Macronutrient and micronutrient levels and phenolic compound characteristics of monofloral honey samples

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Summary
This study aimed to obtain data on specific properties of various monofloral honey samples. Palynological, phenolic component, macroelemental and microelemental analyses were conducted and antimicrobial activities of honey samples were determined. In addition, protocatechuic acid for pine, lavender (from Isparta), heather (from Mugla) and cedar honey; gallic acid for carob, oak, lavender (from Antalya), chestnut, sandalwood and heather (from Antalya) honey; caffeic acid for linden, astragalus, chaste honey; syringic acid for rhododendron honey and the quercetin compound for cornelian cherry-citrus honey were observed to be dominant. As a result of the elemental analyses performed with inductively coupled plasma mass spectrometry, it was determined that elemental profiles of all of the honey samples differed, and the total ratio of macrominerals sodium, potassium, calcium, phosphorus and magnesium was the highest in the oak honey and the lowest in the cornelian cherry-citrus honey. According to antibacterial activity test results, Gram-negative bacteria were found to be more resistant to the honey samples compared to Gram-positive bacteria. In conclusion, the results of this study confirmed that the origin (blossom or honeydew) and plant source of the honey samples had an effect on their elemental content, phenolic component and antimicrobial activity.

Keywords
melissopalynology; phenolic composition; monofloral honey; mineral content; honeydew honey; total pollen number

Honey, which is prepared by collecting natural sugar solutions known as nectar, is transformed from an easily degraded thin, sweet liquid into a durable, dense and high energy food by bees [1]. Honey is categorized as blossom honey or honeydew honey depending on the source of the nectar collected by the bees. While source of blossom honey is nectar of flowers, source of the honeydew honey is a liquid excretion of plants or insects living on the plants [2].

The most traditional method to determine the origin of honey, and from which flowers the nectar is collected by bees, is based on identification of pollen in its composition. It is possible to observe and diagnose pollen using a light microscope, thus making it possible to determine the botanical origin, variety and density of pollen as well as whether a foreign substance has been added to the honey or not [3]. There are many different types of honey on the market, some of which originate in a single plant species, being called monofloral or unifloral honey, and others that originate in several plant species, being called polyfloral or multifloral honey [4]. Honey is classified as monofloral when, according to microscopic analysis, at least 45 % of the pollen grains are determined to

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be from a single plant species. Due to the variety of the botanical sources used by bees in honey production and the various climatic conditions in which the production is carried out, no honey is the same as another, especially in terms of taste and flavour [5]. However, the phenolic compounds present in honey are directly related to botanical resources, such as pollens, nectars, resins and oils, and thus honeys from different floral origins possess distinct bioactive properties [6]. With the growing interest in studies conducted on characterization of honey, researchers have carried out a vast amount of research to determine the floral and geographic origin of honey based on its minerals content. Even though they are found in a small amount in honey compared to other components, trace elements have been widely used in recent years to detect fraudulent honey [7].

Honey has been used for centuries not only as a food, but also for therapeutic purposes. Honey suppresses the development of many types of microorganisms that cause diseases and infections [8]. There are many studies reporting that monofloral honeys, which differ in taste and appearance compared to multifloral honeys, have different therapeutic properties such as antimicrobial and antioxidant properties [9]. In this study, in order to contribute to the identification of monofloral honeys, the degree of botanical origin representation, mineral and phenolic profiles were determined. In addition, the antimicrobial activities of honey samples used for therapeutic purposes were also determined.

MATERIAL AND METHODS

Monofloral honey samples

In this study, lavender (2 samples, lavender 1 from Antalya and lavender 2 from Isparta), cedar (Mugla), cornelian cherry-citrus (Mugla), sandalwood (Antalya), linden (Karabük), heather (2 samples, heather 1 from Antalya and heather 2 from Mugla), chestnut (Samsun), rhododendron (Kastamonu), astragalus (2 samples, astragalus 1 from Elazig and astragalus 2 from Tunceli), carob (Antalya), chaste tree (Aydin), pine (Mugla) and oak (Kirklareli) honey samples in various regions of Turkey were analysed. Honey samples were obtained from the relevant Beekeepers Association in the region where they were produced in 2017. Samples were kept at room temperature until analysis for a maximum of a year.

Authentication of botanical origin of honey

At this stage, melissopalynological analysis method was used to confirm the plant origin of the samples declared by honey producers. The pollen spectra of honey samples were determined according to the methodology described by Louveaux et al. [10].

Total pollen number and honeydew element determination

Total pollen number (*TPN*) and total honeydew element number (*THE*) of honey samples were calculated according to Moar [11] by using tablets of *Lycopodium* spores (batch number 3862; obtained from the Department of Geology, Lund University, Lund, Sweden).

Extraction of phenolic compounds

Extraction methods

Extraction by the non-hydrolysis method was carried out by the modified methods of isolation of phenolic compounds developed by Fischer et al. [12]. A volume of 100 μl of the sample was mixed with 900 μl extraction solution (water, methanol, formic acid 79 : 20 : 1, v/v/v). Afterwards, samples were vortexed for 30 s. Then, the solution was homogenized using sonicator WiseClean (Daihan, Seoul, Korea) at 45 °C for 10 min. Samples were subsequently centrifuged for 5 min at 22400 ×*g*

Tab. 1. Parameters of analysis of phenolic compounds.

High-performance liquid chromatography parameters	
Column	Agilent Zorbax SB-C8 column 150 mm × 3.0 mm, 3.5 μm particle size (Agilent Technologies)
Mobile phase A	0.005 mol·l^{-1} ammonium acetate in water
Mobile phase B	0.1% acetic acid in 1 : 1 acetonitrile-methanol
Autosampler temperature	4 °C
Flow rate	0.7 ml·min^{-1}
Column temperature	35 °C
Injection volume	10 μl
Total run time	12 min
Mass spectrometry parameters	
Ionization mode	Electrospray ionization negative
Gas temperature	300 °C
Gas flow	10 l·min^{-1}
Nebulizer	275790.29 Pa
Sheath gas temperature	400 °C
Sheath gas flow	10 l·min^{-1}
Capillary voltage	3500 V
Nozzle voltage	0 V
Scan type	Dynamic multiple reaction monitoring

and the clear supernatant was used for quantitative analysis by liquid chromatography-tandem mass spectrometry (LC-MS/MS).

Extraction by the hydrolysis method involved mixing of 100 μl of the sample with 200 μl of 2 mol·l^{-1} HCl and vortex-mixing for 30 s. Then, the solution was hydrolysed using a sonicator at 90 °C for 40 min. After adding 700 μl of the extraction solvent, samples were centrifuged for 5 min at 22400 ×*g* and the clear supernatant was used for quantitative analysis by LC–MS/MS.

The reason for using two different sample preparations was to analyse both sugar-containing phenolic acids and basic phenolic acids. No filtration was applied in any method, since polytetrafluoroethylene, nylon and cellulose acetate membrane filters were found to bind some of the phenolic compounds, in particular luteolin, kaempferol, quercetin and rutin.

Liquid chromatography-tandem mass spectrometry

LC–MS/MS was performed using an Agilent 6460 system with a triple quadruple mass spectrometer equipped with an electrospray ionization (ESI) interface (Agilent Technologies, Santa Clara, California, USA). High-performance liquid chromatography (HPLC) and mass spectrometry (MS) parameters are presented in the Tab. 1.

Element analysis of samples

An amount of 0.5 g of honey sample, 9 ml of suprapur nitric acid (Merck, Darmstadt, Germany) and 1 ml of hydrogen peroxide (Sigma Aldrich, St. Louis, Missouri, USA) were mixed. Then, the digestion procedures were carried out in a microwave digestion system (Ethos, Milestone, Italy). The instrumental parameters involved a ramp of 15 min to reach 200 °C and then the system was maintained at 1000 W for additional 15 min. The volume of the samples removed from the microwave digestor was completed to 50 ml with ultra-pure water. Blank solutions were prepared in the same way. Li, Be, B, Na, Mg, Si, P, K, Ca, V, Cr, Mn, Fe, Co, Ni, Cu, Zn, Ga, As, Se, Rb, Sr, Ru, Rh, Pd, Ag, Cd, In, Sn, Sb, Te, Cs, Ba, Hf, Ir, Pt, Au, Hg, Tl, Pb and Bi elements in the honey samples were determined using inductively coupled plasma mass spectrometer (ICP-MS) Agilent 7800 (Agilent Technologies) according to Oroian et al. [13].

Antibacterial activity determination

Three Gram-positive bacteria (*Bacillus cereus* BC 6830, *Enterococcus faecalis* NCTC 12697, *Staphylococcus aureus* NCTC 10788) and four Gram-negative bacteria (*Escherichia coli* NCTC 9001, *Pseudomonas aeruginosa* NCTC 12924, *Salmonella* Typhimurium RSSK 95091, *Yersinia enterocolitica* ATCC 27729) were used. An amount of 1 g of individual honey samples was transferred to a 2 ml sterile microtube. Sterilized distilled water was added and the total volume completed to 2 ml. After that, diluted honey samples were mixed thoroughly with a micropipette. Prepared honey samples were used for determination of antibacterial activity and minimum inhibition concentration (*MIC*) values [14]. Antibacterial activities of the honey samples were determined by agar well diffusion method [15]. *MIC* and minimum bactericidal concentration (*MBC*) values were determined via microbroth dilution method using a modified version of Osés et al. [15]. All details of these methods are presented in our previous article [16].

Statistical analysis

The principal component analysis (PCA) was performed using Minitab software version 17 (Minitab, State College, Pennsylvania, USA). PCA was performed with the multi-element analysis data set in order to discriminate and classify honey samples according to their origin.

RESULTS AND DISCUSSION

In this study, the botanical origin of the honey samples collected from various regions of Turkey and indicated as monofloral by beekeepers was verified by the microscopic analysis of the pollen types. The pollen types and amounts determined in the honey samples are presented in Tab. 2. The total number of pollen grains in 10 g of honey in the samples varied from 3202 to 337362 (Tab. 2), with the highest rate found in chestnut honey and the lowest rate in pine honey. As a result of previous melissopalynological analyses conducted on blossom honeys, it was determined that if the pollen grains of a plant were represented by more than 45 % in the honey, then this honey was mostly produced from that particular plant and, thus, was classified as monofloral. However, certain monofloral honey types require higher represantion of the corresponding pollen to be considered monofloral, e. g. more than 90 % in case of chestnut honey [17]. The results of the present study support this information as the chestnut honey used in this study was represented by *Castanea sativa* pollen at a rate of 98 %. However, various monofloral honeys, namely, *Citrus*, *Lavandula spica*, *L. latifolia*, *Rosmarinus*, *Salvia*, *Robinia*, *Tilia* and *Medicago*, contained less than 45 % of

Tab. 2. Botanical origin of honey samples.

Honey sample	TPN_{10}	THE_{10}	Taxon	Pollen grains share [%]
Lavander 1	235102	–	*Lavandula* spp.	0.5
Lavander 2	48263	–	*Lavandula* spp.	4.0
Astragalus 1	310449	–	*Astragalus* spp.	55.6
Astragalus 2	163443	–	*Astragalus* spp.	53.9
Cornelian cherry-citrus	67275	–	*Cornus* spp.	65.0
			Citrus spp.	15.0
Heather 1	35756	–	*Erica* spp.	28.4
Sandalwood	183763	–	*Arbutus* spp.	14.0
Linden	18102	–	*Tilia* spp.	27.0
Heather 2	85456	–	*Erica* spp.	76.2
Chestnut	337362	–	*Castanea sativa*	98.0
Rhododendron	26850	–	*Castanea sativa*	83.5
			Rhododendron spp.	2.5
Carob	256348	–	*Ceratonia* spp.	39.0
Pine	3202	51866	–	–
Oak	30206	–	–	–
Chaste tree	33535	–	*Vitex agnus-castus*	29.9
Cedar	79976	11917	–	–

TPN_{10} – total pollen number in 10 g honey, THE_{10} – total honeydew element number in 10 g honey.

the pollen of the corresponding plant [17]. Similarly, in the present study, cornelian cherry-citrus honey was found to be represented by 15 % of *Citrus* spp. pollen and linden honey was represented by 27 % of *Tilia* spp. pollen, while lavender honeys were found to be represented by low levels of *Lavandula* spp. pollen, such as 0.5 % and 4.0 %, respectively. Similarly, in a study on European citrus honeys, it was found that the percentage of *Citrus* spp. pollen was between 8 % and 32 % [18]. Other studies in the literature on monofloral honeys reported the pollen rate of *Citrus* spp. to be in the range of 2–42 %, *Arbutus* spp. in the range of 8–20 %, *Lavandula* spp. in the range of 1–42 %, *Calluna* spp. in the range of 10–77 %, *Rhododendron* spp. in the range of 15–77 % and *Tilia* spp. in the range of 1–56 %. In addition, honey samples with such botanic origins were found to be represented by a small proportion of the corresponding pollen. On the other hand, chestnut (> 86 %), eucalyptus (> 83 %) and canola (> 60 %) honeys were reported to be represented by a large proportion of the corresponding pollen [19]. When the different flora in terms of region is considered, the relevant pollen representation rate should be determined within the framework of legal regulations, especially for monofloral honeys that are represented by either low or high pollen grain rates. This is important to prevent the consumers who show interest in monofloral honeys being misled and to ensure correct product labelling.

In this study, qualitative and quantitative determination of 23 different types of phenolic compounds was carried out in the monofloral honey samples. Two different extraction methods, namely, acid hydrolysis and non-hydrolysis, were applied to obtain the phenolic compounds. Results showed that the extraction method was effective at identification and quantification of the phenolic compounds (Tab. 3, Tab. 4). The major phenolic compound types extracted from the monofloral honey samples, excluding the lavender, the heather 1 and cornelian cherry-citrus honey samples, were found to be the same by using both methods. Nevertheless, in all other honey samples, excluding the rhododendron and astragalus honeys, the amounts of major phenolic compounds were higher when extracted with the acid hydrolysis method. It was observed that in particular kaempferol and quercetin were extracted at higher rates by the acid hydrolysis method, while rutin and ethyl gallate were extracted at higher rates by the non-hydrolysis method. Similarly, Biesaga and Pyrzyńska [20] showed that the extraction procedure was important for identification and quantification of some phenolic compounds (rutin, kaempferol, quercetin and others) in honey samples. It was determined that the dominant compound in pine, lavender 2, heather 2 and cedar honeys was protocatechuic acid; in carob, oak, lavender 1, chestnut, sandalwood and heather 1 honeys it was gallic acid, in linden, chaste tree and astragalus honeys it was caffeic acid; in rhododendron honey it was syringic acid, and in cornelian cherry-citrus honey it was

Tab. 3. Results of quantitative analysis of phenolic components of honey samples by non-hydrolysis method.

Compound [ng·ml^{-1}]	Astragalus		Carob	Cedar	Chaste tree	Chestnut	Cornelian cherry-Citrus	Heather		Lavender		Linden	Oak	Pine	Rhodo-dendron	Sandal-wood
	1	2						1	2	1	2					
DHB	18.02	13.73	127.66	366.73	22.23	205.73	22.82	74.02	350.73	159.77	76.97	25.34	1033.10	176.95	48.99	144.82
2-HtC	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd
Caffeic acid	504.72	835.77	243.73	146.75	236.54	223.33	277.42	107.46	143.82	233.70	199.64	642.94	226.59	158.65	218.37	105.37
Catechin	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd
Chlorogenic acid	102.74	96.20	nd	14.24	137.66	37.56	nd	23.51	78.29	nd	nd	nd	nd	nd	nd	nd
Epicatechin	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd
Ethyl gallate	1.98	0.27	4.66	0.32	0.32	0.89	0.56	0.70	0.35	0.62	2.28	2.00	2.27	8.72	0.87	0.86
Gallic acid	18.02	16.63	341.12	19.59	31.23	108.35	27.57	200.06	119.05	101.10	30.20	38.31	2883.37	201.21	46.30	511.03
Isorhamnetin	72.65	73.64	67.36	25.60	24.62	40.36	32.38	39.16	18.88	37.69	103.12	82.93	96.83	21.08	70.55	27.36
Kaempferol	186.31	286.28	nd	nd	179.67	nd	210.96	nd	nd	nd	256.66	nd	153.21	nd	210.73	nd
Lutolein	37.53	40.53	103.06	20.67	31.03	165.36	33.40	86.00	16.61	157.75	96.46	11.66	20.80	32.85	7.63	16.36
Myricetin	nd	4.44	nd	nd	2.92	nd	4.03	nd	nd	nd	nd	nd	nd	nd	nd	nd
Naringin	30.41	33.77	31.50	47.39	33.22	44.81	178.70	32.23	38.07	37.79	33.21	21.07	41.28	37.23	35.33	28.20
p-Coumaric acid	147.53	121.02	140.67	91.11	124.28	110.52	86.61	122.67	84.64	122.59	93.46	208.39	286.96	166.85	336.82	92.39
Phlorizin	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd
Propyl gallate	2.28	nd	8.64	nd	0.55	1.35	0.75	0.72	nd	1.55	6.34	3.73	2.60	12.28	2.05	nd
Protocatechuic acid	102.74	111.13	226.79	576.80	94.45	152.70	191.32	224.93	3109.40	158.31	287.87	424.61	1703.04	3303.33	205.79	382.91
Quercetin	44.17	61.83	125.00	21.05	6.03	77.29	165.57	63.81	31.73	41.42	91.71	65.50	195.75	74.91	51.21	76.94
Resveratrol	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	139.17
Rutin	42.15	56.71	174.09	19.13	68.96	62.58	77.31	71.48	12.79	53.10	44.44	23.76	39.52	32.28	13.49	128.32
Sinapic acid	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd
Syringic acid	69.16	88.37	311.62	300.89	50.17	146.54	126.94	193.77	242.33	193.39	101.44	521.26	166.18	192.89	799.19	176.87
trans-Ferulic acid	47.68	80.32	211.34	39.78	50.86	5.24	30.78	37.21	57.81	35.98	15.61	254.41	307.02	86.81	252.17	41.03

nd – not detected (0 ng·ml^{-1}), DHB – 2.5-dihydroxybenzoic acid, 2-HtC – 2-Hydroxytranscinnamic acid.

Tab. 4. Results of quantitative analysis of phenolic components of honey samples by hydrolysis method.

Compound [ng·ml^{-1}]	Astragalus		Carob	Cedar	Chaste tree	Chestnut	Cornelian cherry-citrus	Heather		Lavender		Linden	Oak	Pine	Rhodo-dendron	Sandal-wood
	1	2						1	2	1	2					
DHB	12.20	7.38	86.28	155.35	9.87	70.93	14.09	29.45	179.23	51.40	43.83	30.07	517.01	234.45	11.30	44.68
2-HtC	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd
Caffeic acid	650.90	791.79	588.93	172.82	420.11	393.56	425.49	156.12	194.91	312.34	266.89	785.94	255.10	233.66	617.84	214.61
Catechin	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd
Chlorogenic acid	145.14	90.68	38.37	11.35	225.37	39.59	13.27	34.90	105.85	39.57	17.65	22.93	14.78	36.49	52.21	17.96
Epicatechin	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd
Ethyl gallate	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd
Gallic acid	45.05	65.72	1603.47	216.54	110.94	483.10	122.32	759.52	464.14	399.62	146.95	138.44	10125.04	781.58	164.21	1629.20
Isorhamnetin	124.97	115.62	94.21	34.20	56.48	78.44	111.60	60.74	34.82	57.45	121.72	72.75	105.04	40.95	105.72	49.52
Kaempferol	471.99	306.21	196.45	166.85	269.06	168.87	285.41	133.76	147.43	162.94	335.99	123.22	186.00	195.24	666.51	350.82
Lutolein	46.87	39.27	121.09	25.04	28.49	167.39	72.54	129.11	17.48	110.34	106.63	12.50	21.08	41.34	29.59	9.88
Myricetin	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd
Naringin	65.25	42.12	29.63	31.45	36.64	25.86	88.71	43.16	32.60	33.62	36.78	18.64	0	45.44	40.42	26.60
p-Coumaric acid	126.98	94.08	116.25	111.46	72.25	51.80	49.93	78.34	92.15	29.82	123.46	132.31	204.18	68.22	190.03	80.79
Phlorizin	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd
Propyl gallate	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd
Protocatechuic acid	160.83	155.98	532.33	1570.15	141.16	359.09	278.24	434.75	4926.93	328.28	697.28	678.02	3739.88	6108.56	251.32	650.48
Quercetin	151.26	146.61	391.09	53.77	189.50	201.14	504.27	228.31	73.84	171.99	128.82	115.31	193.81	112.19	134.12	139.77
Resveratrol	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd
Rutin	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd
Sinapic acid	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd
Syringic acid	181.81	113.50	510.64	211.83	110.48	204.83	179.98	118.82	127.48	152.28	89.16	246.06	41.67	151.72	759.66	175.55
trans-Ferulic acid	15.86	20.37	149.32	29.14	nd	11.29	21.64	nd	38.86	26.18	36.10	89.90	146.08	69.67	203.73	20.61

nd – not detected (0 ng·ml^{-1}), DHB – 2.5-Dihydroxybenzoic acid, 2-HtC – 2-Hydroxytranscinnamic acid.

Tab. 5. Elemental characterization of honey samples.

Element [mg·kg^{-1}]	Astragalus		Carob	Cedar	Chaste tree	Chestnut	Cornelian cherry-citrus	Heather		Lavender		Linden	Oak	Pine	Rhodo-dendron	Sandal-wood
	1	2						1	2	1	2					
Li	0.02	0.04	0.03	0.06	0.02	0.01	0.01	0.02	nd	0.03	0.06	0.02	0.16	0.05	nd	0.01
Be	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd
B	3.91	3.16	5.93	4.02	7.02	2.25	4.65	3.55	4.22	3.48	3.40	4.49	5.13	2.31	2.04	6.95
Na	21.95	46.91	72.61	18.73	27.55	345.24	16.24	51.98	69.96	40.74	23.62	24.48	28.31	17.97	11.51	14.85
Mg	13.47	16.39	162.79	156.23	26.18	50.45	9.36	73.08	84.47	27.91	18.04	40.21	269.78	75.47	37.86	16.77
Si	4.00	5.08	7.72	16.83	13.87	18.59	3.32	25.86	127.40	6.27	5.22	14.57	37.43	172.15	10.36	4.58
P	64.75	69.79	86.62	256.49	76.07	86.28	37.89	75.85	163.42	79.59	62.44	89.50	292.10	200.90	65.80	63.16
K	442.61	514.07	3041.11	5039.98	1321.67	3615.27	438.39	1922.87	4902.19	907.61	767.74	3607.39	5400.75	5043.30	3006.05	2999.30
Ca	11.89	13.03	115.81	18.00	35.12	41.62	11.51	55.03	10.23	20.59	8.21	32.53	29.16	4.97	19.69	8.71
V	nd	nd	nd	0.01	0.01	0.02	nd	nd	nd	0.01	nd	nd	0.01	nd	nd	nd
Cr	nd	nd	0.02	0.02	0.01	0.01	nd	nd	0.02	0.01	nd	0.01	0.03	0.01	0.07	0.01
Mn	0.42	0.54	2.76	4.11	0.47	3.34	0.09	1.18	1.03	0.44	0.31	0.83	27.82	0.98	3.68	0.17
Fe	1.25	0.78	2.75	8.01	2.10	1.28	0.55	1.92	5.86	2.01	1.40	1.84	4.01	3.56	0.43	1.36
Co	0.03	0.02	0.02	0.24	0.04	0.01	0.03	0.07	0.02	0.02	0.02	0.06	0.02	0.08	nd	0.04
Ni	0.03	0.04	0.05	0.59	0.03	0.02	0.02	0.02	0.47	0.07	0.06	0.02	0.26	0.24	0.05	0.05
Cu	0.15	0.20	0.42	2.42	0.48	0.26	0.16	0.15	0.80	0.18	0.23	0.25	1.19	0.99	0.08	0.17
Zn	2.37	0.82	1.01	1.43	0.77	1.06	0.65	0.69	0.61	0.80	0.80	1.28	1.29	0.59	0.69	0.78
Ga	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd
As	nd	nd	nd	0.02	nd	nd	nd	nd	nd	0.01	0.01	0.01	nd	nd	nd	nd
Se	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd
Rb	0.39	0.41	1.46	3.15	0.79	7.28	0.15	0.81	2.06	0.77	0.62	1.59	4.33	2.65	3.30	2.72
Sr	0.17	0.32	0.29	0.24	0.34	1.32	0.08	0.30	0.09	0.21	0.15	0.23	0.34	0.08	0.14	0.03
Ru	nd	0.01	nd	0.01	0.01	nd	0.01	nd	nd	nd	0.01	0.02	nd	nd	nd	0.01
Rh	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd
Pd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd
Ag	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd
Cd	0.02	0.02	0.01	0.09	nd	0.01	nd	nd	0.06	0.28	0.01	0.02	0.01	0.21	0.04	0.03
In	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd
Sn	0.02	0.01	0.05	0.26	0.01	0.01	0.01	0.09	0.19	0.15	0.03	0.01	0.05	nd	nd	0.30
Sb	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd
Te	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd
Cs	nd	nd	nd	0.08	nd	0.03	nd	nd	0.01	nd	nd	nd	0.01	0.01	nd	nd

Tab. 5. *continued*

Element [mg·kg^{-1}]	Astragalus		Carob	Cedar	Chaste tree	Chestnut	Cornelian cherry-citrus	Heather		Lavender		Linden	Oak	Pine	Rhodo-dendron	Sandal-wood
	1	2						1	2	1	2					
Ba	0.02	0.06	0.12	0.14	0.15	0.38	0.01	0.10	0.07	0.11	0.05	0.11	1.45	0.03	0.20	0.04
Hf	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd
Ir	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd
Pt	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd
Au	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd
Hg	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd
Tl	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd
Pb	0.04	0.03	0.01	0.04	0.24	0.03	0.08	nd	0.01	0.52	0.02	0.06	0.01	0.03	0.06	0.05
Bi	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd
Total	567.51	671.73	3 501.59	5 548.37	1 512.95	4 174.77	523.21	2 213.57	5 373.19	1 091.81	892.45	3 819.53	6 103.65	5 526.58	3 162.05	3 120.09

nd – not detected (≤ 0.00 mg·kg^{-1})

quercetin. Although differences were observed in the contents of phenolic compounds in the honey samples, resveratrol was the only compound detected in the sandalwood honey sample. This suggests that this compound could be a marker compound for sandal honey. However, further studies are required to obtain clear information on this subject.

Similar to our study, contents of catechin, myricetin, naringenin, kaempferol, gallic acid, chlorogenic acid and caffeic acid were previously reported in honey samples of different origin [21]. They determined that in the studied Bangladesh honey samples, gallic acid, chlorogenic acid, caffeic acid, benzoic acid, *trans*-cinnamic acid, catechin, myricetin, naringenin and kaempferol were present. Gallic acid, caffeic acid, chlorogenic acid, myricetin, kaempferol, coumaric acid, ferulic acid and quercetin were detected in Australian honey samples [22]. A study conducted on Portuguese honey reported coumaric acid, ferulic acid, quercetin, vanillic acid, rosmarinic acid and kaempferol in the samples [23]. When the results of the studies in the literature were compared with those of the present study, it was observed that the types and ratios of phenolic compounds in the chemical content of the samples differed by region. This may be due to the difference of all plant resources that contribute to honey in a dominant, secondary, minor or trace rate.

Elements are transported to plants and flowers through the root system. From here they pass to the nectar and then to honey. The mineral content of honey is quite low and the amount and variety of elements in honey vary greatly depending on plant sources, climatic conditions of the region and soil composition, but also depend on anthropogenic factors such as environmental pollution, beekeeping practices and honey processing [24]. Increased mineral content causes a sharp aroma and a darker colour of honey [25]. The average concentration of each element that were measured in 16 monofloral honey samples is presented in Tab. 5. The results of the elemental analyses of the honey samples showed that K was the element with the highest rate in all samples, followed by P, Mg, Na, Ca and Si. These results were similar to the results of Bouhlali et al. [26], who investigated 11 different monofloral honey produced in Morocco. In terms of the total content of elements, the honey samples observed in this study can be ranked as follows: oak > cedar > pine > heather 2 > chestnut > linden > carob > sandalwood honey > rhododendron > heather 1 > chaste > lavender 1 > lavender 2 > astragalus 1 > astragalus 2 > cornelian cherry-citrus honey.

Other studies in the literature reported that the mineral content of honeydew honeys or dark coloured honeys was higher than that of blossom honeys [7]. A similar observation was made in the present study as the mineral content of honeydew honeys and dark coloured honeys was higher compared to other honeys.

Many studies were carried out to determine the elemental profile of various monofloral honey types produced in various parts of the world. KARABAGIAS et al. [18] conducted a study on Mediterranean citrus honeys and determined that Ca, P and Mg were the most abundant elements. Similarly, TÜZEN et al. [27] conducted a study to evaluate the trace element content of honey samples of different botanical origin collected across Turkey and reported that while cadmium (Cd) was found at the lowest concentration, Fe had the highest concentration. This result showed that trace element contents of honey produced in different regions correlated with the degree of the trace element contamination of the environment. PISANI et al. [28] and KILIÇ ALTUN et al. [29] carried out mineral substance analyses on multifloral and monofloral honey samples originating from Italy and Turkey, respectively. Their results were in support of those of the present study as they determined that botanical origin affected the mineral profile of the honeys. In those studies, which were conducted to determine the elemental content of honey samples of various origins, it was observed that more significant differences were in the trace element levels, although the macroelement contents were similar. This might have been due to various factors such as the soil structure, floral resources and grade of industrialization of the region where the honey samples were obtained. Thus, the elemental composition of honey can provide information about its nutritional value and can be used in environmental monitoring studies.

Data on mineral composition of honey samples were treated by PCA, results are shown in Fig. 1. Two principal components PC1 and PC2 explained 96.1 % of the total variability. Oak, cedar, pine and heather 2 were separated from other samples by being in the positive region of PC1. Oak, cedar, pine and heather 2 honey samples were well separated from the other samples due to their significantly higher total contents of elements. It is apparent that the oak honey sample was clustered between the positive parts of both PC1 and PC2.

The in vitro antibacterial activity test results and *MIC* values are presented in Tab. 6. Gram-positive bacteria (*Bacillus cereus*, *Enterococcus faecalis* and *Staphylococcus aureus*) were observed to be sensitive to the honey samples, the inhibition zone diameters being found to range from 10 mm to 22 mm. Pine honey showed the highest antimicrobial effect against *B. cereus* with a 22 mm inhibition zone diameter, sandalwood honey against *E. faecalis* with a 12 mm inhibition zone diameter, and pine honey and rhododendron honey against *Staph. aureus* with a 20 mm inhibition zone diameter. The results obtained by the microbroth dilution method showed that the *MIC* values varied in the range of 31.25–250 mg·ml^{-1} for the Gram-positive bacteria, whereas the honey samples had no inhibition effect on the Gram-negative bacteria. When the antibacterial effects of the monofloral honey samples were previously investigated, Gram-negative bacteria were found to be much more resistant compared to Gram-positive bacteria, with only very few samples having inhibition zones, the diameters of which were found to be negligibly low. In a study conducted on three

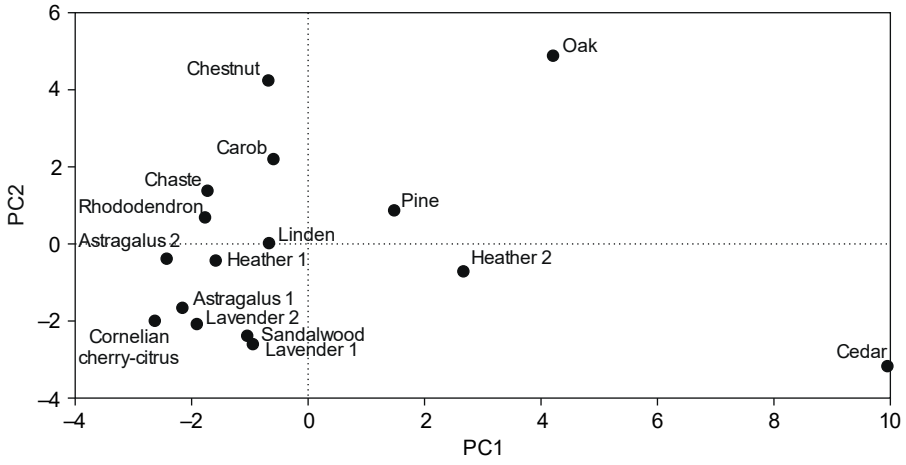

Fig. 1. Principal component analysis of the elemental content parameters loadings of monofloral honeys.

Tab. 6. Antibacterial activity of honey samples.

Honey samples	Inhibition zone diameter [mm]							Minimum inhibitory concentration [mg·ml^{-1}]						
	B1	B2	B3	B4	B5	B6	B7	B1	B2	B3	B4	B5	B6	B7
Astragalus 1	14	–	14	–	–	–	–	250	–	250	–	–	–	–
Astragalus 2	13	–	13	–	–	–	–	250	–	250	–	–	–	–
Carob	15	–	18	11	–	–	–	250	–	125	–	–	–	–
Cedar	14	11	17	–	–	–	11	250	250	125	–	–	–	–
Chaste	12	–	13	–	–	–		–	–	250	–	–	–	–
Chestnut	14	10	15	–	–	–	–	250	–	125	–	–	–	–
Cornelian cherry-citrus	19	–	12	–	–	–	–	62.5	–	250	–	–	–	–
Heather 1	12	–	14	–	–	–	–	250	–	125	–	–	–	–
Heather 2	18	11	17	–	–	10	–	125	250	125	–	–	–	–
Lavender 1	14	11	15	–	–	–	–	250	–	125	–	–	–	–
Lavender 2	16	11	15	–	–	–	–	125	–	125	–	–	–	–
Linden	13	11	13	–	–	–	–	250	–	250	–	–	–	–
Oak	15	11	17	–	10	–	–	250	–	62.5	–	–	–	–
Rhododendron	14	10	20	–	–	–	–	250	–	31.25	–	–	–	–
Sandalwood	18	12	15	–	10	–	–	125	250	125	–	–	–	–
Pine	22	10	20	–	–	–	–	31.25	250	31.25	–	–	–	–
Artificial Honey	–	–	–	–	–	–	–	–	–	–	–	–	–	–

B1 – *Bacillus cereus* BC 6830, B2 – *Enterococcus faecalis* NCTC 12697, B3 – *Staphylococcus aureus* NCTC 10788, B4 – *Escherichia coli* NCTC 9001, B5 – *Pseudomonas aeruginosa* NCTC 12924, B6 – *Salmonella* Typhimurium RSSK 95091, B7 – *Yersinia enterocolitica* ATCC 27729.

different honey samples produced in Anatolia, the samples were found to have a moderate antimicrobial effect against *Helicobacter pylori* ATCC 49503, *Staph. aureus* ATCC 25923, *Bacillus subtilis* ATCC 6633, *Candida tropicalis* ATCC 13803 and *Candida albicans* ATCC 10231 [30]. In another study conducted with honey samples collected from Turkey, it was reported that honey samples inhibited the growth of bacteria *P. aeruginosa* and *Staph. aureus* [31]. Those results are compatible with the results of the present study. Similarly, in a study carried out on chestnut honey produced at Mount Etna, Italy, it was reported that the honey had good antibacterial effect against *E. coli*, *P. aeruginosa* and *E. faecalis* [32]. In another study, to determine the antimicrobial activity of honeys produced in Turkey, chestnut and astragalus honeys were tested against *E. coli*, and clover and mixed flower honeys were tested against *Staph. aureus*. The results showed that the astragalus and mixed flower honeys had high antimicrobial effects against *P. aeruginosa* [33]. However, in the present study, the only determined antimicrobial effects were found to be those of carob honey against *E. coli* at a low rate (11 mm inhibition diameter), pine honey and rhododendron honeys against *Staph. aureus*, and sandalwood and oak honeys against *P. aeruginosa* (10 mm inhibition diameter).

CONCLUSION

In this study, microscopic, phenolic, elemental and antimicrobial analyses were carried out on monofloral honey samples. It was determined that none of the honey samples had the same properties as each other, as they exhibited different phytochemical content and bioactive properties. In addition, it was observed that the source of the honey (honeydew, nectar) or the floral source of the nectar significantly affected the properties of the honey. It is important at this point to define the unique properties of monofloral honey, which is more popular among consumers especially because of its origin and the fact that it is sold at higher prices. Thus, counterfeiting can be prevented by defining the specific characters of honey. However, in order to prevent consumers from being victimized, palynological analyses should be carried out especially for honeys that are sold as monofloral and the rate of representation of the honey sample with the pollen of the relevant plant should be determined.

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