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ANTIBACTERIAL POTENTIAL AND MICROBIOLOGICAL QUALITY OF HONEY FROM SLOVAKIA

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ABSTRACT

In this study, the antibacterial activity of 10 honeys at three concentrations 50%, 25%, and 12.5% was tested against two G^- and two G^+ strains, also the microbiological quality of the honeys in terms of the representation of total count of bacteria, coliforms, yeasts, and filamentous fungi was evaluated, and micromycetes to the species level were identified. Antibacterial activity of the honeys was assayed using well diffusion method, determination of microbial groups by the pour plate method and diversity of mycobiota in honey according to macro- and micromorphological characteristics. Results showed the antibacterial effects of Slovak honey collected from the Spiš region, against bacterial strains *Escherichia coli*, *Pseudomonas fluorescens*, *Enterococcus faecalis*, and *Staphylococcus aureus*, which are among the most common bacteria responsible for nosocomial infections. We found that honeydew honey was very effective against *E. coli* and *S. aureus*, rapeseed honey against *P. fluorescens*, and mixed honey (no. 10) against *E. faecalis*. *Staphylococcus aureus* was the most susceptible bacteria tested for all honeys. The presence of both yeasts and molds was detected in 3 honey samples at concentrations ranging from $<4x10^1$ to $3.6x10^1$ CFU/g, while the total count of bacteria was detected in 9 samples at concentrations ranging from $2.3x10^1$ to $3.6x10^2$ CFU/g (in 1 sample, the occurrence of microorganisms was not recorded even at the lowest dilution of 10^{-1}). Coliforms were not isolated. A total of 3 strains belonging to the *Aspergillus* section *Nigri* were identified. The microbiological analyses of the samples indicates that the honeys were produced, processed and stored in accordance with the rules of good hygiene practice.

Keywords: honey, antibacterial effect, agar-well diffusion, bacteria, filamentous fungi, yeast

INTRODUCTION

Honey is produced by bees from the nectar of various plants or from honeydew (a sticky, sugar-rich secretion of aphids) through the enzymatic processing of these raw materials and the evaporation of water, resulting in a final water content of no more than 20% for most types of honey. Historically, honey was humanity's first sweetener. In addition to its appealing organoleptic properties, ancient people quickly recognized the numerous health benefits of regular honey consumption, such as the alleviation of gastrointestinal disorders and cardiovascular issues related to peptide defensin-1 (**Bucekova *et al.*, 2020**) or other minor components (**Kuropatnicki *et al.*, 2018; Samarghandian *et al.*, 2017**). However, the most significant use of honey was its topical application in treating a wide range of injuries (**Angioi *et al.*, 2021**). Consequently, honey became a crucial remedy in traditional and folk medicine. It is important to note that the components responsible for honey's high therapeutic and prophylactic potential are sensitive to thermal processing, prolonged storage, and heavily depend on the botanical source of the nectar. The chemical composition of honey varies significantly based on its botanical and geographical origin, as well as other factors like climatic conditions and beekeeping practices (**da Silva *et al.*, 2016**). Numerous research studies have highlighted honey's biological properties, including its antioxidant, antimicrobial, antidiabetic, and anticancer effects (**Rao *et al.*, 2016**). Among the various health benefits of honey, its antimicrobial activity has traditionally been attributed to its high osmolarity and acidity (**Bose, 1982**), low water activity, and the disruption of bacterial cell membranes due to antibacterial hydrogen peroxide and phenolic compounds (**Russell *et al.*, 1990**). Enzymes from bee saliva, such as glucose oxidase, and peptides like defensin-1, are crucial for honey's antimicrobial properties and its microbial stability (**Bucekova *et al.*, 2019**). Bee defensin-1, a common yet variable antibacterial component in honey (**Bachanová *et al.*, 2002; Shen *et al.*, 2012**), is particularly effective against Gram-positive bacteria. Few microorganisms can survive or thrive in honey. Those present usually originate from primary contamination sources, such as the honeybee's digestive tract, natural environmental sources like nectar, pollen, propolis, air, flowers, and the hive environment. Secondary sources include contamination during postharvest processing from plants and equipment. While pathogenic bacteria capable of forming spores can persist in honey, they cannot reproduce or form vegetative cells. Filamentous fungi and yeasts can maintain their vegetative forms (**Silva *et al.*, 2017**). Fungal growth, which can lead to mycotoxin production, commonly involves fungi like *Aspergillus* spp. and *Penicillium* spp. (**Foley *et al.*, 2014; Naseer *et al.*, 2015; Sinacori *et al.*, 2014**). However, the presence of fungi does not necessarily indicate mycotoxin presence, as the conditions for fungal growth differ from those for mycotoxin production (**Barkai-Golan and Paster, 2008**). Regarding yeasts, **Sinacori *et al.* (2014)** identified species such as *Debaryomyces hansenii*, *Zygosaccharomyces rouxii*, *Zygosaccharomyces mellis*, *Aureobasidium pullulans*, and *Cryptococcus uzbekistanensis* in honey. Of these, only *Cryptococcus* species have been associated with human pathogenicity. The objective of our study was to investigate the *in vitro* antibacterial effect of honey obtained from the Spiš region of Slovakia against some G^- bacteria (*Escherichia coli*, *Pseudomonas fluorescens*) and G^+ bacteria (*Enterococcus faecalis*, *Staphylococcus aureus*), to investigate the microbiological quality of honey, and their mycological diversity.

MATERIAL AND METHODS

Honey samples

The study was carried out on 10 honey samples obtained from eight local beekeepers in the Spiš region of Slovakia (Tab 1). Each sample was collected in sterile glass container and stored at room temperature in the dark until testing. Samples were labelled according to location and honey type. They were collected in the summer of 2021 (from June to August).

Table 1 Origin and type of honey samples

Location	Type of honey
1. Batizovce	mixed
2. Batizovce	mixed
3. Betlanovce	polyfloral + willow
4. Hranovnica	rapeseed
5. Hranovnica	buckwheat + milk thistle
6. Kežmarok	mixed
7. Levočská dolina	honeydew
8. Slovenský raj	mixed
9. Slovenský raj	mixed
10. Spišský Štiavnik	mixed

Bacterial strains

Four species of bacteria were used to determine the antimicrobial activity. *Enterococcus faecalis* (CCM 1875) and *Staphylococcus aureus* (CCM 299) were used as representatives of Gram-positive bacteria, and *Escherichia coli* (CCM 4225) and *Pseudomonas fluorescens* (CCM 1969) as Gram-negative bacteria. All cultures of microorganisms were obtained from the Czech collection of microorganisms of Masaryk University in Brno.

Well diffusion method

The antimicrobial activity of honey was tested using the well diffusion method. Bacterial inoculum was cultured for 24 hours in Mueller Hinton Broth (MHB, Oxoid, Basingstoke, UK) at 37°C. A total of 100 µL of the inoculum at a concentration of 0.5 McFarland Units (MFU) was applied to Petri dishes containing Mueller Hinton Agar (MHA, Oxoid, Basingstoke, UK). Honey was serially diluted with sterile distilled water to final concentrations of 50%, 25%, and 12.5%. Using a sterile 8 mm diameter cork borer, wells were created in the agar medium, and each honey dilution was added into the wells. Erythromycin, a standard antibiotic (Oxoid, Basingstoke, UK), was used as a control. The plates were incubated at 37°C for 24 hours to allow diffusion. After incubation, inhibition zones were measured from the edge of the well to the border of bacterial growth at three points. All assays were performed in triplicate.

Determination of microbial groups

The cultivation conditions of microorganisms are presented in table 2. Total count of bacteria, coliforms, yeasts, and filamentous microscopic fungi in honey samples were determined by the pour plate method. Ten grams of each honey sample were homogenized with 90 mL of physiological solution. The samples were diluted to 10^{-1}. Further dilution was made using sterile peptone water. From appropriate serial dilutions, a 0.1 mL aliquot was plated on various types of media for microbial counts. We followed the valid Slovak Technical Standards (STN). Analyses were performed in duplicate.

Table 2 Microbiological analyses of microbial groups in honey samples by pour plate method

Microbial groups	Medium	Dilution	Length of cultivation	Temperature of cultivation (°C)	Method
TCB	PCA	10^{-1}, 10^{-2}	72±3 h	30±1	STN EN ISO 4833
CB	VRBA	10^{-1}, 10^{-2}	24±2 h	37±1	STN EN ISO 4832
Yeasts	DG18	10^{-1}, 10^{-2}	5 days	25±1	STN EN ISO 21527-2
FF	DG18	10^{-1}, 10^{-2}	7 days	25±1	STN EN ISO 21527-2

Legend: TCB – Total count of bacteria, CB – Coliform bacteria, FF – Filamentous fungi, PCA - Plate count agar, VRBA - Violet red bile agar, DG18 - Dichloran-glycerol agar

Identification of filamentous fungi

Taxonomic identification of all isolates was conducted through macroscopic and microscopic observation, following the guidelines of **Pitt and Hocking (2009)**. *Aspergillus* strains were incubated on CYA (Czapek Yeast Agar) (**Samson *et al.*, 2010**), MEA (Malt Extract Agar) (**Samson *et al.*, 2010**), and CY20S (Czapek Yeast Extract Agar with 20% Sucrose) (**Pitt and Hocking, 2009**). After seven days of incubation at 25 ± 1°C in darkness, the macroscopic and microscopic characteristics were observed according to relevant mycological literature: **Klich** (**2002**) and **Pitt and Hocking** (**2009**).

RESULTS AND DISCUSSION

Results of antibacterial effect of honey

The antibacterial effect of 10 different honey samples on 4 different bacteria was assessed by the agar well diffusion method. Results and the mean ± SD are presented in table 3 and 4. According to table 3, all honeys were found to exhibit inhibitory effects against Gram-negative bacteria *Escherichia coli* and *Pseudomonas fluorescens* at concentrations of 50%, 25%, and 12.5%, respectively. *E. coli* was chosen to represent species which might became clinically important and responsible for a number of diseases (**Khan *et al.*, 2015**). *Pseudomonas fluorescens* can cause bacteremia in humans, with most reported cases linked to the transfusion of contaminated blood products or the use of contaminated equipment associated with intravenous infusions (**Scales *et al.*, 2014**). These bacteria can also cause nosocomial infections, also known as healthcare-associated infections.

In our study, *Pseudomonas fluorescens* was found to be a more sensitive bacteria than *E. coli*. The antibacterial activity of 50% concentration of honey samples against *E. coli* was found in samples with zones ranging from 23.5 mm (no. 4) to 27 mm. The best antibacterial activity was found in honey sample 7, which was a honeydew honey. Similarly, the best antibacterial activity was observed in the same honey at 25% concentration with an inhibition zone of 23.18 mm. Honey at a concentration of 12.5% also showed antibacterial effect ranging from 10.2 mm (no. 1) to 17 mm. The best antibacterial activity was observed in honey sample 3, which was polyfloral honey with willow.

The strongest antibacterial activity of the honeys against Gram-negative bacteria *P. fluorescens* was found at 50% concentration with an inhibition zone ranging from 34.68 mm (no. 6) to 39 mm. The best antibacterial activity was found in honey sample 2, which was a mixed honey. All samples were also effective at 25% honey concentration with inhibition zones between 25 mm (no. 10) and 36 mm. The best antibacterial activity was found in honey sample no. 4, which was a rapeseed honey. This honey also showed the highest activity against *P. fluorescens* at a concentration of 12.5% with an inhibition zone of 35.5 mm. **Kačániová *et al.* (2022)** investigated the antibacterial activity of 100 honey samples from various floral origins, sourced from Slovakian apiaries and the local market, using the agar well diffusion method. At a 50% concentration, the inhibition zones of the honey samples against *Pseudomonas aeruginosa* ranged from 0.00 to 17.67 mm, with the highest inhibition observed in linden honey obtained from a countryside beekeeper. Sixty-eight honey samples showed no inhibitory effect against this microorganism. At a 25% concentration, 12 honey samples exhibited antibacterial activity against *P. aeruginosa*, with inhibition zones ranging from 6.67 to 10.67 mm, again with linden honey showing the best results. At a 12.5% concentration, antibacterial effects were observed in 12 samples, with inhibition zones ranging from 4.33 to 8.33 mm, the best being in multifloral honey from a town beekeeper. Despite the fact that our samples also came from Slovakia, the honeys in our study showed higher inhibition zones and stronger antibacterial effects at all three concentrations. The antibacterial activity of honey likely depends on the pasture where the bees were raised, climatic conditions, and the natural composition of the floral nectar (**Abd-El Aal *et al.*, 2007**)., the osmolarity of the honey, the H_2O_2 content, the low pH, and the phenolic acid and flavonoid content (**Almasaudi, 2021**). In a study by **Wilkinson and Cavanagh (2005**), the antibacterial activity of 13 honeys against *Escherichia coli* and *Pseudomonas aeruginosa* was assessed using the well diffusion method. All the tested honeys at 10% and 5% concentrations inhibited the growth of both bacteria. No honey exhibited activity at a 1% concentration, and only 5 honeys were active against *E. coli* and 6 against *P. aeruginosa* at a 2.5% concentration. *E. coli* was more susceptible to inhibition by the honeys than *P. aeruginosa*. The results obtained in our study do not align with their findings, as *P. fluorescens* was found to be more sensitive to the honey samples than *E. coli.*

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

Samples	*Escherichia coli*			*Pseudomonas fluorescens*		
	Concentration of honey - diameter of inhibition zones (mm)					
	50%	25%	12.5%	50%	25%	12.5%
1. mixed	26.00±0.55	19.50±0.84	10.20±0.84	38.00±1.00	26.12±1.14	18.50±1.14
2. mixed	26.50±1.30	19.70±0.55	10.50±1.14	39.00±0.84	29.00±0.84	11.18±0.89
3. P + W	26.00±1.00	22.80±1.87	17.00±1.00	36.08±0.75	33.00±0.75	24.62±0.38
4. rape	23.50±0.55	21.00±0.84	15.50±0.55	37.90±0.23	36.00±0.79	35.50±1.12
5. B + T	24.60±1.58	19.00±1.00	11.50±1.30	35.00±0.55	29.70±0.55	19.80±1.30
6. mixed	25.00±2.24	21.80±1.30	15.20±0.86	34.68±0.43	32.40±2.07	20.00±1.58
7. honeydew	27.00±1.00	23.18±0.89	16.50±1.19	38.72±1.58	34.20±0.86	22.50±1.12
8. mixed	25.90±0.23	19.30±0.92	10.68±0.58	37.90±0.74	25.50±0.55	19.20±0.86
9. mixed	25.60±2.10	21.00±1.00	15.90±1.30	37.90±0.23	33.80±1.87	20.32±0.60
10. mixed	25.50±0.55	20.00±0.84	11.00±0.84	38.00±0.79	25.00±1.58	23.72±1.19

Legend: P + W - polyfloral + willow, B + T - buckwheat + thistle

Results showed antibacterial effects of Slovak honey also against Gram-positive bacteria *Enterococcus faecalis* and *Staphylococcus aureus* (Tab 4). Enterococci are often associated with urinary tract and wound infections. *S. aureus* is considered one of the most important pathogens, responsible for nosocomial infections (**Khan *et al.*, 2015**). The antibacterial activity of the honeys at 50% concentration against *E. faecalis* using the well diffusion method ranged from 22.6 mm (mixed honey, no. 6) to 25.78 mm. The highest value of inhibition was found in three samples, no. 7, 9, and 10. No. 7 was a honeydew honey, no. 9 and 10 were mixed honey. At 25 % honey concentration, the antibacterial activity ranged from 15.1 (mixed honey, no. 6) to 24.2 mm (mixed honey, no. 10). Honey with a concentration of 12,5 % showed the highest antibacterial effect again in sample 10. *Staphylococcus aureus* was the most susceptible organism tested for all honey types. The inhibition zones increased with the honey concentration. The antibacterial activity of the honeys at 50% concentration ranged from 37 mm (no. 10) to 49.18 mm (rapeseed honey no. 4). Among the honeys tested, honeydew honey (no. 7) showed interesting antibacterial activity against *S. aureus* at concentrations of 25% and 12.5%. The antibacterial effects of 25% honeys ranged from 32.17 mm (mixed honey, no. 1) to 41.32 mm and at 12.5% concentration from 15.50 mm (no. 1) to 33.64 mm. On the other hand, in a study by **Kačaniová *et al.* (2022)**, the antibacterial activity of honeys with 50% concentration against *S. aureus* was found in only 18% of honey samples, and against *E. faecalis* in 84%. This result implies the best activity of honey against *E. faecalis*. The results of the antibacterial activity of the honey samples at 50 % concentration against *S. aureus* and *E. faecalis* were the same and ranged from 0.00 to 12.33 mm (from beekeeper of multifloral origin collected in a town). The ranges of inhibition zones at 25% honey concentrations against *S. aureus* showed antibacterial activity from 2.38 to 13.00 mm in 13 honey samples, and against *E. faecalis* ranged from 10.00 to 11.00 mm in 4 honey samples (both in multifloral honey from a beekeeper from a town). The antibacterial activity of 12.5% honeys against *S. aureus* was detected in 9 samples, with the best activity in sample from a beekeeper of multifloral origin from a town. The antibacterial effect of 12.5% of the honeys against *E. faecalis* was found in 4 samples and ranged from 5.67 to 8.33 mm (the best in acacia honey from a beekeeper from a town). Our honey samples showed larger inhibition zones against both *E. faecalis* and *S. aureus* at all concentrations. In our study, we found that honeydew honey (no. 7) was very effective against *E. coli* and *S. aureus*, rapeseed honey (no. 4) against *P. fluorescens*, and mixed honey (no. 10) against *E. faecalis*.

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

Samples	*Enterococcus faecalis*			*Staphylococcus aureus*		
	Concentration of honey - diameter of inhibition zones (mm)					
	50%	25%	12.5%	50%	25%	12.5%
1. mixed	24.11±0.92	19.62±0.38	11.00±1.00	45.00±0.84	32.17±0.27	15.50±0.55
2. mixed	24.74±0.98	20.50±0.55	11.64±2.01	47.78±0.26	35.30±0.92	15.70±0.39
3. P + W	24.20±0.84	18.60±1.82	15.00±0.84	43.68±1.22	40.00±1.58	30.38±1.15
4. rapeseed	24.50±0.55	23.00±0.84	15.40±0.55	49.18±0.58	39.10±0.74	31.20±1.30
5. B + T	24.60±1.82	20.00±0.82	15.20±0.81	44.18±0.92	40.50±0.55	33.64±2.14
6. mixed	22.60±0.55	15.10±0.27	12.60±1.14	41.30±1.04	34.00±0.82	18.48±0.88
7. honeydew	25.78±0.46	22.06±0.51	16.00±1.00	47.96±0.30	41.32±0.60	33.18±0.46
8. mixed	24.18±0.92	20.78±0.89	12.62±0.38	38.60±0.55	33.62±0.38	20.00±0.84
9. mixed	25.60±1.82	22.00±1.00	15.50±0.55	45.20±0.84	37.40±0.55	30.98±1.15
10. mixed	25.50±0.55	24.20±0.45	20.00±0.84	37.00±0.84	34.20±0.81	32.52±0.48

Legend: P + W - polyfloral + willow, B + T - buckwheat + thistle

Eleven honeys sourced from Danish flora were assessed for their antimicrobial effects using the agar-well diffusion method (**Matzen *et al.*, 2018**). Some of these honeys exhibited bioactive effects comparable to or greater than those of Manuka (*L. scoparium*) honey, particularly in inhibiting the growth of Gram-negative bacteria such as *P. aeruginosa* and *E. coli*. Gram-positive strains such as *S. aureus* and *S. epidermidis* were the most susceptible to honey, while Gram-negative microbes showed lower sensitivity to all honey samples, including Manuka. This difference in susceptibility between Gram-positive and Gram-negative bacteria may be attributed to differences in cell wall composition. Gram-positive bacteria lack an outer membrane protecting the peptidoglycan layer, making them more vulnerable to antimicrobial agents (**Madigan *et al.*, 2015**). **Sagdic *et al.* (2013)** evaluated the antibacterial activity of 35 multifloral Turkish honeys against 12 bacteria using the agar diffusion method. They found that concentrations of 5%, 10%, and 25% had no inhibitory effect on 14 tested microorganisms, but the highest antimicrobial activity was observed at a 75% concentration against *Escherichia coli* O157:H7, *Salmonella Typhimurium*, *Staphylococcus aureus*, *Listeria monocytogenes*, and *Proteus mirabilis*. However, our findings differ, as our honey samples exhibited antibacterial properties even at low concentrations. **Mercan *et al.* (2007)** investigated the antibacterial activity of 5 honey samples from Turkey at concentrations of 20%, 50%, and 70% using the agar-well diffusion method. They observed that inhibition zones increased with honey concentration, consistent with our study. Each pathogen tested exhibited varying sensitivity to the honey samples. **Fikselova *et al.* (2014)** studied the antimicrobial activity of honeydew honeys from Slovakia, Poland, and Serbia, finding that *Escherichia coli*, *Pseudomonas aeruginosa*, and *Staphylococcus epidermidis* were sensitive to varying degrees, with *Bacillus cereus* being the most resistant. In contrast, **Srećković *et al.* (2019)** reported that honeydew honey was more effective against Gram-positive bacteria than Gram-negative bacteria. Our study showed that honeydew samples exhibited varying antibacterial efficacy against the tested bacteria: *S. aureus* > *P. fluorescens* > *E. coli* > *E. faecalis*.

Results of microbiological testing

Microbiological analyses of 10 honey samples detected the presence of total count of bacteria as well as the presence of yeasts and filamentous fungi. Coliforms were not isolated in any of the samples (Tab 4). Of the total number of samples tested, the presence of both yeasts and filamentous fungi was detected in 3 samples at concentrations $<4x10^1$ to $3.6x10^1$ CFU/g, while the presence of total count of

bacteria was detected in 9 samples (1 to 9). A total of 3 strains belonging to the genus *Aspergillus* were identified. Species of *Aspergillus* section *Nigri* were detected in honey samples 1, 7, and 9. Although the main source of black aspergilli is soil, members of this section have been isolated from various other sources, including honey (**Samson *et al.*, 2004**).

Table 4 Microbial (CFU/g) properties of honey samples

Sample number	TCB	CB	Yeasts	FF
1. mixed	$3.2x10^1$	$<1x10^1$	$<4x10^1$	$<4x10^1$
2. mixed	$2.3x10^1$	$<1x10^1$	$<1x10^1$	$<1x10^1$
3. P + W	$2.7x10^1$	$<1x10^1$	$<4x10^1$	$<1x10^1$
4. rapeseed	$3.6x10^2$	$<1x10^1$	$<1x10^1$	$<1x10^1$
5. B + T	$1.2x10^2$	$<1x10^1$	$<1x10^1$	$<1x10^1$
6. mixed	$8.6x10^1$	$<1x10^1$	$<1x10^1$	$<1x10^1$
7. honeydew	$6.4x10^1$	$<1x10^1$	$<1x10^1$	$<4x10^1$
8. mixed	$9.5x10^1$	$<1x10^1$	$3.6x10^1$	$<1x10^1$
9. mixed	$1.3x10^2$	$<1x10^1$	$<1x10^1$	$<4x10^1$
10. mixed	$<1x10^1$	$<1x10^1$	$<1x10^1$	$<1x10^1$

Legend: TCB – Total count of bacteria, CB – Coliform bacteria, FF – Filamentous fungi, P + W - polyfloral + willow, B + T - buckwheat + thistle

Gradvol *et al.* (2015), in their study conducted in Croatia on 72 honey samples, obtained results similar to ours. They did not detect *Enterobacteriaceae* bacteria, found aerobic mesophilic bacteria within acceptable limits in all honey types, and only detected a low mean value of molds and yeasts. These microbiological findings correlate with a study conducted on 14 honey samples from Bosnia and Herzegovina (**Landeka *et al.*, 2021**). In the latter study, yeasts and molds were found in 35% of honey samples at concentrations ranging from 10 to 100 CFU/g, while aerobic mesophilic bacteria were detected in 15% of samples at concentrations exceeding 100 CFU/g and in 15% at concentrations of 10 to 100 CFU/g. *Enterobacteriaceae* were not isolated or counted**. Sinacori *et al.* (2014)**, in their investigation of 38 honey samples from Italy, reported the presence of yeasts and molds in three samples, none of which exceeded concentrations higher than 10^2 CFU/g, and *Enterobacteriaceae* were detected in two samples. The most frequently isolated species were *Bacillus amyloliquefaciens*, *Zygosaccharomyces mellis*, and *Aspergillus niger*. Multifloral honeys exhibited the highest microbial diversity. In a Polish study involving 245 honey samples, the total number of aerobic bacteria ranged from $1.0x10^1$ CFU/g to $7.5x10^4$ CFU/g. Yeasts and molds counts were low and only sporadically exceeded $1.0x10^2$ CFU/g (**Rozanska, 2011**). Our honey samples also showed low counts of yeasts and filamentous fungi, along with higher bacterial counts. In another study in Croatia in 2018, out of 40 honey samples analyzed, yeasts were present in 25% of the samples and molds in 27.5% of the samples, ranging from 10 to 100 CFU/g (**Kiš *et al.*, 2019**). Isolated molds exceeding the recommended quantitative criterion of 10 CFU/g were identified into seven genera: *Cladosporium*, *Penicillium*, *Alternaria*, *Mucor*, *Acremonium*, *Paeclomyces*, and *Pestalotiopsis*. While our tested samples showed sporadic isolation of black aspergilli (*Aspergillus* section *Nigri*), the mycological diversity was not as wide.

CONCLUSION

In our study, the antibacterial activity of three different concentrations of honey from the Spiš region of Slovakia was investigated. Our results showed that honey samples at 50% concentration had the strongest effect on bacterial growth, especially on *S. aureus*. Similarly, the lowest concentration of honey, 12.5%, had antibacterial effect on all Gram-negative and Gram-positive bacteria tested. The antimicrobial activity suggests that the honeys analysed may have an important role as natural antibacterial products that reduce the effects of bacterial infections and contribute to the improvement of food. Coliforms were not present and the detection of bacteria, yeasts and filamentous fungi was low and did not indicate any hygiene problems associated with the handling or processing of the honey.

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