https://doi.org/10.17221/38/2021-CJFS

Comparison of biochemical and antimicrobial activities of different honey samples

Saban Keskin[1]*, Semiramis Karlidag[2], Nazli Mayda[3], Aslı Ozkok[3]

[1]Vocational School of Health Services, Bilecik Seyh Edebali University, Bilecik, Turkey
[2]Bee and Bee Products Research and Application Center (MTOAGAM), Malatya Turgut Ozal University, Malatya, Turkey
[3]Bee and Bee Products Research and Application Center (HARUM), Hacettepe University, Ankara, Turkey

*Corresponding author: sabankeskin61@hotmail.com

Citation: Keskin S., Karlidag S., Mayda N., Ozkok A. (2021): Comparison of biochemical and antimicrobial activities of different honey samples. Czech J. Food Sci., 39: 273–280.

Abstract: Honey, a natural healing agent and a sweet food, has been used since ancient times. A honey sample could possess many biological activities depending on its chemical composition. The amount and the diversity of these minor components of honey mainly depend on the floral sources. That is why the biological activity of the honey sample obtained in a region should be determined. In this study, total phenolic and flavonoid content, antioxidant activity, melissopalynological analyses and antimicrobial activity of twenty honey samples obtained from Doganyol, Malatya, Turkey were examined. In order to determine the *in vitro* antibacterial activity of honey samples, the agar well diffusion (AWD) method, minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) assays were used. For this purpose, ten Gram-positive bacteria and eight Gram-negative bacteria were used. Total phenolic content was found in the range from 9.68 ± 0.72 to 29.40 ± 1.03 mg GAE g^{-1} sample. Antioxidant activity of honey samples varied from 2.21 ± 0.46 to 6.03 ± 1.11 µmol $FeSO_4$ $7H_2O$ g^{-1}. Honey samples showed moderate antimicrobial activity against tested microorganisms, especially against Gram-positive bacteria. It could be concluded from our findings that there is a good correlation between total phenolic content and the biological activity of honey samples.

Keywords: antioxidant; FRAP; MIC; monofloral honey; phenolics

Honey, a sweet food consumed by humans, is produced by honey bees by collecting the secretion of plants and flowers as nectar. After that, nectar is processed by the bees and stored in the honeycomb cells (Keskin et al. 2020). Honey bees can collect not only the secretion of flowers as nectar sources but also the secretion of some insects living on certain plants. Honey can be classified as blossom honey or honeydew honey according to nectar sources. Turkey has a potential for the production of blossom honey depending on its rich floral sources (Keskin et al. 2020; Kolaylı et al. 2020). The composition of honey varies according to the nectar sources. Besides the nectar sources, climatic conditions have an important effect on the composition of honey (Kolaylı et al. 2020). Honey is the concentrated solution of glucose and fructose. Apart from these monosaccharides, there are some carbohydrates in honey composed of disaccharides and oligosaccharides. Along with these main contents, there are some minor components in varied concentrations such as phenolic acids, flavonoids, vitamins, enzymes, peptides, royal jelly proteins and minerals (Keskin et al. 2020; Kolaylı et al. 2020).

The antioxidant and antimicrobial activity of honey strongly depends on the types and concentration of these minor components. The type and amount of these mi-

https://doi.org/10.17221/38/2021-CJFS

nor ingredients vary depending on the source of nectar (flora) and the geographic origin of honey. Studies have shown in general that dark coloured honeys have more phenolic components and hence better antioxidant activity (Bertoncelj et al. 2007; Keskin et al. 2020; Kolaylı et al. 2020). The nutritional and health-promoting value of honey has been known since ancient times but it has been stated recently that there is a correlation between antioxidant activity and the health-supporting effect of honey. Honeys having higher antioxidant activity are reported as better for the prevention of aging, in degenerative heart and nervous system diseases and in wound healing property (Kolaylı et al. 2020).

Identification and determination of the ratio of the above-mentioned minor components in honey samples of a region are still an attractive research area. For this purpose, in this study total phenolic content, antioxidant activity and antimicrobial activity of twenty honey samples produced in Malatya (Doganyol), Turkey, during the summer season of 2019, were determined. As a result of this study, it was ensured that the data required for the geographical indication of honey produced in Doganyol were obtained.

MATERIAL AND METHODS

Material

Honey samples were obtained from the local beekeepers of Malatya (Doganyol) city, Turkey, in the summer season of 2019. Ethanol, methanol, gallic acid, quercetin, fuchsin, glycerol, acetonitrile, sodium carbonate, $FeCl_3$, $FeSO_4$ $7H_2O$ and Trolox were obtained from Sigma Aldrich Chemie GmbH (Germany). Folin--Ciocalteu phenol reagent was purchased from Fluka Chemie GmbH (Switzerland). All other reagents were of analytical grade.

Palynological analysis

Melissopalynological analyses were conducted to determine the botanical origin characteristics of honeys (Wodehouse 1935; Louveaux et al. 1978). For this, 10 g of honey sample was added to 20 mL of distilled water and made completely homogeneous and centrifuged at 3 000–4 000 rpm for 30–40 min (Micro 220; Carl Roth, France). After centrifugation, the supernatant part was separated from the pellet part on the bottom. With a dissection needle, a piece of glycerin gelatin was taken and the bottom of the tube was touched to the settled part, the precipitated glycerin was placed on a gelatin slide, a coverslip was closed and counted under the microscope (E400; Nikon Eclipse, Japan) (Malkoç et al. 2019). Various literature sources and reference pollen preparations from the collection of Hacettepe University Biology Department were used in pollen diagnosis (Özkök 2019; Mayda and Özkök 2021). According to the census results, the ratios of pollen were determined and the rates were classified as dominant pollen (45% and more), secondary pollen (16–44%), minor pollen (3–15%), trace pollen (3% and less). Samples with pollen grain frequencies of a given plant above 45% were called as monofloral.

Determination of total phenolic content

The total phenolic content of honey samples was determined by using the Folin-Ciocalteu method, gallic acid as standard. The absorbance of test tubes was recorded against a blank solution at 760 nm. The blank contained pure water instead of phenolic compounds. Results were expressed as mg GAE per 100 g of sample (Singleton and Rossi 1965; Singleton et al. 1999).

Determination of antioxidant activity

The antioxidant capacity of the samples was determined by using a ferric reducing antioxidant power (FRAP) assay (Benzie and Strain 1999). The absorbance of the samples was recorded at 593 nm against distilled water blank. Results obtained by the FRAP assay were given as μmol $FeSO_4$ $7H_2O$ per 1 g of sample.

Antibacterial activity assays

Preparation of honey samples for antibacterial activity assays. Initially, a 2-g honey sample was weighed (ATX 224; Shimadzu, Japan) and transferred to a 15 mL sterile Falcon tube (Isolab, Turkey). After that, sterilised distilled water was added to the weighed honey samples and the total volume was completed to 4 mL. Then, honey samples were vortexed and mixed thoroughly; in this way honey samples were diluted to 50% (w/v). Prepared honey samples (50% w/v) were kept in a water bath at 40 °C for 30 min and thus honey samples were made more fluidic for antibacterial activity tests. At the end of these processes, honey samples were used for the determination of antibacterial activity, minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) values (Ecem-Bayram et al. 2019).

Microorganisms and growth conditions. Eighteen different bacterial strains (10 Gram-positive and 8 Gram-negative) were used to determine the *in vitro* antibacterial activities of honey samples. Initially, these pathogen strains were cultured in Mueller Hin-

https://doi.org/10.17221/38/2021-CJFS

ton Broth (MHB) (CM0337; Oxoid, USA) at 37 °C for 24 h. At the end of this incubation period, bacterial suspensions were adjusted to McFarland 0.5 turbidity standard (10^6 CFU mL^{-1}) and used as inoculum (Sherlock et al. 2010). The microorganisms were obtained from the Department of Medical Services and Techniques, Bayburt University Vocational School of Health Services, Turkey.

Determination of antibacterial activity. Agar well diffusion (AWD) method was used to determine the *in vitro* antibacterial activity of honey samples. After the incubation period, observed inhibition zones around the wells were measured with a ruler (KINEX 6001 mechanical ruler; KINEX, Czech Republic) and recorded (Osés et al. 2016). Each assay was carried out in duplicate.

Determination of minimum inhibitory concentration (MIC). Microbroth dilution method was used to determine MIC values of honey samples, and for this purpose, polystyrene 96-well microtiter plates were used (SPL Life Sciences; Gyeonggi-do, Korea). Initially, using a multichannel pipette, 95 µL of sterile MHB medium was added to all wells and then 5 µL of inoculum was added to all wells. Then, 100 µL of previously diluted honey samples (50% w/v) were added to all the first wells and mixed thoroughly by pipetting. After these processes, 100 µL of the sample was taken from the first well and transferred to the second well with a micropipette. This procedure was repeated to the 8th well, respectively, and the concentrations of honey samples in each well were serially diluted. After that, using a plate reader (Multiskan GO, Thermo scientific, USA) 96-well microtiter plates were measured and recorded at 600 nm wavelength. After this spectrophotometric measurement, the microplate was incubated at 37 °C for 24 h. At the end of this incubation period, the microplate was measured again at 600 nm wavelength, and the results were recorded. At the end of all these processes, the lowest concentration that inhibited pathogenic microorganisms was determined as MIC (Sherlock et al. 2010).

Determination of minimum bactericidal concentration (MBC). MBC assays were carried out with minor modifications as previously described by Sherlock et al. (2010).

MBC was determined by transferring 5 µL suspension of MIC assay samples to the Petri dishes separately. Later, the Petri plates were incubated at 37 °C for 24 h. MBC was determined from the corresponding plates where no bacterial colony was formed (Sherlock et al. 2010).

RESULTS AND DISCCUSION

As a result of the palynological examination of 20 honey samples used in the study, 10 of the honeys were found to have monofloral properties (the lowest 46.51% Berberidaceae, the highest 78.95% Asteraceae). Except for honeys such as chestnut, eucalyptus, linden, thyme, and lavender, for a honey to have a monofloral quality, it must contain a minimum of 45% major pollen (Mayda et al. 2019). An amount of 5.62% of the pollen belonging to honeys used in the study was determined as dominant pollen, 13.48% secondary pollen, 33.71% important minor pollen and 47.19% minor pollen. Results of the palynological analysis of honey samples are summarised in Table 1.

The total phenolic content of honey samples was determined by the Folin-Ciocalteu method and results and antioxidant capacity are summarised in Table 2. Total phenolic amounts of honeys were determined spectrophotometrically as GAE. Total amounts of phenolic compounds were found between a minimum of 9.68 ± 0.72 mg GAE 100 g^{-1} and a maximum of 29.40 ± 1.03 mg GAE 100 g^{-1} (Table 2). It was found for the total antioxidant capacity of honey samples that they possessed antioxidant capacity varying between 2.21 ± 0.46 µmol $FeSO_4$ $7H_2O$ g^{-1} and 6.03 ± 1.11 µmol $FeSO_4$ $7H_2O$ g^{-1}.

The amount of compounds varies depending on the honey's flora and geographical origin. The antioxidant capacity of honey is dependent on the flavonoid and vitamin C content, which are the plant-derived polyphenolic compounds it contains. Studies have shown that dark honeys have high phenolic content and antioxidant activity (Bertoncelj et al. 2007; Kolaylı et al. 2020). The concentration and type of phenolic compounds depend on the flower source where the nectar is collected and is mainly responsible for its biological activities (Malkoç et al. 2019). Total amounts of phenolic compounds are determinative for honey's antioxidant capacity (Malkoç et al. 2019). In one study, the total phenolic content of honeys like chestnut (52.4–105.0 mg GAE 100 g^{-1}), agave (42.0–75.1 mg GAE 100 g^{-1}), pine (58.6–74.6 mg GAE 100 g^{-1}) and acacia honey (9.80–12.20 mg GAE 100 g^{-1}) was reported (Kaygusuz et al. 2016). Bertoncelj et al. (2007) reported that the phenolic content was 44.8 mg kg^{-1} in acacia honey, up to 241.4 mg kg^{-1} in fir honey, as GAE. Meda et al. (2005) revealed that the total phenolic content of honeydew honey was found to be the highest in their study with different honeys. Malkoç et al. (2019) calculated the amount of total phenolic matter of honey

https://doi.org/10.17221/38/2021-CJFS

in terms of gallic acid spectrophotometrically and stated that it was between a minimum of 32 mg GAE 100 g^{-1} and a maximum of 85.35 mg GAE 100 g^{-1}. In our study, it is seen that Doganyol honey contains higher phenolic content than acacia honey. Al-Mamary et al. (2002) examined the total phenolic content of five different Yemen honeys by the Folin-Ciocalteu method and reported that the values were between 56.32 mg 100 g^{-1} and 246.21 mg 100 g^{-1}. The total antioxidant activity of five Yemen honey samples was reported between 6.48% and 65.44% (Al-Mamary et al. 2002).

Total phenolic content differs among honey species. One study found that the average phenolic content of honey samples was 462 ± 53 mg kg^{-1}. A high phenolic content for monofloral honey samples like oregano (538 ± 41 mg kg^{-1}) and jujube (609 ± 60 mg kg^{-1}) was determined (Zarei et al. 2019). The total phenolic content of 30 samples of acacia honey from the Croatian territory was analysed. The total phenolic content ranged from 31.72 mg kg^{-1} to 80.11 mg kg^{-1} (Krpan et al. 2009).

Antioxidants are compounds that prevent free radical oxidation of organic compounds at low concentrations. In recent years, the interest of food chemistry and preventive medicine in natural antioxidants of plant origin has increased. The therapeutic role of honey is partly due to its antimicrobial effect and partly to its antioxidant content (Sarı 2014).

In a study, antioxidant activity of chestnut, sunflower, citrus, rhododendron and polyfloral honey was found between 73.20–128.21, 78.09–118.68, 79.77–113.95,

Table 1. Palynological analyses of Doganyol honey

	H1	H2	H3	H4	H5	H6	H7	H8	H9	H10
Asteraceae	3.17	19.35	50.00	3.33	77.86	5.38	7.61	6.38	2.78	–
Astragalus sp.	–	3.23	–	66.67	–	3.23	3.26	2.13	38.89	4.38
Apiaceae	1.59	3.23	0.67	–	–	–	1.09	–	–	–
Anchusa sp.	–	–	–	–	–	–	–	–	–	–
Berberidaceae	–	6.45	33.33	–	0.71	43.01	18.48	8.51	–	53.28
Brassicaceae	1.59	–	–	3.33	0.71	–	2.17	–	–	–
Cistaceae	–	–	–	3.33	–	–	–	–	–	–
Cichorium sp.	–	–	–	–	–	–	–	–	–	–
Caryophyllaceae	–	–	–	–	–	–	–	–	–	–
Centaurea sp.	–	–	–	–	–	–	–	–	–	–
Campanula sp.	–	–	–	–	–	–	–	–	–	–
Echium sp.	–	–	–	–	–	–	–	–	–	–
Fabaceae	15.87	19.35	1.33	–	3.57	8.60	15.22	4.26	19.44	5.84
Geraniaceae	–	–	–	–	–	–	–	–	–	–
Hedysarum sp.	–	–	–	–	–	–	–	2.13	–	–
Lamiaceae	1.59	–	0.67	–	–	–	1.09	–	–	0.73
Liliaceae	–	–	–	–	–	–	1.09	–	–	–
Malvaceae	–	–	–	–	–	–	–	–	–	–
Myosotis sp.	–	–	–	–	–	1.08	–	–	–	–
Onobrychis sp.	–	–	–	–	–	–	–	–	–	–
Poaceae	–	–	–	–	–	–	–	–	–	–
Plantago sp.	–	–	–	–	–	1.08	–	2.13	–	–
Rumex sp.	–	3.23	–	–	0.71	–	–	2.13	–	–
Ranunculaceae	–	–	–	–	–	–	–	–	–	–
Rosaceae	–	3.23	–	1–	–	1.08	1.09	2.13	8.33	–
Sanguisorba sp.	7.94	–	2.67	–	2.14	2.15	14.13	8.51	–	0.73
Salix sp.	–	–	–	–	–	–	2.17	–	5.56	–
Scabiosa sp.	–	–	–	–	–	–	–	–	–	–
Teucrium sp.	19.05	19.35	3.33	–	5.00	11.83	25.00	–	–	7.30
Trifolium sp.	47.62	16.13	6.00	–	5.71	16.13	–	44.68	–	20.44
Total pollen score	39 718	47 041	53 684	5 365	88 190	167 001	59 675	16 618	26 908	10 530

https://doi.org/10.17221/38/2021-CJFS

Table 1. to be continued

	H11	H12	H13	H14	H15	H16	H17	H18	H19	H20
Asteraceae	–	–	31.84	6.98	12	78.95	4.47	–	46.88	17.24
Astragalus sp.	2.91	10.64	1.12	4.65	–	–	9.50	0.88	13.54	4.31
Apiaceae	–	–	1.68	0.58	–	0.48	–	–	2.08	0.86
Anchusa sp.	–	–	–	–	–	–	–	–	–	–
Berberidaceae	51.46	14.89	8.94	46.51	6	0.48	17.32	44.74	3.13	6.90
Brassicaceae	–	–	0.56	0.58	–	0.48	0.56	1.75	–	0.86
Cistaceae	–	2.13	2.23	0.58	–	–	1.68	–	12.50	0.86
Cichorium sp.	–	–	–	–	–	–	–	–	–	–
Caryophyllaceae	–	–	–	–	–	–	–	–	–	–
Centaurea sp.	–	–	–	–	–	–	–	–	–	–
Campanula sp.	–	2.13	–	–	–	0.48	–	–	–	–
Echium sp.	–	–	–	–	–	–	0.56	–	–	–
Fabaceae	1.94	–	24.02	7.56	65	2.39	–	9.65	–	6.03
Geraniaceae	–	–	–	0.58	–	–	0.56	–	1.04	–
Hedysarum sp.	–	–	–	–	–	0.48	0.56	–	–	–
Lamiaceae	–	6.38	5.59	1.74	–	0.48	0.56	–	1.04	–
Liliaceae	–	–	0.56	–	–	–	–	–	–	–
Malvaceae	–	–	–	–	–	–	–	–	–	–
Myosotis sp.	–	–	–	–	–	–	0.56	–	–	–
Onobrychis sp.	–	–	–	0.58	–	–	–	–	–	–
Poaceae	–	–	0.56	–	–	–	–	–	–	–
Plantago sp.	–	–	–	–	–	–	2.79	–	–	–
Rumex sp.	–	–	0.56	0.58	–	–	–	–	–	–
Ranunculaceae	–	–	–	–	–	–	–	–	–	–
Rosaceae	0.97	–	3.91	2.33	–	1.44	1.12	0.88	–	6.03
Sanguisorba sp.	1.94	8.51	–	0.58	–	–	0.56	–	–	12.93
Salix sp.	–	–	–	–	–	–	–	–	–	0.86
Scabiosa sp.	–	–	–	–	–	–	–	0.88	–	–
Teucrium sp.	6.80	2.13	3.91	6.40	2	0.96	32.96	10.53	–	6.03
Trifolium sp.	23.30	27.66	7.26	11.63	–	5.74	16.76	14.91	–	19.83
Total pollen score	11 736	6 787	110 891	217 686	54 941	183 110	127 906	241 133	54 129	56 523

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

Sample No.	Total phenolic content (mg GAE 100g^{-1})	Antioxidant capacity (mol $FeSO_4$ $7H_2O$ g^{-1})	Sample No.	Total phenolic content (mg GAE 100g^{-1})	Antioxidant capacity (mol $FeSO_4$ $7H_2O$ g^{-1})
H1	12.82 ± 1.18	2.78 ± 0.88	H11	12.46 ± 1.21	2.69 ± 0.23
H2	14.98 ± 0.83	3.66 ± 0.21	H12	17.51 ± 1.56	5.16 ± 1.04
H3	20.05 ± 1.12	5.40 ± 0.42	H13	24.98 ± 1.61	5.80 ± 0.96
H4	10.34 ± 0.74	2.31 ± 0.51	H14	29.40 ± 1.03	5.93 ± 0.85
H5	9.68 ± 0.72	2.21 ± 0.46	H15	10.94 ± 0.92	2.37 ± 0.47
H6	14.61 ± 1.15	3.63 ± 0.62	H16	17.63 ± 0.76	5.15 ± 0.28
H7	20.83 ± 1.34	5.51 ± 0.17	H17	29.11 ± 1.47	6.03 ± 1.11
H8	18.73 ± 1.44	5.27 ± 0.33	H18	23.44 ± 1.66	5.65 ± 0.77
H9	25.84 ± 1.61	5.88 ± 0.76	H19	20.41 ± 1.09	5.48 ± 0.47
H10	12.36 ± 1.07	2.73 ± 0.15	H20	21.79 ± 0.88	5.52 ± 0.39

Czech Journal of Food Sciences, 39, 2021 (4): 273–280

https://doi.org/10.17221/38/2021-CJFS

Table 3. *In vitro* antibacterial activity of honey samples: Inhibition zone diameter (IZD) by agar well diffusion method (AWD); minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) (% w/v)

Microorganisms	Assays	Honey samples																			
		H1	H2	H3	H4	H5	H6	H7	H8	H9	H10	H11	H12	H13	H14	H15	H16	H17	H18	H19	H20
Gram-positive																					
Bacillus cereus	IZD	10	12	–	14	–	13	9	15	–	10	11	–	14	–	12	10	13	9	11	–
ATCC 14579	MIC/MBC	50/50	50/50	–	25/25	–	25/25	50/50	25/25	–	50/50	50/50	–	25/25	–	25/50	50/50	25/25	50/50	50/50	–
Bacillus cereus	IZD	12	–	9	13	9	12	–	14	9	–	12	11	10	9	–	10	14	–	13	10
BC 6830	MIC/MBC	25/50	–	50/50	25/25	50/–	50/50	50/50	25/25	50/50	–	25/50	50/50	50/50	50/–	–	50/50	25/25	–	25/50	50/50
Enterococcus faecalis	IZD	11	10	–	12	11	11	–	12	–	11	–	–	–	9	11	–	12	–	10	–
ATCC 49452	MIC/MBC	50/50	50/50	–	50/50	50/50	50/50	–	50/50	–	50/50	–	–	–	50/50	50/50	–	50/50	50/50	50/50	–
Enterococcus faecalis	IZD	–	10	9	10	–	–	–	10	–	–	9	–	10	10	10	9	–	10	–	9
NCTC 12697	MIC/MBC	–	50/50	50/–	50/50	–	–	–	50/50	–	–	50/50	–	50/50	50/50	50/50	50/50	–	50/50	–	50/50
Enterococcus faecium	IZD	–	–	–	–	–	–	9	10	9	–	–	10	–	–	–	–	9	–	9	–
ATCC 700211	MIC/MBC	50/50	–	–	–	–	–	50/50	50/50	50/50	–	–	50/50	–	50/50	–	–	50/50	–	50/50	–
Staphylococus aureus	IZD	–	–	–	9	–	–	–	9	–	–	10	–	9	11	–	9	–	–	–	9
ATCC 25923	MIC/MBC	–	–	–	50/50	–	–	–	50/50	–	–	50/50	–	50/50	25/25	–	50/50	–	–	–	50/50
Staphylococus aureus	IZD	–	11	10	–	9	11	–	–	10	9	–	–	10	–	–	–	11	–	9	–
NCTC 10788	MIC/MBC	–	50/50	50/50	–	50/50	50/50	–	–	50/50	50/–	–	50/–	50/50	–	50/–	–	50/50	–	50/50	–
Staphylococus aureus	IZD	–	10	–	11	–	10	–	12	–	–	9	10	–	9	–	11	–	9	–	–
BC 7231	MIC/MBC	–	50/50	–	25/25	–	50/50	–	25/25	–	50/–	50/–	50/50	–	50/–	–	50/50	–	50/50	–	–
Streptococcus mutans	IZD	9	–	9	–	–	–	–	9	–	–	–	–	10	–	9	–	12	–	9	–
ATCC 35668	MIC/MBC	50/50	–	50/–	–	–	–	–	50/50	50/–	–	–	–	50/50	–	50/–	–	50/50	–	50/50	–
Streptococcus salivarus	IZD	–	9	–	–	–	10	10	–	9	9	–	–	–	–	–	–	9	–	–	9
ATCC 13419	MIC/MBC	–	50/50	–	–	–	50/50	50/50	–	50/–	50/50	–	–	–	–	–	–	50/50	50/50	–	50/50
Gram-negative																					
Acinetobacter baumannii	IZD	–	–	–	9	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
ATCC BA1609	MIC/MBC	–	50/–	–	50/50	–	–	–	–	–	–	–	–	–	–	–	50/–	–	–	–	–
Escherichia coli	IZD	–	–	–	–	–	–	–	9	–	–	–	–	–	–	–	–	–	–	9	–
ATCC BAA 25-23	MIC/MBC	–	–	–	–	–	–	–	50/50	–	–	–	–	–	–	–	–	–	–	50/–	–
Escherichia coli	IZD	–	–	–	–	–	9	–	–	–	–	–	–	–	–	–	–	9	–	–	–
NCTC 9001	MIC/MBC	50/–	–	–	–	–	50/–	–	–	–	–	–	–	–	–	–	–	50/50	–	–	–

https://doi.org/10.17221/38/2021-CJFS

Table 3. to be continued

Microorganisms	Assays	Honey samples																			
		H1	H2	H3	H4	H5	H6	H7	H8	H9	H10	H11	H12	H13	H14	H15	H16	H17	H18	H19	H20
Escherichia coli	IZD	–	–	–	–	–	–	–	10	–	–	–	–	9	–	–	–	–	–	–	–
BC 1402	MIC/MBC	–	–	–	–	–	–	–	50/50	–	–	–	–	50/50	–	–	–	–	–	–	–
Pseudomonas aeruginosa	IZD	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
ATCC 9070	MIC/MBC	–	–	–	–	50/–	–	–	–	–	–	50/–	–	–	–	–	–	–	–	–	–
Pseudomonas aeruginosa	IZD	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
NCTC 12924	MIC/MBC	–	50/–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
Salmonella typhimurium	IZD	–	–	–	9	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
RSSK 95091	MIC/MBC	–	–	–	50/–	–	–	–	–	–	–	–	–	–	–	50/–	–	–	–	–	–
Yersinia enterocolitica	IZD	–	–	–	–	–	–	–	–	9	–	–	–	–	–	–	–	–	–	–	–
ATCC 27729	MIC/MBC	–	–	–	–	–	–	–	–	50/50	–	–	–	50/–	–	–	–	–	–	–	–

(–) Immeasurably low activity

75.80–114.33 and 63.44–98.00 mg ascorbic acid equivalent per 100 g, respectively (Sarı 2014). In an earlier study, antioxidant activity was found to be the lowest in acacia and lime honeys that are light coloured, while the highest was found in fir, spruce and forest dark honeys (Bertoncelj et al. 2007). The antioxidant capacity of honey varies depending on the plant species. In another study in which blackthorn honey samples were examined, it was emphasised that the antioxidant capacity ranged between 560 μmol $FeSO_4$ $7H_2O$ 100 g^{-1} and 1841 μmol $FeSO_4$ $7H_2O$ 100 g^{-1} and the mean value was 1014.91 ± 470.99 μmol $FeSO_4$ $7H_2O$ 100 g^{-1}. According to the results, it was stated that *Paliurus spina-christi* Mill. honey has a much higher antioxidant capacity than other monofloral honeys (Malkoç et al. 2019).

In this study, the *in vitro* antibacterial activity of honey samples against selected pathogenic bacteria was determined using AWD, MIC and MBC assays. Results of AWD, MIC and MBC assays are given in Table 3. Obtained results showed that among the target microorganisms, Gram-negative bacteria were more resistant compared to Gram-positive bacteria at a concentration 500 mg mL^{-1}. As a result of the AWD assay, inhibition zone diameters were measured with a Vernier calliper and it was noted that inhibition zone diameters varied between 9 mm and 15 mm. These results are consistent with the results of the study previously performed by Ecem-Bayram et al. (2019).

In MIC and MBC assays, it has been observed that the majority of the selected target bacteria were not inhibited. In addition, inhibitory concentrations for susceptible strains in MIC and MBC assays were recorded to vary from 25% to 50% (w/v). During this process, especially Gram-negative bacteria were much more resistant against honey samples. In the MIC and MBC assays, the inhibitory concentration and bactericidal concentration values were generally the same.

CONCLUSION

In this study, total phenolic content, antioxidant activity and antimicrobial activity of 20 honey samples obtained from the Doğanyol district of Malatya (Turkey) were determined. Honey samples showed moderate antimicrobial activity especially against Gram-positive bacteria. Total phenolic content and hence the antioxidant activity of the samples were found compatible with literature data. It is seen that honey samples obtained from the Doganyol region are rich in pollen types and the district is suitable for bee breeding and honey production. The results of the study revealed

https://doi.org/10.17221/38/2021-CJFS

that Doganyol has a highly qualified monofloral and polyfloral honey production potential.

Acknowledgement. We thank to Yunus Emre Vural, district governor of Doğanyol, for supplying of honey samples. We also thank to Assist. Prof. Sinan Bayram for the contributions of antimicrobial activity determinations.

REFERENCES

Al-Mamary M., Al-Meeri A., Al-Habori M. (2002): Antioxidant activities and total phenolics of different types of honey. Nutrition Research, 22: 1041–1047.

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.

Bertoncelj J., Doberšek U., Jamnik M., Golob T. (2007): Evaluation of the phenolic content, antioxidant activity and colour of Slovenian honey. Food Chemistry, 105: 822–828

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

Kaygusuz H., Tezcan F., Erim F.B., Yildiz O., Sahin H., Can Z., Kolaylı S. (2016): Characterization of Anatolian honeys based on minerals, bioactive components and principal component analysis. LWT – Food Science and Technology, 68: 273–279.

Keskin Ş., Mayda N., Keskin M., Özkök A. (2020): Investigation of Bilecik honeys in terms of melissopalynology and chemical analyses. Gıda (The Journal of Food), 45: 275–289.

Kolaylı S., Palabiyik I., Atik D.S., Keskin M., Bozdeveci A., Karaoglu S.A. (2020): Comparison of antibacterial and antifungal effects of different varieties of honey and propolis samples. Acta Alimentaria, 49: 515–523.

Krpan M., Marković K., ŠariĆ G., Skoko B., Hruškar M., Vahčić N. (2009): Antioxidant activities and total phenolics of acacia honey. Czech Journal of Food Sciences, 27: 245–247.

Louveaux J., Maurizio A., Vorwohl G. (1978): Methods of melissopalynology. Bee World, 59: 139–157.

Malkoç M., Kara Y., Özkök A., Ertürk Ö., Kolaylı S. (2019): Characteristic properties of Jerusalem thorn (*Paliurus spina-christi* Mill.) honey. Uludag Bee Journal, 19: 69–81.

Mayda N., Keskin M., Keskin Ş., Özkök A. (2019): Determination of botanic origins, total phenolic and flavonoid contents of bee pollen collected from the Bilecik province. Uludag Bee Journal, 19: 152–160.

Mayda N., Özkök A. (2021): Determination and comparison of melissopalynological and some chemical characters of raw and processed honeys. Progress in Nutrition, 23: e2021240.

Meda A., Lamien C.E., Romito M., Millogo J., Nacoulma O.G. (2005). Determination of the total phenolic, flavonoid and proline contents in Burkina Fasan honey, as well as their radical scavenging activity. Food Chemistry, 91: 571–577.

Osés S.M., Pascual-Maté A., de la Fuente D., de Pablo A., Fernández-Muiño M.A., Sancho M.T. (2016): Comparison of methods to determine antibacterial activity of honeys against *Staphylococcus aureus*. NJAS – Wageningen Journal of Life Sciences, 78: 29–33.

Özkök A. (2019): Determination of antioxidant capacities, chemical composition and melissopalynological characterization of *Verbascum* spp. and *Euphorbia* spp. honeys. Fresenius Environmental Bulletin, 28: 2644–2649.

Wodehouse R.P. (1935): Pollen Grains. New York, USA, McGraw-Hill: 106–109.

Sarı E. (2014): Determination of some biological and physicochemical properties of honey produced in Turkey. (Türkiye'de üretilen bazı balların biyolojik ve fizikokimyasal özelliklerinin belirlenmesi) [Ph.D. Thesis.] Kayseri, Turkey, Erciyes University, Institute of Science, Department of Biology. (in Turkish).

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*. BMC Complementary and Alternative Medicine, 10: 47

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.

Zarei M., Fazlara A., Tulabifard N. (2019): Effect of thermal treatment on physicochemical and antioxidant properties of honey. Heliyon, 5: 01894.

Received: February 2, 2021
Accepted: June 17, 2021