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Physicochemical properties, antioxidant and antimicrobial activity of seven honey samples collected in the Nišava district

Original Article

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Abstract:

Honey is a functional food used in traditional medicine to treat numerous human ailments. The health-promoting properties of honey depend on its physicochemical characteristics, chemical composition, and antioxidant potential, which result from the botanical and geographical origin of honey. In this study, the physicochemical properties, antioxidant and antimicrobial potential of seven honey samples from the Nišava region were analyzed. The honeydew sample had the highest content of phenols and flavonoids and the best scavenging and antimicrobial activities (MIC 6.25-25%). The content of flavonoids was also high in meadow honey, which demonstrated excellent scavenging activity against the DPPH radical. Acacia and lavender honey samples showed good antibacterial activity (MIC 12.5-25%), but only honeydew inhibited the growth of yeast *Canida albicans* (MIC 12.5%). The results presented in this paper revealed differences in the biological activity of honey samples, indicating the necessity of analyzing each honey sample individually before using it as a functional food.

Key words:
blossom honey, honeydew, antioxidant, antimicrobial, physicochemical properties

Apstrakt:

Fizičko-hemijska svojstva, antioksidativna i antimikrobna aktivnost sedam uzoraka meda prikupljenih na teritoriji nišavskog okruga

Med je funkcionalna hrana koja se koristi u tradicionalnoj medicini za lečenje brojnih ljudskih bolesti. Biološka aktivnost meda zavisi od njegovih fizičko-hemijskih karakteristika, hemijskog sastava i antioksidativnog potencijala, koji proizilaze iz botaničkog i geografskog porekla meda. U ovoj studiji analizirana su fizičko-hemijska svojstva, antioksidativni i antimikrobni potencijal sedam uzoraka meda iz nišavskog regiona. Uzorak medljike je imao najveći sadržaj fenola i flavonoida i najbolju antioksidativnu i antimikrobnu aktivnost (MIK 6,25-25%). Sadržaj flavonoida je takođe bio visok u livadskom medu, koji je pokazao odličnu aktivnost neutralizacije DPPH radikala. Bagremov med i med od lavande su pokazali dobru antibakterijsku aktivnost (MIK 12,5-25%), ali je samo medljika inhibirala rast kvasca *Canida albicans* (MIK 12,5%). Rezultati prikazani u ovom radu su pokazali velike razlike u biološkoj aktivnosti uzoraka meda, što ukazuje na neophodnost analiziranja svakog uzorka meda pojedinačno pre njegove upotrebe kao funkcionalne namirnice.

Ključne reči:
cvetni med, medljika, antioksidativna aktivnost, antimikrobna aktivnost, fizičkohemijske karakteristike

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Introduction

Honey is a bee product that is one of the most important functional foods in the world, with an annual global production of 1.83 million tons in 2022 (FAOSTAT, 2024). Blossom (floral) honey and honeydew are the two types of honey, classified according to the kind of sugar sources collected by the bees (Bergamo et al., 2019). Floral honey is made from the secretions of living plants (nectar), while honeydew is the product of collecting the excretions of insects that live on plants. These two types of honey differ in their physicochemical, chemical, and biological properties (Pita-Calvo & Vázquez, 2017).

The consumption of honey is popular with consumers all over the world for its nutritional and health-promoting properties, which have been known since ancient times in many traditional

medicines (Nikhat & Fazil, 2022). Various studies have reported antimicrobial, antioxidant, anti-inflammatory, antidiabetic, and anticarcinogenic effects of different honey samples (Erejuwa et al., 2012; Meo et al., 2017; Pasupuleti et al., 2020; Tafere, 2021). Clinical studies have confirmed that honey positively affects human health in healing wounds and burns, as well as cardiovascular, gastrointestinal, and diabetes diseases (Samarghandian et al., 2017; Palma-Morales et al., 2023). However, the chemical composition and biological activities of honey vary greatly from sample to sample and depend primarily on the geographical, seasonal, and botanical origin of the samples as well as the conditions under which the honey is collected, processed, and stored (Da Silva et al., 2016; Obey et al., 2022).

The aim of the present study was to compare the physicochemical properties, antioxidant and antimicrobial activity of seven honey samples collected in the Nišava district. One of the selected samples was honeydew and the other six were blossom honey. The blossom honey samples had different botanical origins. Acacia and lavender honey were selected as monofloral honey samples, while mixed acacia and sage honey and meadow honey were selected as polyfloral honey samples.

Materials and Methods

Honey samples

This study analyzed seven honey samples collected on the territory of the Nišava district. The samples were obtained from the Association of Beekeepers „Suva planina" from Niška Banja. **Table 1** lists the types of honey and the localities where the samples were collected.

Table 1. Honey samples used in the study

Samples	Abv.	Localities
Acacia honey	A1	Zaplanje (G. Barbeš)
Acacia honey	A2	Sićevo
Acacia honey (crystallized)	AC	Sićevo
Acacia and sage honey	AS	Sićevo
Lavender honey	L	Tamnjanica
Meadow honey	M	Niš surroundings
Honeydew honey	H	Niš surroundings

Chemicals and reagents

Butylated hydroxyanisole (BHA) and DPPH were obtained from Sigma Chemicals Co (St Louis, MO, USA) and Folin-Ciocalteu phenol reagent from Merck (Darmstadt, Germany). Na_2CO_3, $C_2H_3KO_2$, $K_2O_8S_2$, and L(+)-ascorbic acid were purchased from AnalaR Normapur (VWR, Leuven, Belgium) and $Al(NO_3)_3x9H_2O$ from Fluka Chemie AG (Buchs, Switzerland). ABTS and quercetin hydrate (Qu) were obtained from TCI Europe NV (Boerenveldsweg, Belgium). All chemicals were of analytical grade.

Physicochemical analysis

The physicochemical analysis of the honey samples was carried out according to the methods listed in the Harmonized Methods of the International Honey Commission (International Honey Commission, 2009). The water content was determined two spaces with a refractometer at 20 °C using Wedmore tables. The pH value and acidity of honey samples were determined by dissolving 10 g of honey in 75 ml of distilled water. A pH meter (LLG pH meter 7, LLG, Germany) was used to measure the pH values of the samples at room temperature. The total acidity of honey samples was determined as the sum of free and lactone acidity. The free acidity was determined by adding 0.05M NaOH to pH 8.5. The lactone acidity was determined by adding 10 ml of 0.05M NaOH to pH 8.5 and subsequent titration with 0.05M HCl to pH 8.3. A honey solution for the analysis of the hydroxymethylfurfural (HMF) content was prepared by dissolving 5 g of honey in 25 ml of water. Subsequently, 0.5 ml of Carrez reagents I and II were added to the solution for clarification and the samples were additionally diluted with water. Alcohol was also added to prevent foaming. The absorbance of the sample was measured at 284 nm and 336 nm using a UV-VIS spectrophotometer (Shimadzu 1650 PC, Kyoto, Japan). The HMF content is expressed in mg/kg and was calculated using the following equation: $HMF=(A_{284}-A_{336})x149.7$. Diastase activity was determined using a buffered solution of soluble starch and honey incubated at 40 °C. The absorbance of 1 ml of solution was measured at 660 nm at 5-minute intervals. The amount of reducing sugars and sucrose was determined by titration of Fehling's solution containing methylene blue with a honey solution.

Antioxidative activity

To test the antioxidant potential of honey samples, a honey solution was prepared by dissolving 10 g of honey in 10 ml of distilled water. The solution was then filtered, and the resulting samples were used to determine the total phenolic and flavonoid contents and to test the potential of the samples to neutralize DPPH (2,2-diphenyl-1-picrylhydrazil) and ABTS (2,2'-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid)) radicals.

Total phenolic content

The total phenolic content (TPC) was determined using the Folin-Ciocalteu method (Singleton et al., 1999). Concentrated commercial Folin-Ciocalteu reagent (Merck, Germany) was diluted with distilled water at a ratio of 1:10. 1500 µl of the reagent was added to 300 µl of a diluted honey sample with concentrations of 1 and 0.5 g/ml. After the mixture was kept in the dark for 6 minutes, a 7.5% Na_2CO_3 solution was added. The contents were mixed with a vortex, the test tubes were covered and left in a dark place for 2 hours. The absorbance of the mixtures was measured with a spectrophotometer at a wavelength of 740 nm. The TPC is expressed in mg gallic acid equivalents (GA)/kg honey calculated using the standard curve for gallic acid.

Total flavonoid content

The total flavonoid content (TFC) was measured according to the method described by Woisky and Salatino (Woisky & Salatino, 1998). The reaction mixture was prepared by adding 4.1 ml of 80% C_2H_5OH, 0.1 ml of 10% $Al(NO_3)_3x9H_2O$ and 0.1 ml of 1M $C_2H_3KO_2$ to 600 µl of a diluted honey sample with a concentration of 1 and 0.5 g/ml. The contents were mixed with a vortex, the test tubes were covered and left in a dark place for 40 minutes. The absorbance of the mixtures was measured with a spectrophotometer at a wavelength of 415 nm. The TFC was calculated using the standard curve for quercetin and expressed in mg equivalents of quercetin (QE)/kg honey.

DPPH scavenging activity

The determination of the potential antioxidant capacity by DPPH assay was performed spectrophotometrically using the Blois method (Blois, 1958). DPPH dissolved in methanol at a 0.04 mg/ml concentration was used as the reagent. The concentrations of the honey solution were selected based on experiments with the DPPH solution according to the procedure indicated. The reaction mixture, prepared by mixing 200 µl of the tested sample with 1800 µl of the DPPH solution, was gently shaken and kept at room temperature in the dark for 30 minutes. The absorbance of the solution was then measured at 517 nm. The percentage of DPPH radical scavenging was calculated according to the following equation:

$$\textbf{Scavenging activity (\%)} = (A_0\text{-}A_1) \times 100/A_0$$

where A_0 represents the absorbance of the initial DPPH solution and A_1 represents the absorbance of the samples.

The results are presented as the IC_{50} value, which indicates the concentration of the sample that scavenges 50% of the DPPH radicals. Two known antioxidants, vitamin C and butylated hydroxyanisole (BHA), were used as positive controls.

ABTS radical scavenging activity

The ABTS radical scavenging test was performed according to the modified method of Miller and Rice-Evans (Miller & Rice-Evans, 1997). The ABTS reagent was prepared by dissolving 19.2 mg of ABTS in 5 ml of 2.46 mM $K_2O_8S_2$, and the solution was allowed to stand in the dark at room temperature for 12-16 hours. The reaction mixture was prepared by mixing 75 µl of diluted honey sample and 3 ml of ABTS reagent. The contents were mixed with a vortex, then the test tubes were covered and allowed to stand for 30 minutes at 30 ºC in a water bath. The absorbance was measured at a wavelength of 734 nm. ABTS scavenging activity was calculated based on the calibration curve for vitamin C and expressed in mg vitamin C equivalents (VitC)/g honey.

Antimicrobial activity
Microbial strains

The antimicrobial activity of honey samples was determined against Gram-positive bacteria *Kocuria rhizophila* ATCC 9341 and *Staphylococcus aureus* ATCC 6538, and Gram-negative bacteria *Escherichia coli* ATCC 8739, *Proteus mirabilis* ATCC 12453 and *Pseudomonas paraeruginosa* ATCC 9027. Two fungal strains, the yeast *Candida albicans* ATCC 10231 and the mold *Aspergillus brasiliensis* ATCC 16404 were also used for the antimicrobial test. The cultures of the bacterial strains were grown on Nutrient agar (Torlak, Serbia) at 37 °C, while the fungal cultures were incubated on Sabouraud dextrose agar (Torlak, Serbia) at 30 °C.

Antimicrobial assay

The antimicrobial activity of the honey samples was tested using the micro/well dilution method (Clinical and Laboratory Standards Institute, 2012.). The bacterial cultures were incubated at 37 °C on Mueller-Hinton agar, while the fungi were incubated at 30 °C on Sabouraud dextrose agar. The bacterial suspensions were prepared in Mueller-Hinton broth, fungal suspensions were made in Sabouraud dextrose broth, and their turbidity was standardized using a McFarland densitometer (DEN-1B, Biosan). The final density of the bacterial and yeast inocula was $5x10^5$, while the final density of the mold inoculum was $1x10^4$. The stock solutions of the honey samples were prepared in distilled sterile water (initial concentration 0.5 g/mL = 50%) and then serially diluted (dilution factor 2). The final concentration of the tested samples in the medium was 250.00-0.1 mg/ mL (25-0.01%). After dilution,

the inoculum was added to all wells of the microtiter plates and then the plates were incubated at 37 °C for 24 hours (bacterial strains) and at 30 °C for 48 hours (fungi). Chloramphenicol and Nystatin were used as a positive control, while a non-inoculated well without an antimicrobial substance was used to confirm the sterility of the medium. Artificial honey prepared by dissolving 40 g fructose, 30 g glucose, 8 g maltose and 2 g sucrose in 100 ml distilled water and sterilized at a temperature of 121 °C for 15 minutes served as a control (AL-Waili et al., 2013). The minimum inhibitory concentration (MIC) is defined as the minimum concentration of honey that inhibits the visible growth of cell cultures. All experiments were done in triplicate.

Statistical analysis

All measurements in antioxidant activity tests were performed in triplicate, and the results are presented as averages ± standard deviation (SD). The results were statistically analyzed using one-way analysis of variance (ANOVA) followed by Tukey's HSD test ($p \leq 0.05$) using the Minitab®17 software (Minitab, LLC, State College, Pennsylvania, USA).

Results and discussion

Physicochemical properties of honey samples

The physicochemical properties of honey as important indicators of the quality and authenticity are determined by European and Serbian regulations (Codex Alimentarius Commission, 2001; Vranić et al., 2017). **Tab. 2** shows the values of physicochemical parameters for all analyzed honey samples and the reference values prescribed by Serbian regulations (Vranić et al., 2017).

Most of the physicochemical parameters of the analyzed honey samples complied with the regulations (**Tab. 2**), which indicates the good quality of the collected honey samples. Only the content of hydroxymethylfurfural (HMF), an organic compound formed during the dehydration of sugars in honey, was higher in one sample of crystallized acacia honey AC (68.47 mg/kg) than the maximum value of 40 mg/kg specified in the Codex Alimentarius standard. In the AC sample, the activity of the enzyme diastase, which converts starch into sugars with shorter chains, was also the lowest (**Tab. 2**). The obtained values of diastase activity and HMF content of the AC sample could indicate improper preservation of the honey and its possible exposure to sunlight or another heat source during processing and storage. The sample of acacia and sage honey (AS) had an extremely low HMF content and the lowest water content so this honey could be described as the freshest of all analyzed honey samples according to the Codex Alimentarius standard. The diastase activity was the highest in the AS sample, which also indicates the exceptional quality of this honey sample.

Several physicochemical parameters are important for the differentiation between blossom honey and honeydew (Pita-Calvo & Vázquez, 2017). Manzanares et al. (2011) concluded that acidity, pH, electrical conductivity, proline, invertase, and glucose are the most important differentiating factors between these two kinds of honey based on a multivariate analysis of the physicochemical parameters and sugar composition of 77 honey samples. The honeydew honey in our study had the highest pH and acidity, but the lowest reducing sugar content (**Tab. 2**). In addition, the color of sample H was darker compared to the other honey samples (data not shown), which is also characteristic of this honey type (Kesić et al., 2020).

Antioxidant activity

One important parameter for the biological activity of honey is its antioxidant activity, which

Table 2. Physicochemical properties of analyzed honey samples

Parameters	Samples							Referent value
	A1	A2	AC	AS	L	M	H	
Moisture (%)	15.64	15.24	16.73	14.9	16.32	15.43	15.06	<20
Diastase activity (degrees of the Goethe scale)	23.75	25.69	11.63	32.78	39.45	30.9	37.71	>8
HMF (mg/kg)	4.79	7.84	68.47	1.87	6.99	4.8	9.11	<40
pH	4.15	4.23	3.79	4.12	3.54	4.33	4.51	
Acidity (meq/kg)	10.55	7.36	10.41	13.44	32.88	23.8	33.25	<50
Reducing sugar content (%)	76.4	73.95	70.19	72.94	74.05	67.15	66.71	>60
Sucrose content (%)	2.15	2.03	2.82	2.92	2.48	2.62	2.77	<5

was evaluated in this study by determining the content of total phenols (TPC) and flavonoids (TFC) and measuring the ability of honey samples to trap DPPH and ABTS radicals.

Total phenol and flavonoid contents were significantly different ($p<0.05$) for most honey samples (**Fig. 1**). The highest TPC and TFC values were determined in the honeydew sample (sample H), while the acacia honey sample A2 had the lowest phenol and flavonoid content. Apart from sample H, TPC was quite high in samples of acacia crystal (AC), lavender (L), and meadow (M) honey (**Fig. 1a**). On the other hand, TFC was two or ten times higher in sample H compared to other analyzed honey samples (**Fig. 1b**). High TFC was also determined in the samples of meadow honey (55.78±0.018) and acacia crystal honey (36.54±0.038).

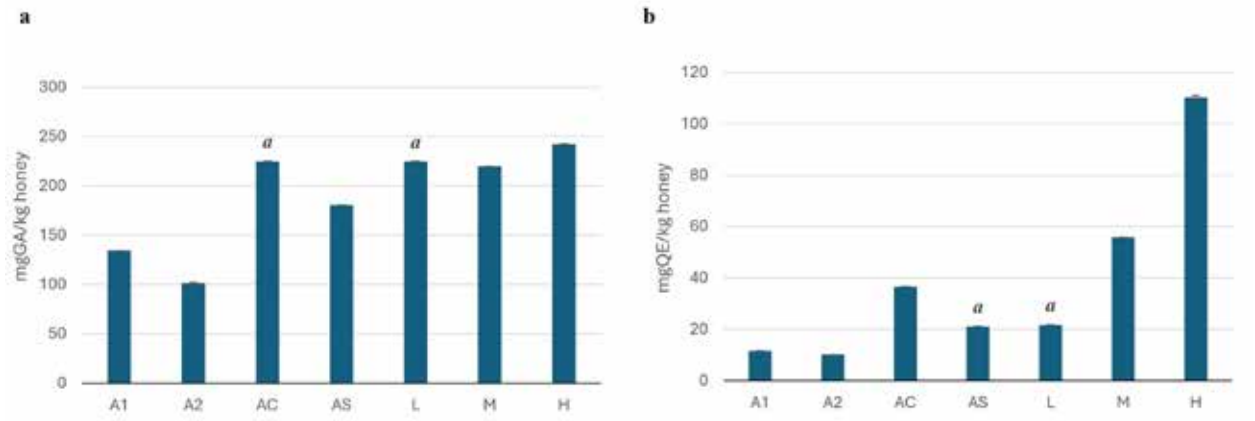

Fig. 1. Total phenol (**a**) and flavonoid (**b**) contents in honey samples. Results are presented as mean±SD. Values marked with the same letters are not significantly different ($p>0.05$)

Phenolic acids and flavonoids are components found in honey in varying amounts depending on the biological origin of the honey and the geographical area from which the honey was collected (Becerril-Sánchez et al., 2021). However, the amount of these biologically active compounds is higher in honeydew than in floral honey samples (Pita-Calvo & Vázquez, 2017). Also, polyfloral honey such as meadow honey had higher TPC and TFC and better antioxidant activity compared to monofloral honey (Atanacković Krstonošić et al., 2019). The results obtained in this study for TPC and TFC are similar to previously published results for different honey samples collected on the territory of Serbia (Savatović et al., 2011; Gašić et al., 2014; Čanadanović-Brunet et al., 2014; Velimirović et al., 2023).

The results of the scavenging activity of honey samples are presented in **Fig. 2**. Honeydew and meadow honey showed the best antioxidant activity in the DPPH method with IC_{50} values of 0.26 mg/ml and 0.44 mg/ml, respectively. Low and similar IC_{50} values were also found for AC and AS samples (0.87 mg/ml and 0.89 mg/ml, respectively), while the A2 sample exhibited the weakest DPPH scavenging activity with an IC_{50} value of 3.45 mg/ml. Phenolic compounds from various biological sources are one of the most important radical scavengers, so the DPPH scavenging activity of the sample is directly correlated with TPC (Lewoyehu & Amare, 2019). The sample of honeydew and meadow honey, which in this work had the lowest IC values, also had high TPC and TFC, indicating that flavonoids are the main carriers of scavenging activity. Honey is rich in various flavonoids among which pinocembrin, apigenin, kaempferol, quercetin, pinobanksin, luteolin, galangin, hesperetin, and isorhamnetin are the most important compounds that affect the antioxidant activity of honey (Da Silva et al., 2016).

The obtained values for antioxidant activity in the ABTS method showed that all honey samples, except sample A1, had a better antioxidant potential than the positive control BHA (**Fig. 2b**). The difference between the samples was small but statistically significant between certain honey samples (**Fig. 2b**) and the best activity was found in samples AS and H (3.81 mgVitC/g). Sample A1 had the lowest activity in the ABTS method of 1.46 mgVitC/g.

In the ABTS assay, there was no such clear correlation between the content of phenols and flavonoids and the ABTS scavenging activity as in the DPPH assay. Stagos et al. (2018) showed by examining the antioxidant activity of honey samples from Mount Olympus in Greece that there was no positive correlation between TPC and scavenging activity in DPPH and ABTS assays although some honey samples with high TPC had the best DPPH activity (Stagos et al., 2018). Apart from phenolic acids and flavonoids, ascorbic acid and other vitamins, carotenoids, catalase, peroxidase,

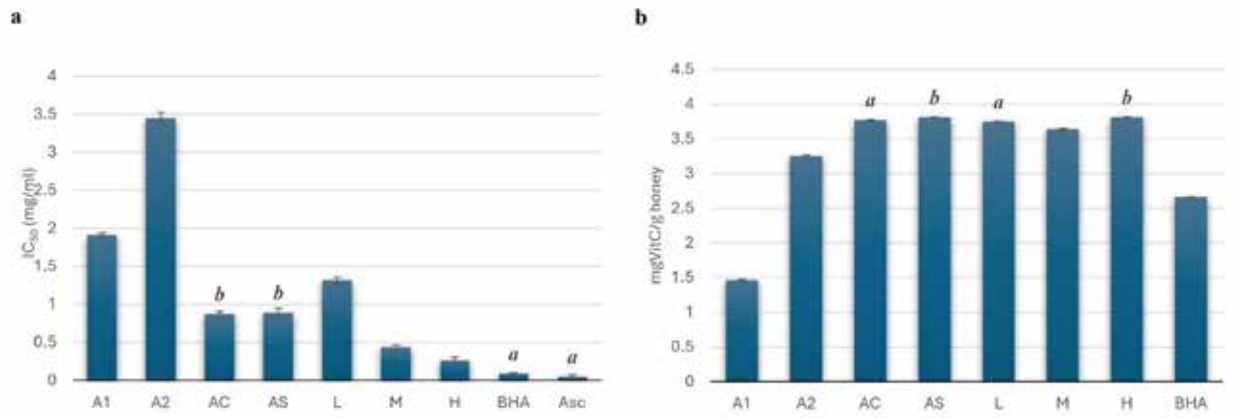

Fig. 2. Antioxidant activity measured by the DPPH (**a**) and ABTS (**b**) methods in honey samples. The antioxidant activity was compared to butylated hydroxyanisole (BHA) and vitamin C as standards. Results are presented as mean±SD. Values marked with the same letters are not significantly different (p>0.05)

and Maillard reaction products found in honey can contribute to the antioxidant activity of honey (Chua et al., 2013; Pauliuc et al., 2020).

Antimicrobial activity of honey

The antimicrobial activity of honey is a well-known therapeutic property of this functional food which has been used in many parts of the world since ancient times (Majtan et al., 2021). Considering that honey is an antimicrobial agent with a wide spectrum of action, it was used in clinical studies for the treatment of postoperative infection, burns, necrotizing fasciitis, infected and nonhealing wounds and ulcers, boils, pilonidal sinus, venous ulcers, and diabetic foot ulcers (Al-Waili et al., 2011).

The antimicrobial activity of seven honey samples was evaluated against Gram-positive and Gram-negative bacteria, the yeast *C. albicans* and the mold *A. brasiliensis* (**Tab. 3**). Sample H (honeydew) showed the best antimicrobial activity, acting on three bacterial strains (*K. rhizophila, P. mirabilis*, *P. paraeruginosa*) with MIC values of 6.25-12.5% and was the only honey sample that inhibited the growth of the yeast *C. albicans*. This sample had the lowest MIC of 6.25% against two bacterial strains, *K. rhizophila* and *P. mirabilis*. The better antimicrobial activity of honeydew compared to blossom honey samples was also evident in other studies (Nedić et al., 2022). Sample A1 (acacia honey) also had good antibacterial activity (MIC 12.5%) against all three tested strains of Gram-negative bacteria (*E. coli*, *P. mirabilis*, *P. paraeruginosa*) and the Gram-positive bacterium *K. rhizophila*. Similar MIC values as in this work were reported for honey samples of different botanic and geographic origins (Basson & Grobler, 2008; Živković et al., 2019; Obey et al., 2022). Lavender honey also showed similar activity to sample A1 but was less effective against *E. coli*. The crystallized acacia honey (AC) showed the weakest antimicrobial activity, exhibiting the same pattern of antimicrobial activity as the control sample of artificial honey (AH).

The antimicrobial activity of honey can be attributed to various chemical components and enzymes contained in honey and to its specific physico-chemical properties. Low water content, high osmolarity due to a high sugar concentration, low pH due to organic acids in honey, and the concentration of hydrogen peroxide are considered the most important antimicrobial factors (Almasaudi, 2021). Phenolic compounds, including phenolic acids and polyphenols, or some specific compounds, such as methylglyoxal in Manuka honey or the peptide bee-defensin-1 in Revamil honey, can also contribute to the antimicrobial properties of honey (Kwakman et al., 2011; Majtan et al., 2021).

Sample H, which showed the best antimicrobial activity, had the highest acidity, the highest content of phenols and flavonoids, and the best scavenging activity for DPPH and ABTS radicals. The synergistic effect of all these potential antimicrobial factors probably influenced the good antimicrobial activity of this honey sample. The total phenolic content was also high in lavender honey (sample L), and this sample had the lowest measured pH (3.54), suggesting that phenolics and acidity are probably the main contributors to antimicrobial activity. On the other hand, the sample of acacia honey A1 with the best antibacterial activity had a low content of phenols and flavonoids but the highest concentration of reducing sugars.

The weakest antimicrobial effect of all honey samples analyzed was against the mold *A. brasiliensis*, while only sample H had an inhibitory effect on the growth of the yeast *C. albicans*. The better antimicrobial effect of honey on bacteria

Table 3. Minimal inhibitory concentration (MIC) of honey samples against different microorganisms expressed in %

Microorganisms	ATCC number	Samples							
		A1	A2	AC	AS	L	M	H	AH
Gram + bacteria									
Kocuria rhizophila	9341	12.5	25	>25.00	25	12.5	25	6.25	>25.00
Staphylococcus aureus	6538	25	25	>25.00	25	25	25	25	>25.00
Gram - bacteria									
Esherichia coli	8739	12.5	25	>25.00	25	25	25	25	>25.00
Proteus mirabilis	12453	12.5	12.5	25	12.5	12.5	12.5	6.25	>25.00
Pseudomonas paraeruginosa	9027	12.5	25	>25.00	25	12.5	25	12.5	>25.00
Yeast									
Candida albicans	10231	>25.00	>25.00	>25.00	>25.00	>25.00	>25.00	12.5	>25.00
Mold									
Aspergillus brasiliensis	16404	>25.00	>25.00	>25.00	>25.00	>25.00	>25.00	>25.00	>25.00

compared to fungi has been established in many studies (Basson & Grobler, 2008; AL-Waili et al., 2013; Kolayli et al., 2020) Among the bacterial strains, *S. aureus* ATCC 6538 showed the highest resistance, while *P. mirabilis* ATCC 12453 was the most sensitive bacterial strain, which was affected by all honey samples except the AC sample.

Conclusion

The results presented in this paper show that the seven honey samples collected in the Nišava district were of good quality. The honeydew sample differed from the other honey samples regarding its physicochemical properties and had the best antioxidant and antimicrobial activity. The content of phenols and flavonoids, the scavenging activity of DPPH and ABTS radicals, and the antimicrobial activity of the other honey samples were diverse, indicating the need to test each honey sample individually before using it as a functional food.

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References

Almasaudi, S. (2021). The antibacterial activities of honey. *Saudi Journal of Biological Sciences, 28*(4), 2188–2196. https://doi.org/10.1016/j.sjbs.2020.10.017

AL-Waili, N., Al Ghamdi, A., Ansari, M.J., Al-Attal, Y., Al-Mubarak, A., & Salom, K. (2013). Differences in Composition of Honey Samples and Their Impact on the Antimicrobial Activities against Drug Multiresistant Bacteria and Pathogenic Fungi. *Archives of Medical Research*, *44*(4), 307–316. https://doi.org/10.1016/J.ARCMED.2013.04.009

Al-Waili, N.S., Salom, K., Butler, G., & Al Ghamdi, A.A. (2011). Honey and microbial infections: A review supporting the use of honey for microbial control. *Journal of Medicinal Food*, *14*(10), 1079–1096. https://doi.org/10.1089/jmf.2010.0161

Atanacković Krstonošić, M.T., Cvejić Hogervorst, J.M., Krstonošić, V.S., & Mikulić, M.P. (2019). Phenolic content and in vitro antioxidant capacity of mono- and polyfloral honeys originating from Serbia. *Food and Feed Research*, *46*(1), 83–90. https://doi.org/10.5937/FFR1901083A

Basson, N.J. & Grobler, S.R. (2008). Antimicrobial activity of two South African honeys produced from indigenous *Leucospermum cordifolium* and *Erica* species on selected micro-organisms. *BMC Complementary and Alternative Medicine*, *8*, 41. https://doi.org/10.1186/1472-6882-8-41

Becerril-Sánchez, A.L., Quintero-Salazar, B., Dublán-García, O., & Escalona-Buendía, H.B. (2021). Phenolic compounds in honey and their

relationship with antioxidant activity, botanical origin, and color. *Antioxidants*, *10*(11), 1700. https://doi.org/10.3390/antiox10111700

Bergamo, G., Seraglio, S.K.T., Gonzaga, L.V., Fett, R., de Mello Castanho Amboni, R.D., Dias, C.O., & Costa, A.C.O. (2019). Differentiation of honeydew honeys and blossom honeys: a new model based on colour parameters. *Journal of Food Science and Technology*, *56*(5), 2771-2777. https://doi.org/10.1007/s13197-019-03737-2

Blois, M.S. (1958). Antioxidant Determinations by the Use of a Stable Free Radical. *Nature*, *181*, 1199–1200.

Čanadanović-Brunet, J., Ćetković, G., Šaponjac, V.T., Stajčić, S., Vulić, J., Djilas, S., Štajner, D., & Popović, B. (2014). Evaluation of phenolic content, antioxidant activity and sensory characteristics of Serbian honey-based product. *Industrial Crops and Products*, *62*, 1–7. https://doi.org/10.1016/j.indcrop.2014.08.009

Chua, L.S., Rahaman, N.L.A., Adnan, N.A., & Eddie Tan, T.T. (2013). Antioxidant activity of three honey samples in relation with their biochemical components. *Journal of Analytical Methods in Chemistry*, *2013*(1), 313798. https://doi.org/10.1155/2013/313798

Clinical and Laboratory Standards Institute (2012). M07-A9; Methods for Dilution Antimicrobial Susceptibility Tests for Bacteria That Grow Aerobically. Approved Standard. Ninety Edition, Wayne, PA, USA, Volume 32.

Codex Alimentarius Commission (2001). Revised Codex Standard for Honey, Codex STAN 12-1981. Rev. 1 (1987), Rev. 2 (2001). *Codex Standard, 12*, 1-7.

Da Silva, P.M., Gauche, C., Gonzaga, L.V., Costa, A.C.O., & Fett, R. (2016). Honey: Chemical composition, stability and authenticity. *Food Chemistry*, *196*, 309–323. https://doi.org/10.1016/J.FOODCHEM.2015.09.051

Erejuwa, O.O., Sulaiman, S.A., & Ab Wahab, M.S. (2012). Honey: A novel antioxidant. *Molecules, 17*(4), 4400–4423. https://doi.org/10.3390/molecules17044400

FAOSTAT, Food and Agriculture Organization of the United Nations, Statistics Division. https://www.fao.org/faostat/en/#data/QCL (accessed September 2024).

Gašić, U., Kečkeš, S., Dabić, D., Trifković, J., Milojković-Opsenica, D., Natić, M., & Tešić, Z. (2014). Phenolic profile and antioxidant activity of Serbian polyfloral honeys. *Food Chemistry*, *145*, 599–607. https://doi.org/10.1016/j.foodchem.2013.08.088

International Honey Commission (2009). Harmonized methods of the International Honey Commission. 1-63.

Kesić, A., Ćelan, S., Mehmedinović, N.I., & Šestan, A. (2020). Optical activity, total phenolic content and color intensity of nectarian honey and honeydew. *European Journal of Food Science and Technology*, *8*(1), 12-33.

Kolayli, 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*(4), 515–523. https://doi.org/10.1556/066.2020.49.4.18

Kwakman, P.H.S., te Velde, A.A., de Boer, L., Vandenbroucke-Grauls, C.M.J.E., & Zaat, S.A.J. (2011). Two major medicinal honeys have different mechanisms of bactericidal activity. *PLoS ONE*, *6*(3), e17709. https://doi.org/10.1371/journal.pone.0017709

Lewoyehu, M. & Amare, M. (2019). Comparative evaluation of analytical methods for determining the antioxidant activities of honey: A review. *Cogent Food and Agriculture*, *5*(1). https://doi.org/10.1080/23311932.2019.1685059

Majtan, J., Bucekova, M., Kafantaris, I., Szweda, P., Hammer, K., & Mossialos, D. (2021). Honey antibacterial activity: A neglected aspect of honey quality assurance as functional food. *Trends in Food Science and Technology*, *118B*, 870–886. https://doi.org/10.1016/j.tifs.2021.11.012

Manzanares, A.B., García, Z.H., Galdón, B.R., Rodríguez, E.R., & Romero, C.D. (2011). Differentiation of blossom and honeydew honeys using multivariate analysis on the physicochemical parameters and sugar composition. *Food Chemistry*, *126*(2), 664–672. https://doi.org/10.1016/j.foodchem.2010.11.003

Meo, S.A., Al-Asiri, S.A., Mahesar, A.L., & Ansari, M.J. (2017). Role of honey in modern medicine. *Saudi Journal of Biological Sciences*, *24*(5), 975–978. https://doi.org/10.1016/j.sjbs.2016.12.010

Miller, N.J. & Rice-Evans Catherine, A. (1997). Factors influencing the antioxidant activity determined by the ABTS radical cation assay. *Free Radical Research*, *26*, 195–199.

Nedić, N., Nešović, M., Radišić, P., Gašić, U., Baošić, R., Joksimović, K., Pezo, L., Tešić, Ž.,

& Vovk, I. (2022). Polyphenolic and Chemical Profiles of Honey From the Tara Mountain in Serbia. *Frontiers in Nutrition*, *9*, 941463. https://doi.org/10.3389/fnut.2022.941463

Nikhat, S. & Fazil, M. (2022). History, phytochemistry, experimental pharmacology and clinical uses of honey: A comprehensive review with special reference to Unani medicine. *Journal of Ethnopharmacology*, *282*, 114614. https://doi.org/10.1016/j.jep.2021.114614

Obey, J.K., Ngeiywa, M.M., Lehesvaara, M., Kauhanen, J., von Wright, A., & Tikkanen-Kaukanen, C. (2022). Antimicrobial activity of commercial organic honeys against clinical isolates of human pathogenic bacteria. *Organic Agriculture*, *12*(2), 267–277. https://doi.org/10.1007/s13165-022-00389-z

Palma-Morales, M., Huertas, J.R., & Rodríguez-Pérez, C. (2023). A Comprehensive Review of the Effect of Honey on Human Health. *Nutrients*, *15*(13), 3056. https://doi.org/10.3390/nu15133056

Pasupuleti, V.R., Arigela, C.S., Gan, S.H., Salam, S.K.N., Krishnan, K.T., Rahman, N.A., & Jeffree, M.S. (2020). A review on oxidative stress, diabetic complications, and the roles of honey polyphenols. *Oxidative Medicine and Cellular Longevity, 2020*(1), 8878172. https://doi.org/10.1155/2020/8878172

Pauliuc, D., Dranca, F., & Oroian, M. (2020). Antioxidant activity, total phenolic content, individual phenolics and physicochemical parameters suitability for Romanian honey authentication. *Foods*, *9*(3), 306. https://doi.org/10.3390/foods9030306

Pita-Calvo, C. & Vázquez, M. (2017). Differences between honeydew and blossom honeys: A review. *Trends in Food Science and Technology, 59*, 79–87. https://doi.org/10.1016/j.tifs.2016.11.015

Samarghandian, S., Farkhondeh, T., & Samini, F. (2017). Honey and health: A review of recent clinical research. *Pharmacognosy Research, 9*(2), 121–127. https://doi.org/10.4103/0974-8490.204647

Savatović, S.M., Dimitrijević, D.J., Djilas, S.M., Ĉanadanović-Brunet, J.M., Ćetković, G.S., Tumbas, V.T., & Štajner, D.I. (2011). Antioxidant activity of three different Serbian floral honeys. *Acta Periodica Technologica*, *42*, 145–155. https://doi.org/10.2298/APT1142145S

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. https://doi.org/10.1016/S0076-6879(99)99017-1

Stagos, D., Soulitsiotis, N., Tsadila, C., Papaeconomou, S., Arvanitis, C., Ntontos, A., Karkanta, F., Adamou-Androulaki, S., Petrotos, K., Spandidos, D.A., Kouretas, D., & Mossialos, D. (2018). Antibacterial and antioxidant activity of different types of honey derived from Mount Olympus in Greece. *International Journal of Molecular Medicine*, *42*(2), 726–734. https://doi.org/10.3892/ijmm.2018.3656

Tafere, D.A. (2021). Chemical composition and uses of Honey: A Review. *Journal of Food Science and Nutrition Research*, *4*(3), 194-201. https://doi.org/10.26502/jfsnr.2642-11000072

Velimirović, D., Tošić, S., Mitić, S., Pavlović, A., Rašić Mišić, I., & Stojanović, G. (2023). Mineral, phenolic content and antioxidant activity of selected honey samples consumed in Serbia. *Journal of Apicultural Research*, *62*(4), 850–862. https://doi.org/10.1080/00218839.2021.1898783

Vranić, D., Petronijević, R., Dinović Stojanović, J., Korićanac, V., Babić Milijašević, J., & Milijašević, M. (2017). Physicochemical properties of honey from Serbia in the period 2014-2016. *IOP Conference Series: Earth and Environmental Science*, *85*(1). https://doi.org/10.1088/1755-1315/85/1/012058

Woisky, R.G. & Salatino, A. (1998). Analysis of propolis: Some parameters and procedures for chemical quality control. *Journal of Apicultural Research*, *37*(2), 99–105. https://doi.org/10.1080/00218839.1998.11100961

Živković, J., Sunarić, S., Stanković, N., Mihajilov-Krstev, T., & Spasić, A. (2019). Total phenolic and flavonoid contents, antioxidant and antibacterial activities of selected honeys against human pathogenic bacteria. *Acta Poloniae Pharmaceutica - Drug Research*, *76*(4), 671–681. https://doi.org/10.32383/appdr/105461