

Chestnut Honey as a Complementary Medicine: Determination of Antibacterial Activity, Heavy Metal Residue and Health Risk Assessment

Research Article

Mesut Ertan GÜNEŞ

Department of Food
Processing, Vocational
School of Technical
Science, Bursa Uludağ
University Turkey

ORCID-

[0000-0002-9347-8307](https://orcid.org/0000-0002-9347-8307)

Correspondence

Mesut Ertan GÜNEŞ

egunes@uludag.edu.tr

Article info

Submission: 01-05-2021

Accepted: 28-08-2021

Online First: 09-08-2021

e-ISSN: 2548-1150

doi prefix: 10.31797/vetbio

• <http://dergipark.org.tr/vetbio>

This work is licensed under a
Creative Commons Attribution
4.0 International License



ABSTRACT

In this study, residue levels for heavy metal content and antimicrobial effects of 27 chestnut honey samples obtained from member beekeepers of Yalova Bee Association are examined. Honey samples are digested under high temperature and pressure in a microwave oven and Arsenic (As), Lead (Pb), Cadmium (Cd), Mercury (Hg) limits are determined using ICP-OES. Using Agar gel diffusion method, 19 of the honey samples' antibacterial activity was tested on strains of *Staphylococcus aureus* (ATCC 25923), *Escherichia coli* (ATCC 25922), *E. coli* O 157: H7, *Bacillus cereus* (ATCC 6633), *Salmonella Typhimurium* (NCTC 12416) and *Listeria monocytogenes* (ATCC 7644). As, Pb, Cd and Hg residue levels in all honey samples were below the designated limits by Turkish Food Codex Regulation. The non-carcinogenic health risk associated with heavy metal contamination in honey is found to be very low. None of the 19 honey samples show antibacterial effect on *L. monocytogenes* strains. At 100% honey concentration, in 18 out of 19 honey samples, antibacterial effect is observed on strains of *S. Typhimurium*, *E. coli* O 157: H7 while all 19 samples show antibacterial activity on strains of *E. coli* and *B. cereus*. The antibacterial activity on these bacteria is recorded to be maintained at 50% honey concentration. At 25% honey concentration, the antibacterial effect show a decline in most of the honey samples.

Keywords: Chestnut honey, antibacterial activity, heavy metals, health risk

INTRODUCTION

Chestnut honey produced in Turkey is rich in bioactive substances (Küçük et al., 2007). Chestnut honey forests are located across Black Sea, Marmara and Aegean regions (Sarıkaya et al., 2009).

With its dark color and strong aromatic flavor chestnut honey is rich in glucose oxidase, catalase, ascorbic acid, carotenoid derivatives, organic acids, Maillard reaction products, amino acids and proteins, phenolic compounds, flavonoids and minerals such as tannin, potassium, magnesium, manganese and barium (Dağ et al., 2017). In different studies conducted on Anatolian chestnut honey, significant antioxidant compounds with high antibacterial activity are reported (Ayvaz et al., 2018; Güneş et al, 2017; Sarıkaya et al., 2019).

Honeybees are exposed to environmental pollutant as they gather plant nectar, pollen and water (De Oliveira et al. 2016). Bee products are important not only for their rich nutritional value but also together with the bees for being significant agents in monitoring environmental pollution (Yarsan et al., 2007).

How to cite this article

Güneş, ME. (2021). Chestnut Honey as a Complementary Medicine: Determination of Antibacterial Activity, Heavy Metal Residue and Health Risk Assessment. *Journal of Advances in VetBio Science and Techniques*, 6(2), 82-89. <https://doi.org/10.31797/vetbio.931144>

Metals such as arsenic, cadmium, lead and mercury may be present in soil, water and atmosphere at certain levels. In addition, metals may contaminate food products as a result of pollution by human activities such as vehicle exhaust, industrial waste, or irresponsible farming. Individuals may be exposed to such heavy metals through the environment, consumption of contaminated products and/or water. Accumulation of metals in the body may, in time, lead to several health issues and risks (Tolgahan et al., 2018).

In this study, residue levels for heavy metal content and antimicrobial effects of 27 chestnut honey samples obtained in 2014 from member beekeepers of Yalova Bee Association are examined.

MATERIAL and METHOD

Honey samples

Locations where honey samples are collected: 27 raw honey samples are collected from 7 different spots where the bee yards of Yalova Beekeepers Association members are commonly located. The collected samples are stored in glass jars in a dark and cool environment. All 27 samples are assessed in terms of their heavy metal profiles. Although no crystallization was expected for chestnut honey, 8 of the 27 samples were crystallized, therefore antibacterial activity is tested for the 19 non-crystallized honey samples.

Antimicrobial analysis

In this study, antibacterial activity is assessed on strains of *S. aureus* (ATCC 25923), *E. coli* (ATCC 25922), *E. coli* O 157: H7, *B.cereus* (ATCC 6633), *S.Typhimirium* (NCTC 12416) and *L.monocytogenes* (ATCC 7644). Agar-well diffusion method was used to assess antibacterial activity (Perez et al.,1990). Standard strains enriched in Mueller Hinton Broth are adjusted to 0.5 McFarland and cultivated into 1 ml. Mueller Hinton agar (Oxoid, CM 0337). Samples with a concentration of 100%, 50%(v/v) and 25% (v/v)

are prepared from all 19 honey samples. Parallel honey samples of 50 µl at each concentration level are added into holes with a diameter of 6 mm on the Petri dish. Plates are incubated at 37°C for 24 hours. The average of the inhibition zone diameters formed at the end of the incubation period is recorded. Zone diameters of <5.5 – 9 mm, 9 – 12 mm, 12 – 15 mm and > 15 mm indicate very low, low, average and high inhibition respectively (Küçük et al., 2007). All analyses are carried out two times as parallel processes.

Heavy metal analysis

Crystallized honey samples are kept in a water bath at 70°C to dissolve. 10 ml HNO₃ (65%, Merck) and 1 ml H₂O₂ (30%, Merck) are added on samples of 0.5 gr and resulting samples are burned in a microwave oven (Berghof MWS-3+ / Germany) with gradually increasing temperature (160-200 C/max. 53 min). At the end of the combustion, 5 ml of ultrapure water (18.3 MΩ ultrapure water - Human, Korea) was added to each sample. Then, the concentrations of Pb, Cd, As and Hg are determined with a calibrated Plasma Optical Emission Spectrometer (ICP-OES-Perkin Elmer Optima 2100DV). Heavy metal analyses are conducted in the Food Research laboratory of Turkish Ministry of Agriculture and Forestry. The settings of the ICP-OES device are presented in Table 1.

Potential health risk assessment of the honey samples

Health risk of honey samples collected from Yalova province is determined by Target Hazard Quotient (THQ) analysis. THQ indicates the ratio between heavy metal exposure and the reference exposure dose. The potential health risk resulting from a daily consumption of 3.3 g (FAO, 2017) of honey is calculated for a seventy-year-old adult weighing seventy kg (Magna et al., 2018).

Table 1. Operating Conditions of the ICP-OES (Perkin Elmer Optima 2100 DV).

Parameter	
RF power	1450 watts
Plasma gas flow	15 L/min
Auxillary gas flow	0.2 L/min
Nebulizer Flow	0.65 L/min
Sample Pump Flow	1.5 mL/min
Plasma Viewing	Dual View
Elements	Wavelength (nm)
Pb	220.353
Cd	228.802
As	193.696
Hg	253.652

$$THQ = \left[\frac{(EFr \cdot EDtot \cdot IFR \cdot C)}{(RfDo \cdot BWa \cdot ATn)} \right] \cdot 10^{-3}$$

Formula variables are as follows; EFr, indicates the exposure frequency (365 days/year); EDtot, the exposure duration (70 year); IFR, the food ingestion rate (g day⁻¹); C, the concentration (µg g⁻¹); RfDo, the oral reference dose (µg g⁻¹ day⁻¹) (Cd: 0.001; Pb: 0.004; As: 0.003, day⁻¹, Hg: 0,0016 mg/kg-1/day declared by US-EPA, 2009; BWa, the

adult body weight (70 kg) and ATn, is the average time for non-carcinogens which is equal to EFr multiplied by EDtot.

RESULTS

Antibacterial effects on selected Gr (-) and Gr (+) bacteria are presented in Table 2. None of the 19 honey samples show antibacterial effect on strains of *L. monocytogenes*.

Table 2. Heavy Metal Levels of Honey Samples (µg/kg), Health Risk due to intake (THQ) and tolerable daily intake limits (% PTDI)

µg/kg	Pb (ppb)	Cd (ppb)	As (ppb)	Hg (ppb)
Lowest	0.096	0.076	0.032	0.240
Highest	39.067	1.198	0.950	44.546
Average	21.301	1.183	0.516	21.490
Measurement uncertainty	±0.004	±0.001	±0.001	±0.003
THQ	2.5x10 ⁻⁴	5.56x 10 ⁻⁵	8.1x10 ⁻⁵	6.45x10 ⁻⁴
PTDI (%)	4.4	15.4	4.71	4.4

One hundred percent concentrations of all honey samples are recorded to have high antibacterial activity with wide zone diameters on strains of *S. Typhimurium*. Except for one sample, at 100% concentration level, all samples show antibacterial effect on strains of *E. coli* and *B. cereus*. Nevertheless, only 15 samples form antibacterial effect zones on *S. aureus* strains.

The antibacterial activity of samples at 50% concentration level is found to be in line with that of 100% concentration. However, partial decreases in activity zones are recorded. When the concentration of honey samples is reduced to 25%, a decline and even loss of antibacterial activity has been detected in many samples. At this concentration level, 8 samples are observed

to show no antibacterial effect on *S. Typhimirium* strains while only 10 samples achieve to form activity zones on *S. aureus* and *E. coli* O 157:H7 strains. The number of samples with activity zones at 25% concentration decreases to 7 samples on *E. coli* (ATCC 25922) and 3 samples on *B.cereus* strains. At all concentration levels, the highest antibacterial activity is detected on strains of *S. Typhimirium*. This is followed by *E. coli* O 157: H7 and *E. coli*, *S. aureus* and *B. cereus*, respectively.

Levels of Lead (Pb), Cadmium (Cd), Arsenic (As), Mercury (Hg) in honey samples are determined by ICP-MS. Heavy metal contents of samples are recorded as \bar{x} 21.301348 ppb for Pb, \bar{x} 1.18381 ppb for Cd, \bar{x} 21.4909 ppb for Hg and \bar{x} 0.51687 ppb for As (Table 3).

When the potential health risk associated with heavy metal residue levels in Yalova chestnut honey samples are examined, exposure values are determined to be 2.5×10^{-4} for Pb, 5.5×10^{-5} for Cd, 8.1×10^{-5} for As and 6.45×10^{-4} for Hg. It is noted that the recorded levels are below the designated reference limits of daily consumption. (US-EPA 2009) Daily tolerable level of heavy metal intake (PTDI) determined by European Food Safety Authority (EFSA-2012) and FAO/WHO Expert Committee on Food Additives (JEFCA 2011) are 0.357; 2.1; 0.185; 0.357 mg/kg/day for Cd, As, Hg, Pb respectively. Taking these limits in consideration, a 70 kg adult's 3.3 gr of Yalova honey consumption per day corresponds to %15.4 Cd > % 4.7 As >%4.4 Pb >% 4.3 Hg of the daily tolerable intake limits per each heavy metal. (Table 3)

Tablo 3. Heavy Metal Profiles of Yalova Honey and Samples in Previous Literature (mg/kg)

Origin of Samples	Pb (ppm)	Cd (ppm)	As (ppm)	Hg (ppm)	Literature
Yalova	0.021	0.0011	0.0005	0.0214	Our values
Anatolia	0.0003	0.000038			Altunatmaz, 2018
Tokat	0.0018	0.000041			Arıkan, 2010
Elazığ	0,409	337.9	4.8		Aygün, 2020
Bingöl	Nd	nd	nd	nd	Bengü, 2020
Trakya	Bdl- 0,48	Bdl-0,01			Citak, 2012
Mid-Anatolia	1.5	0.242			Leblebici, 2008
China	0.033	0.001	0.013	0.0016	Mei-ru, 2013
Sicily	0.170	0.0153			Naccari, 2014
Karadeniz	0.04- 0.73	nd- 1.28			Nisbet, 2013
Muğla	0.005-0.016	Bdl			Silici, 2013
Comercial	1.101	0.343		0.618	Şireli,2015
Anatolian	0.008-0.106	0.9-17.9			Tuzen, 2007
Hatay	0.39	0.03			Yücel, 2013

DISCUSSION

It was first suggested in 1892 by Van Ketel et al. (1892) that honey may have antibacterial effects (Dustman, 1979). The antibacterial activity of honey was first described in 1937, by Duo and Dziao (1937) and the substance in honey enabling for bacteria inhibition was named as inhibin. It is later argued that the antibacterial efficacy may also be influenced by the floral

sources from which the honey is obtained (Molan et al., 1988).

In a study focusing on honey samples from Turkey, the antimicrobial activity of chestnut, rhododendron, and multifloral honey samples is examined against the strains of *Helicobacter pylori*, *S. aureus*, *B. subtilis*, *C. tropicalis* and *C. albicans* and the highest antimicrobial activity is

recorded for chestnut honey (Küçük et al., 2007). Results of the studies referring to a correlation between the antibacterial activity of chestnut honey and its antioxidant capacity have shown that the types of honey with high antioxidant content, especially chestnut honey, also exhibit significantly high antibacterial efficacy (Ayvaz et al., 2018; Güneş et al., 2016; Yıldız et al., 2013).

S.aureus is the most vulnerable and sensitive bacteria against the antibacterial effect of honey. Güneş et al. (2017) recorded antibacterial activity of chestnut honey at 100%, 50% and 25% concentrations on strains of *S.aureus*. In this study, strong antibacterial activity against *S. aureus* strains is determined for chestnut honey samples from Yalova province.

Ayvaz et al. (2018) reported that the most sensitive microorganisms to chestnut honey samples from the Black Sea region are *C. albicans*, *S. aureus* and *E. coli*, while the most resistant microorganism is *B. subtilis*. The antibacterial activity of honey samples from Yalova province on *E. coli* strains is in line with this study. Combarros-Fuertes et al. (2020) investigated the chestnut, avocado and polyfloral honey samples' mechanism of action on *E. coli* and *S. aureus* strains and pointed out that all honey types caused significant alterations on the cell structures of *E. coli* and *S. aureus*. The study especially highlighted the remarkable metabolic deformations caused on *S. aureus* by honey samples' antibacterial effect.

In their studies on chestnut honey and its antibacterial and antifungal activity on strains of *E. coli* ATCC (25922), *Yersinia pseudotuberculosis* ATCC (911), *Pseudomonas aeruginosa* ATCC (43288), *S. aureus*, ATCC (25923), *Enterococcus faecalis* ATCC (29212), *B. cereus* (709 Roma), *Mycobacterium smegmatis* (ATCC607), *Candida albicans* ATCC (60.193) and *Saccharomyces cerevisiae* (RSKK 251), Kolaylı et al. (2016) recorded the highest inhibition levels against *S. aureus*, *E.*

faecalis, *Y. pseudotuberculosis* and *E. coli* strains.

The antimicrobial properties of honey are due to its acidity, osmolarity, H₂O₂, low water activity (aW), aromatic acids, different chemical compounds; phenolics and flavonoids content (Bogdanov et al., 2008). Chestnut honey is rich in terms of mineral content and phenolic compounds (Güneş et al., 2017; Kolaylı et al., 2016). The highest antibacterial activity for honey samples from Yalova province is observed on *S.Typhimirium* followed by *E. coli* O 157:H7 and *E.coli*, *S.aureus* and *B.cereus*, respectively. Studies have shown that chestnut honey accelerates the treatment of wound infections and, especially, shortens the healing process of open wounds caused by diabetes (McLoone et al., 2020). The therapeutic efficacy of Yalova chestnut honey should be considered and utilized for the same purpose.

Heavy metals such as lead, cadmium, arsenic and mercury are taken into the human body through food products, drinking water and respiration. Such metals cannot be excreted through the body's excretory system and accumulate in the soft tissues of the body. Accumulation of each metal at levels above the effective doses for children and adults may cause organ poisoning, and several other severe health issues (i.e. thyroid, neurological, autism and infertility) and even death (Özpolat, 2016).

Yalova province is surrounded by the regions where heavy industrial organizations operate. In the study, pollution levels and potential health risks are revealed by the heavy metal profiles of honey samples obtained from the region.

The average lead level of 27 honey samples analyzed in the study is found to be 0.021 ppm. This is below the levels recorded in all previous studies mentioned above. (Table 4) However, the lead levels of honey samples from Yalova are found to be above the levels recorded by Tuzen et al. (2007) for honey samples from different cities in Anatolia, Arıkan (2010) for samples

from Tokat province, Silici and et al. (2013) for samples from Muğla, Nisbet et al. (2013) for samples from Black Sea region, Altunalmaz et

al. (2018) for samples from Anatolia and Bengü (2011) for samples from Bingöl province.

Table 4. Antimicrobial Activity of Chestnut Honey from Yalova

Con. v/v (%)	Bacteria	Honey Samples (Inhibition zone diameter mm)																		
		1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19
100%	<i>B.cereus</i>	27	28	27	31	26	29	26	-	28	29	30	32	28	27	27	29	26	30	33
	<i>S.aureus</i>	20	-	15	21	18	-	-	-	14	15	17	15	13	15	15	19	18	14	14
	<i>E.coli</i>	29	29	28	33	32	30	-	30	32	31	35	33	30	31	34	30	31	31	32
	<i>E.coli O157 H:7</i>	29	33	30	-	32	28	29	26	30	30	30	32	31	30	31	31	30	32	32
	<i>S. Typhimirium</i>	30	32	30	38	30	28	36	41	32	34	32	36	36	40	35	32	28	38	32
50%	<i>B.cereus</i>	23	24	19	-	20	23	23	-	24	26	24	25	26	22	19	25	-	24	24
	<i>S.aureus</i>	12	-	13	14	14	-	-	-	11	13	14	11	-	14	-	14	15	12	11
	<i>E.coli</i>	25	24	26	28	20	24	-	22	27	23	29	24	24	22	27	28	29	24	27
	<i>E.coli O157 H:7</i>	26	23	27	-	25	27	27	22	27	25	23	28	28	27	28	23	26	29	26
	<i>S. Typhimirium</i>	23	18	22	26	20	19	18	21	23	20	20	18	23	24	19	22	22	20	27
25%	<i>B.cereus</i>	15	-	-	-	14	-	-	-	-	-	-	20	-	-	-	-	-	-	-
	<i>S.aureus</i>	10	-	10	12	11	-	-	-	-	11	11	-	-	-	-	12	11	9	-
	<i>E.coli</i>	22	-	-	24	-	19	-	20	-	19	-	19	-	-	-	-	-	19	-
	<i>E.coli O157 H:7</i>	22	-	24	-	-	22	-	18	23	-	16	-	-	15	-	-	19	23	-
	<i>S. Typhimirium</i>	18	17	19	22	18	-	-	19	22	-	17	-	-	-	-	18	18	16	-

The average cadmium level of honey samples from Yalova is recorded to be 0.0011 ppm. Considering the previous literature, a higher cadmium level is observed compared to honey samples collected from Tokat (Arıkan, 2010), Trakya (sunflower honey samples) (Çıtak, 2012), Muğla (Silici, 2013), Anatolian region Altunalmaz, 2018) and Bingöl (Bengü, 2020). Compared to the previously published studies, lower cadmium levels are observed. In a study conducted by Naccari et al.(2016) on 3 different monofloral honey samples in Sicily, higher lead and cadmium levels were recorded compared to the present stud

The average arsenic residue levels for honey samples collected from Yalova is determined to be 0,0005 ppm. This is a lower level compared to honey samples for Elazığ (Aygün, 2020).

In the study, the average mercury residue level of 30 chestnut honey samples is found to be 0.0214 ppm. The observed level is much lower

compared to the residue limits recorded by Şireli et al. (2015) for commercial honey samples. The mercury residue level obtained by Ru et al. (2013) in their study on 48 monofloral honey samples is lower than that of Yalova honey. The cadmium values recorded in the same study are similar to the findings of this study while the lead and arsenic levels are higher than the values observed for chestnut honey samples from Yalova.

There is no designated maximum limit on the heavy metal contamination of honey in the Turkish Food Codex Regulation (TGK-29.12.2011). It is concluded that the target hazard quotient and health risk factors associated with lead, cadmium, arsenic and mercury residues in honey calculated for a seventy-year-old adult, weighing seventy kg with a 3.3 g of daily honey consumption, is below the reference limits set by US-EPA (US-EPA 2009). When the level of heavy metal residues in the honey samples are evaluated for consumer health and in

terms of tolerable weekly intake limits (EFSA 2012, JEFCA 2011), it is determined that a 70 kg adult's 3.3 gr of Yalova honey consumption per day corresponds to 15.4%, 4.7%, 4.4%, 4.3% of the daily tolerable intake limit for Cd, As, Pb, Hg, respectively. Adjusting for individual differences, potential health risk factors associated with a daily consumption of even 15 g of honey collected from Yalova province would be below the designated reference limits.

CONCLUSION

In conclusion, pure chestnut honey has a potential to be a complementary product in protective and therapeutic applications for human health. It has been concluded that honey samples obtained from Yalova region is a valuable source in terms of their potential use in treatments of both traditional and complementary medicine (apitherapy). period.

ACKNOWLEDGMENT

The authors kindly thank Dr. Evren Erköse and appreciate his contributions during the research

Ethical approval: It has been declared that it is not subject to ethics committee approval.

Conflict of interest: The authors declare that they have no conflict of interest.

KAYNAKLAR

Altunatmaz, S.S., Tarhan, D., Aksu, F., Özsoğacı, N.P, Erman, M., & Barutçu, U.M. (2018). Levels of Chromium, Copper, Iron, Magnesium, Manganese, Selenium, Zinc, Cadmium, Lead and Aluminium of honey varieties produced in Turkey. *Food Science and Technology*, 39, 392-397. Doi:10.1590/fst.19718

Arıkan A. (2010). The effect of traffic on heavy metal accumulation in bee products. Ms Thesis, Gaziosmanpaşa University, Graduate School of Natural and Applied Sciences, Tokat.

Aygün, O. (2020). Elazığ'da Üretilen Balların Bazı Toksik Ağır Metal Düzeyleri. *Fırat Üniversitesi Mühendislik Bilimleri Dergisi*, 32(1), 119-125.

Ayvaz, M. Ç., Ömür, B., Ertürk, Ö., & Kabakçı, D. (2018). Phenolic profiles, antioxidant, antimicrobial, and DNA damageinhibitory activities of chestnut honeys from Black Sea Region of Turkey. *Journal of Food Biochemistry* 42:e12502

Bengü, A.S., & Kutlu, M.A. (2020). Bingöl'den temin edilen ballarda icp-ms ile bazı temel ve toksik elementlerin analizi. *Uludağ Arıcılık Dergisi*, 20 (1), 1-12.

Bogdanov, S., Jurendic, R.S., Sieber, R., & Gallmann, P. (2008). Honey for Nutrition and Health: A Review. *Journal of the American College of Nutrition*, 27 (6), 677-689.

Citak, D., Silici, S., Tuzen, M., & Soylak, M. (2012). Determination of toxic and essential elements in sunflower honey from Thrace Region, Turkey. *International Journal of Food Science and Technology*, 47, 107-113. Doi:10.1111/j.1365-2621.2011.02814.x

Combarros-Fuertes, P., Estevinho, L.M., Teixeira-Santos, R., Rodrigues, A.G., Pina-Vaz, C., Fresno, J.M., & Eugenia Tornadijo, M. (2020). Antibacterial Action Mechanisms of Honey: Physiological Effects of Avocado, Chestnut, and Polyfloral Honey upon *Staphylococcus aureus* and *Escherichia coli*. *Molecules*, 25, 1252. Doi:10.3390/molecules25051252

Dağ, B., Sıralı, R., & Tarakçı, Z. (2017). Investigation of Some Properties of Chestnut Honey Produced in Black Sea Region of Turkey. *Batman University Journal of Life Sciences*, 7(2/2), 118-123.

De Oliveira, R.C., Queiroz, S.C.D.N., Da Luz, C.F.P., Porto, R.S., & Rath S. (2016). Bee pollen as a bioindicator of environmental pesticide contamination. *Chemosphere*, 163, 525-534. doi:10.1016/j.chemosphere.2016.08.022

Duo, D.H., & Dziao, S.T. (1937). Nachweis antibakterieller, hitze- und lichtempfindlicher Hemmungsstoffe Inhibine im Naturhonig Blatenhonig. *Zeitschrift für Hygiene und Infektionskrankheiten*, 120, 155-167.

Dustmann, J. H. (1979). Antibacterial effect of honey. *Apiacta*, 14(1), 7-11.

EFSA (2012) Scientific Committee. Scientific panels and units in the absence of actual measured data. *European Food Safety Authority Journal*, 10: 2579. doi: 10.2903/j.efsa.2012.2579

FAO/WHO Expert Committee on Food Additives (JECFA) (2011). Safety evaluation of certain food additives and contaminants. WHO Food Additives. 63, 605-685.

F.A.O. (Food and Agriculture Organization of the United Nations) (2017). Largest consumers of honey. 1-2. accessed 2020 Oct.1 <http://www.fao.org/3/ca4657en/ca4657en.pdf>.

Güneş, M. E., Şahin, S., Demir, C., Borum, E., & Tosunoglu, A. (2017). Determination of phenolic compounds profile in chestnut and floral honeys and their antioxidant and antimicrobial activities. *Journal of Food Biochemistry*, 41(3), 1-12.

Idris, Y. M. A., Mariod, A. A., & Hamad, S. I. (2011). Physicochemical properties, phenolic contents and antioxidant activity of Sudanese honey. *International Journal of Food Properties*, 14, 450-458.

- Kolayli, S., Can, Z., Yildiz, O., Sahin, H., & Karaoglu, S.A. (2016).** A comparative study of the antihyaluronidase, antiurease, antioxidant, antimicrobial and physicochemical properties of different unifloral degrees of chestnut (*Castanea sativa* Mill.) honeys. *Journal of Enzyme Inhibition and Medicinal Chemistry*, 31(S3), 96–104. <https://doi.org/10.1080/14756366.2016.1209494>
- Küçük, M.; Kolaylı, S.; Karaoğlu,Ş; Ulus,Y,E; Baltacı,C; & Candan,F (2007).** Biological activities and chemical composition of three honeys of different types from Anatolia. *Food Chemistry*, 100(2), 526-534
- Leblebici, Z. (2006).** Kayseri Yöresinde Bulunan Bazı Bal Örneklerinde Ağır Metal Kirliliğinin Belirlenmesi, Yüksek Lisans Tezi, Erciyes Üniversitesi Fen Bilimleri Enstitüsü, Kayseri, 71.
- Magna, E.K., Dabi, M., Badu, E., & Owusu, P. (2108).** Determination of Heavy Metals and Potential Health Risk Assessment of Honey Harvested from the Tamale Metropolis of Ghana Using Atomic Absorption Spectrophotometer (AAS). *Elixir Pollution*, 121, 51522-51525.
- McLoone, P., Tabys, D., & Fyfe, L. (2020).** Honey Combination Therapies for Skin and Wound Infections: A Systematic Review of the Literature. *Clinical, Cosmetic and Investigational Dermatology*, 13, 875–888. doi: 10.2147/CCID.S282143.
- Molan, P.C., Smith, I.M., & Reid, G.M. (1988).** A comparison of the antibacterial activity of some New Zealand honeys. *Journal of Apicultural Research*, 27(4): 252-256.
- Naccari, C., Macaluso, A., Giangrosso, G., Naccari, F., & Ferrantelli, V. (2014).** Risk Assessment of Heavy Metals and Pesticides in Honey from Sicily (Italy). *Journal of Food Research*, 3(2), 107-117. doi:10.5539/jfr.v3n2p107
- Nisbet, C., Guler, A., Yarim, G.F., Cenesiz, S., & Ardalı, Y. (2013).** Çevre ve flora kaynaklarının arı ürünlerinin mineral madde içerikleri ile ilişkisi. *Türk Biyokimya Dergisi*, 38 (4), 494–498.
- Özbolat, G., & Tuli, A. (2016).** Ağır metal toksisitesinin insan sağlığına etkileri. *Archives Medical Review Journal*, 25(4), 502-521. doi:10.17827/akt.253562
- Perez, C., Pauli, M., & Bazerque, P. (1990).** An antibiotic assay by the agar well diffusion method. *Acta Biologia et Medicina Experimentalis*, 15, 113–115.
- Ru, Q.M., Feng, Q., & He, H. J. (2013).** Risk assessment of heavy metals in honey consumed in Zhejiang province, southeastern China. *Food and Chemical Toxicology*, 53: 256-262. <https://doi.org/10.1016/j.fct.2012.12.015>
- Sarıkaya, A. O., Ulusoy, E., Ozturk, N., Tunçel, M., & Kolaylı, S. (2009).** Activity and phenolic acidconstituents of chestnut (*Castania sativamill.*) honey and propolis. *Journal of Food Biochemistry*, 33, 470–481.
- Seven, T., Can, B, Darende, B.N, & Ocak, S. (2018),** Hava ve Toprakta Ağır Metal Kirliliği. *Ulusal Çevre Bilimleri Araştırma Dergisi*, 1(2), 91-103.
- Silici, S., Uluoğlu, O.Z., Tuzen, M., & Soylak, M. (2013).** Honeybees and honey as monitors for heavy metal contamination near thermal power plants in Mugla, Turkey. *Toxicology and Industrial Health*, 32(3), 507–516. DOI: 10.1177/0748233713503393
- Şireli, U.T., Çil, G.İ., Dikmen, B.Y, Filazi, A., & Ülker, H. (2015).** Detection of Metals in Different Honey Brands. *Kafkas Üniversitesi Veteriner Fakültesi Dergisi*, 21(6), 915-918.
- Tuzen, M., Silici, S., Mendil, D., & Soylak, M. (2007).** Trace element levels in honeys from different regions of Turkey. *Food Chemistry*, 103, 325–330. [Doi:10.1016/j.foodchem.2006.07.053](https://doi.org/10.1016/j.foodchem.2006.07.053)
- Türk Gıda Kodeksi Bulaşanlar Yönetmeliği (2011).** Resmi Gazete, 29.12.2011, Sayı: 28157 (3. Mükerrer), Ankara
- USEPA. (2009).** Risk-based Concentration table. Philadelphia PA. Washington DC, USA, Environmental Protection Agency
- Yarsan, E., Karacal, F., Ibrahim, I.G., Dikmen, B., Koksall, A., & Das, Y.K. (2007).** Contents of Some Metals in Honeys from Different Regions in Turkey. *Bulletin of Environmental Contamination and Toxicology*, 79, 255–258. DOI: 10.1007/s00128-007-9034-9
- Yıldız, O., Can, A., Saral, O., Yuluğ, E., Ozturk, F., Aliyazicioglu, R., Canpolat, S., & Kolaylı, S. (2013).** Hepatoprotective potential of chestnut bee pollen on carbon tetrachloride-induced hepatic damages in rats. *Journal of Evidence Based Complementary and Alternative Medicine, Special Issue*, Article ID 461478, 9 p. <http://dx.doi.org/10.1155/2013/461478>
- Yücel, Y., & Sultanoglu, P. (2013).** Characterization of Hatay honeys according to their multi-element analysis using ICP-OES combined with chemometrics. *Food Chemistry*, 140, 231-237. <https://doi.org/10.1016/j.foodchem.2013.02.046>