https://doi.org/10.17221/63/2021-CJFS

Honey: Determination of volatile compounds, antioxidant and antibacterial activities

Semiramis Karlidag[1,2], Merve Keskin[3,4], Sinan Bayram[5], Nazli Mayda[6], Asli Ozkok[7]*

[1]*Bee and Bee Products Research and Application Center (MTOAGAM), Malatya Turgut Ozal University, Malatya, Turkey*
[2]*Akcadag Vocational School, Malatya Turgut Ozal University, Malatya, Turkey*
[3]*Vocational School of Health Services, Bilecik Seyh Edebali University, Bilecik, Turkey*
[4]*Biotechnology Application and Research Center, Bilecik Seyh Edebali University, Bilecik, Turkey*
[5]*Vocational School of Health Services, Bayburt University, Bayburt, Turkey*
[6]*Department of Pharmaceutical Botany, Pharmacy Faculty, Trakya University, Edirne, Turkey*
[7]*Bee and Bee Products Research and Application Center (HARUM), Hacettepe University, Ankara, Turkey*

**Corresponding author: merveozdemirkeskin@gmail.com*

Citation: Karlidag S., Keskin M., Bayram S., Mayda N., Ozkok A. (2021): Honey: Determination of volatile compounds, antioxidant and antibacterial activities. Czech J. Food Sci., 39: 208–216.

Abstract: Honey is an important functional food for human health and nutrition that is collected by honey bees and stored in the honeycombs. In this study, total phenolic content, antioxidant activity and volatile compounds of 13 different honey samples collected from various districts of Malatya province were investigated. As a result of this study, it was determined that the total phenolic content varied between 8.50 mg GAE 100 g^{-1} and 73.90 mg GAE 100 g^{-1} and it was observed that the honey samples were rich in aldehydes, aliphatic acid and esters, alcohols, hydrocarbons, carboxylic acid esters, ketones, terpenes, fatty acids and esters. In addition, the antibacterial effects of honey samples were determined against 18 different pathogenic bacteria using agar well diffusion (AWD) method, minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC). In AWD assay, it was recorded that inhibition zone diameters varied between 9 mm and 14 mm and honey samples were found to have a partial inhibitory effect against selected target pathogens.

Keywords: minimum bactericidal concentration; phenolic compounds; minimum inhibitory concentration; antioxidant activity

Honey is a bee-derived natural food item and the nutritive value of this supersaturated natural product as well as its prophylactic medicinal value have been known for many years (Kassim et al. 2010; Mohamed et al. 2010). This unique food product is rich in minerals, carbohydrates, organic acids, phenolic acids, flavonoids, vitamins, enzymes and other proteins (Pyrzynska and Biesaga 2009; Lachman et al. 2010). Its phenolic acids and flavonoids act as natural antioxidants (Pyrzynska and Biesaga 2009; Lachman et al. 2010). Due to its composition, honey is important for human nutrition and traditional medicine (Al-Mamary 2002). It is reported that honey is effective in the treatment of many diseases such as gastrointestinal disorders, treatment of wounds and burns, and providing stomach protection against acute and chronic gastric lesions (Al-Mamary 2002). The composition of honey differs primarily depending on the herbal source from which the nectar was collected. Therefore, in this study, honey samples were collected in Malatya. It is a city located in the east of Turkey and rich in botanical sources. The districts of Malatya are presented in Figure 1. The total phenolic content, antioxidant activity and chemical profile of different honey samples from different locations of Malatya were investigated. Effects of honey samples on 18 different microorganisms were determined (10 gram-positive bacteria and 8 gram-negative bacteria).

https://doi.org/10.17221/63/2021-CJFS

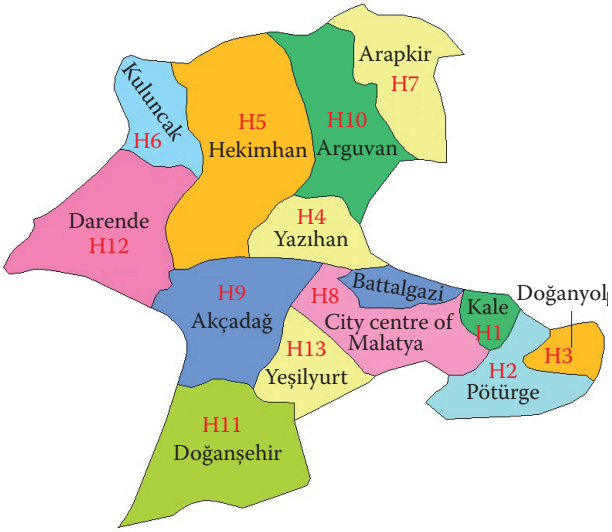

Figure 1. Districts of Malatya province where samples were obtained

MATERIAL AND METHODS

Honey samples were obtained directly from beekeepers in Malatya (Turkey) in 2019. The honey samples were stored in a dark place at room temperature until they were analysed.

Determination of botanical origin. Samples were prepared for analysis by making a slight modification of the Sorkun (2008) method for botanical origin determination that was used for sample preparation. Microscopic slides were examined with Nikon Eclipse E400 microscope (Nikon, Japan).

Determination of total pollen grains. Microscopic preparations started to be examined from the upper left corner, and the whole preparation was scanned and the pollen and *Lycopodium* sp. spores were counted. The total number of pollen grains was determined by calculating the obtained values (Sorkun 2008).

Determination of total phenolic content. Total phenolic content of honey samples was determined according to the Folin-Ciocalteu method (Singleton and Rossi 1965; Singleton et al. 1999).

Determination of antioxidant activity. Antioxidant capacity of honey samples was measured by using ferric reducing antioxidant power (FRAP) (Benzie and Strain 1999).

Determination of chemical composition by gas chromatography-mass spectrometery (GS/MS). To determine chemical composition of honey samples, the Barcarolo et al. (1998) method was used with minor modifications. Analysis was performed using Agilent brand 5973N Selective Mass Detector, 6890N Network GC System (GC-MS; Agilent, USA). DB-5MS column (30 m × 25 mm and 0.25 µm film thickness) was used. In the gas chromatography section, after keeping the temperature at 50 °C for 1 min, the temperature was increased to 150 °C with the increasing rate 10 °C min^{-1} and after this period it was kept at this temperature for 2 min. Finally, the temperature was increased to 280 °C with the increasing rate 20 °C min^{-1}, the injection temperature was determined as 280 °C and the analysis time was 49.5 min.

Determination of antibacterial activity. Honey was weighed (1 g) (ATX 224; Shimadzu, Japan) and transferred to 2 mL sterile Eppendorf tube (Isolab, Turkey). Sterilized distilled water was added to the weighed honey and the total volume completed to 2 mL. After that, diluted honey samples were vortexed and mixed thoroughly (REAXtop; Heidolph, Germany), resulting in a concentration of 50% (w/v). Diluted honey samples (50% w/v) were used for determination of antibacterial activity, minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) values (Ecem Bayram et al. 2019). To determine the *in vitro* antibacterial activity of honey samples, agar well diffusion (AWD) method was used (Sherlock et al. 2010; Osés et al. 2016). Each assay was carried out in duplicate.

Determination of minimum inhibitory concentration (MIC). In order to determine MIC values, microbroth dilution method was used and for this purpose, 96-well microtiter plates (SPL Life Sciences; Gyeonggi-do, Korea) were used (Huttunen et al. 2013). At the end of processes, the lowest concentration inhibiting pathogenic microorganisms was determined as MIC (Sherlock et al. 2010).

Determination of minimum bactericidal concentration (MBC). MBC values were determined with small modifications as previously described by Sherlock et al. (2010). After determining the MIC values, 5 µL suspension samples were taken with a micropipette from each well of 96-well microplate and transferred to the Petri dishes in sequence. After these procedures, the Petri plates were incubated at 37 °C for 24 h. At the end of the incubation period, the lowest concentration that did not form any bacterial colony was accepted as MBC (Sherlock et al. 2010).

RESULTS AND DISCCUSION

In this study, botanical origin of honey samples was determined and the results are given in Table 1. Following the results, it is clear that nine honey samples

https://doi.org/10.17221/63/2021-CJFS

Table 1. Pollen type and frequency of honey samples

Sample No.	Predominant pollen (≥ 45%)		Secondary pollen (16–44%)		Minor pollen (3–15%)		Trace pollen (< 3%)		TPN
	scientific classification	value (%)	scientific classification	value (%)	scientific classification	value (%)	scientific classification	value (%)	
H1	Fabaceae	49.50	Ranunculaceae	27.72	*Hedysarum* sp. Rosaceae	11.88 9.90	–	–	19 539
H2	–	–	Fabaceae *Teucrium* sp.	32.71 35.51	Berberidaceae Rosaceae *Sanguisorba* sp.	11.21 10.28 5.61	Brassicaeae Cistaceae Lamiaceae	0.93 0.93 0.93	136 998
H3	*Teucrium* sp.	46.59	Fabaceae	30.68	Brassicaceae Rosecae	14.77 3.41	Lamiaceae Rumex sp. *Sanguisorba* sp.	2.27 1.14 1.14	275 604
H4	*Teucrium* sp.	78.81	–	–	Fabaceae Berberidaceae	6.78 8.47	Asteraceae *Onobrychis* sp.	1.69 0.85	125 656
H5	Berberidaceae	45.87	–	–	Asteraceae Fabaceae Caryophyllaceae *Campanula* sp. Lamiaceae *Onobrychis* sp.	10.09 4.59 3.67 5.50 15.60 8.26	*Cichorium* sp. Rosaceae *Sanguisorba* sp. *Scabiosa* sp.	0.92 2.75 0.92 0.92	176 301
H6	Fabaceae	52.58	Berberidaceae	16.50	*Plantago* sp. Rosaceae *Teucrium* sp.	6.19 10.31 8.25	Asteraceae Lamiaceae Liliaceae Poaceae *Rumex* sp.	1.03 1.03 1.03 1.03 2.06	209 624
H7	Fabaceae	45.75	Asteraceae *Teucrium* sp.	17.56 21.28	Rosaceae	5.32	Brassicaeae Cistaceae Liliaceae *Plantago* sp.	1.06 1.06 6.38 2.13	98 555
H8	Fabaceae	57.00	Berberidaceae *Teucrium* sp.	18.87 16.88	Rosaceae	3.12	Asteraceae Lamiaecae	1.00 1.03	67 802
H9	Fabaceae	43.69	Berberidaceae	30.10	Asteraceae	11.65	Apiaceae *Plantago* sp. *Teucrium* sp. Rosaceae	1.94 0.97 2.91 1.94	243 153
H10	Fabaceae	44.30	Ranunculaceae Rosaceae	18.99 17.72	*Anchusa* sp. Berberidaceae	3.80 7.59	Asteraceae *Salix* sp.	2.53 1.27	163 967
H11	Fabaceae	46.34	Berberidaceae	19.51	*Anchusa* sp. Lamiaceae Ranunculaceae *Salix* sp.	12.20 4.88 9.76 4.88	Rosaceae	2.44	87 937
H12	Fabaceae	41.82	Asteraceae Rosecaeae	27.27 20.00	Ranunculaceae	5.45	Brassicaeae Poaceae *Plantago* sp.	1.82 1.82 1.82	208 129
H13	Fabaceae	76.92	–	–	Berberidaceae Roseceae	7.69 15.38	–	–	37 166

TPN – total pollen number; dominant (D) (≥ 45%), secondary pollen (S) (16–44%), minor pollen (M) (3–15%) and trace pollen (T) (< 3%)

https://doi.org/10.17221/63/2021-CJFS

are monofloral honey samples. The amount of total phenolic content and iron reduction capacity are documented in Table 2 and GC-MS analysis results were shown in Table 3. It was found that honey samples are rich in aldehydes, alcohols, carboxylic acids and esters. When we compare the results, it is clear that the families Fabaceae and Berberidaceae, and *Teucrium* sp. had dominant pollens. Total phenolic content of honey samples ranged between 73.90 mg GAE 100 g^{-1} and 11.88 mg GAE 100 g^{-1}. Thus, sample No. H3 had the highest antioxidant capacity. Another observation was that honey samples had a more effective antibacterial effect against gram-positive bacteria compared to gram-negative bacteria (at 500 mg mL^{-1} concentration). In the AWD assay, it was recorded that inhibition zone diameters varied between 9 mm and 14 mm. In addition, honey samples were found to have a partial inhibitory effect against selected target pathogens. As a result of antibacterial activity tests, no honey sample was found to inhibit all target pathogens. In the MIC and MBC assays, it was observed that the majority of pathogenic microorganisms were not inhibited. However, for sensitive bacteria, it was observed that the inhibitory concentration and bactericidal concentrations varied between ~125 mg mL^{-1} and 250 mg mL^{-1} (25–50% w/v). It was also observed that the inhibitory concentrations and bactericidal concentrations of the honey samples were generally the same but, in some samples, it was recorded that the MBC concentrations were higher than the MIC value. The results of *in vitro* antibacterial activity assays (AWD, MIC and MBC) are given in Table 4. Bobiș et al. (2013) reported that antimicrobial activity of unifloral honey samples lies between 0 mm and 12 mm for *Escherichia coli*. The analysis of pollen types can be helpful in determining the flower and geographical origins of honeys (Pyrzynska and Biesaga 2009). In another study with 24 honey samples collected from the Siirt region, the average pollen value was found 22 506 and the total pollen number between 2 086 and 55 710, and the pollen composition of the honey samples was defined as relatively rich (Gürbüz et al. 2019). Total phenolic content of the honey samples collected from various cities in Turkey ranged from 25 mg GAE 100 g^{-1} to 125 mg GAE 100 g^{-1} (Mayda et al. 2013). In a study, total phenolic content of different Czech honey samples was determined. It was reported that total phenolic content of honey samples ranged between 89.9 mg GAE kg^{-1} to 215.2 mg GAE kg^{-1} (Lachman et al. 2010). It was reported that total phenolic content and FRAP values of different Polish honey samples ranged between 121.6 mg GAE kg^{-1} and 1 173.8 mg GAE kg^{-1} and from 0.6 mmol Fe^{2+} kg^{-1} to 5.7 mmol Fe^{2+} kg^{-1}, respectively (Kuś et al. 2014). Dezmirean et al. (2015) reported that total phenolic content of heather honey samples varied between 59.46 mg GAE 100 g^{-1} and 72.13 mg GAE 100 g^{-1}. It was found that total phenolic content of different Romanian honey samples ranged from 20.01 mg GAE 100 g^{-1} to 274.65 mg GAE 100 g^{-1} (Tamas-Krumpe et al. 2019). It was stated in another study that honey samples contained groups of compounds like aldehydes, aliphatic acid and esters, alcohols, hydrocarbons, carboxylic acid esters, ketones,

Table 2. Total phenolic content and antioxidant capacity of honey samples (n = 3)

Sample No.	Total phenolic content (mg GAE 100 g^{-1})	Antioxidant capacity (μmol $FeSO_4.7H_2O$ g^{-1})
H1	23.69 ± 0.76	1.52 ± 0.02
H2	20.48 ± 0.68	1.34 ± 0.02
H3	73.90 ± 1.66	3.76 ± 0.08
H4	16.01 ± 0.13	0.78 ± 0.02
H5	23.86 ± 0.58	1.55 ± 0.46
H6	17.53 ± 0.27	0.88 ± 0.02
H7	16.90 ± 0.22	0.80 ± 0.02
H8	46.84 ± 0.61	2.17 ± 0.11
H9	17.02 ± 0.33	0.84 ± 0.05
H10	22.12 ± 0.39	1.48 ± 0.06
H11	8.50 ± 0.11	0.27 ± 0.01
H12	11.88 ± 0.14	0.36 ± 0.02
H13	16.79 ± 0.35	0.81 ± 0.02

Czech Journal of Food Sciences, 39, 2021 (3): 208–216

https://doi.org/10.17221/63/2021-CJFS

Table 3. Volatile compounds of honey samples

Compounds	Sample No.												
	H1	H2	H3	H4	H5	H6	H7	H8	H9	H10	H11	H12	H13
2-Methylbutyraldehyde	3.21	–	1.74	1.83	–	0.37	2.02	0.35	2.60	2.11	0.26	0.98	1.11
Butyraldehyde	0.95	0.14	–	–	–	0.71	0.08	–	–	0.95	–	–	–
Isobutyraldehyde	1.74	1.68	0.76	1.77	7.81	2.41	3.69	0.67	0.16	4.65	0.24	–	–
1-octen-3-ol	–	1.07	3.11	1.67	–	–	–	0.88	1.16	–	–	–	3.13
Furfuryl alcohol	0.96	2.92	2.52	3.5	3.78	2.67	3.47	3.37	3.27	4.81	–	3.04	2.76
2-Ethyl-1-hexanol	–	–	0.11	0.85	–	0.09	–	–	–	–	0.91	–	–
n-Nonane	0.31	–	–	0.93	–	–	–	1.57	2.63	–	–	–	–
Hexanoic acid	–	–	2.32	3.89	3.97	–	–	–	–	–	0.68	2.24	–
4-Methylpentanoic acid	6.46	2.46	–	1.71	–	–	12.09	–	0.27	3.06	1.90	–	1.70
2-Methyl-2-pentanoic acid	0.46	0.39	–	–	–	–	–	–	0.37	0.60	–	–	–
Benzoic acid	0.62	2.09	1.69	3.75	1.73	2.57	3.19	3.12	0.25	1.13	2.67	–	2.20
Valeric acid	2.01	2.87	–	–	–	–	2.59	–	–	–	1.93	–	1.98
Pyruvic acid	13.82	3.02	15.74	4.18	3.14	20.85	1.45	–	10.00	0.77	0.59	8.31	–
Propionic acid	1.90	–	0.99	–	–	–	3.03	–	3.91	3.43	2.60	1.15	–
5-Methyl-2,3-hexanedione	–	–	–	–	–	–	–	0.74	1.31	–	–	1.26	1.86
6-Methyl-3,5-heptadien-2-one	0.42	–	1.04	–	0.41	–	1.11	0.48	–	–	0.40	–	0.25
2-Octanone	–	0.70	0.65	–	0.56	–	0.09	–	–	–	1.11	–	–
3,5-Dimethyl-1,2-cyclopentadione	1.63	2.43	2.48	0.10	1.92	0.29	2.03	1.93	2.17	2.95	0.64	0.29	0.30
Homofuronol	5.97	9.23	3.59	10.79	9.64	2.40	4.21	11.44	30.70	2.58	6.76	3.11	6.68
Limonene	0.75	0.53	0.52	–	0.82	0.36	0.52	0.60	0.97	1.10	–	–	0.55
Isoborneol (isomer 1)	–	1.80	–	1.88	–	2.31	–	–	1.95	–	0.76	–	–
Stearic acid	17.53	9.52	10.53	6.24	1.71	11.93	6.73	32.03	0.87	2.26	0.62	3.96	4.12
Myristic acid	2.21	6.88	9.49	0.50	0.36	9.25	1.12	3.06	–	1.07	16.44	22.02	7.76
Heptanoic acid	–	0.34	2.29	–	–	2.71	1.07	1.86	–	1.41	–	–	0.65
Nonanoic acid	–	1.41	0.85	–	–	–	1.18	–	–	1.01	0.78	–	–
Isovaleric acid	2.10	–	–	–	–	–	–	2.13	1.94	–	–	0.36	–
Decyl acetate		0.02	1.73	2.05	1.20	1.11	0.86	0.36	–	4.10	0.26	0.98	0.23
Ethylacetoacetate	2.04	–	–	–	0.29	–	0.28	–	–	–	–	0.30	–
Nonyl acetate	–	–	–	0.73	–	2.01	0.19	0.19	–	1.09	0.11	1.52	0.79
Lauryl acetate	–	5.54	–	–	–	–	–	–	–	–	0.88	1.35	0.98

https://doi.org/10.17221/63/2021-CJFS

Table 3. to be continued

Compounds	Sample No.												
	H1	H2	H3	H4	H5	H6	H7	H8	H9	H10	H11	H12	H13
n-Propyl acetate	6.67	15.50	7.34	18.95	19.00	21.63	24.40	7.68	11.17	6.94	14.78	26.36	17.60
Isopropyl acetate	5.33	0.11	1.91	–	–	–	2.87	1.82	0.11	–	0.52	–	–
2-Ethylbutyl acetate	1.41	0.30	1.46	0.32	–	–	0.24	0.66	0.34	0.62	1.06	–	–
Hexyl acetate	1.15	1.99	0.67		6.02	1.61	–	0.07	–	8.45		1.58	0.82
n-Butyl acetate	–	–	–	0.49	–	0.49	2.36	–	–	–	1.68	5.41	0.29
1-penten-3-ol	–	–	–	–	0.94	1.22	–	1.66	–	0.54	–	–	–
Diethyl succinate	–	1.06	2.96	2.16	–	1.30	–	1.36	4.33	5.09	1.65	–	0.55

Table 4. *In vitro* antibacterial activity of honey samples

Microorganisms	Assays	Sample No.												
		H1	H2	H3	H4	H5	H6	H7	H8	H9	H10	H11	H12	H13
Gram positive														
Bacillus cereus ATCC 14579	IZD	–	13	10	10	9	–	10	–	10	12	11	–	–
	MIC/MBC	–	25/50	50/50	50/50	50/–	–	50/50	–	50/50	25/50	50/50	–	–
Bacillus cereus BC 6830	IZD	10	14	9	–	10	9	13	9	13	–	10	10	10
	MIC/MBC	50/50	25/25	50/–	–	50/50	50/50	25/25	50/50	25/25	–	50/50	50/50	50/50
Enterococcus faecalis ATCC 49452	IZD	–	12	–	–	–	–	14	10	–	13	–	–	12
	MIC/MBC	–	50/50	–	–	50/–	–	25/25	50/50	50/–	25/25	–	–	25/50
Enterococcus faecalis NCTC 12697	IZD	–	11	–	9	–	11	–	–	10	–	10	–	–
	MIC/MBC	–	50/50	50/–	50/–	–	50/50	–	–	50/50	–	50/50	–	–
Enterococcus faecium ATCC 700211	IZD	9	10	9	–	–	–	12	–	–	10	–	11	–
	MIC/MBC	50/–	50/50	50/50	–	–	–	50/50	–	–	50/50	–	50/50	–
Staphylococus aureus ATCC 25923	IZD	–	–	–	–	9	–	–	9	–	–	10	–	–
	MIC/MBC	–	50/–	–	–	50/–	–	–	50/50	–	–	50/50	–	–
Staphylococus aureus NCTC 10788	IZD	–	9	11	–	–	9	–	–	10	–	–	–	9
	MIC/MBC	–	50/50	50/50	–	–	50/50	–	–	50/50	50/–	–	–	50/50
Staphylococus aureus BC 7231	IZD	9	–	–	9	–	10	9	–	11	–	9	9	–
	MIC/MBC	50/50	–	–	50/50	–	50/50	50/–	50/–	50/50	–	50/50	50/50	–
Streptococcus mutans ATCC 35668	IZD	–	9	–	–	10	–	9	–	9	–	–	–	–
	MIC/MBC	50/–	50/50	–	–	50/50	–	50/50	–	50/50	–	–	–	50/–

https://doi.org/10.17221/63/2021-CJFS

Table 4. to be continued

Microorganisms	Assays	Sample No.												
		H1	H2	H3	H4	H5	H6	H7	H8	H9	H10	H11	H12	H13
Gram positive														
Streptococcus salivarus ATCC 13419	IZD	–	–	9	–	–	–	11	–	–	–	10	–	–
	MIC/MBC	–	–	50/50	–	–	–	50/50	–	–	–	50/50	–	50/–
Gram negative														
Acinetobacter baumannii ATCC BA1609	IZD	–	–	–	–	–	–	–	–	9	–	–	–	–
	MIC/MBC	–	50/–	–	–	–	–	–	–	50/50	–	–	–	–
Escherichia coli ATCC BAA 25-23	IZD	–	–	–	–	–	–	–	–	–	–	–	–	–
	MIC/MBC	–	–	–	50/–	–	–	–	–	–	–	–	–	–
Escherichia coli NCTC 9001	IZD	–	–	–	–	–	9	–	–	–	–	–	–	–
	MIC/MBC	–	–	–	–	–	50/–	–	–	–	–	–	–	–
Escherichia coli BC 1402	IZD	9	–	–	–	–	–	–	–	–	–	–	–	–
	MIC/MBC	50/–	–	–	–	–	–	–	–	–	–	–	–	50/–
Pseudomonas aeruginosa ATCC 9070	IZD	–	–	–	–	–	–	–	–	–	–	–	–	–
	MIC/MBC	–	50/–	–	–	–	–	50/–	–	–	–	–	–	–
Pseudomonas aeruginosa NCTC 12924	IZD	–	–	–	–	–	–	–	–	–	–	–	–	–
	MIC/MBC	–	–	–	–	–	–	–	–	–	–	–	–	–
Salmonella typhimurium RSSK 95091	IZD	–	–	–	–	–	–	–	–	–	–	9	–	–
	MIC/MBC	–	–	50/–	–	–	–	–	–	–	–	50/50	–	–
Yersinia enterocolitica ATCC 27729	IZD	–	–	–	9	–	–	–	–	–	–	–	–	–
	MIC/MBC	–	–	–	50/50	–	–	–	–	–	–	–	–	–

IZD – inhibiton zone diameter by agar well diffusion (AWD) method; MIC – minimum inhibition concentration (% w/v); MBC – minimum bactericidal concentration (% w/v)

https://doi.org/10.17221/63/2021-CJFS

terpenes, fatty acids and esters (Keskin et al. 2020). Mădaş et al. (2019) reported that honey samples are rich in alcohols, aldehydes, esters, ketones, sulphur compounds, aliphatic hydrocarbons, nitrogen compounds, carboxylic acids, aromatic acids and ethers. It is clear that our results are consistent with the literature.

CONCLUSION

Honey is an important bee product with the high nutritional value. It shows antioxidant and antimicrobial properties thanks to the components it contains. In this study total phenolic content, antioxidant activities and antimicrobial activities of different honey samples were compared. It has been observed that gram-negative bacteria are highly resistant to honey samples and gram-positive bacteria are more sensitive compared to gram-negative bacteria. These results support the hypothesis that the minor components like phenolics, aliphatic acid and esters, ketones, terpenes, fatty acids and esters contribute greatly to the nutritional value of honey.

Acknowledgement. We would like to thank to head of Malatya beekeepers union for supplying honey samples.

REFERENCES

Al-Mamary M.A. (2002): Antioxidant activity of commonly consumed vegetables in Yemen. Malaysian Journal of Nutrition, 8: 179–189.

Barcarolo R., Centeleghe M., Zanatta P., Conte L.S. (1998): GC-MS coupled with head space sampling with reverse carrier flow in sampling step applied to honey characterization. In: 5th International Symposium of Hyphenated Technique in Chromatography, Bruges, Belgium, Feb 1, 1998: 11–21.

Benzie I.F., Strain J.J. (1999): Ferric reducing/antioxidant power assay: Direct measure of total antioxidant activity of biological fluids and modified version for simultaneous measurement of total antioxidant power and ascorbic acid concentration. Methods in Enzymology, 299: 15–27.

Bobiş O., Mărghitaş L.A., Dezmirean D., Gherman B., Chirilă F. (2013): *In vitro* antibacterial activity of unifloral honeys against honeybee pathogens *Paenibacillus larvae* and *Escherichia coli*. Lucrări Stiințifice – Seria Zootehnie, 55: 160.

Dezmirean D., Mărghita, L.A., Fiț N., Chirilă F., Gherman B., Mărgăoan R., Aurori A., Bobiș O. (2015): Antibacterial effect of heather honey (*Calluna vulgaris*) against different microorganisms of clinical importance. Bulletin of University of Agricultural Sciences and Veterinary Medicine Cluj-Napoca (UASVM), Animal Science and Biotechnologies, 72: 72–77.

Ecem Bayram N., Yüzer M.O., Bayram S. (2019): Melissopalynology analysis, physicochemical properties, multielement content and antimicrobial activity of honey samples collected from Bayburt, Turkey. Uludag Bee Journal, 19: 161–176.

Gürbüz S., Özenirler Ç., Mayda N., Çelemli Ö.G., Özkök A. (2019): Pollen spectrum of some honey samples produced in Siirt-Turkey. Hacettepe Journal of Biology and Chemistry, 47: 295–303.

Huttunen S., Riihinen K., Kauhanen J., Tikkanen Kaukanen C. (2013): Antimicrobial activity of different Finnish monofloral honeys against human pathogenic bacteria. Apmis, 121: 827–834.

Kassim M., Achoui M., Mustafa M.R., Mohd M.A., Yusoff K.M. (2010): Ellagic acid, phenolic acids, and flavonoids in Malaysian honey extracts demonstrate *in vitro* anti-inflammatory activity. Nutrition research, 30: 650–659.

Keskin Ş., Mayda N., Keskin M., Özkök A. (2020): Investigation of Bılecık honeys in terms of melissopalynology and chemical analyses (Bilecik ballarinin melissopalinolojik ve kimyasal özellikleri açisindan değerlendirilmesi). Food (Gıda), 45: 275–289. (in Turkish)

Kuś P.M., Congiu F., Teper D., Sroka Z., Jerković I., Tuberoso C.I.G. (2014): Antioxidant activity, color characteristics, total phenol content and general HPLC fingerprints of six Polish unifloral honey types. LWT – Food Science and Technology, 55: 124–130.

Lachman J., Orsák M., Hejtmánková A., Kovářová E. (2010): Evaluation of antioxidant activity and total phenolics of selected Czech honeys. LWT – Food Science and Technology, 43: 52–58.

Mădaş N.M., Mărghitaş L.A., Dezmirean D.S., Bonta V., Bobiş O., Fauconnier M.L., Francis F., Haubruge E., Nguyen K.B. (2019): Volatile profile and physico-chemical analysis of acacia honey for geographical origin and nutritional value determination. Foods, 8: 445.

Mayda N., Keskin M., Keskin Ş., Ozkok A. (2019): Determination of botanic origins, total phenolic and flavonoid contents of bee pollen collected from the Bilecik province (Bilecik ilinden toplanan ari polenlerinin botanik orijinleri ile toplam fenolik ve flavonoid içeriklerinin belirlenmesi). Uludag Bee Journal (Uludağ Arıcılık Dergisi), 19: 152–160. (in Turkish)

Mohamed M., Sirajudeen K.N.S., Swamy M., Yaacob M., Sulaiman S. (2010): Studies on the antioxidant properties of Tualang honey of Malaysia. African Journal of Traditional, Complementary and Alternative Medicines, 7: 59–63.

https://doi.org/10.17221/63/2021-CJFS

Osés S.M., Pascual-Mate A., de la Fuente D., de Pablo A., Fernandez-Muino M.A., Sancho M.T. (2016): Comparison of methods to determine antibacterial activity of honeys against *Staphylococcus aureus*. Journal of the Royal Netherlands Society for Agricultural Sciences (NJAS) – Wageningen Journal of Life Sciences, 78: 29–33.

Pyrzynska K., Biesaga M. (2009): Analysis of phenolic acids and flavonoids in honey. TrAC – Trends in Analytical Chemistry, 28: 893–902.

Sherlock O., Dolan A., Athman R., Power A., Gethin G., Cowman S., Humphreys H. (2010): Comparison of the antimicrobial activity of Ulmo honey from Chile and Manuka honey against methicillin-resistant *Staphylococcus aureus*, *Escherichia coli* and *Pseudomonas aeruginosa*. BioMed Central (BMC) Complementary and Alternative Medicine, 10: 1–5.

Singleton V.L., Rossi J.A. (1965): Colorimetry of total phenolics with phosphomolybdic-phosphotungstic acid reagents. American Journal of Enology and Viticulture, 16: 144–158.

Singleton V.L., Orthofer R., Lamuela-Raventós R.M. (1999): Analysis of total phenols and other oxidation substrates and antioxidants by means of folin-ciocalteu reagent. Methods in Enzymology, 299: 152–178.

Sorkun K. (2008): Turkey's Nectar Plants, Pollen and Honey (Türkiye'nin Nektarlı Bitkileri, Polenleri ve Balları). Ankara, Turkey, Palme Yayıncılık: 341. (in Turkish)

Tamas-Krumpe O., Bobış O., Mărgăoan R., Chırılă F., Lațiu C., Ognean L. (2019): Correlative research regarding the total polyphenolic content, antioxidant and antibacterial activity of three types of Romanian honey. Scientific Papers. Series D. Animal Science, LXII: 282–288.

Received: March 10, 2021
Accepted: June 7, 2021