

ANTIBACTERIAL AND ANTIOXIDANT ACTIVITIES OF HONEY FROM SOUTHWESTERN SLOVAKIA

Soňa Felšöciová^{*1}, Simona Kunová², Eva Ivanišová^{2,3}

Address(es): doc. Ing. Soňa Felšöciová, PhD.,

¹ Slovak University of Agriculture in Nitra, Faculty of Biotechnology and Food Sciences, Institute of Biotechnology, Department of Microbiology, Trieda Andreja Hlinku 2, 949 76 Nitra, Slovakia, phone number: +421 37 641 4435.

² Institute of Food Sciences, Faculty of Biotechnology and Food Sciences, Slovak University of Agriculture in Nitra, Tr. A. Hlinku 2, 949 76 Nitra, Slovakia.

³ Food Incubator, AgroBioTech Research Centrum, Slovak University of Agriculture in Nitra, Tr. A. Hlinku 2, 949 76 Nitra, Slovakia; +421 37 641 4421.

*Corresponding author: sona.felsociova@uniag.sk

<https://doi.org/10.55251/jmbfs.13948>

ARTICLE INFO

Received 10. 2. 2026

Revised 6. 3. 2026

Accepted 25. 3. 2026

Published 1. 4. 2026

Regular article



ABSTRACT

This study evaluated the physicochemical characteristics, antibacterial and antioxidant activity of ten honey samples collected from the Žitný ostrov region in southwestern Slovakia. Physicochemical parameters, including water activity and moisture content, were analyzed. All tested honey samples complied with EU regulations for moisture content, and water activity values were below 0.60, indicating good microbiological stability. The antibacterial effect was assessed using the standard well diffusion method. All samples were tested at four concentrations (100%, 50%, 25%, and 12.5% v/v) against *Staphylococcus aureus*, *Enterococcus faecalis*, and *Escherichia coli*, and the inhibition zones were measured. All tested honeys exhibited inhibitory activity against the selected bacteria, however, a reduction in efficacy was observed at lower concentrations. At 25%, one sample lost activity against *E. coli*, and three samples were ineffective against *E. faecalis*. At 12.5%, three honeys lost activity against *E. coli*, and four samples showed no inhibition against *E. faecalis*. In addition to antimicrobial effects, the antioxidant activity (DPPH method) and total polyphenols (spectrophotometrically) of honey samples were also evaluated. Autumn honey showed the highest antioxidant activity (1.03 mg TEAC/g), while linden honey exhibited the highest polyphenol content (8.97 mg GAE/g).

Keywords: honey, antibacterial activity, antioxidant activity, agar well diffusion method, *Staphylococcus aureus*, *Enterococcus faecalis*, *Escherichia coli*

INTRODUCTION

Honey has long been recognized not only as a natural sweetener but also for its medicinal properties, particularly its antimicrobial activity. This biological effect is attributed to a combination of physicochemical and biochemical factors, including low pH, high osmolarity, the presence of hydrogen peroxide, and various phytochemicals, such as phenolic acids and flavonoids (Mandal & Mandal, 2011; Al-Waili *et al.*, 2012). The composition and potency of these components can vary significantly depending on the botanical and geographical origin of the honey, as well as storage conditions and processing methods (Estevinho *et al.*, 2008; Alvarez-Suarez *et al.*, 2010).

Among the wide range of bacteria affected by honey, both Gram-positive and Gram-negative species have been shown to be susceptible. Particularly relevant in food safety and health contexts are *Staphylococcus aureus*, *Enterococcus faecalis*, and *Escherichia coli*, which can be responsible for foodborne illnesses and hospital-acquired infections (Cooper *et al.*, 2002; Kačaniová *et al.*, 2022). While *S. aureus* is well known for its ability to develop resistance to multiple antibiotics, *E. coli* is often used as an indicator of fecal contamination and hygiene standards (Snowdon & Cliver, 1996). *Enterococcus faecalis* is a leading cause of nosocomial infections, frequently implicated in healthcare-associated urinary tract infections, bacteremia, and endocarditis. It is also notable for its ability to acquire multiple antibiotic resistance (Brinkwirth *et al.*, 2021).

The antimicrobial activity of honey is concentration-dependent, often decreasing with dilution (Badet & Quero, 2011). Nevertheless, certain monofloral honeys, such as manuka or thyme honey, retain significant activity even at lower concentrations due to the presence of unique non-peroxide factors (Kwakman & Zaat, 2012).

In addition to its well-documented antimicrobial properties, honey also exhibits significant antioxidant activity, which contributes to its overall therapeutic potential. This antioxidant capacity is primarily attributed to the presence of phenolic compounds, flavonoids, enzymes, such as catalase and glucose oxidase, as well as organic acids, ascorbic acid, and Maillard reaction products (Gheldof & Engeseth, 2002; Bertoneclj *et al.*, 2007). These compounds are capable of scavenging reactive oxygen species (ROS), chelating metal ions, and inhibiting lipid peroxidation, thereby helping to reduce oxidative stress and associated cellular damage (Alvarez-Suarez *et al.*, 2010). The total antioxidant capacity of honey is strongly influenced by its floral source, with darker honeys generally

exhibiting higher levels of phenolic content and antioxidant activity than lighter varieties (Ferreira *et al.*, 2009; Alvarez-Suarez *et al.*, 2013). Consequently, antioxidant profiling of honey provides valuable insight into its quality, authenticity, and potential health benefits, especially in the context of functional foods and nutraceuticals. Given the variability in honey composition related to botanical and geographical origin, a comprehensive evaluation of physicochemical, antibacterial, and antioxidant properties is required.

Therefore, the aim of this study was to evaluate the physicochemical properties, antibacterial activity, and antioxidant potential of ten honey samples collected from the Žitný ostrov region in southwestern Slovakia. Water activity and moisture content were determined, antibacterial activity was assessed against two Gram-positive bacteria (*Staphylococcus aureus* and *Enterococcus faecalis*) and one Gram-negative bacterium (*Escherichia coli*) at four honey concentrations (100%, 50%, 25%, and 12.5% v/v), and antioxidant activity together with total polyphenol content were analysed.

MATERIAL AND METHODS

Honey samples

Table 1 provides an overview of ten honey samples of different botanical origins, produced in 2024 by two beekeepers from Baloň and Jurová, located in the Žitný ostrov region of southwestern Slovakia. Žitný ostrov, the largest river island in Europe, is known for its fertile soil, diverse flora, and favorable microclimatic conditions. These unique environmental features may influence the botanical origin and chemical composition of local honeys, potentially affecting their antimicrobial and physicochemical properties. The selection of samples includes both monofloral honeys (e.g., acacia, phacelia, linden) and polyfloral honeys (e.g., wildflower, spring, autumn), providing a representative cross-section of the region's honey production. The samples were packed in glass containers and stored at room temperature (22 ± 1 °C) in the dark until analysis.

Chemicals

All chemicals used for antioxidant activity and total polyphenol analyses were analytical grade and were purchased from Rechem (Slovakia) and Sigma Aldrich (USA).

Table 1 Summary of monofloral and polyfloral honeys collected in 2024

Sample No.	Type of honey	Origin
1.	Acacia honey	Baloň
2.	Phacelia honey	Baloň
3.	Wildflower honey	Baloň
4.	Linden honey	Baloň
5.	Rapeseed honey	Baloň
6.	Acacia honey	Jurová
7.	Spring honey	Jurová
8.	Rapeseed honey	Jurová
9.	Sunflower honey	Jurová
10.	Autumn honey	Jurová

Bacterial strains

Three bacterial species were used to determine antimicrobial activity. *Enterococcus faecalis* (CCM 1875) and *Staphylococcus aureus* (CCM 299) were used as representatives of Gram-positive bacteria, whereas *Escherichia coli* (CCM 4225) represented Gram-negative bacteria. All microbial cultures were obtained from the Czech Collection of Microorganisms, Masaryk University, Brno.

Physicochemical Parameters

Water activity was measured using a LabMaster instrument (Novasina, Pfäffikon, Switzerland). The samples were placed in plastic sample cups, equilibrated at 25 °C, and the water activity values were recorded directly from the instrument display.

The moisture content of the honey samples was determined using an optical refractometer (HHR-2N, ATAGO, Japan).

Well diffusion method

The antimicrobial properties of the honey samples were evaluated using the agar well diffusion method. Bacterial strains were first cultured in Mueller-Hinton Broth (MHB, Oxoid, Basingstoke, UK) at 37 °C for 24 hours. Subsequently, 100 µL of a bacterial suspension standardized to 0.5 McFarland turbidity was uniformly spread onto the surface of Mueller Hinton Agar (MHA, Oxoid, Basingstoke, UK) plates. Honey samples were diluted in sterile distilled water to obtain final concentrations of 50%, 25%, and 12.5% (v/v). Wells with a diameter of 8 mm were created in the agar using a sterile cork borer. One well was filled with undiluted honey (100%), while the remaining wells were filled with the respective honey dilutions. The plates were then incubated at 37 °C for 24 hours. After incubation, the inhibition zones were measured from the outer edge of the well to the point of visible bacterial growth and then converted to total inhibition zone diameters (mm), in accordance with standard practice. Each measurement was performed at three different points to ensure accuracy. Each test was conducted in triplicate to ensure reproducibility.

Control samples

To ensure correct interpretation of the antibacterial effects, three types of controls were included:

Negative control: sterile distilled water applied into a single agar well to confirm that any inhibition was not caused by the solvent or handling.

Positive control (antibiotic): a standard susceptibility disk (ampicillin 10 µg; Oxoid) placed on Mueller–Hinton agar as a reference for growth inhibition.

Osmotic control (“artificial honey”): a sugar solution mimicking the osmolarity of honey (40% fructose, 30% glucose, 10% sucrose, 20% distilled water, w/v). The artificial honey was tested at 100%, 50%, 25%, and 12.5% (v/v), identical to the honey dilutions.

All controls were processed under the same conditions as the honey samples: Mueller–Hinton agar seeded with 0.5 McFarland inoculum, incubation at 37 °C for 24 hours, and measurement of inhibition zones using the same procedure as for honey.

Sample preparation for antioxidant activity and total polyphenols

One gram of the sample was extracted with 20 mL of distilled water for 1 hour. After centrifugation at 4000 x g (Rotofix 32 A, Hettich, Germany) for 10 min, the supernatant was collected and used for determination of antioxidant activity and total polyphenol content. The extraction was performed in triplicate.

Radical scavenging activity – DPPH method

Radical scavenging activity of the extracts was measured using 2,2-diphenyl-1-picrylhydrazyl (DPPH) according to Sánchez-Moreno et al. (1998). The sample (0.4 mL) was mixed with 3.6 mL of DPPH solution (0.025 g DPPH in 100 mL of methanol). The absorbance of the reaction mixture was measured at 515 nm using a Jenway 6405 UV/Vis spectrophotometer (England). Trolox (6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid) (10–100 mg/L; R²=0.989) was used as the

standard and the results were expressed in mg Trolox equivalents per g of sample (mg TEAC/g).

Total polyphenol content

Total polyphenol content of the extracts was determined by the method of Singleton and Rossi (1965) using Folin-Ciocalteu reagent. A 0.1 mL aliquot of each sample was mixed with 0.1 mL of the Folin-Ciocalteu reagent, 1 mL of 20% (w/v) sodium carbonate, and 8.8 mL of distilled water. After 30 min in the dark, the absorbance at 700 nm was measured using a Jenway 6405 UV/Vis spectrophotometer (England). Gallic acid (25–300 mg/L; R²=0.998) was used as the standard, and the results were expressed as mg gallic acid equivalents per g of sample (mg GAE/g).

Statistical analysis

All measurements were performed in triplicate, and the results are expressed as mean ± standard deviation (SD). Statistical analysis was carried out using XLSTAT (Addinsoft, Paris, France). Differences among honey samples were evaluated using one-way analysis of variance (ANOVA) followed by Tukey’s post hoc test to determine statistically significant differences between the means. Differences were considered statistically significant at p < 0.05. The relationship between antioxidant activity (DPPH) and total polyphenol content was assessed using Pearson’s correlation coefficient (r).

RESULTS AND DISCUSSION

Physicochemical parameters

Among the physicochemical parameters, water activity and moisture content were determined and are presented in Table 2. Water activity ranged from 0.396 (wildflower honey) to 0.452 (rapeseed honey from Jurová). Although water activity is not directly regulated by current Slovak or European honey legislation, expert recommendations suggest that the water activity of stable honey should be below 0.60 to minimize the risk of yeast growth and fermentation. The values observed in this study were well below this threshold, indicating good microbiological stability of the analyzed samples. Moisture content ranged from 14.2% (linden honey) to 19.6% (sunflower honey). All samples complied with the maximum moisture content limit of 20%, as defined by the Codex Alimentarius Commission (1981/2022), confirming their suitability for storage and the reduced risk of microbial growth.

Table 2 Physicochemical parameters of different types of honey

Sample No.	Type of honey	Water activity	Moisture content (%)
1.	Acacia honey	0.405 ^c	14.8 ^c
2.	Phacelia honey	0.403 ^{cd}	15.5 ^c
3.	Wildflower honey	0.396 ^c	14.9 ^c
4.	Linden honey	0.399 ^{de}	14.2 ^c
5.	Rapeseed honey	0.401 ^{ed}	15.4 ^c
6.	Acacia honey	0.450 ^a	18.3 ^b
7.	Spring honey	0.423 ^b	19.5 ^a
8.	Rapeseed honey	0.452 ^a	19.2 ^a
9.	Sunflower honey	0.445 ^a	19.6 ^a
10.	Autumn honey	0.447 ^a	18.3 ^b

Legend: ^{a,b,c,d,e} Different letters within the same column denote significant differences (p < 0.05).

Antimicrobial activity

The negative control (sterile distilled water) produced no inhibition. The positive control (antibiotic disk) yielded a clear, expected inhibition zone and served as a benchmark for the assay. The osmotic control (artificial honey) showed negligible to low inhibition across the tested dilutions, consistently lower than the activity of natural honeys. These findings confirm that the antibacterial effects observed for the tested honeys cannot be attributed solely to high osmolarity.

The antimicrobial activity of the 10 honey samples against *Staphylococcus aureus*, *Enterococcus faecalis* and *Escherichia coli* was evaluated at four concentrations (100%, 50%, 25%, and 12.5%; Tables 3 and 4). Overall, the results revealed a clear concentration-dependent inhibitory effect, with *Staphylococcus aureus* and *Escherichia coli* generally more sensitive than *Enterococcus faecalis*.

Against *S. aureus*, the strongest inhibition at 100% concentration was observed in rapeseed honey (No. 5, 13.60±0.58 mm), followed by sunflower honey (No. 9, 12.50±0.73 mm), linden honey (No. 4, 12.25±0.50 mm), and phacelia honey (No. 2, 11.50±1.29 mm). At 50%, phacelia honey maintained high activity (10.75±1.50 mm), as did rapeseed honey from Jurová (No. 8, 9.25±0.50 mm), while other samples, such as autumn honey (No. 10), showed moderate inhibition (5.75±0.96 mm). At 25%, inhibition generally declined, though sunflower honey (No. 9, 7.67±0.49 mm) and phacelia honey (No. 2, 7.07±0.65 mm) retained notable activity. At the lowest concentration (12.5%), most honeys exhibited minimal inhibitory effects, except for sunflower honey (No. 9), which retained measurable

activity (6.00±0.00 mm). These results indicate that *S. aureus* is highly sensitive to honey, and that floral origin, likely related to phenolic content, hydrogen peroxide, and other bioactive compounds, strongly influences antibacterial potency (Kwakman & Zaat, 2012; Almasaudi, 2021).

Overall, inhibitory activity against *E. faecalis* was lower. The highest inhibition at 100% concentration was observed in phacelia honey (No. 2, 11.5±0.57 mm) and acacia honey from Baloň (No. 1, 10.75±0.96 mm). Phacelia honey retained measurable activity down to the 12.5% concentration (1.83±0.41 mm), while several other samples, including rapeseed (No. 8), sunflower (No. 9), and autumn honeys (No. 10), lost activity below 50%, with no detectable inhibition at 25% and 12.5%. This pattern aligns with previous findings indicating that *E. faecalis* is generally more resistant to honey than *S. aureus*, due to its capacity to survive harsh conditions, including high salt and bile concentrations (Arias & Murray, 2012; Silva et al., 2023). Nevertheless, the retained activity in phacelia and acacia honeys highlights the potential contribution of floral origin and bioactive constituents to antibacterial efficacy (Irish et al., 2011; Sakač et al., 2022).

Table 3 Antibacterial effect of honey on growth of Gram-positive bacteria

Sample No.	<i>Staphylococcus aureus</i>				<i>Enterococcus faecalis</i>			
	Honey concentrations (v/v) and inhibition zone diameters (mm)							
	100%	50%	25%	12.5%	100%	50%	25%	12.5%
1.	10.50±0.57 ^{bc}	8.75±0.96 ^{ab}	5.72±0.49 ^{ab}	4.50±0.57 ^a	10.75±0.96 ^a	7.50±0.41 ^{ab}	4.75±0.96 ^{ab}	1.00±0.82 ^a
2.	11.50±1.29 ^{abc}	10.75±1.50 ^a	7.07±0.65 ^a	5.00±1.41 ^a	11.50±0.57 ^a	9.87±0.85 ^a	6.50±1.38 ^a	1.83±0.41 ^a
3.	10.25±0.50 ^c	6.00±1.26 ^c	4.12±0.86 ^{bc}	1.25±0.50 ^b	9.00±0.00 ^b	6.83±0.41 ^{ab}	4.44±0.45 ^{ab}	1.00±0.00 ^a
4.	12.25±0.50 ^{ab}	7.87±0.85 ^b	3.75±0.50 ^c	1.37±0.48 ^b	7.33±0.52 ^c	5.75±0.96 ^{bc}	3.00±0.00 ^{bc}	1.83±0.41 ^a
5.	13.60±0.58 ^a	7.67±0.57 ^b	3.75±0.50 ^c	1.25±0.50 ^b	7.25±1.26 ^c	5.25±0.50 ^c	2.50±0.57 ^c	0.00 ^b
6.	10.25±0.5 ^{bc}	7.00±0.81 ^{bc}	4.00±0.82 ^{bc}	1.00±0.00 ^b	7.75±0.50 ^c	4.00±0.00 ^d	1.25±0.50 ^d	1.00±0.00 ^a
7.	10.75±0.50 ^{bc}	6.50±1.38 ^c	4.00±0.82 ^{bc}	2.75±0.96 ^c	7.00±0.82 ^c	5.50±0.57 ^{bc}	3.0±0.82 ^{bc}	1.25±0.85 ^a
8.	10.50±0.57 ^{bc}	9.25±0.50 ^{ab}	4.50±1.29 ^{bc}	3.25±0.50 ^{ac}	6.25±0.64 ^d	1.00±0.00 ^c	0.00 ^c	0.00 ^b
9.	12.50±0.73 ^{ab}	9.00±1.15 ^{ab}	7.67±0.49 ^a	6.00±0.00 ^a	7.67±0.48 ^c	2.37±0.48 ^c	0.00 ^c	0.00 ^b
10.	10.25±0.50 ^c	5.75±0.96 ^c	4.50±0.57 ^{bc}	2.27±0.49 ^c	7.00±0.00 ^c	4.33±0.57 ^{cd}	0.00 ^c	0.00 ^b

Legend: 1, 6 - acacia honeys; 2 - phacelia honey; 3 - wildflower honey; 4 - linden honey; 5, 8 - rapeseed honeys; 7 - spring honey; 9 - sunflower honey; 10 - autumn honey; samples 1-5 originated from Baloň; samples 6-10 from Jurová; ^{a,b,c,d,e} Different letters within the same column denote significant differences (p < 0.05).

Our study demonstrates a clear dose-dependent antibacterial effect of the honey samples against *Escherichia coli* (Table 4), in line with previous reports showing that honey's efficacy increases with concentration (Mudenda et al., 2023). At 100% concentration, strong inhibition was observed, particularly for acacia honey from Baloň (12.67±0.53 mm), wildflower honey (12.50±0.50 mm), and spring honey (12.00±0.00 mm). Similar patterns have been reported in other studies, where natural honeys exhibited pronounced activity against *E. coli*, in some cases even exceeding their effectiveness against *Staphylococcus aureus* (Mudenda et al., 2023). For example, unpasteurized and untreated natural honey produced inhibition zones of 22 mm against *E. coli* and 20 mm against *S. aureus* at full concentration, while at 50% concentration the zones decreased to 18 mm and 16 mm, respectively.

The concentration-dependent decline in activity across both bacterial species emphasizes that dilution reduces osmotic pressure and the effectiveness of peroxidase-related mechanisms and inhibitory phenolic compounds, suggesting a threshold below which honey's antimicrobial effect is limited (Kwakman & Zaat, 2012; Ahmed & Othman, 2013). Variability among samples further underscores the role of floral source, harvesting region, and processing conditions in shaping the antimicrobial potential of honey. It should be noted that due to the high viscosity of honey, diffusion in agar may be partially limited, and inhibition zones should therefore be interpreted with this methodological constraint in mind. However, this study is based on ten honey samples obtained from only two beekeepers within a single geographic area (Žitný ostrov). Therefore, the results should be interpreted as region-specific and cannot be generalized to broader populations of honeys. Broader multi-regional sampling would be necessary to robustly evaluate geographic influences.

Upon dilution to 50%, the antibacterial activity of the honey samples declined but remained clearly detectable in the most active honeys, namely acacia honey from Baloň (8.33±0.53 mm), phacelia honey (6.33±0.57 mm), and wildflower honey (6.67±0.58 mm). Several other samples exhibited only moderate inhibition, with zones ranging approximately from 3 to 5 mm. At 25% concentration, acacia, phacelia, and wildflower honeys from Baloň maintained moderate inhibitory effects (approximately 5.0–5.6 mm), whereas the remaining samples showed only minimal activity (about 1.0–2.6 mm). Notably, rapeseed honey from Jurová exhibited no detectable inhibition at this concentration, which aligns with reports indicating that antibacterial activity often diminishes or disappears below 5–10% honey concentration (Wilkinson & Cavanagh, 2005).

Table 4 Antibacterial effect of honey on growth of Gram-negative bacteria

Sample No.	Type of honey	<i>Escherichia coli</i>			
		Honey concentrations (v/v) and inhibition zones diameter (mm)			
		100%	50%	25%	12.5%
1.	Acacia honey	12.67±0.53 ^a	8.33±0.53 ^a	5.00±1.00 ^a	4.33±0.57 ^a
2.	Phacelia honey	11.67±0.57 ^{ab}	6.33±0.57 ^b	5.00±1.00 ^a	3.00±1.00 ^{ab}
3.	Wildflower honey	12.50±0.50 ^a	6.67±0.58 ^b	5.60±1.51 ^a	4.33±0.57 ^a
4.	Linden honey	8.37±1.48 ^c	4.83±1.04 ^c	2.60±1.51 ^b	2.00±0.75 ^{bc}
5.	Rapeseed honey	10.53±0.50 ^b	4.57±0.51 ^c	2.00±0.00 ^{bc}	1.00±0.00 ^{cd}
6.	Acacia honey	10.63±0.55 ^b	3.00±0.00 ^d	2.00±0.00 ^{bc}	0.00 ^c
7.	Spring honey	12.00±0.00 ^{ab}	4.57±0.51 ^c	3.00±0.00 ^b	2.00±0.00 ^{bc}
8.	Rapeseed honey	10.07±1.01 ^b	5.60±1.22 ^{bc}	0.00 ^d	0.00 ^c
9.	Sunflower honey	10.55±0.51 ^b	5.83±1.04 ^{bc}	1.00±0.00 ^c	0.00 ^c
10.	Autumn honey	8.93±0.11 ^c	3.27±0.46 ^d	2.00±0.00 ^{bc}	0.77±0.25 ^d

Legend: samples 1-5 originated from Baloň; samples 6-10 from Jurová; ^{a,b,c,d,e} Different letters within the same column denote significant differences (p < 0.05).

At 25% concentration, acacia honey, phacelia honey and wildflower honey from Baloň maintained moderate inhibition (~5.0–5.60 mm), while the remaining samples showed only minimal zones (~1.0–2.6 mm). Rapeseed honey from Jurová exhibited no detectable inhibition, echoing literature showing that inhibition often vanishes below 5%–10% concentrations (Wilkinson & Cavanagh, 2005).

At the lowest tested concentration (12.5%), most honey samples lost detectable antibacterial activity, with only samples 1, 2, and 3 retaining weak inhibitory effects (approximately 3.0–4.33 mm). These findings support the concept that honey's antimicrobial activity results from the combined effects of high osmolarity, low pH, hydrogen peroxide production, and the presence of phenolic and flavonoid compounds, all of which are more effective at higher concentrations (Almasaudi, 2021). Mechanistic studies on honeys such as manuka and Yemeni Sidr further demonstrate that honey can disrupt bacterial cell membranes, inhibit protein synthesis, and induce DNA damage in *E. coli* (Al-Sayaghi et al., 2022), suggesting similar modes of action may underlie the antibacterial effects observed

in the most active samples. These mechanistic effects were described in previous studies but were not investigated in the present work, therefore, our conclusions are limited strictly to the observed inhibition patterns.

Radical scavenging activity – DPPH method

Among the tested samples, autumn honey exhibited the highest antioxidant activity (1.03 ± 0.02 mg TEAC/g), followed by sunflower honey (0.95 ± 0.03 mg TEAC/g) and both samples of rapeseed honey (0.89–0.91 mg TEAC/g) (Tab. 5). In contrast, linden honey demonstrated the lowest antioxidant potential (0.82 ± 0.03 mg TEAC/g), with acacia honey also showing relatively low values (0.79–0.86 mg TEAC/g). The variation in antioxidant activity among different honey types is primarily attributed to their botanical origin and associated phenolic content. Generally, darker honeys, such as autumn and sunflower varieties tend to contain higher levels of phenolic compounds and flavonoids, which are responsible for their enhanced radical scavenging activity (Bertoncelj et al., 2007; Al-Mamary

et al., 2002). The high value of autumn honey may also reflect a greater diversity of floral sources and a longer maturation period, which could contribute to the accumulation of antioxidant compounds. In contrast, lighter honeys, such as acacia and linden are typically associated with lower phenolic content and, consequently, lower antioxidant capacity (Ferreira et al., 2009). However, despite their lighter color, certain varieties like rapeseed honey demonstrated comparatively high antioxidant activity. This suggests that factors beyond color – such as floral origin, climate conditions, and storage – also significantly influence the antioxidant profile (Bogdanov et al., 2008).

Interestingly, phacelia and wildflower honeys showed moderate antioxidant capacities (0.88–0.91 mg TEAC/g), aligning with values reported for multifloral honeys in other European studies (Khalil et al., 2010). This indicates that the combination of nectar sources can yield synergistic antioxidant effects.

Total polyphenol content

The highest total polyphenol content was observed in linden honey (8.97±0.01 mg GAE/g), followed by rapeseed honey (5.32±0.04 mg GAE/g) (Tab. 5). In contrast, rapeseed honey (2) showed an unexpectedly low polyphenol content (0.31±0.03 mg GAE/g), indicating significant variation even within the same floral type, possibly due to differences in environmental factors, processing, or storage conditions. Honeys, such as acacia, wildflower, phacelia, and spring honey exhibited relatively low polyphenol levels (0.39–0.58 mg GAE/g), consistent with findings reported in the literature for lighter, monofloral honeys (Bertoncelj et al., 2007; Alvarez-Suarez et al., 2010). The values for sunflower (0.79±0.01 mg GAE/g) and autumn honey (0.74±0.02 mg GAE/g) suggest moderate phenolic content, which may contribute to their observed antioxidant activity. The high polyphenol level in linden honey is notable and may be attributed to the high concentration of specific phenolic compounds such as flavonoids and phenolic acids, which are characteristic of *Tilia* species (Gheldof & Engeseth, 2002). The discrepancy between the two rapeseed honey samples suggests that polyphenol content can vary widely depending on geographic origin, seasonal conditions, and nectar composition. These findings support the widely accepted notion that the botanical and geographical origin of honey strongly influences its phenolic profile, which in turn impacts both its antioxidant capacity and potential health benefits (Ferreira et al., 2009). Moreover, the relatively low polyphenol content in acacia honeys aligns with their light color and mild flavor, typically associated with low levels of bioactive compounds (Khalil et al., 2010). Interestingly, while some samples such as autumn and sunflower honeys showed moderate polyphenol content, they exhibited higher antioxidant activity in the DPPH assay (Tab. 5), suggesting that antioxidant potential is not solely dependent on total polyphenol levels. This supports previous research indicating that antioxidant activity is also influenced by the presence of other bioactive substances such as enzymes, organic acids, and Maillard reaction products (Bogdanov et al., 2008).

Table 5 Antioxidant activity and total polyphenol content of tested honey

Sample No.	Type of honey	DPPH (mg TEAC/g)	Total polyphenols (mg GAE/g)
1.	Acacia honey	0.86±0.02 ^{cd}	0.53±0.01 ^d
2.	Phacelia honey	0.91±0.12 ^b	0.44±0.02 ^c
3.	Wildflower honey	0.88±0.02 ^c	0.39±0.03 ^f
4.	Linden honey	0.82±0.03 ^d	8.97±0.01 ^a
5.	Rapeseed honey	0.89±0.01 ^c	5.32±0.04 ^b
6.	Acacia honey	0.79±0.04 ^e	0.58±0.02 ^c
7.	Spring honey	0.86±0.01 ^{cd}	0.41±0.02 ^{ef}
8.	Rapeseed honey	0.91±0.02 ^b	0.31±0.03 ^g
9.	Sunflower honey	0.95±0.03 ^b	0.79±0.01 ^c
10.	Autumn honey	1.03±0.02 ^a	0.74±0.02 ^c

Legend: TEAC – Trolox equivalent antioxidant capacity; GAE – gallic acid equivalent; mean ± standard deviation; samples 1–5 originated from Balón; samples 6–10 from Jurová; ^{a,b,c,d,e,f,g} Different letters within the same column denote significant differences (p < 0.05).

To evaluate the relationship between antioxidant capacity and phenolic compounds, a Pearson correlation analysis was performed between DPPH radical scavenging activity and total polyphenol content. The analysis revealed a weak negative correlation (r = -0.31) between these parameters across the analyzed honey samples. This result indicates that higher total polyphenol content did not necessarily correspond to higher antioxidant activity in the DPPH assay. A notable example is linden honey, which exhibited the highest polyphenol content but relatively low antioxidant activity. This discrepancy suggests that antioxidant capacity in honey is influenced not only by the total amount of phenolic compounds but also by their specific composition and interactions with other bioactive constituents, such as enzymes, organic acids, peptides, and Maillard reaction products. Similar observations have been reported in previous studies,

which emphasize that the antioxidant potential of honey depends on the synergistic effect of multiple compounds rather than solely on total polyphenol concentration (Ferreira et al., 2009). Therefore, while phenolic compounds contribute significantly to antioxidant properties, they cannot be considered the only determinant of radical scavenging activity.

CONCLUSION

The present study confirmed that all analysed honey samples met the physicochemical quality requirements, with moisture content within EU limits and water activity values below 0.60, indicating good microbiological stability. The results demonstrated a clear concentration-dependent antibacterial effect of honey against *Staphylococcus aureus*, *Enterococcus faecalis*, and *Escherichia coli*, with notable variability among different honey types. Phacelia honey exhibited the most consistent antibacterial activity across all tested concentrations, while acacia and wildflower honeys showed strong effects particularly against *E. coli*. The reduced sensitivity of *E. faecalis* compared to *S. aureus* highlights differences in bacterial susceptibility to honey. Significant differences observed between honeys of the same botanical origin but different geographical locations underline the influence of floral source, environmental conditions, and local microclimate on antimicrobial efficacy. Antioxidant analyses revealed that darker honeys, particularly autumn honey, possessed higher radical scavenging activity, whereas total polyphenol content did not always correlate directly with antioxidant capacity, as demonstrated by linden honey. Overall, the findings emphasize the multifactorial nature of honey’s biological activity and confirm that botanical origin, geographical location, and concentration play a crucial role in determining both antibacterial and antioxidant properties. Further research focused on the identification of specific bioactive compounds and their mechanisms of action could provide deeper insight into the therapeutic and functional potential of honey. Given the limited number of samples and the single-region sourcing, these results should be considered preliminary and geographically bounded, warranting confirmation in larger, multi-regional datasets.

Acknowledgments: This research was funded by the Science Grant Agency VEGA 1/0454/25 and KEGA 030SPU-4/2024.

REFERENCES

Ahmed, S., & Othman, N. H. (2013). Review of the medicinal effects of tualang honey and a comparison with manuka honey. *The Malaysian journal of medical sciences: MJMS*, 20(3), 6.

Al-Mamary, M., Al-Meer, A., & Al-Habori, M. (2002). Antioxidant activities and total phenolics of different types of honey. *Nutrition research*, 22(9), 1041-1047. [https://doi.org/10.1016/S0271-5317\(02\)00406-2](https://doi.org/10.1016/S0271-5317(02)00406-2)

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-Sayaghi, A. M., Al-Kabsi, A. M., Abduh, M. S., Saghir, S. A. M., & Alshawsh, M. A. (2022). Antibacterial mechanism of action of two types of honey against *Escherichia coli* through interfering with bacterial membrane permeability, inhibiting proteins, and inducing bacterial DNA damage. *Antibiotics*, 11(9), 1182. <https://doi.org/10.3390/antibiotics11091182>

Alvarez-Suarez, J. M., Tulipani, S., Romandini, S., Bertoli, E., & Battino, M. (2010). Contribution of honey in nutrition and human health: a review. *Mediterranean journal of nutrition and metabolism*, 3(1), 15-23. <https://doi.org/10.1007/s12349-009-0051-6>

Alvarez-Suarez, J., Giampieri, F., & Battino, M. (2013). Honey as a source of dietary antioxidants: structures, bioavailability and evidence of protective effects against human chronic diseases. *Current medicinal chemistry*, 20(5), 621-638. <https://doi.org/10.2174/092986713804999358>

Al-Waili, N., Salom, K., Al-Ghamdi, A., & Ansari, M. J. (2012). Antibiotic, pesticide, and microbial contaminants of honey: human health hazards. *The scientific world journal*, 2012(1), 930849. <https://doi.org/10.1100/2012/930849>

Arias, C. A., & Murray, B. E. (2012). The rise of the *Enterococcus*: beyond vancomycin resistance. *Nature reviews microbiology*, 10(4), 266-278. <https://doi.org/10.1038/nrmicro2761>

Badet, C., & Quero, F. (2011). The in vitro effect of manuka honeys on growth and adherence of oral bacteria. *Anaerobe*, 17(1), 19-22. <https://doi.org/10.1016/j.anaerobe.2010.12.007>

Bertoncelj, J., Doberšek, U., Jamnik, M., & Golob, T. (2007). Evaluation of the phenolic content, antioxidant activity and colour of Slovenian honey. *Food chemistry*, 105(2), 822-828. <https://doi.org/10.1016/j.foodchem.2007.01.060>

Bogdanov, S., Jurendic, T., Sieber, R., & Gallmann, P. (2008). Honey for nutrition and health: a review. *Journal of the American college of nutrition*, 27(6), 677-689. <https://doi.org/10.1080/07315724.2008.10719745>

Brinkwirth, S., Ayobami, O., Eckmanns, T., & Markwart, R. (2021). Hospital-acquired infections caused by enterococci: a systematic review and meta-analysis, WHO European Region, 1 January 2010 to 4 February 2020. *Eurosurveillance*, 26(45), 2001628. <https://doi.org/10.2807/1560-7917.es.2021.26.45.2001628>

- Codex Alimentarius Commission. (1981). *Standard for honey CXS 12-1981* (Adopted in 1981; revised in 1987, 2001; amended in 2019, 2022). Food and Agriculture Organization of the United Nations (FAO) & World Health Organization (WHO).
- Cooper, R. A., Molan, P. C., & Harding, K. G. (2002). The sensitivity to honey of Gram-positive cocci of clinical significance isolated from wounds. *Journal of applied microbiology*, 93(5), 857–863. <https://doi.org/10.1046/j.1365-2672.2002.01761.x>
- Estevinho, L., Pereira, A. P., Moreira, L., Dias, L. G., & Pereira, E. (2008). Antioxidant and antimicrobial effects of phenolic compounds extracts of Northeast Portugal honey. *Food and chemical toxicology*, 46(12), 3774-3779. <https://doi.org/10.1016/j.fct.2008.09.062>
- Ferreira, I. C., Aires, E., Barreira, J. C., & Estevinho, L. M. (2009). Antioxidant activity of Portuguese honey samples: Different contributions of the entire honey and phenolic extract. *Food chemistry*, 114(4), 1438-1443. <https://doi.org/10.1016/j.foodchem.2008.11.028>
- Gheldof, N., & Engeseth, N. J. (2002). Antioxidant capacity of honeys from various floral sources based on the determination of oxygen radical absorbance capacity and inhibition of in vitro lipoprotein oxidation in human serum samples. *Journal of agricultural and food chemistry*, 50(10), 3050-3055. <https://doi.org/10.1021/jf0114637>
- Irish, J., Blair, S., & Carter, D. A. (2011). The antibacterial activity of honey derived from Australian flora. *PloS one*, 6(3), e18229. <https://doi.org/10.1371/journal.pone.0018229>
- Kačaniová, M., Borotová, P., Galovičová, L., Kunová, S., Štefániková, J., Kowalczewski, P. L., & Šedík, P. (2022). Antimicrobial and antioxidant activity of different Honey samples from beekeepers and Commercial Producers. *Antibiotics*, 11(9), 1163. <https://doi.org/10.3390/antibiotics11091163>
- Khalil, M. I., Sulaiman, S. A., & Boukraa, L. (2010). Antioxidant properties of honey and its role in preventing health disorder. *The open nutraceuticals journal*, 3(1), 6-16. <https://doi.org/10.2174/1876396001003010006>
- Kwakman, P. H., & Zaat, S. A. (2012). Antibacterial components of honey. *IUBMB life*, 64(1), 48-55. <https://doi.org/10.1002/iub.578>
- Mandal, M. D., & Mandal, S. (2011). Honey: Its medicinal property and antibacterial activity. *Asian Pacific journal of tropical biomedicine*, 1(2), 154–160. [https://doi.org/10.1016/S2221-1691\(11\)60016-6](https://doi.org/10.1016/S2221-1691(11)60016-6)
- Mudenda, S., Hikaambo, C. N., Chabalenge, B., Mfuno, R. L., Mufwambi, W., Ngazimbi, M., Matafwali, S., & Daka, V. (2023). Antibacterial activities of honey against *Escherichia coli* and *Staphylococcus aureus*: A potential treatment for bacterial infections and alternative to antibiotics. *Journal of Pharmacognosy and Phytochemistry*, 12(3), 06-13. <https://doi.org/10.22271/phyto.2023.v12.i3a.14655>
- Sakač, M., Jovanov, P., Marić, A., Četojević-Simin, D., Novaković, A., Plavšić, D., ... & Kovač, R. (2022). Antioxidative, antibacterial and antiproliferative properties of honey types from the Western Balkans. *Antioxidants*, 11(6), 1120. <https://doi.org/10.3390/antiox11061120>
- Sánchez-Moreno, C., Larrauri, A., & Saura-Calixto, F. (1998). A procedure to measure the antioxidant efficiency of polyphenols. *Journal of Science and Food Agriculture*, 76(2), 270-276. [https://doi.org/10.1002/\(sici\)1097-0010\(199802\)76:2<270::aid-jsfa945>3.3.co;2-0](https://doi.org/10.1002/(sici)1097-0010(199802)76:2<270::aid-jsfa945>3.3.co;2-0)
- Silva, I. C. D., Conceição, E. O. A., Pereira, D. S., Rogez, H., & Muto, N. A. (2023). Evaluation of the antimicrobial capacity of bacteria isolated from stingless bee (*Scaptotrigona aff. postica*) Honey cultivated in Açai (*Euterpe oleracea*) monoculture. *Antibiotics*, 12(2), 223. <https://doi.org/10.3390/antibiotics12020223>
- Singleton, V. L., & Rossi, J. A. (1965). Colorimetry of total phenolics with phosphomolybdic-phosphotungstic acid reagents. *American journal of Enology and Viticulture*, 16(3), 144-158. <https://doi.org/10.5344/ajev.1965.16.3.144>
- Snowdon, J. A., & Cliver, D. O. (1996). Microorganisms in honey. *International Journal of Food Microbiology*, 31(1–3), 1–26. [https://doi.org/10.1016/0168-1605\(96\)00970-1](https://doi.org/10.1016/0168-1605(96)00970-1)
- Wilkinson, J. M., & Cavanagh, H. M. (2005). Antibacterial activity of 13 honeys against *Escherichia coli* and *Pseudomonas aeruginosa*. *Journal of medicinal food*, 8(1), 100-103. <https://doi.org/10.1089/jmf.2005.8.100>