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Research Article

The Antimicrobial Effects of Kyrgyz Honey

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ABSTRACT

Honey is a sweet and versatile substance produced by the complex interactions between bees and flowers. Honey is not only a nutrient, but also a bee product with antibacterial properties. Kyrgyzstan's geographical location, including the Tien Shan and Altai mountains, has made it one of the most important beekeeping regions in Central Asia. The rich flora and natural environment of these regions give Kyrgyz honey unique properties. The aim of this study is the determination of the antibacterial activity of honey samples on sale in the Chuy region of Kyrgyzstan. In this context, the antimicrobial effects of different honeys against *Staphylococcus aureus* and *Escherichia coli* were investigated using the agar-well diffusion and disc diffusion methods, with the aim of comparing the effectiveness of these methods. This study, conducted on white honey, buckwheat honey and three different multifloral honeys, provides important information for understanding the microbial effects of honey. Although the zones formed were more clearly visible when analysed using the agar-well diffusion method, it was found to be more sensitive than the disc diffusion method. As a result, it has been observed that different types of honey have different antimicrobial effects.

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Kırgız Balının Antimikrobiyal Etkileri

ÖZET

Bal, arılar ve çiçekler arasındaki kompleks etkileşimler sonucunda üretilen tatlı ve çok yönlü bir maddedir. Bal sadece bir besin maddesi değil, aynı zamanda antibakteriyel özelliklere sahip bir arı ürünüdür. Kırgızistan'ın coğrafi konumu, Tien Shan ve Altay Dağları'nı içermesi nedeniyle, Orta Asya'nın önemli arıcılık bölgelerinden biri olmasını sağlamıştır. Bu bölgelerin zengin florası ve doğal ortamı, Kırgızistan balının benzersiz özellikler kazanmasına yardımcı olmaktadır. Bu çalışmanın amacı, Kırgızistan'ın Chuy bölgesinde satılan bal örneklerinin antibakteriyel aktivitesini belirlemektir. Bu kapsamda *Staphylococcus aureus* ve *Escherichia coli*'ye karşı çeşitli balların antimikrobiyal etkileri agar-well difüzyon ve disk düfizyon yöntemleri kullanılarak araştırılmış ve bu yöntemlerin etkinliğinin karşılaştırılması amaçlanmıştır. Beyaz bal, Karabuğday balı ve üç farklı multifloral bal kullanılarak gerçekleştirilen bu çalışma, balın mikrobiyal etkilerini anlamak adına önemli bilgiler sunmaktadır. Agar-well difüzyon yöntemi kullanılarak yapılan analizlerde oluşan zonlar daha net görünürken, bu yöntem disk difüzyon yöntemine göre daha duyarlı olduğu belirlenmiştir. Sonuç olarak, farklı tür ballarda farklı antimikrobiyal etkilere sahip olduğu gözlemlenmiştir.

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INTRODUCTION

Honey, a sweet and viscous substance, is mainly produced by honeybees (Apis mellifera) (Hossain et al., 2022). Honey is a food product known and appreciated throughout the world. Flower origin, colour, aroma and taste are important factors in determining the quality of honey (Piana et al.,2023). In addition to being an important animal product, honey has become an important antimicrobial food because it has antibacterial properties and does not cause microbial resistance (Acaroz et al.,2024; Asma et al.,2022; İplikçioğlu-Çil et al., 2020). There is great interest in destroying foodborne pathogens or preventing their multiplication in food (Çakmak et al.,2023; İplikçioğlu-Çil et al.,2020; Schell et al.,2022). It strongly suggests that the antimicrobial components of honey have the potential to inhibit the proliferation and spread of pathogenic micro-organisms (İstanbullugil et al.,2023). There have been many studies of the antibacterial effects of honey on bacteria associated with wound healing (Balazs et al.,2023; Israili,2014; Skadins et al., 2023; Yupanqui Mieles et al.,2022). Recently there has been a growing demand for monofloral honeys with unique properties (Tananaki et al.,2021). Most of the honeys produced in Kyrgyzstan are polyfloral honeys, and it is difficult to produce monofloral honeys. For this reason, Kyrgyz honeys are often named after the places where they are produced; they are distinguished by the names of mountainous regions, for example, Toktogul, Sary-Chelek, Kara-Soro, At-Bashy (Mazhitova and Smanalieva,2022). Honey is produced in many regions of Kyrgyzstan (Kadyrova and Smanalieva,2017; İşenbaeva et al.,2021a). Buckwheat honey is a monofloral type of honey obtained from the buckwheat plant grown in Kyrgyzstan. Buckwheat honey is usually dark in colour and has a characteristic flavour and pleasant aroma. Buckwheat honey is believed to have many health benefits and is often consumed as a source of natural healing (Şabdanova et al.,2020). White honey is the honey obtained from the pollen of the sainfoin plant (Onobrychis sp.). Because it does not cause allergies, this honey is in demand on the international market (İşenbaeva et al.,2021b). In this study, the in vitro antibacterial activity of honey produced in Kyrgyzstan was investigated against important food-borne pathogens such as *Escherichia coli* and *Staphylococcus aureus*. For this purpose, two common methods such as agar-well diffusion and disc diffusion were selected and the effectiveness of these methods in the detection of antibacterial activity was compared.

MATERIAL AND METHODS

This research was carried out in the laboratories of the Department of Food Hygiene and Technology, Faculty of Veterinary Medicine, Kyrgyz-Turkish Manas University.

Honey samples: This study was carried out on 15 freshly harvested, unpasteurized, unprocessed and natural honey samples from local beekeepers operating in Kyrgyzstan. For this purpose, white honey and buckwheat honey were collected from monofloral honeys and Toktogul, Sary-Chelek and At-Bashy honey were collected from polyfloral honeys. Each sample was collected from 3 different beekeepers. The samples were placed in sterile containers and stored at room temperature in the dark until testing.

Bacterial strains: *Staphylococcus aureus* (ATCC 29213), one of the Gram-positive bacteria, and *Escherichia coli* (ATCC 25922), one of the Gram-negative bacteria, which are among the most important food-borne pathogens, were used. Bacterial cultures were incubated in Tryptic Soy Broth medium for 24 hours at 37°C and then adjusted to 0.5 McFarland turbidity (1×10^8 cfu/mL). 0,1 mL of the adjusted cultures were taken and transferred to Mueller Hinton Agar medium and spread with a sterile drigalski loop.

Disc diffusion method: The collected honey samples were weighed as 0.5 g and dissolved thoroughly by mixing with 1 ml of sterile distilled water. Samples (500 mL^{-1} dilution) were prepared in this way. Pre-prepared *E. coli* (ATCC 25922) and *S. aureus* (ATCC 29213) were inoculated with a sterile drigalski loop on Mueller-Hinton agar using the drop plate method, adjusted to 0.5 McFarland. Subsequently, 40 µL of the prepared honey sample was transferred to 6 mm diameter blank paper discs (Oxoid, England). All discs were then placed in sheets and gently pressed to ensure full contact. Sterile blank discs and discs saturated with methylene blue were used as controls. Plates were incubated for 24 hours at 37°C and the resulting zones were measured. (Patton et al.,2006).

Agar-Well diffusion method: 0,5 McFarland adjustments of each test pathogen were inoculated onto Mueller-Hinton agar using the drop plate method (Suerdem et al.,2018). Wells of 8 mm diameter were

drilled on the surface of the agar and 180 microlitres of each sample was added. Plates were incubated at 37 °C for 24 hours. The inhibition zones were measured after incubation.

Statistical analysis: The SPSS statistical analysis program (IBM SPSS, Statistics 20) was used to determine the minimum, maximum, mean and standard error using descriptive statistics.

RESULTS AND DISCUSSION

The antimicrobial activity of 15 honey samples from the Chuy region of Kyrgyzstan was analysed. The antimicrobial zones of honey by disc diffusion method are shown in Table 1. When the zones in the honeys analyzed by disc diffusion method were measured, it was found that Sary-Chelek, Buckwheat honey, Toktogul and At Bashy honey showed similar antimicrobial effects against *S. aureus*, while white honey showed the least effect. It was found that honeys analyzed by disc diffusion method showed different zones against *E.coli*. Buckwheat honey creates a zone of 9.00± 0.58 mm, Sary-Chelek honey creates a zone of 8.00± 0.58 mm, Toktogul honey creates a zone of 6.00± 0.58 mm, and the least effect is found in At Bashy honey with 4.33± 0.33 mm. Yalazi and Zorba (2020) reported that the secretion honey they collected in the Kaz Mountains region had an antimicrobial effect on *S. aureus* and *E. coli* but no effect on *Bacillus cereus, Candida albicans* and *Saccharomyces cerevisiae*. İplikçioğlu-Çil et al., (2020) reported that honey collected from different regions of Turkey had antibacterial activity against *E. coli, Listeria monocytogenes, Salmonella* Typhimurium and *S. aureus*. These findings support our research. Ramos et al., (2018) the antibacterial activity of 24 honey samples collected in Argentina was evaluated against microorganisms isolated from contaminated food. The researchers observed antimicrobial effects for most strains of *E. coli, Salmonella* spp, *S. aureus, Pseudomonas aeruginosa* and *Bacillus cereus* at 1:2 dilutions. In the same study, they reported that some honeys had no antibacterial effect at a dilution of 1:4. Çakır and Dervişoğlu (2022) prepared different concentrations (500, 250 and 125 mg mL^{-1}) of honey collected from four different districts of Bingöl province and their antimicrobial effects were investigated using the disc diffusion method. Researchers have reported that honey has antibacterial activity against *S. aureus* but not against *L. monocytogenes*. They also noted that only honey samples from Genç and Yedisu districts showed antibacterial activity against *E. coli* at a concentration of 500 mg mL-1, while other concentrations did not show antimicrobial effects. In our study, we observed antimicrobial activity against *S. aureus* in all honey samples. However, one sample from the white honey variety analysed by disc diffusion method did not show antimicrobial activity against *E. coli*, while antimicrobial effects were observed in other samples. The antibacterial activity of honey is significantly influenced by the geographical location and the botanical diversity from which the honey is obtained. Engin et al., (2022) their research found that the antimicrobial activity of monofloral honey was highest in sunflower honey. They noted that the highest inhibitory effect of monofloral honey was against S. typhimurium and that the antimicrobial effect of heat-treated honey stored in areas exposed to light decreased over time. İşenbaeva et al., (2021b) reported antibacterial activity against *E. coli, S. aureus* and *Shigella flexneri* in white honey collected in Kyrgyzstan.

Table 1. *The antimicrobial zones of honey by disc diffusion method*

	S.aureus Inhibition Zones (mm)			***E.coli Inhibition Zones (mm)***		
Type	min	max	Mean± SE	min	max	Mean± SE
Buckwheat honey	7.00	10.00	8.67±0.88	8.00	10.00	9.00± 0.58
White honey	0.00	7.00	2.33± 2.33	0.00	5.00	3.00± 1.53
Toktogul	7.00	10.00	8.33± 0.88	5.00	7.00	6.00± 0.58
At-Bashy	8.00	10.00	9.00± 0.58	4.00	5.00	4.33± 0.33
Sary-Chelek	7.00	9.00	8.00± 0.58	7.00	9.00	8.00± 0.58

The antimicrobial effects of honey using the agar well diffusion method is shown in Table 2. When the table was examined, it was found that buckwheat honey had the best effect against *S.aureus* with 18.00±0.58 mm and white honey had the least effect with 11.00±0.58 mm. Using the agar-well diffusion method, it was found that buckwheat honey, Sary Chelek and Toktogul honey had high antimicrobial effects against *E.coli*, while white honey and At-Bashy honey had the least effect.

Table 2. *The antimicrobial zones of honey by Agar-Well diffusion method*

	S.aureus Inhibition Zones (mm)			*E.coli Inhibition Zones (mm)*		
Type	min	max	Mean± SE	min	max	Mean± SE
Buckwheat honey	17.00	19.00	18.00±0.58	12.00	15.00	13.67±0.88
White honey	10.00	12.00	11.00±0.58	8.00	10.00	9.00±0.58
Toktogul	15.00	17.00	16.00±0.58	10.00	14.00	12.33±1.20
At-Bashy	14.00	16.00	15.00±0.58	10.00	11.00	10.33±0.33
Sary-Chelek	9.00	14.00	12.00±1.53	11.00	16.00	13.33±1.45

The antimicrobial effects of honeys against *S. aureus* were demonstrated using the disc diffusion and agar-well methods in Figure 1, while their antimicrobial effects against *E. coli* were shown in Figure 2. By examining both graphs, it can be observed that the honeys exhibited distinct antimicrobial effects against the selected food pathogens. This finding supports the results obtained by other researchers (Çakır and Dervişoğlu, 2022; Hulea et al.,2022; İplikçioğlu-Çil et al.,2020; Yalazi and Zorba,2020).

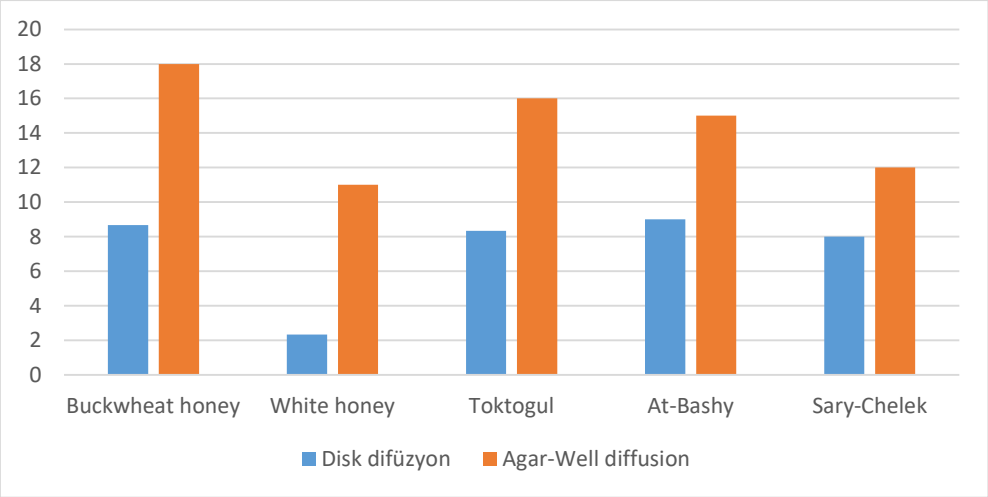

Figure 1. *Antimicrobial activity of honey against S. aureus by disc diffusion and agar-well method*

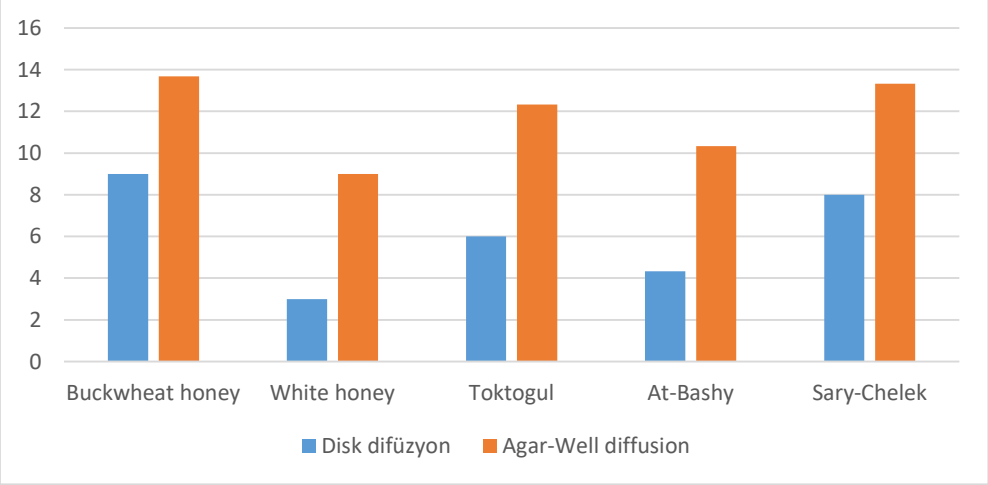

Figure 2. *Antimicrobial activity of honey against E. coli by disc diffusion and agar-well method*

CONCLUSION

Studies show that honeys from different geographical areas have significant and variable antibacterial activity against Gram-negative and Gram-positive bacteria. In the research, it was observed that both methods had antimicrobial activity against *E. coli* and *S. aureus*, which are food pathogens of choice. As a result of the analysis, the least amount of protection against pathogens was recorded in white honey samples. It is thought that this is due to the pollen collected by the bees during the production of white honey, which is a monofloral honey, and the components of the sainfoin plant (Onobrychis sp.).

Buckwheat honey showed high antimicrobial activity against both *E. coli* and *S. aureus*, as evidenced by the zones measured by both methods. This is thought to be due to the high antimicrobial activity of the constituents found in buckwheat plants. It has been concluded that the polyfloral honeys Sary-Chelek, Toktogul and At Bashy create different zones against *E. coli* and *S. aureus* due to the differing flora of the region where these honeys are collected. It can be seen that the zones formed in the disc diffusion and agar-well diffusion analyses are best obtained by the agar-well method. As a result, it is necessary to carry out research that will show the effects of honey made from the natural vegetation of Kyrgyzstan on other food-borne pathogens. The literature review revealed a limited number of scientific studies on bees and bee products in Kyrgyzstan. There is a need for further research in these areas.

CONFLICT OF INTEREST

The authors declared no conflict of interest.

AUTHOR CONTRIBUTION

All authors contributed equally.

ETHICAL APPROVAL

Scientific rules, ethics and citation rules were followed during the writing process of the study titled "**The Antimicrobial Effects of Kyrgyz Honey**". There was no tampering with the data collected and this study was not sent to any other academic publication environment for evaluation. Our study does not involve data from living animals. Therefore, ethical committee approval is not required.

NOTE

This research abstract was presented virtually as an oral presentation at the II. International Congress of Bee Science (ICbees) on 14-16 June 2023 in Kyrgyzstan.

REFERENCES

Acaroz, U., Kurek-Gorecka, A., Olczyk, P., Tas, N., Alı, A., Paramanya, A., Balyan, P., Noor, A., Kamaraj, S., Malekıfard, F., Hosseını, A., Istanbullugıl, F. R., Arslan-Acaroz, D., Asma, S. T., Seguenı, N., Ceylan, A. B., & Jın, X. (2024). The Role of Bee Products in the Control of Antimicrobial Resistance and Biofilm Formation. *Kafkas Univ Vet Fak Derg.* 30 (2): 131-153 https://doi.org/10.9775/kvfd.2023.30966

Asma, S. T., Bobi¸sbobi¸s, O., Victori¸ta Bonta, Acaroz, U., Rizwan, S., Shah, A., Istanbullugil, F. R., and Arslan-Acaroz, D. (2022). General nutritional profile of bee products and their potential antiviral properties against mammalian viruses. *Nutrients*, 14(17), 3579. https://doi.org/10.3390/nu14173579

Balazs, V. L., Nagy-Radvanyi, L., Bencsik-Kerekes Erika and Koloh, R., Szabo, D., Kocsis, B., and Kocsis Marianna and Farkas, A. (2023). Antibacterial and Antibiofilm Effect of Unifloral Honeys against Bacteria Isolated from Chronic Wound Infections. *Microorganisms*, 11(2), 509. https://doi.org/10.3390/microorganisms11020509

Çakır, Y., & Dervişoğlu, G. (2022). Antimicrobial Effect of Honeys Collected in Bingol Region. *MAS Journal of Applied Sciences*, 7(2), 537–544. https://doi.org/10.52520/masjaps.v7i2id206

Çakmak, Ö., TAŞ, N., Acaröz, U., Arslan-Acaroz, D., İstanbullugil, F. R., Evrenkaya, S., and Gürler, Z. (2023). Inhibitory effects of honey on Staphylococcus aureus and Escherichia coli: A current review. *Veteriner Farmakoloji ve Toksikoloji Derneği Bülteni,* 14(3), 133-148. https://doi.org/10.38137/vftd.1385968

Engin, A. S., Ceylan, Ö., and Duru, M. E. (2022). Monofloral Balların Saklama Koşullarına Göre Antimikrobiyal Aktivite Üzerine Etkisi. *Süleyman Demirel Üniversitesi Fen Bilimleri Enstitüsü Dergisi*, 26(2),299-304. https://doi.org/10.19113/sdufenbed.1062571

Hossain, M. L., Lim, L. Y., Hammer, K., Hettiarachchi, D., and Locher, C. (2022). A Review of Commonly Used Methodologies for Assessing the Antibacterial Activity of Honey and Honey Products. *Antibiotics*,11(7), 975. https://doi.org/10.3390/antibiotics11070975

Hulea, A., Obistioiu, D., Cocan, I., Alexa Ersilia and Negrea, M., Neacsu, A.-G., Hulea, C., Pascu, C., Costinar, L., Iancu, I., and Tirziu Emil and Herman, V. (2022). Diversity of Monofloral Honey Based on the Antimicrobial and Antioxidant Potential. *Antibiotics*, 11(5),595. https://doi.org/10.3390/antibiotics11050595

İplikçioğlu-Çil, G., Küplülü, Ö., Cengiz, G., Dicle Korkmaz, S., Arslan, B., and Gürcan, S. (2020). Antibacterial activity of Turkish honey against selected foodborne pathogens. *Ankara Univ Vet Fak Derg*, 67, 413–418. https://doi.org/10.33988/auvfd

Israili, Z. H. (2014). Antimicrobial Properties of Honey. *American Journal Of Therapeutics*, 21(4), 304–323. https://doi.org/10.1097/MJT.0b013e318293b09b

İstanbullugil, F. R., Taş, N., Acaröz, U., Arslan-Acaröz, D., Çakmak, Ö., Evrenkaya, S., and Gürler, Z. (2023). Review on the Antimicrobial Effect of Honey on Salmonella and Listeria monocytogenes: Recent Studies. *Manas Journal of Agriculture Veterinary and Life Sciences*, 13(2), 210–225. https://doi.org/10.53518/MJAVL.1379465

Kadyrova, K., & Smanalieva, J. (2017). Determination of Physical and Chemical Properties of Honey from the Mountainous and Lowland Regions of Kyrgyzstan. *MANAS Journal of Engineering*, 5(1), 29–34.

Mazhitova, A., and Smanalieva, J. (2022). Amino acid composition and some physicochemical parameters of multi-floral honey from mountainous regions of Kyrgyzstan. *Uludağ Arıcılık Dergisi*, 22(2), 188-202.

Patton, T., Barrett, J., Brennan, J., & Moran, N. (2006). Use of a spectrophotometric bioassay for determination of microbial sensitivity to manuka honey. *Journal of microbiological methods*, 64(1), 84-95.

Piana, M. L., Cianciabella, M., Daniele, G. M., Badiani, A., Rocculi, P., Tappi, S., Gatti, E., Marcazzan, G. L., Magli, M., Medoro, C., and Predieri, S. (2023). Influence of the Physical State of Two Monofloral Honeys on Sensory Properties and Consumer Satisfaction. *Foods*, 12(5), 986. https://doi.org/10.3390/foods12050986

Ramos, O. Y., Salomón, V., Libonatti, C., Cepeda, R., Maldonado, L., and Basualdo, M. (2018). Effect of botanical and physicochemical composition of Argentinean honeys on the inhibitory action against food pathogens. *LWT*, 87, 457-463. https://doi.org/10.1016/j.lwt.2017.09.014

Schell, K. R., Fernandes, K. E., Shanahan, E., Wilson, I., Blair, S. E., Carter, D. A., and Cokcetin, N. N. (2022). The Potential of Honey as a Prebiotic Food to Re-engineer the Gut Microbiome Toward a Healthy State. *Frontiers in nutrition*, 9,957932 https://doi.org/10.3389/fnut.2022.957932

Skadins, I., Labsvards, K. D., Grava, A., Amirian, J., Tomsone, L. E., Rusko, J., Viksna, A., Bandere, D., and Brangule, A. (2023). Antimicrobial and Antibiofilm Properties of Latvian Honey against Causative Agents of Wound Infections. *Antibiotics*, 12(5)816 https://doi.org/10.3390/antibiotics12050816

Suerdem, T. B., Akyalcin, H., Altan, S., and Tosun, M. N. (2018). Screening of antimicrobial and antioxidant activity of different types of honey samples obtained from Geyikli (Çanakkale) province. *Communications Faculty of Sciences University of Ankara Series C Biology*, 27(2), 69-78. https://doi.org/10.1501/commuc_0000000199

Tananaki, C., Liolios, V., Kanelis, D., and Rodopoulou, M. A. (2021). Investigation of volatile compounds in combination with multivariate analysis for the characterization of monofloral honeys. *Applied Sciences*, 12(1), 264. https://doi.org/10.3390/app12010264

Yalazi, E., ve Zorba, M. (2020). Kazdağları Bölgesi Salgı Ballarının Antioksidan ve Antimikrobiyal Özellikleri. *Turkish Journal of Agriculture - Food Science and Technology*, 8(3), 587–593. https://doi.org/10.24925/turjaf.v8i3.587-593.3022

Yupanqui Mieles, J., Vyas, C., Aslan, E., Humphreys, G., Diver, C., and Bartolo, P. (2022). Honey: An advanced antimicrobial and wound healing biomaterial for tissue engineering applications. *Pharmaceutics*, 14(8), 1663. https://doi.org/10.3390/pharmaceutics14081663

Işenbaeva, N. N., Kerimaliev, J. K., & Abdurasulov, A. H. (2021a). Palinologiçeskaya harakteristika monoflornıh medov Respubliki Kırgızstan. *Vestnik Başkirskogo gosudarstvennogo agrarnogo universiteta*, (1), 68-72. https://doi.org/10.31563/1684-7628-2021-57-1-68-72

Işenbaeva, N. N., Kurmanov, R. G., Kerimaliev, J. K., & Giniyatullin, M. G. (2021b). Issledovanie Pokazateley Kaçestva Razliçnıh Vidov Meda Kırgızstana. *Vestnik BGAU*, (4), 72–80. https://doi.org/10.31563/1684-7628-2021-60-4-72-80

Şabdanova, N. K., & Kaynaeva, J. A. (2020). Diversity Of Honey Species In Kyrgyzstan And Their Features. *Nauka, novıe tehnologii i innovatsii Kırgızstana,* (6), 30-33.