EVALUATION OF ANTIOXIDANT PROPERTIES AND PHYTOCHEMICAL COMPOSITION IN VARIETAL HONEYS

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ABSTRACT

A total of fourteen honey samples, including monofloral, polyfloral, and honeydew honeys, collected from Bulgaria, Turkiye, and Morocco were analyzed with the aim to investigate the influence of floral origin on the biological properties of honey.

The antioxidant activities of the samples were estimated using the DPPH, ABTS, FRAP, and CUPRAC methods, as well as analyzing their phytochemical composition by measuring the total phenolic content through the Folin-Ciocalteu method and individual polyphenols by HPLC.

The results demonstrated that honeydew honey exhibited the highest total phenolic content and antioxidant activity among all the samples tested. It was followed by linden and forest honeys, which also demonstrated significant biological activity. In contrast, acacia and ziziphus honeys displayed the lowest levels of phenolic content and antioxidant properties among the samples.

Our results indicated that while the botanical origin of honey had a significant influence on its biological properties, other factors such as geographical location also played a role in determining its quality. The observed variations among the honey samples from the same botanical origin could be attributed to factors such as specific landscape conditions, including whether the honey was sourced from an urban or rural area, the timing of harvest, beekeeping practices, and storage conditions.

Keywords: honey; antioxidant activity; phenolic content; radical scavenging activity.

INTRODUCTION

The investigation of bioactive foods and their chemical composition has gained significant research interest in the field of human health and nutrition. Biologically active components in diet such as polyphenols, minerals, and vitamins have been widely studied, along with their synergistic or antagonistic interactions, so that the antioxidant, microbiological, and antifungal activities to be evaluated. Among foods abundant in bioactive compounds, honey holds significant importance due to the presence of numerous redox-active substances. The properties of honey are attributed to its minor components, such as enzymes, ascorbic acid, carotenoid-like substances, organic acids, amino acids, proteins, minerals, and polyphenols, particularly flavonoids and phenolic acids [1].

Phenolics stand as one of a highly prevalent group of secondary plant metabolites, which contain one or more phenolic units in their structure. They can be classified into several categories, including simple phenols, phenolic acids (e.g. benzoic acid and cinnamic acid.), flavonoids, stilbenes and tannins, which are the main phenolic compounds found in the diet [2].

Moreover, those compounds have been proposed as putative markers for the estimation of botanical origin of honey [1]. Phenolic compounds possess redox properties that tightly correlate with their antioxidant potential, allowing them to function as reducing agents, hydrogen donors, or singlet oxygen quenchers [3]. As the plants differ by their naturally synthesized radical scavenging compounds, the biochemical profile of honey varies with the floral source used by bee for food. Genetic and physiological factors of the plants reflect on the composition of bioactive compounds in honey. Furthermore, honey originating from the same floral source but different locations may exhibit variations in composition due to environmental factors and soil properties [4]. Consequently, the quantity and type of bioactive components can vary widely based on the floral and geographical origin of honey, thus causing variations in AOA (antioxidant activity) observed in different honeys.

The main goal of this investigation was to analyze honey samples collected from fourteen specific floral sources in Bulgaria, Turkiye, and Morocco. The study aimed to assess their antioxidant properties and phytochemical composition. It is worth noting that there is limited existing data on the biological activity of the honeys being studied in this research. Therefore, this study contributes to fill this gap by providing new insights into understanding the relationship between the floral origin and the chemical and biological properties of the honey samples under analysis.

EXPERIMENTAL

Materials and methods

Honey samples

The present study involved analysis of monofloral, polyfloral and honeydew honeys collected from Bulgaria, Turkiye, and Morocco **(**Table 1**)**.

Five monofloral honeys from lavender, coriander, acacia, linden and tistle, as well as honeydew, forest and polyfloral honeys were purchased from Bulgarian manufacturers. The acacia and linden honey were collected in the region of Kazanlak. The lavender honey is collected between Maglizh and Gurkovo. Honeydew honey is obtained along the Karaagach River, near Kiten area. The polyfloral, forest and the coriander honey were harvested from the central Bulgaria in the Sredna Gora region. The tistle honey is from northeast Bulgaria in the Ludogorie region.

Tree polyfloral honeys were purchased from different regions of Turkiye, namely Yüksekova, in the Hakkari region of Turkiye, which is a mountainous area located in the most south-eastern part of Turkiye where grow number of endemic plants. Another Turkish honey is from Kayseri, a large industrialized city in Central Turkiye, rich in plants with high level of endemism. The third Turkish honey is from Muş region in eastern Turkiye, obtained from various flowers that grow in the plain of Muş and the mountains surrounding the plain.

The Morocco samples from euphorbia (*Euphorbia sp.*) and ziziphus honey (*Ziziphus lotus L.*) were harvested from the north-east part of the country. Argan honey was harvested from the region of Essaouira, Marakesh-Safi region, on the Atlantic coast, where is the location of the argan tree forests.

The varieties of honey were determined by the beekeepers based on availability of floral sources near the hive location.

Chemicals

6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid (Trolox) was obtained from Acros

Table 1. Description of botanical and geographical origin of honey varieties studied.

Sample	Botanical origine	Country
1	Lavender	Bulgaria
2	Acacia	Bulgaria
3	Linden	Bulgaria
4	Forest	Bulgaria
5	Coriander	Bulgaria
6	Honeydew	Bulgaria
7	Polyfloral	Bulgaria
8	Tistle	Bulgaria
9	Polyfloral	Turkiye, Muş
10	Euphorbia	Marocco, Nador
11	Argan	Marocco, Essaouira
12	Ziziphius	Marocco, Nador
13	Polyfloral	Turkiye, Yüksekova
14	Polyfloral	Turkiye, Kayseri

Organics, USA. 2,2′-azinobis-3-ethylbenzothiazoline-6-sulfonate (ABTS), 2-diphenyl-2-picrylhydrazyl hydrate (DPPH), 2,4,6-tri(2-pyridyl)-s-triazine (TPTZ), and GA (gallic acid) were acquired from Sigma Aldrich, Germany. The Fe (III) chloride salt, neocuproine, and potassium peroxodisulfate were obtained from Merck, Germany. 96 % Ethanol and HPLC grade methanol were sourced from J.T. Baker, Netherlands. Merck, Germany was also the provider of copper (II) chloride, sodium acetate, ammonium acetate, sodium carbonate, hydrochloric acid, acetic acid and the Folin Ciocalteu's reagent. Purified water (18 MΩcm), prepared by a MicroMed purification system (TKA Wasseraufbereitungssysteme GmbH, Niederelbert, Germany), was used to prepare all samples and standards.

Instruments

For the analysis of individual polyphenols, an Agilent 1100 Series chromatograph was utilized. This chromatograph was equipped with a degasser, a binary pump, a column Zorbax Eclipse XDB-C18 with specifications 4.6 × 150 mm, 5 μm, and a UV/Vis detector. Absorbance measurements and spectra recording were conducted using a PerkinElmer Lambda 15 UV-Vis spectrophotometer (PERKIN-ELMER, USA) with 1 cm optical path cuvettes. A Hanna Instruments, USA pH-meter was used for the pH measurements.

Determination of honey antioxidant activities and phenolic content

Sample preparation

2.5 g of honey was extracted with 25 mL of water in a shaking water bath until the honey was dissolved completely. After extraction step, the samples were filtered through a PTFE membrane filters 0.45 μm and analyzed on phenolic content and antioxidative activity [5].

Total polyphenols

The total polyphenolic content (TPC) was determined following the reaction with Folin-Ciocalteu (FC) reagent measured with the PERKIN-ELMER spectrophotometer at 760 nm against a gallic acid solutions calibration curve ranging from 1 to 10 μg mL^{-1} [6, 7]. A 5 mg mL^{-1} stock solution of gallic acid was prepared in ethanol. The measurements expressed in μg GAE g^{-1} were executed in triplicate.

Antioxidant activity evaluation

To evaluate the radical scavenging activity DPPH method was used as proposed from Brand-Williams et al. with slight modification [8]. A 2-diphenyl-2-picrylhydrazyl hydrate (DPPH) solution with concentration of 1.10^{-4} mol L^{-1} in methanol was prepared. An aliquot of 5.0 mL was put into 10 mL volumetric flask together with 0.4 mL of honey sample. The flask was filled to the mark with methanol. Thirty minutes after the start of the reaction the DPPH radical color change was registered at spectrophotometrically at 520 nm. A calibration curve with Trolox solutions was constructed based on the decrease in absorbance (ΔA = Ablank – A) corresponding to the DPPH radical scavenging activity. The results were expressed as microgram of Trolox equivalents (TE) per gram of honey sample (μg TE g^{-1}).

The ABTS activity was assessed following the protocol outlined by Re et al. and Arts et al. [9, 10]. Specifically, an aliquot of 0.2 mL of honey was blended with 3.9 mL of prepared ABTS solution. After allowing the reaction to proceed at room temperature for 6 min, the absorbance change was recorded at 734 nm. A calibration curve with Trolox solutions was constructed based on the decrease in absorbance (ΔA = Ablank - A) corresponding to the ABTS radical cation scavenging activity. The results were expressed as microgram of Trolox equivalents (TE) per gram of honey sample (μg TE g^{-1}).

The FRAP analysis was conducted following the Benzie and Strain`s procedure [11]. In summary, an aliquot of 1.0 mL of freshly prepared TPTZ reagent was combined with 0.2 mL of the honey sample and 2.8 mL of water to reach a volume of 4 mL. The absorbance was then measured at 595 nm after incubating for 5 minutes at 37°C. FRAP activity was reported as micrograms of iron (II) equivalents (FE) for gram of honey sample (μg Fe g^{-1}).

CUPRAC assay was performed according to procedure proposed by Moharram and Youssef [12]. To a test tube were added 1 mL each of Cu (II), neocuproine and ammonium acetate buffer solution, 0.3 mL honey sample and deionized water to final volume 4.1 mL. After 30 min, the absorbance at 450 nm was recorded against a reagent blank. The results were expressed as microgram of Trolox equivalents (TE) per gram of honey sample (μg TE g^{-1}).

Extraction of individual polyphenols

The extraction of individual polyphenols was performed according to the previously published procedure [13]. Around 3 g of honey samples were mixed with 4.5 mL of acidified demineralized water (pH 2.0 with concentrated HCl), put in the ultrasound bath until the liquefication. Then, these samples were put on the previously prepared (6 mL of the mixture consisting of methanol and demineralised water with their volumetric ratio 1:1) STRATA-X SPE cartridges (60 mg, 3 mL). After poring samples through these cartridges, the cartridges were washed with 2 mL acidified water and 5 mL ultrapure water to remove sugars and other polar honey constituents. Then the samples were dried using a vacuum for 13 min. The phenolic fraction remained on the cartridges and was washed with 2 mL of a mixture consisting of methanol and acetonitrile in the volumetric ratio 2:1. Phenolic fractions were then diluted twice with 10 mM sulfuric acid. Obtained clear solutions were transferred into glass bottles and left in the fridge.

HPLC-DAD analysis of individual polyphenols

For the HPLC analysis of individual polyphenols, the mobile-phase components were the same as used by Bertoncelj et al.: 1 % aqueous solution of formic acid (A), and acetonitrile (B) [13]. The mobile-phase gradient was as follows: 0 - 5 min: 10 % B; 5 - 50 min: 10 - 60 % B; 50 - 52 min: 60 - 80 % B; 52 - 60 min: 80 % B; 60 - 70 min: 80 - 10 % B; 70 - 80 min: 10 % B. Flow of the mobile phase was 0.5 mL min^{-1}, the column was thermostated at 25ºC, and the injected volume was 20 µL.

Individual polyphenols were identified by comparisons of their retention times and spectral characteristics with the standards.

RESULTS AND DISCUSSIONS

Since the honey is an ancient food which is considered as vital source of antioxidants, herein we assessed the putative antioxidant activity of different honeys.

Total polyphenolic content (TPC)

Phenolic compounds have gained significant attention with their role in balancing the deleterious effects of oxidants, and thus, to combat the oxidative stress-mediated disorders.

However, many researchers have found that the total phenolic content in honey is typically low and depends on the composition of nectar from the predominant plants involved in its production [14 - 16].

Table 2 displays the TPC measured in honey varieties examined in this study. Based on the results present, it can be observed that the Folin-Ciocalteu method revealed variations in the total phenolic content among the tested honeys. The polyphenol content of the honeys under consideration varied between 3.6 to 7.9 µg GAE g^{-1}. Among the samples tested, honeydew honey exhibited the highest total phenolic content. Forest and linden honeys followed closely behind it. Conversely, samples from ziziphus, acacia and argan honeys displayed the lowest total phenolic content. Other researchers have also observed that linden samples contained higher total phenolic compounds compared to acacia honeys [17]. These findings indicate that acacia honey can be categorized as food with a relatively low polyphenol content.

The TPC values obtained in our study were lower than those reported by other researchers who analyzed honey from different sources. For instance, Gheldof et al. [18] provided information that the total phenols in acacia honey were 4.6 mg GAE/100 g, whereas

Table 2. Total phenolic content of tested honeys.

Sample No	Honey variety	Total polyphenols	
		µg GAE g^{-1}	RSD%
1	Lavender, Bulgaria	5.30	1.89
2	Acacia, Bulgaria	3.71	1.08
3	Linden, Bulgaria	5.80	1.72
4	Forest, Bulgaria	6.80	2.94
5	Coriander, Bulgaria	4.80	6.25
6	Honeydew Bulgaria	7.90	1.27
7	Polyfloral, Bulgaria	5.40	1.85
8	Tistle, Bulgaria	5.45	1.28
9	Polyfloral, Muş	4.30	2.33
10	Euforbia, Morocco	5.20	1.92
11	Argan, Morocco	3.87	2.33
12	Ziziphus, Morocco	3.65	0.55
13	Polyfloral, Yüksekov	4.59	0.87
14	Polyfloral, Kayseri	5.09	1.38

Marghitaş et al. [19] showed the values ranging from 2 to 39 mg GAE/100 g. If we take in account the variations in extraction methods, detection techniques, and instruments employed in different published studies, it is not surprising that comparing honeys based on their phenolic profiles poses a challenge. However, certain patterns can be observed in some instances, particularly regarding the ranking order of honey varieties regarding TPC content [20].

It is important to note that the Folin-Ciocalteu reagent (FCR) is not exclusively specific to phenolic compounds because it can be reduced by nonphenolic compounds such as vitamin C and reducing sugar present in honey [21]. Additionally, phenolic compounds react with FCR under basic conditions, typically adjusted to pH ~10 using a sodium carbonate solution.

In our work we observed that in general, darker honeys had higher phenolic content compared to pale ones. Pale honeys (lavender and acacia) typically contained approximately 3.7 mg GAE per gram, while dark honeys (honeydew, forest, and linden) have notably higher phenolic content. This trend has been observed in honeys from various regions and botanical sources [23 - 25].

Additionally, several studies have found a positive correlation between honey color and its total mineral concentration. Darker honeys tend to have higher mineral content compared to pale ones which suggest that minerals may play a role in the antioxidant activity of honey. From the other hand, Kähkönen et al. showed that antioxidant activity does not correlate with TPC [26].

Antioxidant activity

In addition to TPC, another aim of this study was to investigate the potential antioxidant activity of the honey collection. To achieve this objective, four methods were employed, primarily based on electron transfer (ET) or hydrogen atom transfer (HAT) reactions. Electron transfer reaction assays include the Trolox equivalent antioxidant capacity (TEAC/ABTS) assay, the Ferric Reducing Antioxidant Power (FRAP) assay, and the Copper Reducing Antioxidant Capacity (CUPRAC). The 2,2-diphenyl-1-picrylhydrazyl (DPPH) radical scavenging capacity assay involves both electron and proton transfer mechanisms.

As explained by many authors, a singular method could not adequately assess the antioxidant capacity of compounds. Presently, there is no universally acknowledged technique for evaluating the antioxidant activity, given that variations in experimental conditions result in significant differences across results with utilized methodologies [27 - 29]. Since the antioxidant activity correlates with reducing capacity, the conditions affecting the redox equilibrium in the solution can change measured AOA sensibly [30].

Electron transfer based assays, though based on a common mechanism, frequently exhibit differences in measured antioxidant capacities that could be due to variations in the working pH values required for different methods.

The pH values exert a significant influence on the antioxidant capacity. In the CUPRAC test, the redox reaction occurs at nearly neutral pH, in contrast to the acidic conditions (pH 3.6) of the FRAP test or the basic conditions (pH 10) of the Folin-Ciocalteu (FCR) assay. In acidic media, the reducing ability may be suppressed due to the protonation of phenolics. Conversely, under basic conditions, proton dissociation of phenolics could enhance the sample's reducing capacity [22].

Therefore, a clear correlation between Antioxidant Activity and Total Phenolic Content results may not always be apparent.

In addition to the influence of acidity, the electron transfer based assays are sensitive to redox potential of the solution. This sensitivity arises from the diverse chromogenic redox reagents employed in ABTS, FRAP, and CUPRAC methods, each possessing distinct standard potentials. Consequently, the number of compounds that can undergo oxidation by ABTS, FRAP, and CUPRAC varies according to the potential of the reagent. Furthermore, the Folin and FRAP technics quantify only hydrophilic antioxidants, whereas methods like DPPH target hydrophobic antioxidants [29, 31].

In summary, factors such as redox potentials, solvent, and pH influence electron transfer-based methods, resulting in discrepancies in the measured AOA values.

However, from the results obtained in this study we can note some general trends. All four methods consistently demonstrated that honeydew honey exhibited the highest AOA among all the honey varieties, while acacia and ziziphus honeys displayed the lowest AOA as can be seen in Fig. 1.

In general, honey samples that displayed greater

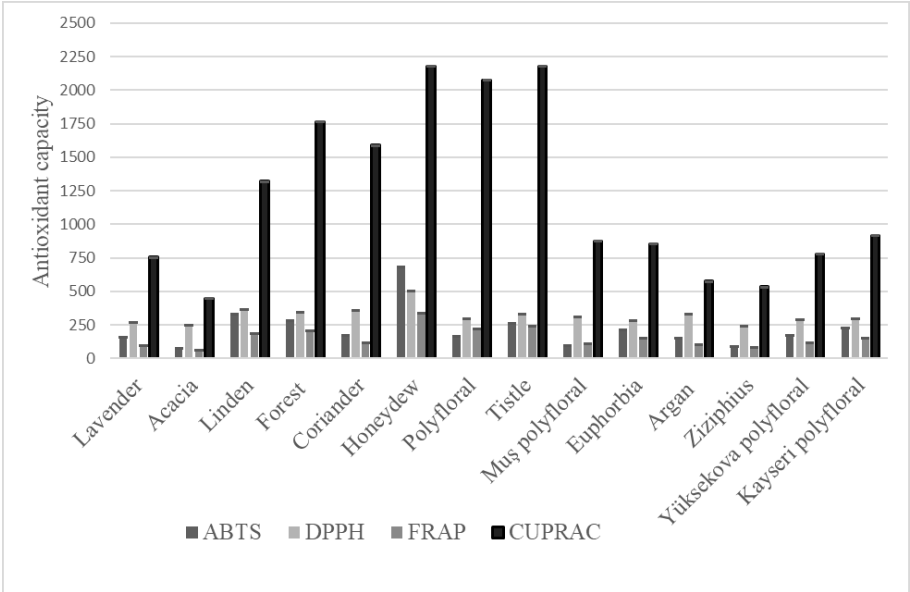

Fig. 1. Evaluated antioxidant activity of fourteen honey samples with variable origin, ABTS [μg TE g^{-1}], DPPH [μg TE g^{-1}], CUPRAC [μg TE g^{-1}], FRAP [μg Fe g^{-1}].

effectiveness in the DPPH reaction system also exhibited higher inhibition in the ABTS, FRAP, and CUPRAC systems.

The scavenging activity of honey samples was assessed using the DPPH assay and Trolox was used as a positive control. In this assay, the unpaired electron of DPPH combines with a hydrogen atom provided by the antioxidant present in honey, resulting in the conversion of the purple-colored DPPH radical to its reduced form, which appears yellow. To quantify the extent of decolorization, a UV-Visible spectrophotometer was employed. The results presented in Fig. 1 demonstrate that honeydew honey exhibited the highest scavenging activity, followed by linden, forest, coriander and argan honeys. It was found that lavender, acacia and ziziphus honeys displayed the lowest free radical scavenging activity [32]. Our results are consistent with the literature data where the highest DPPH radical scavenging activity was also determined in the honeydew honeys [33]. Additionally, Wilczyńska found that the lowest radical scavenging activity was reported for two samples of acacia honeys [25]. Moreover, another investigation also reveals that the acacia honey sample demonstrated the lowest values of total phenols and antioxidant activity [34].

The ferric reducing antioxidant power (FRAP) assay was employed to further analyze the antioxidant activities of honey samples. Unlike other analysis methods, the FRAP assay evaluates antioxidant power by measuring the capability of a sample to reduce $[Fe\ (III)\ (TPTZ)_2]^{3+}$ to $[Fe\ (II)\ (TPTZ)_2]^{2+}$, (TPTZ = 2,4,6-tripyridyl-s-triazine).

The FRAP activity for honey varieties ranged from 58.1 ± 0.06 μg TE g^{-1} to 335.1 ± 0.2 μg TE g^{-1}. Among the samples tested, honeydew honey exhibited the highest reducing antioxidant power, as measured by the FRAP test, with a value of 335.1 ± 0.2 μg TE g^{-1}. On the other hand, acacia honey showed the lowest reducing antioxidant power, with a measurement of 58.1 ± 0.06 μg TE g^{-1}. These results align with previous studies where the antioxidant activity, measured using the FRAP method, ranged from 95 to 2705 μmol TE kg^{-1} [35, 36]. Tomczyk et al. measured FRAP activity of honey varieties ranging from 0.64 mmol TE kg^{-1} for acacia honey to 2.32 mmol TE kg^{-1} for forest honey [37]. Similarly, Ibrahimi and Hajdari evaluated that forest honey exhibited a highest FRAP activity of 22.39 mg TE/100 g, while acacia honey, conversely, demonstrated the lowest activity with a value of 3.65 mg TE/100 g [5]. The analysis encompassed seven distinct honey varieties: forest, meadow, mixed, chestnut, acacia, lime, and pine. Additionally, Bertoncelj et al. reported FRAP values ranging from 71.0 ± 10.2 μM Fe(II) to 478.5 ±

95.5 μM Fe(II) for acacia, lime, chestnut, fir, spruce, multifloral, and forest honeys from Slovenia with highest values for forest and fir honeys and lowest value for acacia honey [23].

The radical scavenging activity of almost all honey types assessed by ABTS reaction system was considerably lower compared to the DPPH reaction, except for honeydew sample (Fig. 1). This observation suggests that the reducing capacity of honeydew honey may has a stronger influence compared to the hydrogen atom donating capacity of the active compounds present in this particular variety of honey. The sample with the lowest ABTS radical scavenging activity was identified to be acacia honey with 75.1 ± 0.01 μg TE g^{-1}, while honeydew honey exhibited the highest activity with 685.8 ± 0.07 μg TE g^{-1}.

The results obtained from CUPRAC assay confirmed the findings obtained from the other electron transfer assays, namely ABTS and FRAP. Among the honey varieties tested, honeydew honey exhibited the highest reducing activity, followed by thistle and polyfloral honeys, while acacia honey demonstrated the lowest activity. Similarly, ziziphus and argan honey showed relatively low CUPRAC potential.

It is worth noting that the CUPRAC results displayed the highest values compared to the DPPH, FRAP, and ABTS assays used in this study. This trend has been observed by Minkova et al. in the analysis of wine samples [38]. One possible explanation is that the calibration graph for the CUPRAC test is linear over a wider concentration range, compared to the other methods [27]. Moreover, the redox reactions exhibit different kinetics due to the different inertness of the complexes. For instance, Cu (II) labile complexes exhibits faster kinetics compared to inert complexes of iron ions, which result in differences in the measured AOA with CUPRAC and FRAP methods [27].

Previous studies have observed a correlation between the color of honey and its antioxidant activity. Typically, darker honeys such as forest and honeydew exhibit higher antioxidant activity, while paler honeys like acacia display lower antioxidant activity. Ferreira et al. found that the highest DPPH scavenging activity was observed in dark honey, followed by amber and light honey [39]. Additionally, the total phenol content displayed a similar pattern.

Moreover, minerals, when complexed with phenolic compounds, can demonstrate synergistic effects on antioxidant capacity. Certain metals can serve as electron donors and stabilize their charges through polyphenolic structures [40]. Therefore, honeydew honey, which possesses high contents of phenolics, proteins, and minerals, exhibits the highest antioxidant capacity [33].

It is relevant to mention that a correlation between the phenolic content and antioxidant activity of the honey types was observed by Wilczyńska [25]. This can be attributed to the structural characteristics of phenolic compounds which include the number and positions of hydroxyl groups and the nature of substitutions in the aromatic rings which characteristics explain the interaction with free radicals [20].

Individual phenol compounds

The honey samples studied exhibited a wide variation of polyphenolic compounds. The predominant phenolic compounds found in some of samples have been gallic, caffeic, maleic acids and benzoic acid derivative whereas luteolin, naringenin, vanilic acid and galangin showed very low distribution across the honey samples Table 3.

Larsen et al. found gallic acid content in raw honey to be 1.86 ± 0.71 mg kg^{-1} [41]. Zhu et al. found a concentration of 0.657 ± 0.023 mg kg^{-1} for gallic acid in acacia honey and 1.450 ± 0.036 mg kg^{-1} for gallic acid in litchi honey [42].

Regarding the current research in the monofloral lavender honey, a total of eight phenolic compounds have been discovered. Between these compounds we found luteolin, naringenin, apigenin, and anthocyanin which play a crucial role in combatting cardiac toxicity by employing diverse mechanisms. These mechanisms primarily involve diminishing reactive oxygen species (ROS), curtailing lipid peroxidation, regulating mitochondrial permeability, and suppressing apoptosis [43, 44]. In Turkish polyfloral honeys, rutin has been found to be the prevalent phenolic compounds (Table 2). Rutin shows several pharmacological activities including antiallergic, anti-inflammatory and vasoactive, antitumor, antibacterial, antiviral, and antiprotozoal properties. Moreover, it has also been reported that rutin has hypolipidaemic, anticarcinogenic and antidiabetic effect [45].

Differences in the concentrations of polyphenol observed in various literature sources can be elucidated by the utilization of diverse pesticides, which have

detrimental effects on bee health. Furthermore, the extensive practice of supplementing bees with bee breads of varying quality contributes to these variations [46, 47]. In a similar way, Fakhlaei et al. discussed various forms of adulteration, highlighting the prevalence of sugar-based adulterants [48].

CONCLUSIONS

The assessment of total phenol content and antioxidant activity has become an essential tool for evaluating the quality of honey, considering its nutritional value and sensory characteristics.

Herein, the results obtained demonstrate variations in phenolic compositions and antioxidant capacity across examined collection of honey samples. Additionally, the correlation between the floral source of honey and its biological potency has been also revealed.

When evaluating the antioxidant properties of individual honey varieties, honeydew honey stands out for its significant content of phenolic compounds and strong AOA as assessed by testing systems.

By comparison of the honeydew honey with acacia and argan honeys, all employed methods indicate a higher level of Antioxidant Activity (AOA) and TPC in honeydew honey.

Additionally, darker honeys exhibited higher concentrations of polyphenols, leading to a correspondingly increased antioxidant capacity.

The consistent findings from our researches revealed correlations between the total phenolic profiles and antioxidant activity of the examined honey samples which is not surprising considering the similar chemistry of assessment tests applied.

It is noteworthy that methods like DPPH, ABTS, FRAP, and CUPRAC provide the opportunity to compare the antioxidant properties of the examined honey varieties, obtaining their relative antioxidant activity. Given the absence of one universal method for assessing the absolute antioxidant activity, it is advisable to evaluate it using several methods that assess different aspects, including electron- and proton-transfer capabilities.

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