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Floral origin, Antioxidant and Antimicrobial Activity of Some Floral Honey

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Keywords
Antimicrobial activity, Antioxidant activity, *Apis mellifera*, Honey, Physicochemical analysis

Abstract: The study was aimed to assess botanic origin, antioxidant activity, antimicrobial activity and physicochemical properties of four honey samples were collected in Turkey. The melissopalynologic results revealed that Sample 1 and 2 are polyfloral, and others are unifloral. Total contents of phenolics (116.47-1711.13 mg GAE/100g) and flavonoids (1.46-27.25 mg CAE/100g) were analyzed, and the antioxidant activities measured by ferrous ion chelating activity (FICA) (71.32-93.35%), hydrogen peroxide scavenging activity (HPSA) (SC_{50}: 217.8-1238.39 µg/mL), 2,2'-azino-bis (3-ethylbenzothiazoline-6-sulfonic acid) (ABTS) radical scavenging (SC_{50}: 20.15-27.14 µg/mL), 2,2-diphenyl-1-picrylhydrazyl (DPPH) radical scavenging activity (SC_{50}: 22.93-308.17 µg/mL) and ferric reducing antioxidant power (FRAP) (71.03-99.54%) differed notably. For comparison of the samples, butylated hydroxy anisol (BHA), rutin (RUT) and trolox (TRO) were used as standard antioxidant compounds. According to these results, samples showed more efficient antioxidant activity than TRO except for ABTS assay. Also, Sample 1 and 4 have higher FICA activity and Sample 2 and 3 demonstrated more FRAP activity than standards. Honey samples demonstrated an inhibitory effect on the growth of *Staphylococcus aureus* and *Escherichia coli*, according to agar diffusion assays and minimum inhibitory concentration (MIC) values.

Bazı Çiçek Balların Botanik Çeşitliliği, Antioksidan ve Antimikrobiyal Aktivitesi

Anahtar Kelimeler
Antimikrobiyal aktivite, Antioksidan aktivite, *Apis mellifera*, Bal, Fizikokimyasal analiz

Öz: Çalışma, Türkiye'de toplanan dört bal örneğinin botanik kökenini, antioksidan aktivite, antimikrobiyal aktivite ve fizikokimyasal özelliklerini değerlendirmek amacıyla yapılmıştır. Melissopalinolojik sonuçlar, Örnek 1 ve Örnek 2'nin polifloral, diğerlerinin ise unifloral olduğunu ortaya çıkardı. Toplam fenolik (116,47- 1711,13 mg GAE/100g) ve flavonoid (1,46-27,25 mg CAE/100g) miktarları belirlendi. Ayrıca, antioksidan aktiviteler demir iyon şelatlama aktivitesi (FICA) (%71,32-93,35), hidrojen peroksit giderme aktivitesi (HPSA) (SC_{50}: 217,8-1238,39 µg/mL), 2,2'-azino-bis (3-etilbenzotiazolin-6-sülfonik asit) (ABTS) radikal giderme (SC_{50}: 20,15-27,14 µg/mL), 2,2-difenil-1-pikrilhidrazil (DPPH) radikal giderme aktivitesi (SC_{50}: 22,93-308,17 µg/mL) ve demir indirgeme gücü (FRAP) (%71,03-99,54) ile değerlendirildi. Örneklerin karşılaştırılması için standart antioksidan bileşikler olarak bütil hidroksi anizol (BHA), rutin (RUT) ve troloks (TRO) kullanıldı. Bu sonuçlara göre, örnekler ABTS analizi dışında TRO'dan daha verimli antioksidan aktivite göstermiştir. Ayrıca, Örnek 1 ve 4 daha yüksek FICA aktivitesine sahiptir ve Örnek 2 ve 3, standartlardan daha fazla FRAP aktivitesi göstermiştir. Bal örneklerinde yapılan agar difüzyon analizi ve minimum inhibitör konsantrasyon (MIC) değerleri; bu örneklerin *Staphylococcus aureus* ve *Escherichia coli*'nin gelişiminde inihibe edici etkisi olduğunu göstermiştir.

1. INTRODUCTION

Honey is a crucial and unique substance derived from bees and plants. Turkey is located at the geographic crossroads of Europe, and Asia and has wide range climates (temperate climate, arid type climate, continental climate) [1]. Therefore, plant and animal diversity is also quite high. There are five different honeybee races (*Apis mellifera carnica*, *Apis mellifera anatoliaca*, *Apis mellifera meda*, *Apis mellifera caucasica* and *Apis mellifera syriaca*) in Turkey and these races adapted to the several climates and territorials [2]. In addition, Anatolia is one of the very fertile area of the world in terms of the plant species on it. The number of taxa in Turkey is around 11.400. The number of endemic taxa is about 3.700 and the rate of endemism is around 32% according to Flora of Turkey [3].When the consideration of this biodiversity in Turkey is not surprising that a wide variety of honey produced [4].

Honeys are natural food with antioxidant and antimicrobial activities, which has been used for decades for different curative purposes [5, 6]. Several studies have been reported the relationships between antibacterial and antioxidant activities and the content of phenolic and flavonoid compounds in honey [6]. Previous studies have found out that the botanical origin of honey is a significant point regulating honey's antioxidant and antimicrobial activities [7, 8]. For this reason, pollen analysis was performed from four Turkish honeys by mellisopalynological method. In addition, we determined the antioxidant and antimicrobial activities of honey. We investigated it to contribute to better perception of the relationship between antioxidant and antimicrobial activities of honey in terms of botanic origin. The study also aims to evaluate the use of honey samples in food or medicine as a result of physicochemical, antioxidant and antimicrobial analyzes.

2. MATERIAL AND METHODS

Four honey samples from different localities in Turkey (İspir-Erzurum; Sample 1, Fethiye-Muğla; Sample 2 and 3, and Bulancak-Giresun; Sample 4) were provided by individual beekeepers and kept at room temperature. These samples were collected from places where beekeeping activities are intense in Turkey (Fig. 1). All the reagents and chemicals used in the experiments were of analytical grade.

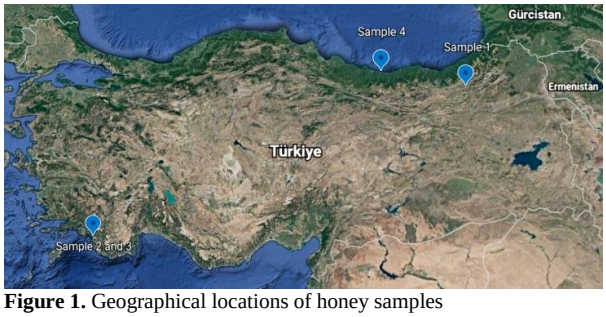

Figure 1. Geographical locations of honey samples

2.1. Mellisopalynological Analysis

10 g sample of each honey was analyzed following the conventional method without acetolysis [9]. Floristic arrangements of each honey sample were determined using Nikon Eclipse Ci. The next terms were used for density groups: >45%; predominant pollen, 16–45%; secondary pollen, 3–15%; important minor pollen and <3%; trace pollen [10]. The following equation (Eq. 1) was used to obtain the frequency per taxon.

$$Frequency = \frac{Total\ number\ of\ pollen\ of\ particular\ species}{Total\ number\ of\ observed\ pollen} x100 \quad (1)$$

2.2. Physicochemical Analysis

The pH of honey was measured directly using a pH meter (Ohaus, Starter 3100, USA). Refractometer (Abbe 60 Refractometer, Bellingham Stanley, Kent, UK) was used to measure directly the refractive index and total soluble solids (°Brix) at 20 °C. The moisture content was measured based on the refractometric method [11]. The analysis was performed in duplicate for each sample. The g/100 g moisture content values corresponding to the corrected refractive index values were calculated using Wedmore's table.

2.3. Preparation of Honey Sample Solutions

A 10 g of honey samples were added to 50 ml of distilled water and kept in a water bath until the samples dissolved. Finally, total volumes are adjusted to 100 mL by using distilled water.

2.4. Antioxidant Analysis

2.4.1. Total flavonoids content (TFC) assays

Total flavonoid contents of honey samples were assigned via colorimetric method with minor modifications [12]. In this assay, sample solutions (0.5 mL) were mixed with 1.5 mL of absolute ethanol. And then $AlCl_3.6H_20$ (0.1 mL, 10.0%) and potassium acetate (0.1 mL, 1.0 mol/L) were added. Distilled water was used for bringing the total volume to 5.0 mL. 30 min later, the absorbance values were read at 415 nm. The obtained results were calculated as µg catechin equivalent by using catechin standard calibration graphic (R^2=0.9979).

2.4.2. Total phenolics content (TPC) assays

Total phenolic content assays were done according to previous method [13]. For this assay, sample solutions (0.5 mL) were added to distilled water (7.0 mL) and Folin C reagent (0.5 mL), respectively. 3 min later, sodium carbonate solution (3.0 mL, 2.0 %) was mixed with this solution. Color development expected during 1 h and the absorbance values were recorded at 760 nm. For expression of the total phenolic contents, standard gallic acid graphic was used (R^2= 0.9995).

2.4.3. 2,2-Diphenyl-1-picrylhydrazyl (DPPH) radical scavenging activity assays

The 2,2-Diphenyl-1-picrylhydrazyl (DPPH) radical scavenging activity assays were carried out according to previous method with minor modifications [14]. For this reason, the diluted samples (3.0 mL) at the different concentrations (10-100 µg/mL) were mixed with stable DPPH radical solutions (1.0 mL, 0.2 mM in ethanol). The obtained mixtures were shaken vigorously and left to stand for 30 min at room temperature and in an unlit environment. Finally, absorbance values were measured at 517 nm. The radical scavenging activity results were calculated as SC_{50} (µg/mL).

2.4.4. Hydrogen peroxide scavenging activity (HPSA) assays

The hydrogen peroxide scavenging activities (HPSA) of samples were tested out by using spectrophotometric method [15]. According to this common method, the samples (3.4 mL) were added to hydrogen peroxide (0.6 mL, 40 mM prepared in the phosphate buffer solution (PBS)). The results were given as SC_{50} values (µg/mL) via absorbance values at 230 nm.

2.4.5. Ferrous ion chelating activity (FICA) assays

Ferrous ions chelating activities (FICA) were examined by using previous method [16] with slowly modifications. Briefly, the tested solutions (0.4 mL) added to $FeCl_2$ solution (0.05 mL, 2 mM) and kept at the room temperature for 10 min. Finally, ferrozine solution (0.2 mL, 5 mM) and pure ethanol (3.3 mL) were mixed with this solution, respectively and all tubes were vortexed about 5 min. After these mixing processes, absorbance values were recorded at 562 nm and FICA values were expressed as activity (Eq. 2).

$$Ferrous\ Ions\ Chelating\ Activity\ (\%) = \left[1 - \left(\frac{As}{Ac}\right)\right] \times 100 \quad (2)$$

2.4.6. 2,2'-azino-bis (3-ethylbenzothiazoline-6-sulfonic acid) (ABTS) radical scavenging assays

The 2,2'-azino-bis (3-ethylbenzothiazoline-6-sulfonic acid) (ABTS) radical cation scavenging assays were performed via spectrophotometric method [17]. In this experimental method, ABTS (2.0 mM) and potassium per sulfate (2.45 mM) was stirred to product ABTS•+. The fresh prepared solution was stand up for 16 h at room temperature and in an unlit environment. The absorbance of this solution was fixed to 0.750 ± 0.020 at 734 nm. Therefore, dilution process was realized by using the PBS (0.1 M, pH 7.4). The obtained ABTS radical cation solution is stable for 2 days. For determination of activity, the adjusted ABTS•+ (1.0 mL) was added to serially diluted sample solutions (3.0 mL) at the different concentrations (1-10 µg/mL in PBS). The activities were enounced as SC_{50} values (µg/mL).

2.4.7. Ferric reducing antioxidant power (FRAP) assays

The ferric reducing antioxidant power assays (FRAP) were exerted according to Oyaizu method [18] with minor modifications. Firstly, PBS (2.5 mL, pH 6.6, 0.2 M) and potassium ferricyanide (2.5 mL, 1.0%) were stirred with samples (2.5 mL), respectively. Later, the colored mixture solutions were incubated for 20 min at 50°C. Then, TCA (2.5 mL, 10%) were mixed with this solution. Lastly, this solution (2.5 mL) was stirred with $FeCl_3$ (0.5 mL, 0.1%) and distilled water (2.5 mL). The absorbance value of the obtained solution was recorded at 700 nm. The ferric reducing antioxidant power results were given as % (Eq. 3).

$$FRAP\ (\%) = \left[{}^{As}/_{Ac}\right] \times 100 \quad (3)$$

2.5. Antimicrobial Activity

2.5.1. Microorganisms and culture

Antimicrobial activities of pollen extracts were tested against *Staphylococcus aureus* (ATCC 29213), *Escherichia coli* (ATCC 25922), *Bacillus cereus* (ATCC 10876), *Candida albicans* (ATCC 10231) strains. The strains were stored in media containing 15% concentration of glycerol and kept at -80 °C before use. Bacterial and yeast cultures were incubated in Mueller-Hinton Broth (MHB) for 6 hours at 37°C. Each culture was then transferred to MHB and incubated at 37 °C for 24 h. A culture solution containing a 0.5 McFarland concentration was prepared.

2.5.2. Agar diffusion assay

Antimicrobial activities of pollen extracts was done by agar diffusion method [19]. Pollen extracts were prepared at a concentration of 83.3 mg/mL in ethanol. Ethanol was used as negative control. Each strain was inoculated on Muller-Hinton Agar (MHA) medium with sterile swabs. Each pollen extract solution (100 µL) and controls were dropped into 8 mm diameter wells in the medium. Microbial cultures were incubated at 37 °C for 24 h. After incubation, the diameter of the inhibition zone occurred around each well was measured and evaluated.

2.5.3. Minimum inhibitory concentration (MIC) assay

Minimum inhibitory concentration (MIC) was determined using the microplate method [20]. Each pollen extract (83.3 mg/ml) was diluted 50% with Muller-Hinton Broth medium and 50 µl of a microorganism culture solution was added to 12 wells of a 96-well microplate. Microplates were incubated at 37 °C for 24 h. The absorbance values of the samples after incubation were measured at 450 nm using an automated microplate reader (Multiskan FC, Thermo).

3. RESULTS AND DISCUSSION

3.1. Mellisopalynological Analysis

The palynological analysis of four honey samples produced in different biogeographical regions of Turkey was performed. Findings indicated huge diversity in the floral origin of samples. In addition, two samples were polyfloral honey, two were unifloral honey from distinctive floral sources such as *Medicago* and *Melilotus* sp. from Fabaceae. Pollen analysis supplied the description of 23 taxa belonging to 14 botanical families (Table 1). The most common are the ones from Fabaceae and Asteraceae family.

Table 1. The pollen spectrum in four honey samples collected from Turkey

Taxa	Sample 1	Sample 2	Sample 3	Sample 4
Asteraceae				
Achillea	2.7			
Anthemis		15.7		0.8
Artemisia	4.5	7.4	17.6	3.1
Circium			3.5	2.3
Senecio	4.5	1.9		
Taraxacum		5.6		
Apiaceae				
Daucus		2.8	2.4	0.8
Brassicaceae	3.6	5.6		1.5
Sisymbrium				0.8
Betulaceae				
Betula				0.8
Boraginaceae	2.7	2.8	1.2	6.9
Cynoglossum				4.6
Chenopodiaceae		0.9		
Cupressaceae				
Juniperus			1.2	0.8
Fabaceae	18			
Astragalus	19.8			
Lathyrus		29,6		
Lotus				16.9
Medicago			65.9	
Melilotus				57.7
Trifolium		6.5		
Vicia	15.3	1.9	5.9	
Juglandaceae			2.4	
Lamiaceae				
Stachys	6.3			
Thymus	22.5	3.7		0.8
Oleaceae		12		
Poaceae				0.8
Rosaceae				
Amygdalus		3.7		
Sanguisorba				0.8
Scrophulariaceae				
Verbascum				
Undetermined				0.8

3.2. Physicochemical Analysis

The moisture, refractive index (nd), brix, pH values of honey samples are showed in Table 2. The values obtained for the moustiure and pH of honeys are within the range reported previously for several honey types from different regions [21-23]. However, the brix results were lower than those reported studies. The high sugar concentration and low water content in honey are known to contribute to honey's antibacterial activity by helping to provide a protective barrier. Furthermore, mild acidity content in honeys have antimicrobial effects [8].

Table 2. The moustiure, nd, brix, pH values of honey samples

Honey samples	Moisture (%/100 g)	nd	Brix (10 g/L)	pH
Sample 1	21.8	1.482±0.003	76.96±1.27	3.93±0.16
Sample 2	20.4	1.485±0.004	78.43±1.53	4.6±0.04
Sample 3	19.8	1.487±0.008	78.9±2.16	4.15±0.2
Sample 4	23.8	1.477±0.002	74.93±0.58	3.64±0.08

The moisture content in all honey samples ranges between 19.8% and 23.8% (Table 2). The moisture content of honey can also vary between different types of honey. The low moisture content in honey can have a protective effect against microbial strains [24].

3.3. Antioxidant Analysis

The antioxidant activity of four honey samples was determined by various methods and was showed in Table 3. The total phenolic amount of samples was defined from 116.47 to 1322.22 mg GAE/100g. Rababah et al. (2014) [25] determined that total phenolic amount of honey samples from Jordan found 33.7- 86.3 mg GAE/100g. Gül and Pehlivan [26] declared that total phenolic amount of the 23 different monofloral honey samples from Turkey varied between 34.37 to 470.70 mg GAE/100g. The total phenolic compound content of the 67 honey samples from 14 municipalities of western Paraná ranged from11.39-61.27 mg GAE/100g [27]. Phenolic components are important in protecting various biomolecules of living cells. For this reason, it is recommended that these phenolic components be taken daily in the diet.

Table 3. The antioxidant activity of the four honey samples ([1] mg GAE/100g, [2] mg CAE/100g, [3] %, [4] SC_{50} µg/mL)

	TPC[1]	TFC[2]	FICA[3]	HPSA[4]	ABTS[4]	DPPH[4]	FRAP[3]
Sample 1	374.04	2.39	91.35	217.94	20.15	22.93	83.28
Sample 2	1711.13	27.25	71.32	247.43	27.14	24.74	99.16
Sample 3	1322.22	21.59	77.32	1238.39	25.11	25.01	99.54
Sample 4	116.47	1.46	93.35	232.8	20.3	308.17	71.03
BHA*			84.03	192.78	8.42	8.24	71.94
RUT*			86.17	122.07	15.54	16.27	92.1
TRO*			68.97	444.47	4.18	26.03	52.91

*Butylated Hydroxy Anisol (BHA), Rutin (RUT) and Trolox (TRO)

The flavonoid amount of samples was found between 1.46 to 27.25 mg CAE/100 g. Glahardo et al. [27] flavonoid contents of the honey samples analyzed varied from 7.97 to 44.99 mg QE/100g Temizer et al. [28]showed that the total flavonoid content of fifteen honey samples from Ordu in Turkey found between 1.65 and 38.75 mg CAE/100g. Bayram et al. [29] indicated total flavonoid content of sixty different honey samples

collected from Bayburt, ranged from 31.29 to 118.7 mg CAE/kg. İbrahimi and Hadjari [30] claimed that total flavonoid content of the one-hundred honey samples of different botanic origin ranged from 1.11 ± 0.62 to 7.51 ± 3.75 mg CE/100g. Flavonoids constitute the main class of polyphenols, one of the most researched compounds recently. Flavonoids have several important biological activities such as antioxidant, antimutagenic, anticancer, antiviral and anti-inflammatory [31]. Nowadays, many ingredients belonging to this class have been defined and many foods consumed daily are available [32].

We detected that the honey samples and standards showed between 22.93 to 308.14 and 8.24 to 26.03 µg/mL for DPPH activity, respectively. Sample 1, 2 and 3 have more DPPH activity than TRO Table 3). This activity experiment is very important in terms of measuring the activity of removing both endogenous and exogenous radicals in the body. Kıvrak and Kıvrak [33] disclosed that DPPH of 60 Turkish honey samples of nineteen different floral origins found between SC50: 54.33–99.40 µg/mL. Temizer et al. [22] claimed that DPPH activity of *Castanea sativa* honeys varied from 584.86±0.06 to 595.04±0.29 µg/mL. Alzahrani et al. [34] stated that DPPH activity of Manuka, Acacia, and Wild carrot honey ranged from 0.004±0.001 to 53.31±0.084 µg/mL in terms of SC_{50}.

ABTS activity in this study found between 20.15 to 27.14 µg/mL and the studied honeys showed less ABTS activity than the standards. All used standards showed influental ABTS activity than our samples (Table 3). The results obtained from this experiment are also important in terms of reducing the effect of radicals in living cells, as in DPPH radical scavenging activity. Kıvrak and Kıvrak [33] found 10.33–41.20 µg/mL in terms of SC_{50} for ABTS activity of honey samples. Alzahrani et al. [34] determined that ABTS activity of those honeys varied from 0.083±0.005 to 202.26±1.033 µg/mL in terms of SC_{50}.

FICA, FRAP and HPSA are very important values for antioxidant activities, but literature deal with these parameters of honey are not enough. Antioxidant activity of honeys in this study showed 71.32-93.35% for FICA, 217.94-1238.39% for HPSA and 71.03-99.54% for FRAP.

3.4. Antibacterial Activity

It was determined that all honey samples showed high antimicrobial activity against *E. coli* ATCC 25922 and *S. aureus* ATCC 29213 (Table 4). Among all microorganisms, *B.cereus* ATCC 10876 and *C.albicans* ATCC 10231 was most resistant against all honey samples. In the present study, Sample 2 (Muğla) and 3 determined high antibacterial activity on *E.coli* and *S.aureus.* Molan and Cooper [35] reported that the differences in antimicrobial effects between honeys may be related to their geographical, seasonal and botanical source. Moreover, some studies have reported that the variation in plant source in honey significantly affects honey's antibacterial activity [8].

Table 4. A diameter of inhibition zone (mm) and MIC (mg/ml) of four honey samples.

	C.albicans		*B.cereus*		*E.coli*		*S.aureus*	
	MIC (mg/ml)	Zone diameter (mm)	MIC (mg/ml)	Zone diameter (mm)	MIC (mg/ml)	Zone diameter (mm)	MIC (mg/ml)	Zone diameter (mm)
Sample 1	-	-	-	-	41.7	12	41.7	12
Sample 2	-	-	-	-	83.3	12	83.3	18
Sample 3	-	-	-	-	20.8	14	83.3	14
Sample 4	-	-	-	-	41.7	12	83.3	12

4. CONCLUSION

In this study found that honey content is rather variable, depending mainly on floral sources, providing its antioxidant and antimicrobial ability. Most of honey's antioxidant and antimicrobial activities are related to the floral origin, climate, and topographic diversity. In the present study, Sample 3 with the greatest inhibition power against *E.coli* strains showed a floral association of *Artemisia* typ and *Medicago*, *Medicago* being the dominant pollen. Sample 1 with the biggest barrier ability against *S. aureus* strains indicated being preeminent sources Fabaceae family and *Thymus* sp. While FRAP activity is higher in Sample 3, HPSA activity is higher in Sample 1 than others. Sample 2 had the hugest total phenolic and flavonoid amount, however showed not higher effect against *E. coli* and *S.aureus* strain than others. These results suggest that these honey samples have the potential for use in the human diet with physicochemical, antioxidant, and antimicrobial properties.

REFERENCES

[1] Öztürk MZ, Çetinkaya G, Aydin S. Climate Types of Turkey According to Köppen-Geiger Climate Classification. J Geog. 2017;35:17-27.

[2] Palmer M, Smith D, Kaftanoglu O. Brief communication. Turkish honeybees: genetic variation and evidence for a fourth lineage of Apis mellifera mtDNA. J. Hered.. 2000;91(1):42-6.

[3] Davis PH. Flora of Turkey. Flora of Turkey. 1965.

[4] Kılıç Altun S, Dinç H, Paksoy N, Temamoğulları FK, Savrunlu M. Analyses of Mineral Content and Heavy Metal of Honey Samples from South and East Region of Turkey by Using ICP-MS. Int. J. Anal. Chem.. 2017;2017:6391454.

[5] Israili ZH. Antimicrobial properties of honey. Am. J. Ther.. 2014;21(4):304-23.

[6] Bueno-Costa FM, Zambiazi RC, Bohmer BW, Chaves FC, da Silva WP, Zanusso JT, et al. Antibacterial and antioxidant activity of honeys from the state of Rio Grande do Sul, Brazil. LWT.. 2016;65:333-40.

[7] Taormina PJ, Niemira BA, Beuchat LR. Inhibitory activity of honey against foodborne pathogens as influenced by the presence of hydrogen peroxide and level of antioxidant power.Int. J. Food Microbiol.. 2001;69(3):217-25.
[8] Lusby PE, Coombes AL, Wilkinson JM. Bactericidal activity of different honeys against pathogenic bacteria. Arch Med Res.. 2005;36(5):464-7.
[9] Louveaux J, Maurizio A, Vorwohl G. Methods of melissopalynology. Bee world. 1978;59(4):139-57.
[10] Pound M, Dalgleish A, McCoy J, Partington J. Melissopalynology of honey from Ponteland, UK, shows the role of Brassica napus in supporting honey production in a suburban to rural setting. Palynology. 2018;42(3):400-5.
[11] AOAC. Association of Official Agricultural Chemists. Official methods of analysis (15th ed.): Arlington, VA; 1990.
[12] Chung Y-C, Chang C-T, Chao W-W, Lin C-F, Chou S-T. Antioxidative activity and safety of the 50 ethanolic extract from red bean fermented by Bacillus subtilis IMR-NK1. J. Agric. Food Chem. 2002;50(8):2454-8.
[13] Slinkard K, Singleton VL. Total phenol analysis: automation and comparison with manual methods. AJEV. 1977;28(1):49-55.
[14] Blois MS. Antioxidant determinations by the use of a stable free radical. Nature. 1958;181(4617):1199-200.
[15] Ruch RJ, Cheng S-j, Klaunig JE. Prevention of cytotoxicity and inhibition of intercellular communication by antioxidant catechins isolated from Chinese green tea. Carcinogenesis. 1989;10(6):1003-8.
[16] Dinis TC, Madeira VM, Almeida LM. Action of phenolic derivatives (acetaminophen, salicylate, and 5-aminosalicylate) as inhibitors of membrane lipid peroxidation and as peroxyl radical scavengers. Arch. Biochem. Biophys. 1994;315(1):161-9.
[17] Gökce H, Alpaslan YB, Zeyrek CT, Ağar E, Güder A, Özdemir N, et al. Structural, spectroscopic, radical scavenging activity, molecular docking and DFT studies of a synthesized Schiff base compound. J. Mol. Struct.. 2019;1179:205-15.
[18] Oyaizu M. Studies on products of browning reaction antioxidative activities of products of browning reaction prepared from glucosamine. J Nutr Diet. 1986;44(6):307-15.
[19] Rios J, Recio M, Villar A. Screening methods for natural products with antimicrobial activity: a review of the literature. J Ethnopharmacol. 1988;23(2-3):127-49.
[20] Wiegand I, Hilpert K, Hancock RE. Agar and broth dilution methods to determine the minimal inhibitory concentration (MIC) of antimicrobial substances. Nat. Protoc. 2008;3(2):163.
[21] Karabagias IK, Maia M, Karabagias VK, Gatzias I, Badeka AV. Quality and origin characterisation of Portuguese, Greek, Oceanian, and Asian honey, based on poly-parametric analysis hand in hand with dimension reduction and classification techniques. Eur Food Res Technol. 2020;246(5):987-1006.
[22] Temizer IK, Güder A, Temel FA, Cüce H. Antioxidant activities and heavy metal contents of Castanea sativa honey. Global Nest J 2018;20(3):541-50.
[23] Albu A, Simona-Maria C-M, Pop IM. Physicochemical parameters and spectral structure (ft-ir) of honey from iasi county (North-Eastern Romania). Scientific Papers: Series D, Animal Science-The International Session of Scientific Communications of the Faculty of Animal Science. 2019;62(2).
[24] Chirife J, Zamora MC, Motto A. The correlation between water activity and% moisture in honey: Fundamental aspects and application to Argentine honeys. J. Food Eng.. 2006;72(3):287-92.
[25] Rababah TM, Al-Omoush M, Brewer S, Alhamad M, Yang WD, Alrababah M, et al. Total Phenol, Antioxidant Activity, Flavonoids, Anthocyanins and Color of Honey as Affected by Floral Origin Found in the Arid and Semiarid Mediterranean Areas. J Food Process Pres. 2014;38(3):1119-28.
[26] Gül A, Pehlivan T. Antioxidant activities of some monofloral honey types produced across Turkey. Saudi J. Biol. Sci.. 2018;25(6):1056-65.
[27] Galhardo D, Garcia RC, Schneider CR, Braga GC, Chambó ED, França DLBD, et al. Physicochemical, bioactive properties and antioxidant of Apis mellifera L. honey from western Paraná, Southern Brazil. Food Sci. Technol.. 2020.
[28] Temizer İK, Güder A, Türkmen Z. Assessment of Palynological Characterization and Total Phenol-Flavonoid Content of Some Honeys from Ordu in Turkey. Erzincan University Journal of Science and Technology. 2019;12(3):1275-82.
[29] Bayram NE, Kara HH, Can AM, Bozkurt F, Akman PK, Vardar SU, et al. Characterization of physicochemical and antioxidant properties of Bayburt honey from the North-east part of Turkey. J. Apic. Res.. 2020:1-11.
[30] Ibrahimi H, Hajdari A. Phenolic and flavonoid content, and antioxidant activity of honey from Kosovo. J. Apic. Res.. 2020;59(4):452-7.
[31] Brodowska KM. Natural flavonoids: classification, potential role, and application of flavonoid analogues. Eur. J. Biol. Res. 2017;7(2):108-23.
[32] Terahara N. Flavonoids in foods: a review. Natural product communications. 2015;10(3):1934578X1501000334.
[33] Kıvrak Ş, Kıvrak İ. Assessment of phenolic profile of Turkish honeys. Int J Food Prop.. 2017;20(4):864-76.
[34] Alzahrani HA, Boukraa L, Bellik Y, Abdellah F, Bakhotmah BA, Kolayli S, et al. Evaluation of the antioxidant activity of three varieties of honey from different botanical and geographical origins. Glob J Health Sci. 2012;4(6):191-6.
[35] Molan P, Cooper R. Honey and sugar as a dressing for wounds and ulcers. Tropical Doctor. 2000;30(4):249-50.