an imbalance between free radicals and antioxidants is generally responsible for cellular damage and the activation of various mechanisms, which are important causative agents of many human diseases, including cancer and atherosclerosis, as well as the aging process itself [20–22]. Thus, antioxidants that alleviate oxidative stress constitute a major line of defense regulating general health status [23]. In this context, the potential role of micronutrients as antioxidants (vitamin C, vitamin E, carotenoids, and phenolic compounds) has stimulated intense research efforts [24, 25]. Antioxidants in different types of honey demonstrate effectiveness in preventing the risk of several complications, such as heart disease, cancer, immune diseases, and various inflammatory processes [26].

Glycation plays a central mechanistic role in diabetes and accelerated aging disorders. The initiation and propagation phases of glycation reactions generate a significant amount of free radicals and carbonyl species such as methylglyoxal (MG). MG is considered a highly reactive carbonyl group that can induce dicarbonyl stress with damaging effects on normal physiological functions. This can lead to the development of diabetes, micro- and macroangiopathy, thickening of the

basement membrane due to collagen bridging, and other complications at the endothelial and immune levels [27, 28]. Glycation products are generally responsible for the production of toxic compounds for humans, such as malonaldehyde and acetaldehyde. These compounds can form abnormal complexes with biological substances, including DNA and RNA [29]. Recently, many authors have investigated the potential ability of polyphenols to delay or reduce the formation of MG and advanced glycation end–products (AGEs) [30].

In order to contribute significant information to the field regarding Moroccan honeys knowledge, the present paper compares the antioxidant capacities of phenolic extracts of eight honeys—Zantaz (*Bupleurum* sp.), fennel, thistle, eucalyptus, spurge, thyme, jujube, and carob—harvested from central and eastern parts of Morocco. We also evaluated for the first time the capacity of honey phenolic extracts to attenuate in vitro protein glycation.

2 Materials and methods

2.1 Honey samples

The eight Moroccan honey types were selected based on their reputation and popularity in Morocco. Honey samples were collected directly from beekeepers located in various regions of Morocco (Table 1) under the supervision of a bee expert from the National Institute of Agronomic Research (INRA) in Marrakech, Morocco. The number of collected honey samples was determined based on multiple criteria: (1) the availability of honey beekeepers producing the targeted honey type in each region, (2) the abundance or rarity of the selected honey type, and (3) the authenticity of the collected sample. Honey beekeepers were carefully selected from each region to ensure that the collected honey samples were sourced from the target flower species, ensuring their monofloral nature. The eight reputed Moroccan honey samples were stored in sealed containers at $25 \pm 2\,°C$ until analysis.

2.2 Moisture, color, and browning index

The moisture content and color of the honey samples were determined using standard IHC methods [31]. The color was evaluated spectrophotometrically at 635 nm. The mm Pfund values were obtained using the following formula:

$$mm\ Pfund = -38.7 + 371.39 \times Absorbance$$

The browning index was determined by measuring the absorbance at two wavelengths, 720 and 450 nm [32]. This parameter reflects the amount of melanoidins in the honey samples.

2.3 Melissopalynological analysis

Pollen analysis was carried out using the method of the International Commission of Bee Botany as previously described by Louveaux et al. [33]. A minimum of 500 pollen grains were counted and analyzed for each honey sample. The results expressed in terms of frequency allowed the classification of the analyzed samples into four classes according to the expression results proposed by the International Commission of Bee Botany: predominant pollen (more than 45% of the specific pollen detected), secondary pollen (16–45%), important minor pollen (3–15%), and minor pollen (less than 3%). The identification of pollen grains was performed using a reference collection and different bibliographic sources [34, 35]

2.4 Phenolic substances

The bioactive phenolic compounds present in the eight Moroccan honey samples were extracted using the method of Azar et al. [36] and Wahdan [37] with some modifications. Approximately 5 g of each honey sample was dissolved in 10 mL of acidified water (pH 2 with 0.1 M HCl) and added to 10 mL of ethyl acetate, the mixture was then stirred for 10 min and centrifuged for 20 min at 5000 rpm. This ethyl acetate extraction process was repeated three times. The top organic layer was collected and concentrated at 40 °C using a rotary evaporator. The resulting extracts were collected in methanol/water (v/v, 80%) except for the extracts used for the antiglycation study, which were recovered in reaction buffer instead of methanol/water.

Table 1 Geographical origin and main physicochemical properties of different Moroccan honey samples

Samples	No. samples	Harvest year	Geographical origin	Moisture (%)	Melanoidins (A.U.)	Pfund scale (mm)
Zantaz	2	2019	Ait bazza (North–east Morocco)	18.1 ± 1.12 b	1.031 ± 0.12 ab	166.90 ± 6.43 c
Fennel	3	2018	Rural municipality of Jaidat (Central Morocco)	17.2 ± 0.82 ab	1.417 ± 0.15 b	137.78 ± 3.05 cd
Thistle	2	2018	Rural community Bourrous (Central Morocco)	17.6 ± 0.29 b	1.440 ± 0.09 b	139.27 ± 4.89 cd
Eucalyptus	2	2018	Essaouira (West-central Morocco)	19.9 ± 0.21 c	0.650 ± 0.05 a	63.13 ± 3.87 ab
Spurge	2	2018	Mirleft (South Morocco)	19.5 ± 0.34 c	0.941 ± 0.06 ab	143.48 ± 3.96 cd
Thyme	1	2018	Rich (East Morocco)	16.3 ± 0.56 a	1.26 ± 0.13 ab	126.15 ± 6.85 c
Jujube	1	2019	Al Haouz (Central Morocco)	16.4 ± 0.47 a	0.60 ± 0.046 a	75.51 ± 8.41 b
Carob	2	2018	Demnat (Central Morocco)	17.5 ± 0.1 b	0.61 ± 0.021 a	30.94 ± 7.42 a
p value	–	–	–	*< 0.001*	*< 0.05*	*< 0.001*

The values are the means of at least two determinations ± standard deviation. Differences were not statistically significant if p > 0.05. Values in the same column with different letters are significantly different according to Tukey's multiple range test. Differences were considered to be statistically significant if p < 0.05, highly significant if p < 0.01, and very highly significant if p < 0.001

2.4.1 Determination of the total phenolic content

The phenolic content of the eight honey samples was evaluated using the Folin-Ciocalteu method as described by Singleton et al. [38] and expressed as gallic acid equivalents per kilogram of honey (mg GAE/kg).

2.4.2 Determination of total flavonoid content (TFC)

The flavonoid content of the eight honey samples was estimated using the method described by Zhishen et al. [39] with catechin serving as the reference compound. The total flavonoid content (TFC) was expressed as catechin equivalents (CE) per kilogram of honey.

2.5 Antioxidant capacities

To evaluate the antioxidant activity of the different honey extracts, we tested various methods with different mechanisms of action. For instance, we evaluated the inhibitory effect of our honey extracts on free radicals (DDPH and ABTS), the β-carotene bleaching capacity, the ferric-reducing antioxidant power (FRAP), and the oxygen radical absorbance capacity (ORAC). According to the results of the β-carotene bleaching test, linoleic acid can react with oxygen and reactive oxygen species, leading to the production of peroxyl radicals (LOO–). These peroxyl radicals can reduce the amount of β-carotene, resulting in the formation of a stable β-carotene radical [40]. On the other hand, the FRAP method evaluates the potential of honey extracts to reduce Fe^{3+} to intense blue Fe^{2+} in an acidic medium. The ORAC test involves measuring the decrease in protein fluorescence resulting from the loss of its conformation due to oxidative damage caused by a peroxyl radical source (ROO). This assay is commonly used to evaluate antioxidant capacity [41].

2.5.1 DPPH assay

The activity of the honey extracts in neutralizing DPPH free radicals was evaluated using the method previously described by Brand-Williams et al. [42]. In brief, 200 μL of the honey extracts was mixed with 3.800 μL of a methanolic solution of DPPH (0.06 mM) and incubated for 45 min. The absorbance of the obtained solutions was measured at 515 nm, and the results are expressed as the percentage of DPPH radical inhibition using the following equation:

$$DPPH\% = \frac{\left(A_{control} - A_{sample}\right)}{A_{control}} \times 100$$

2.5.2 ABTS assay

The activity of the extracts against ABTS free radicals was evaluated using the method of Rice-Evans [43] with some modifications. The free radical ABTS solution (0.007 M) was prepared using $K_2S_2O_8$ (2.45 mM). The absorbance of the different reactions was measured at 734 nm against a blank. The inhibition of ABTS radicals are expressed as a percentage of inhibition.

$$ABTS\% = \frac{\left(A_{control} - A_{sample}\right)}{A_{control}} \times 100$$

2.5.3 β-Carotene bleaching activity

The bleaching assay was performed according to the method described by Ou et al. [44]. To evaluate the bleaching activity of ß-carotene, an emulsion was formed by mixing ß-carotene, linoleic acid, and Tween 40, which was then incubated with honey extract at 50 °C. The percentage of ß-carotene was determined by measuring the absorbance at 470 nm.

$$\beta \text{ carotene\%} = \frac{\beta \text{ carotene content after 2h assay}}{\text{Initial } \beta \text{ carotene content}} \times 100$$

2.5.4 Ferric-reducing antioxidant assay

The ferric-reducing antioxidant capacity (FRAP) test was conducted following the method described by Yen and Chen [45], which involves the use of potassium ferricyanide, trichloroacetic acid, and $FeCl_3$. The absorbance was measured at 700 nm, and a higher absorbance indicates a greater ferric-reducing capacity of the honey extract.

2.5.5 Oxygen radical antioxidant capacity

The oxygen radical antioxidant capacity (ORAC) of the honey samples was estimated using the method described by Ou et al. [46]. The different extracts were recovered after drying in phosphate buffer to avoid fluorescein bleaching when using methanol, which resulted in a kinetic curve without decay. The ORAC assay was performed by mixing 1500 μl of fluorescein (32.5 nM) prepared in phosphate buffer (PBS) (12.6 mM, pH 7.2) with 750 μL of honey extract, blank or standard (Trolox, 0.02 mM) at 37 °C for 20 min. This was followed by adding 750 μL of 2,20-azobis (2-amidinopropane) dihydrochloride (AAPH) (400 mM in PBS), and the reaction was carried out at 30 °C. The fluorescence intensity was measured using a Shimadzu RF-6000 spectrofluorometer at 485 nm and 535 nm, corresponding to the excitation and emission wavelengths, respectively. ORAC values are expressed as mM Trolox equivalents (mM-TE) and were determined using the following equation:

$$ORAC\ (mM\ TE) = \frac{C_{Trolox} \times \left(AUC_{Sample} - AUC_{Blank}\right)}{\left(AUC_{Trolox} - AUC_{Blank}\right)} \times k$$

where C_{Trolox} is the Trolox concentration, AUC is the area below the fluorescence curve recorded, and K represents the dilution factor of each sample.

2.6 In vitro antiglycation activity

The capacity of honey extracts to minimize the glycation of bovine serum albumin (BSA) was evaluated using the method described by Foray et al. [47], with modifications in the excitation and emission wavelengths. Briefly, 1 mL of each honey extract (four concentrations: 1 to 8 mg/mL), 4 mL of fructose (1 M in 200 mM PBS, pH 7.4), 5 mL of BSA (20 mg/mL in PBS, pH 7.4), and 10 μL of sodium azide were mixed to form the reaction mixture, which was subsequently brought to a final volume of 20 mL with phosphate buffer. The reaction mixture was kept at 37 °C for one week, after which the fluorescence was measured at excitation and emission wavelengths of 250 and 345 nm, respectively. The percentage of AGE inhibition was calculated as follows:

$$Inhibition\% = \left(1 - \frac{FI_{extract}}{FI_{blank}}\right) \times 100$$

2.7 Phenolic compound analysis

A Shimadzu LC20 HPLC system equipped with a C18 column (4.6 × 250 mm, 5.0 μm) and a diode array detector for UV−visible absorption spectra was used for the analyses. The method performed most effectively in a linear gradient elution profile with a binary mixture of methanol (A) and formic acid diluted in water (1:19) (B) at a flow rate of 1 mL/min. Initially, the composition of the elution solution was 5% A maintained for 1 min, then increased linearly to 100% in 55 min and maintained for 4 min, the injection volume was 20 μL. Detection and chromatograms were recorded at 280 nm. The detected phenolic compounds were identified using standards.

2.8 Statistical analysis

The results are presented as the means ± standard deviations. All the assays were performed at least in triplicate. Statistical analysis, including correlations analysis and one-way analysis of variance (ANOVA), and Tukey's post-hoc test was performed using SPSS 18.0.0. The results were also subjected to a multivariate analysis (principal component analysis). Differences between means were considered to be statistically significant at the 95% confidence level ($p < 0.05$).

3 Results and discussion

3.1 Physicochemical characteristics

The moisture content of the honey samples ranged from 16.3 to 19.9%, which met the standard values specified by international commissions (moisture percentages below 20%). The maximum percentage of moisture in honey is generally controlled for safety purposes to prevent fermentation. This is the only parameter that, according to honey standards, must be respected for all honeys marketed worldwide [31].

In the present study, the colors of the honey samples ranged from extra white for thistle honey (30.94 ± 7.42 mm Pfund) to dark amber for Zantaz honey (166.9 ± 6.43 mm Pfund). The melanoidins content varied from 0.6 ± 0.046 for jujube honey to 1.26 ± 0.05 for thyme honey (Table 1). These parameters are important characteristics of honey that indicate the presence of certain bioactive components A higher Pfund scale value for a honey sample indicates a greater potential for bioactivity due to the presence of phenolic compounds [48]. The analysis of melanoidins in honey samples has received the attention of several studies. These studies aim to support the hypothesis, based on preliminary observations, that the production of melanoidins in honey is a fundamental process that partly explains its bioactivity [49]. In fact, all substances that are known to actively support honey bioactivities (sugars, polyphenols, and amino acids/proteins) are involved in the formation of melanoidins [49].

3.2 Melissopalynological analysis

Palynological analysis of the selected Moroccan honey samples revealed eleven pollen taxa (Table 2, Fig. 1); eight pollen taxa were very predominant, and three were considered secondary pollen. The pollen quantitative analysis indicated that the analyzed honey samples had pollen percentages greater than 45% for a single pollen type, as in the example of spurge honey, which presented 70% *Euphorbia resinifera*, these findings highlighted the monoflorality of the honey samples according to the recommendation of Louveaux et al. [33].

The results of the present work, showed that the eucalyptus and spurge honey samples are rich in different types of pollen. Four types of pollen were identified in the mentioned honeys, which are *Eucalyptus* sp. (66%), *Foeniculum vulgare* (1%), *Bupleurum* sp. (30%), *and Erigeron uniflorus* (1%) for the eucalyptus honey, *and Euphorbia resinifera* (70%), *Erigeron*

Table 2 Main pollen taxa in the different types of Moroccan honeys

Pollen Taxa	Main pollen types of Moroccan honey samples (%)							
	Zantaz	Fennel	Thistle	Eucalyptus	Spurge	Thyme	Jujube	Carob
Thymus sp.	–	–	–	–	–	76	–	–
Eucalyptus sp.	–	–	–	66	11	21	–	23
Ceratonia siliqua	–	–	–	–	–	–	–	62
Foeniculum vulgare	–	80	–	1	11	–	–	10
Ziziphus lotus	–	–	–	–	–	–	84	–
Eryngium ilicifolium	–	–	66	–	–	–	–	–
Bupleurum sp.	62	–	8	30	–	–	–	–
Fam.: Asteraceae	–	10	–	–	–	–	–	–
Artemisia sp.	5	4	–	–	–	–	–	–
Euphorbia resinifera	–	–	–	–	70	–	–	–
Erigeron uniflorus	23	–	–	1	4	–	–	–
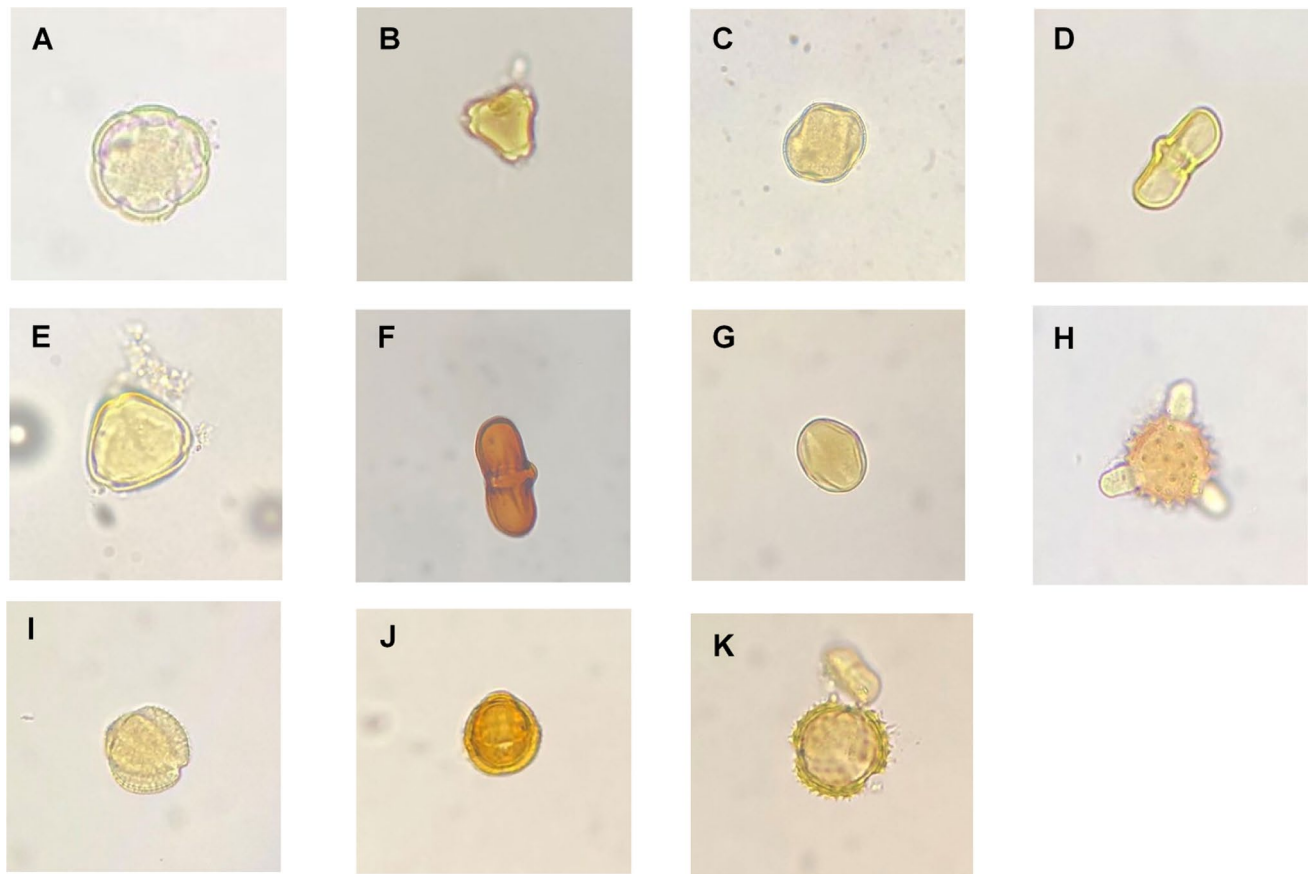
Fig. 1 Main pollen species identified in the selected Moroccan honey samples: **A** *Thymus* sp.; **B** *Eucalyptus* sp; **C** *Ceratonia siliqua*; **D** *Foeniculum vulgare*; **E** *Ziziphus lotus*; **F** *Eryngium ilicifolium*; **G** *Bupleurum* sp.; **H** Fam.: Asteraceae; **I** *Artemisia* sp.; **J** *Euphorbia resinifera*; **K** *Erigeron uniflorus*

uniflorus (4%), *Foeniculum vulgare* (11%)*, and Eucalyptus* sp. (11%) detected in the spurge honey sample. In addition, the occurrence of other important minor pollens was also observed, namely, those from the family Asteraceae and from *Artemisia* sp. (Fig. 1).

According to the palynological analysis, the examined honey samples were considered natural honey, and these samples were classified as monofloral with very frequent pollen grains characteristic of each type of the selected Moroccan honey samples.

3.3 Bioactive compounds and antioxidant activities

Natural antioxidants, particularly those aimed at reducing the potential damaging effects of free radicals, have received great attention[50]. Various methods have been employed to assess the antioxidant capacity of bioactive molecules obtained from the ethyl acetate extracts [51, 52].

Due to the lack of a standardized test for evaluating the antioxidant potential, the use of various methods to assess the behavior of honey bioactive compounds under different oxidation conditions is recommended. Each of these methods may provide specific information regarding the antioxidation mechanisms of our extracts. The antioxidant activities of all the honey extracts are expressed as IC50 (Table 3), which is the concentration that inhibits 50% of the oxidation. The DPPH assay was used to determine the scavenging capacity of the different extracts of the studied honey samples, showing IC50 values ranging from 9.24 ± 2.52 mg/mL for the Zantaz honey to 54.41 ± 5.14 mg/mL for carob tree honey. On the other hand, the eucalyptus honey extract exhibited the lowest scavenging activity for the ABTS radical (40.65 ± 30.41 mg/mL), while the highest activity was observed for the Zantaz honey. Compared to the DPPH assay, the Zantaz honey extract exhibited the highest ABTS activity (IC50 of 8.53 ± 1.44 mg/mL). The honey extracts inhibited the ABTS radical more effectively than the DPPH radical (Table 3). This could be due to the specific activity of the ABTS test,

Table 3 The total phenolic content (mg GAE/kg of honey), total flavonoids content (mg CE/kg of honey), and antioxidant activities (IC50, mg/ml) of the different honey extracts

Honey sample	TPC (mg GAE/kg of honey)	TFC (mg CE/kg of honey)	DPPH IC50 (mg/ml)	ABTS IC50 (mg/ml)	β-Carotene IC50 (mg/ml)	FRAP IC50 (mg/ml)	ORAC values (mM TE/g of honey)
Zantaz	163.83 ± 1.84 [f]	84.44 ± 1.20 [c]	9.24 ± 2.52 [a]	8.53 ± 1.44 [a]	3.02 ± 0.78 a	2.07 ± 0.15 [a]	4.65 ± 0.40 [a]
Fennel	107.35 ± 6.08 [de]	52.22 ± 11.02 [abc]	19.63 ± 2.94 [b]	8.62 ± 3.27 [a]	5.04 ± 2.40 [ab]	3.44 ± 0.33 [a]	0.36 ± 0.03 [b]
Thistle	86.17 ± 5.79 [cd]	60.89 ± 2.86 [abc]	32.22 ± 10.39 [b]	20.41 ± 5.06 [b]	17.40 ± 11.14 [bc]	7.30 ± 3.58 [ab]	1.29 ± 0.24 [c]
Eucalyptus	55.95 ± 5.79 [ab]	46.46 ± 2.34 [abc]	38.77 ± 29.13 [bcd]	40.65 ± 30.41 [bcd]	36.69 ± 11.41 [cd]	16.89 ± 3.34 [b]	0.25 ± 0.09 [b]
Spurge	63.15 ± 1.41 [bc]	40.50 ± 11.17 [ab]	45.16 ± 3.09 [c]	33.63 ± 2.72 [c]	8.77 ± 2.67 [b]	7.37 ± 2.66 [ab]	0.64 ± 0.38 [b]
Thyme	115.96 ± 8.48 [e]	71.15 ± 3.62 [bc]	16.59 ± 4.66 [b]	16.42 ± 0.96 [b]	4.69 ± 0.43 [ab]	3.57 ± 0.4 [a]	0.71 ± 0.26 [b]
Jujube	67.10 ± 2.55 [bc]	51.22 ± 10.42 [abc]	26.61 ± 9.91 [b]	19.38 ± 6.41 [bd]	11.80 ± 7.55 [b]	7.13 ± 0.34 [ab]	0.59 ± 0.07 [b]
Carob	35.62 ± 1.56 [a]	23.83 ± 4.07 [a]	54.41 ± 5.14 [d]	34.10 ± 8.83 [c]	15.14 ± 9.53 [bc]	8.48 ± 0.28 [ab]	0.68 ± 0.34 [b]
P value	< 0.001	< 0.01	< 0.01	< 0.01	< 0.01	< 0.05	< 0.05

The values are the means of at least two determinations ± standard deviation. Differences were not statistically significant if p > 0.05. Values in the same column with different letters are significantly different according to Tukey's multiple range test. Differences were considered to be statistically significant if p < 0.05, highly significant if p < 0.01, and very highly significant if p < 0.001

which assesses the radical scavenging capacity of compounds with both hydrophobic and hydrophilic properties [43]. Generally, the structural characteristics of the bioactive compounds in each honey extract are considered key elements in their bioactivity, as they can donate electrons or hydrogen ions [53].

The antioxidant proprieties of the Moroccan honeys determined using the β-carotene bleaching test and FRAP test are expressed as IC50 (mg/mL) and are depicted in Table 3. The lowest IC50 values were obtained for Zantaz, fennel, and thyme honey. However, the highest value was found in eucalyptus honey. It should be noted that the IC50 values obtained for our honey samples were lower than the IC50 values reported by Dine et al. [54] for various Moroccan honey samples. Similarly, the ORAC method showed that Zantaz honey has a significant protective potential for the fluorescein molecule compared to the other samples, as shown in the fluorescence decay curves in Fig. 2. As indicated in Table 2, the highest ORAC value was found for the Zantaz honey extract (4.65 mM TE/g), which confirmed its high antioxidant capacity. In general, the key advantage of the ORAC test over other assays is the production of peroxyl radicals, which have a redox potential and reactivity pathways that are similar to those of physiological conditions, and the use of physiological temperature and pH. As a result, antioxidant agents react with a charge and level of protonation that are comparable to those in the human body [55].

Correlation coefficients (r) were calculated to evaluate any potential link between the antioxidant tests results and the polyphenol content. These coefficients were calculated using 1/IC50 for the antioxidant activity tests. Transforming IC50 values into their reciprocal values (1/IC50) is a common practice in correlation analyses because it allows for a better comparison of the antioxidant capacities of different honey samples. By transforming the IC50 values, the higher antioxidant capacities are represented by higher values, which are more intuitive and easier to interpret in the context of correlation analysis.

High positive correlations were detected between the phenolic concentrations of the Moroccan honeys and two antioxidant activity assays: the DPPH assay ($r=0.917$) and the ABTS assay ($r=0.834$). Relatively weak correlations were found between the phenolic concentrations and the results of the other tests: β-carotene radical inhibition capacity ($r=0.605$), FRAP test ($r=0.729$), and ORAC test ($r=0.742$) (Table 4). These correlations suggest that phenolic compounds play a crucial role in the antioxidant capacity of the Moroccan honey samples.

Several significant positive correlations were detected between the color of the honey samples and their phenolic content, flavonoid content, and melanoidins, with correlation coefficients of 0.790, 0.732, and 0.760, respectively (Table 4). These results suggest that the greater the number of phenolic compounds is, the greater the intensity of the color. These findings are consistent with previous studies, such as those conducted by Adgaba et al. [56] and Ranneh et al. [57] on Cuban honey.

Fig. 2 Fluorescence decay curve during the ORAC assay in the presence of various types of honey
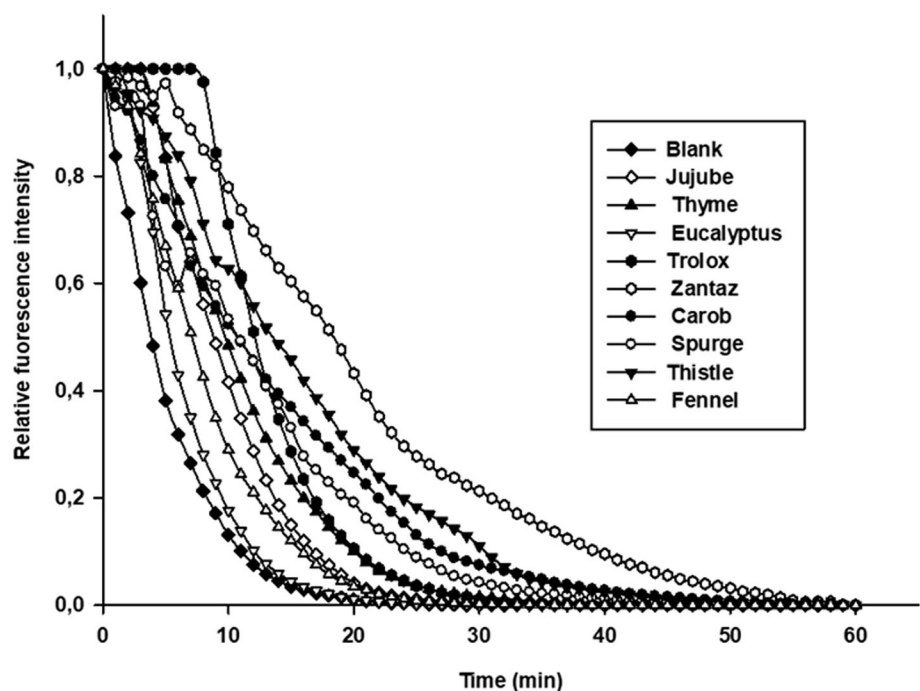
Table 4 Pearson correlation of phenolic content with antioxidant activity, color intensity, and melanoidins content

	TPC	TFC	DPPH	ABTS	β-Carotene	FRAP	Color	Melanoidins	ORAC
TPC	1								
TFC	0.929**	1							
DPPH	0.917**	0.916**	1						
ABTS	0.834*	0.727*	0.873**	1					
β-Carotene	0.605	0.411	0.523	0.759*	1				
FRAP	0.729*	0.559	0.659	0.884**	0.962**	1			
Color	0.790*	0.732*	0.655	0.651	0.596	0.641	1		
Melanoidins	0.576	0.521	0.518	0.634	0.454	0.576	0.760*	1	
ORAC	0.742*	0.676	0.528	0.495	0.386	0.481	0.513	0.105	1

*Statistically significant ($p < 0.05$), ** highly significant ($p < 0.01$) and *** very highly significant ($p < 0.001$). TPC: total phenolic content. TFC: total flavonoid content. Antioxidant activities (DPPH, ABTS, β-carotene, and FRAP) are expressed as 1/IC50 except for the ORAC assay which is expressed in mM TE/g of honey for correlation analysis

3.4 In vitro antiglycation activity

An important aspect of our study was the assessment of the potential inhibitory effect of various honey extracts on the generation of advanced glycation end-products (AGEs). Typically, the main source of highly reactive carbonyl species, such as methylglyoxal (MG), is the spontaneous modification of amino acids or proteins by reducing sugars. Excessive AGEs in food have been linked to many common diseases, including kidney disorders and diabetes mellitus [58, 59].

In our study, we were interested in determining the fluorescence intensities corresponding to the production of advanced glycation products under our conditions. In this context, we studied the fluorescence properties of AGEs formed after incubation of fructose and BSA solution for 7 days at 37 °C under experimental conditions similar to those of the physiological environment. Under these conditions, the variations in the emission spectrum relative to the excitation wavelength were determined and are presented in a 2D plot (Fig. 3). The plot shows a high emission fluorescence

Fig. 3 2D plot of the variation in the emission spectrum as a function of the excitation wavelength
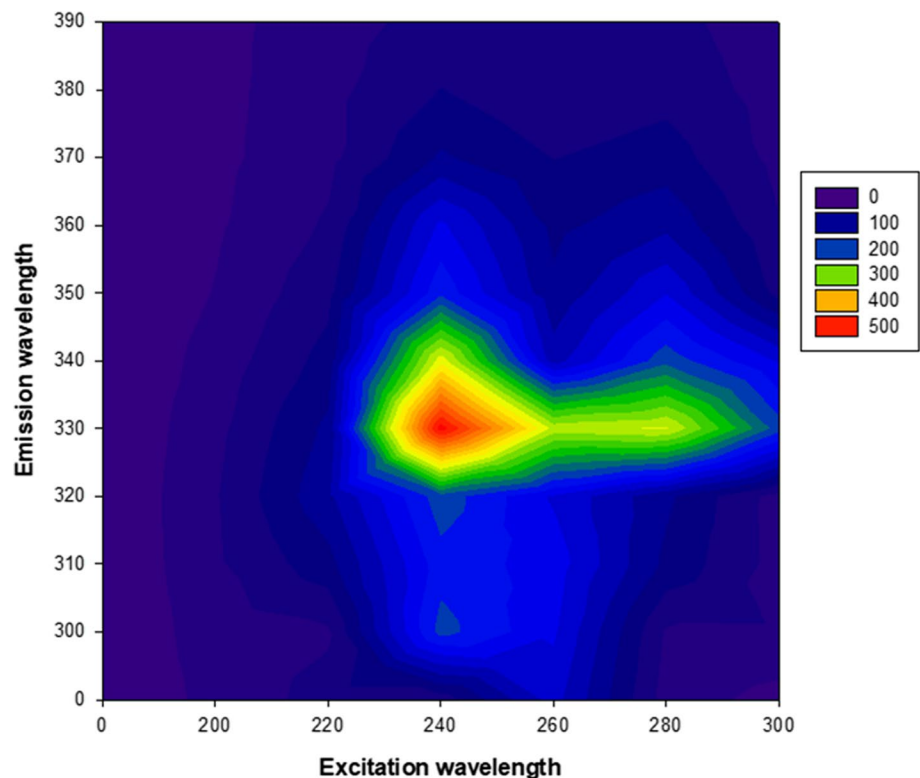
intensity between 320 and 350 nm during excitation between 220 and 295 nm, but the highest emission signal was obtained between 340 and 350 nm when using an excitation wavelength between 245 and 250 nm. Accordingly, to assess the antiglycation activity of our honey samples, we chose 250 nm as the excitation wavelength and 345 nm as the emission wavelength (λexc, 250 nm; λem, 345 nm).

These findings are similar to those reported by Aminjafari et al. [60], whose reported a maximum fluorescence emission intensity at AGEs between 320 and 345 nm during 270 nm excitation. However, several studies have been carried out to assess the formation of AGEs at excitation and emission wavelengths different than those reported in the present study [61–63]. These disagreements could be explained by the difference in the monosaccharide used (fructose or glucose) and, more specifically, by the difference in the proteins/amino acids involved in the formation of heterocycles via intra- and intermolecular crosslinking of AGEs [64]. Indeed, the type of BSA used in the glycation reaction can affect the nature of the formed AGEs. There are so many types of BSA commercially available and manufactured by different processes which could be confusing for scientists: which BSA fraction would be more suitable for a specific application?. Additionally, several methods exist for isolating BSA proteins from cow blood, which leads to two types of BSA (BSA without fatty acids and fatty acids). A study conducted by Ma et al. [65] demonstrated differences in the performance of different types of isolated BSA, which are due to the presence of bound fatty acids that improve the conformational stability of the BSA protein and give a negative charge to BSA protein, which influence its reactivity.

The results of our study on the capacity of honey extracts to inhibit the formation of AGEs are shown in Fig. 4. At a concentration of 8 mg/mL, the extracts of thistle honey (96%), fennel honey (88%), and Zantaz honey (77%) were found to be the most effective inhibitors of glycation, while the jujube honey extract had the lowest percentage of inhibition. Furthermore, we observed that increasing the concentration of Zantaz honey extract led to a proportional decrease in the fluorescence intensity at 345 nm, which is a characteristic wavelength of advanced glycation end-products, as shown in Fig. 5. The antiglycation activity observed in this study can be attributed to the bioactive compounds present in the analyzed honey samples, particularly polyphenols, which are abundant in honey [66]. Recent studies have attempted to explain the antiglycation capacity of polyphenolic substances based on their chemical and biological characteristics [67, 68]. For example, Yen [69] evaluated the ability of common flavonoids to inhibit AGEs (generation of hemoglobin Amadori products) and reported that quercetin, luteolin, and rutin were the most effective flavonoid compounds. Similarly, Xia et al. [67] reported that syringic acid had a greater anti-glycation effect than chlorogenic acid due to its high binding capacity to the amino groups of lysine, which can be attributed to differences in the molecular size of these compounds. These in vitro studies support the notion that phenolic compounds are effective at inhibiting the formation of AGEs. An important finding of this study was that honeys with high melanoidins content were more effective inhibitors of glycation

Fig. 4 The percentages of AGE inhibition by different honey extracts. The bars represent the standard deviation of the mean. Values with different letters are significantly different ($P < 0.05$)
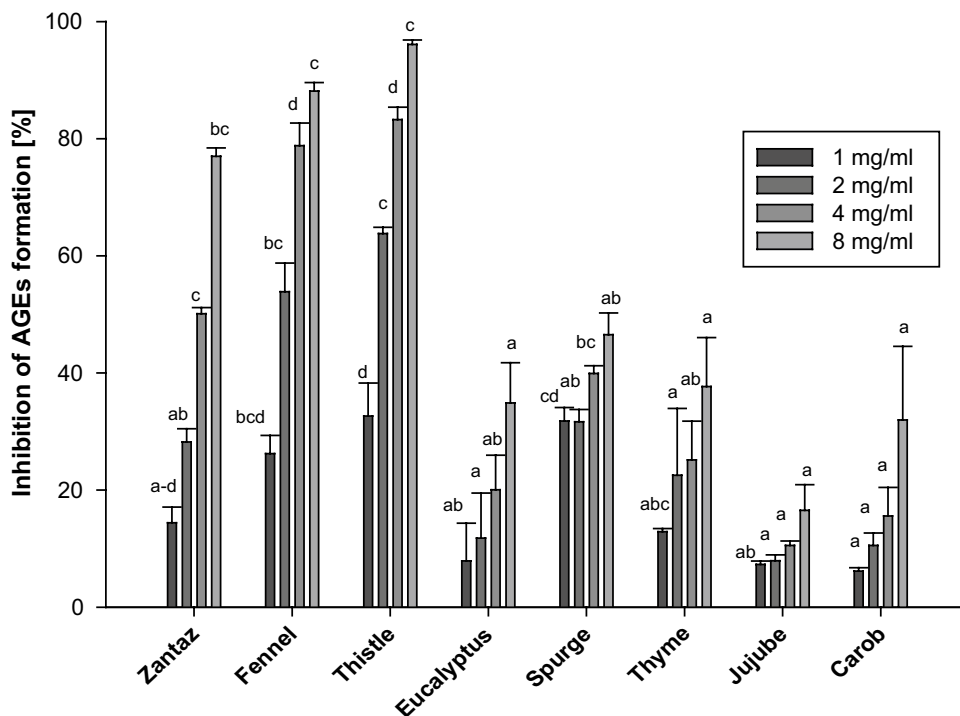
Fig. 5 Effect of different concentrations of Zantaz honey extract on the formation of fluorescent advanced glycation end-products at 345 nm
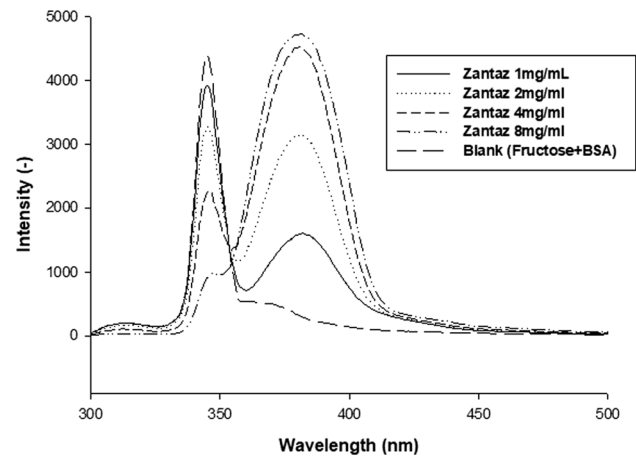
than those with low melanoidins content. This result emphasizes that although melanoidins are typically considered products of glycation reactions, their presence in honey does not necessarily negate their antiglycation activity. In our study, the observed antiglycation effect of honey with high melanoidins content can be explained by the involvement of polyphenols in the formation of honey melanoidins. Therefore, melanoidins acquire antioxidant and antiglycation activity because of the presence of polyphenols [70].

Good-quality honeys are known to contain higher amounts of melanoidins [71]. The antioxidant and antimicrobial activities of honey are significantly correlated with its melanoidin, phenolic, and flavonoid contents [72–74]. Additionally, melanoidins have been associated with the beneficial effects of nonenzymatic browning processes [75], which include the development of color and flavor in dried fruits [76]. Thus, the presence of melanoidins in honey contributes to its beneficial biological activities, including anti-glycation [77]. It is possible to use honey as a natural alternative to sulfites for controlling enzymatic browning during fruit and vegetable processing, as for juices and other products [78]. These scientific findings support the beneficial effects of melanoidins in honey; however, further studies are needed to draw consistent conclusions on the effects of these compounds in vivo.

3.5 HPLC–DAD analysis

HPLC analysis of the honey phenolic compounds revealed that the most abundant compound in all samples was ellagic acid except for thyme honey (Table 5). The obtained phenolic profiles are different from those reported in previous studies [79, 80]. Variations in the phenolic profiles of honeys from the same botanical origin can originate from several factors, including environmental factors, botanical variability, bee behavior and foraging, processing and storage, and the analytical methods used to assess the phenolic profile of honey [81]. Even within the same botanical origin, different

Table 5 Phenolic compound content in different Moroccan honey samples (mg/100 g)

Compound	Retention Time (min)	Zantaz	Fennel	Thistle	Eucalyptus	Spurge	Thyme	Jujube	Carob
Gallic acid	5.30	0.38 ± 0.01	–	–	0.14 ± 0.02	–	–	0.15 ± 0.02	0.19 ± 0.02
Catechin	12.30	–	1.30 ± 0.03	0.19 ± 0.01	0.09 ± 0.01	0.23 ± 0.01	0.26 ± 0.01	0.47 ± 0.03	–
Hydroxyphenyl acetic acid	14.50	0.66 ± 0.03	0.30 ± 0.02	2.17 ± 0.07	–	0.19 ± 0.01	0.38 ± 0.01	0.97 ± 0.02	1.20 ± 0.99
Caffeic acid	15.82	0.12 ± 0.01	0.05 ± 0.01	0.26 ± 0.02	0.13 ± 0.02	2.13 ± 0.25	0.11 ± 0.09	0.13 ± 0.01	0.24 ± 0.01
Vanillic acid	16.10	0.83 ± 0.03	0.26 ± 0.01	0.69 ± 0.05	0.80 ± 0.02	–	0.63 ± 0.01	–	0.44 ± 0.05
Epicatechin	16.60	–	0.30 ± 0.01	0.33 ± 0.02	–	0.03 ± 0.01	0.15 ± 0.02	–	–
Vanillin	18.55	0.33 ± 0.01	3.37 ± 0.44	0.74 ± 0.05	–	0.64 ± 0.03	0.26 ± 0.02	0.05 ± 0.01	0.20 ± 0.01
Ferulic acid	22.96	0.41 ± 0.01	0.54 ± 0.44	2.54 ± 0.05	0.19 ± 0.01	0.06 ± 0.01	0.07 ± 0.01	0.20 ± 0.01	0.07 ± 0.01
Ellagic acid	27.20	6.69 ± 0.13	3.68 ± 0.08	4.05 ± 0.05	2.39 ± 0.13	2.66 ± 0.10	0.97 ± 0.09	1.19 ± 0.04	1.28 ± 0.08
Quercetin	32.50	0.98 ± 0.03	0.61 ± 0.08	2.73 ± 0.24	1.24 ± 0.09	–	4.27 ± 0.17	0.25 ± 0.01	–

plant varieties or subspecies may produce nectar with distinct chemical compositions, leading to differences in phenolic content. The comparison of the phenolic compound profiles of the studied honey samples (Fig. 6 and Table 5) which exhibited high antioxidant and anti-glycation activity, such as those of Zantaz, thyme, and thistle honeys, provided some evidence that vanillin, ferulic acid, ellagic acid, and quercetin which dominate the phytochemical content of these honeys may be mainly responsible for their high bioactivity. In an in vitro experiment that simulated the initial glycation phase, the authors found that phenolic acids can all considerably prevent the initial reaction of glycation [30]. These phenolic compounds are present in relatively high concentrations, especially in thistle, Zantaz, and fennel honey samples. For example, the highest concentrations of ellagic acid were 6.69 ± 0.13, 3.68 ± 0.08, and 4.05 ± 0.05 mg/100 g in these three types of honeys, respectively. In addition, an experiment on protein crosslinking showed that ferulic acid and vanillic acid can reduce the production of AGEs [82]. Fennel honey is rich in ferulic acid, as shown in Table 4. Ferulic acid reduces the products that arise from the initiation of the Maillard reaction, as demonstrated in an in vitro fructose-protein model system [83].

3.6 Principal component analysis (PCA) for honey samples

PCA was also performed to determine the similarities and differences between antioxidant activities (DPPH, ABTS, β-carotene, FRAP, and ORAC assays), melanoidins content, in vitro anti-glycation activity, and phenolic compounds identified using HPLC–DAD. The combination of PCA accounted for 43.89% of the variance in F1 and 21.91% of the variance in F2 explaining 65.80% of the total data variance. Starowicz et al. [71] achieved a higher total data variance (82.13%), which could be attributed to the difference in parameters analyzed by these authors. The correlations between the original variables and the obtained principal components are shown in Fig. 7. Each of the variables is represented by a vector. The direction and length of the vectors indicate the extent to which the given variables affected the principal components. The melanoidins content, DPPH, ORAC, ABTS, β-carotene and FRAP results and concentrations of the main identified phenolic compounds were strongly correlated with the Zantaz, thyme, thistle and fennel honeys. The honey types most strongly correlated with anti-glycation activity were Zantaz, fennel and thistle honeys which are characterized by high melanoidins content as well as high concentrations of ferulic acid, ellagic acid and quercetin. PCA revealed two distinct honey groups: (1) Zantaz, thyme, fennel and thistle and (2) eucalyptus, carob, jujube and spurge. The first group is characterized by high biological activities, however, the second group is characterized by low concentrations of phenolic compounds and consequently low biological activities except
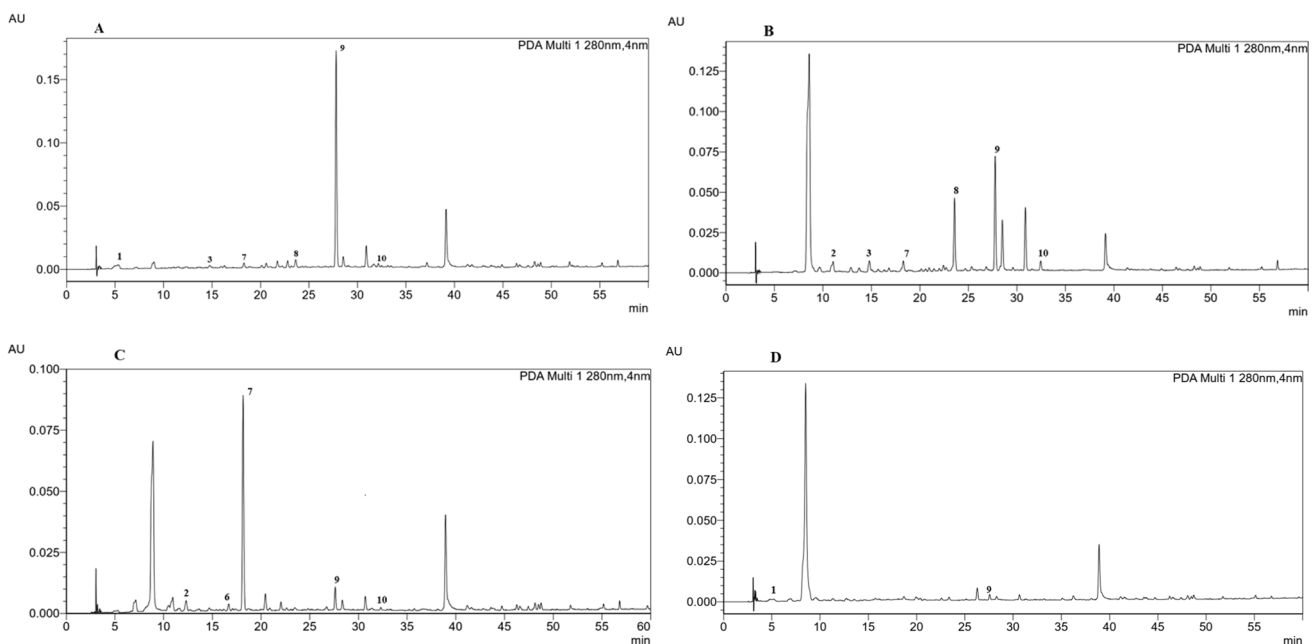
Fig. 6 HPLC chromatograms of different honey extracts detected at 280 nm: A chromatogram of Zantaz honey extract; B chromatogram of Thistle honey extract; C chromatogram of Fennel honey extract; D chromatogram of Carob honey extract. Peaks: 1. gallic acid; 2. (+)-catechin; 3. hydroxyphenylacetic acid; 4. caffeic acid; 5. vanillic acid; 6. epicatechin; 7. vanillin; 8. ferulic acid; 9. ellagic acid; 10. Quercetin
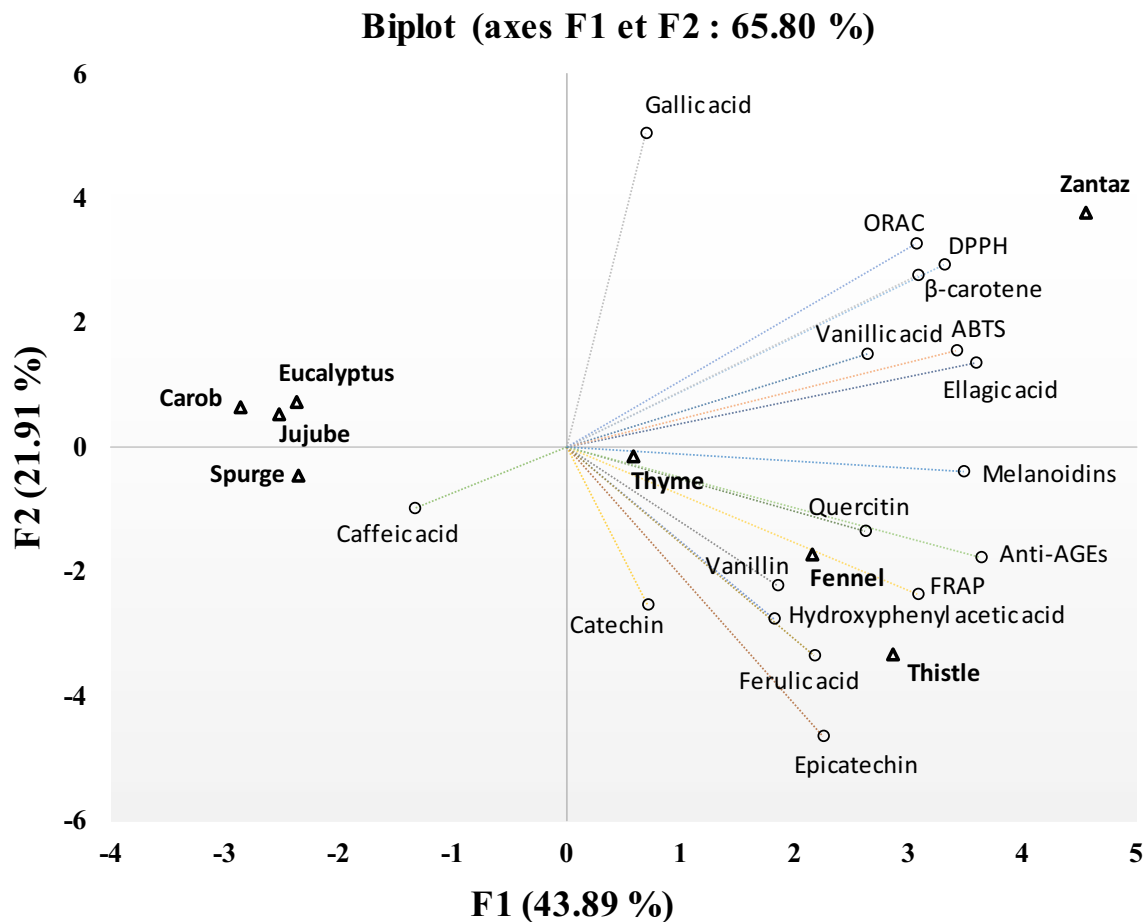
Fig. 7 PCA biplot for the Moroccan honey samples with the identified phenolic compounds and all the studied biological activities

for spurge honey which has significantly greater concentration of caffeic acid than to the other honey extracts. As previously reported by Laaroussi et al. [10], we also proved that melanoidins and phenolic contents are correlated with the biological activities of honey.

4 Conclusion

The analysis of the phenolic profiles of the samples that exhibited high antioxidant and antiglycation activities, such as those of Zantaz and thistle honey, revealed the dominance of vanillin, ferulic acid, ellagic acid, and quercetin. These phenolic compounds might be the main contributors to the high bioactivity of these honey samples. Additionally, the greater phenolic contents in darker-colored honey samples than in lighter-colored ones could provide valuable information for those who use honey for therapeutic purposes, as it could help classify different honey samples based on their color. The present study demonstrated that phenolic compounds found in Moroccan honey can inhibit the formation of advanced glycation end-products, which could potentially prevent the development of several metabolic diseases, such as diabetes, atherosclerosis, and Alzheimer's disease. The anti-glycation properties of honey could be beneficial for individuals with diabetes or those at risk of developing diabetes. It might help in managing blood sugar levels and reducing the formation of advanced glycation end products (AGEs), which are associated with diabetic complications. Moreover, our findings suggest that melanoidins may contribute to the antiglycation properties of honey by competing with proteins for binding sites on reducing sugars or by acting as scavengers of reactive carbonyl species that are involved in the formation of AGEs. However, further studies are required to fully understand the mechanism behind this phenomenon.

Declarations

Competing interests Authors has no conflict of interest to declare.

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