



## Research Article



# Evaluation of the *in vitro* antibacterial activity of natural multifloral honeys against selected gram-positive and gram-negative bacterial strains

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The increasing emergence of antibiotic-resistant bacteria has raised concerns about the effectiveness of conventional antimicrobial therapies, prompting the search for alternative treatments. Honey, especially multifloral honey, has gained attention for its potential antibacterial properties. The aim of this study was to evaluate the *in vitro* antibacterial activity of different natural multifloral honeys against selected Gram-positive and Gram-negative bacterial strains. The antibacterial activity was evaluated using the Kirby-Bauer disc diffusion method on six bacterial strains: Gram-positive strains such as *Staphylococcus aureus* subsp. *aureus* Rosenbach ATCC®25923™, *Enterococcus faecalis* (Andrewes and Horder) Schleifer and Kilpper-Balz ATCC®29212™, vancomycin-susceptible *Enterococcus faecalis* (Andrewes and Horder) Schleifer and Kilpper-Balz ATCC®51299™, and Gram-negative strains such as *Pseudomonas aeruginosa* (Schroeter) Migula ATCC®27853™, *Escherichia coli* (Migula) Castellani and Chalmers ATCC®25922™ and *Escherichia coli* (Migula) Castellani and Chalmers ATCC®35218™. Honey samples were obtained from different Polish and Hungarian apiaries. The results showed that while the honeys showed varying degrees of antibacterial activity, some strains showed resistance, particularly *Pseudomonas aeruginosa* and *Staphylococcus aureus*. However, all the honeys tested showed significant inhibitory effects against *Escherichia coli* and *Enterococcus faecalis*, with significant increases in the zone of inhibition compared to the control groups. These results suggest that natural multifloral honeys may have promising antibacterial properties, especially against Gram-negative bacteria, and could serve as potential adjuncts to conventional antimicrobial therapies. Further research is needed to explore the specific compounds responsible for this antibacterial activity and its clinical implications.

**Keywords:** antibacterial activity, multifloral honey, *in vitro*, gram-positive bacteria, gram-negative bacteria, antimicrobial resistance, Kirby-Bauer disc diffusion technique

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## Introduction

Honey has been recognised for its medicinal properties since ancient times and is valued for its natural antibacterial, anti-inflammatory and antioxidant effects (Samarghandian et al., 2017). Interest in honey as a therapeutic agent has increased in recent years, particularly due to the rise of antibiotic resistance, which is considered a major global health challenge (Mandal and Mandal, 2011; Nolan et al., 2019). Natural honey, especially multifloral varieties, contains a complex composition of bioactive compounds such as phenolic acids, flavonoids, hydrogen peroxide and antimicrobial peptides such as defensin-1, all of which contribute to its antimicrobial potential (Combarros-Fuertes et al., 2020; Luca et al., 2024). Despite the known health benefits, there is a need for more comprehensive studies on the efficacy of different types of natural honey against specific bacterial pathogens (Almasaudi, 2021).

Natural multifloral honey is obtained from the nectar of different plant species, resulting in a unique composition that can vary according to geographical location, floral source and environmental conditions. These factors influence the antimicrobial properties of honey, making multifloral honey an interesting subject for antibacterial studies (Laallam et al., 2015; Ben Amor et al., 2022; Vijan et al., 2023). Honey from different regions is known to have different antimicrobial profiles due to differences in floral sources, which can affect the concentration and activity of their bioactive compounds (Alzahrani et al., 2012; Elbanna et al., 2014). Therefore, understanding the antimicrobial efficacy of multifloral honey from different geographical regions, such as Poland and Hungary, can provide valuable insights into its potential as a natural antibacterial agent.

The mechanisms by which honey exerts its antibacterial effects are diverse (Combarros-Fuertes et al., 2020). Honey's low water content and high sugar concentration create a hyperosmotic environment that inhibits microbial growth by dehydrating bacterial cells (Olaitan et al., 2007). In addition, the acidity of honey (typically a pH of 3.2 to 4.5) provides an unfavourable environment for bacterial survival (Mandal and Mandal, 2011). The enzymatic production of hydrogen peroxide and the presence of non-peroxide antibacterial agents also contribute to honey's antimicrobial activity (Almasaudi, 2021). These properties make honey effective against a wide range of bacteria, including both gram-positive and gram-negative strains, which have different structural characteristics that influence their susceptibility to

antibacterial agents (Almasaudi et al., 2017; Almasaudi, 2021).

Previous studies have demonstrated the efficacy of honey against several pathogens, including *Staphylococcus aureus*, *Escherichia coli* and *Pseudomonas aeruginosa*, which are common causes of infection (Tkaczenko et al., 2023, 2024). *S. aureus*, a gram-positive bacterium, is often associated with skin infections, respiratory infections and food poisoning (Tong et al., 2015), while *E. coli*, a gram-negative bacterium, can cause gastrointestinal and urinary tract infections (Zhou et al., 2023). *P. aeruginosa*, also a Gram-negative bacterium, is known for its multidrug resistance and is a common pathogen in hospital-acquired infections (Pachori et al., 2019). The ability of honey to inhibit these bacterial strains highlights its potential as an alternative or complementary antibacterial treatment, particularly in wound care and infection prevention.

However, the antibacterial efficacy of honey can vary widely depending on the bacterial strain, type of honey and geographical origin (Albaridi, 2019; Almasaudi, 2021). Studies comparing the *in vitro* antibacterial activity of honeys from different regions are limited and there is a need for standardised assessments of their efficacy. The aim of this study was to evaluate the *in vitro* antibacterial activity of natural multifloral honeys from different Polish and Hungarian producers against selected gram-positive and gram-negative bacterial strains, including *Staphylococcus aureus*, *Enterococcus faecalis*, *Escherichia coli* and *Pseudomonas aeruginosa*. By investigating the inhibition zones and antibacterial potency of these honey samples, we aim to contribute to the growing body of knowledge on honey's potential as a natural antibacterial agent and to identify those varieties that may hold the most promise for clinical and pharmaceutical applications.

## Materials and methodology

**Natural multifloral honey.** The various natural multifloral honeys from Polish producers such as the "Pszczółka" apiary (Ustka, Poland; 54°34'43"N 16°52'09"E), the "Sądecki Bartnik" apiary (Stróże, Poland; 49°39'21"N 20°58'22"E), Fulmer GmbH Magyarországi (Dunavarsány, Hungary; 47°17'N 19°04'E), "Karolczak Cezary" Beekeeping Farm (Sławno, Poland; 54°21'44"N 16°40'49"E) and "Zaczarowany Ogród" Beekeeping Farm (Złocieniec, Poland; 53°31'45"N 16°00'43"E) were used in the current study. Samples were stored in resealable

bottles at 5°C in the dark, but allowed to reach room temperature before analysis. Nutritional values of multifloral honey: Energy value – 300-320 kcal, carbohydrates – 78-83 g, including sugars – 82 g, proteins – 0.3 g, sodium – 4 mg, potassium – 52 mg, calcium – 6 mg, iron – 0.4 mg, magnesium – 2 mg, ascorbic acid – 0.5 mg.

**Determination of antibacterial activity of honey samples by disc diffusion method.** The antibacterial activity of honeys was evaluated *in vitro* using the Kirby-Bauer disc diffusion method (Bauer et al., 1966). In the present study, Gram-positive strains such as *Staphylococcus aureus* subsp. *aureus* Rosenbach ATCC®25923™, *Enterococcus faecalis* (Andrewes and Horder) Schleifer and Kilpper-Balz ATCC®29212™, vancomycin-susceptible *Enterococcus faecalis* (Andrewes and Horder) Schleifer and Kilpper-Balz ATCC®51299™, and Gram-negative strains including *Pseudomonas aeruginosa* (Schroeter) Migula ATCC®27853™, *Escherichia coli* (Migula) Castellani and Chalmers ATCC®25922™ and *Escherichia coli* (Migula) Castellani and Chalmers ATCC®35218™ were used.

The bacterial strains were inoculated onto Mueller-Hinton (MH) agar dishes and sterile filter paper discs impregnated with multifloral honey samples were placed on each culture dish. The dishes containing the bacterial isolates and multifloral honey samples were incubated at 37°C for 24 hours. After incubation, the zones of inhibition produced by the antibacterial action of the honeys were observed and measured in millimetres using a calliper. A control plate containing 96% ethanol was included in each experiment for comparison. Eight replicates (n = 8) were performed for each bacterial strain. Photographs were also taken to document the results.

A clear zone of inhibition around each honey-impregnated disc indicated the susceptibility of the bacterial strains to the multifloral honeys, using the diameter of this zone as an indicator of bacterial susceptibility. Bacterial responses were categorised on the basis of zone diameter: susceptible (S)  $\geq 15$  mm, intermediate (I) 10-15 mm, and resistant (R)  $\leq 10$  mm, following the criteria established by Okoth et al. (2013).

**Statistical analysis.** The diameters of the inhibition zones were measured, averaged and expressed as the mean  $\pm$  standard error of the mean (S.E.M.). Statistical analysis of the data was performed to evaluate the

antibacterial activity of each honey sample. Each honey variety was treated as an independent data set and statistical calculations were performed separately for each. The data were analysed using one-way analysis of variance (ANOVA) with Statistica v. 13.3 software (TIBCO Software Inc., USA), following the methodology described by Zar (1999).

## Results and discussion

Figures 1 and 2 present the results showing the average diameters of inhibition zones around Gram-positive and Gram-negative bacterial growth, induced by various natural multifloral honeys produced by Polish and Hungarian manufacturers.

The results of our study showed that the *S. aureus* subsp. *aureus* Rosenbach ATCC®25923™ strain was resistant to several natural multifloral honeys produced by Polish and Hungarian producers. When different honeys were applied to this strain, we observed a statistically non-significant increase in the inhibition zone: 7.4% (p > 0.05) for honey from the "Zaczarowany Ogród" apiary (Złocieniec, Poland), 15.8% (p > 0.05) for honey from the "Karolczak Cezary" apiary (Sławno, Poland), and 1.1% (p > 0.05) for honey from both Fulmer Ltd. Magyarországi (Dunavarsány, Hungary) and "Sądecki Bartnik" apiary (Stróże, Poland) compared to the control samples (9.54  $\pm$  0.85 mm) (Fig. 1).

The *E. faecalis* (Andrewes and Horder) Schleifer and Kilpper-Balz ATCC®51299™ strain also showed resistance to the various natural multifloral honeys tested. Compared to the control (32.82  $\pm$  2.56 mm), all honey samples led to a reduction in the inhibition zone: 27.53  $\pm$  1.89 mm for honey from the "Pszczółka" Apiary (Ustka), 31.44  $\pm$  2.45 mm for honey from the "Sądecki Bartnik" apiary (Stróże, Poland), 28.79  $\pm$  1.87 mm for honey from Fulmer Ltd. Magyarországi (Dunavarsány, Hungary), 32.43  $\pm$  2.12 mm for honey from the "Karolczak Cezary" apiary (Sławno, Poland), and 26.78  $\pm$  2.56 mm for honey from the "Zaczarowany Ogród" apiary (Złocieniec, Poland). The respective percentage decreases were 16.2% (p > 0.05), 4.3% (p > 0.05), 12.2% (p > 0.05), 1.2% (p > 0.05), and 18.3% (p > 0.05) (Fig. 1).

Conversely, the *E. faecalis* (Andrewes and Horder) Schleifer and Kilpper-Balz ATCC®29212™ strain demonstrated susceptibility to some of the honey samples tested. All honey samples increased the inhibition zone compared to the control (11.51  $\pm$  1.12 mm): 22.09  $\pm$  1.62 mm for honey from the "Pszczółka" Apiary (Ustka), 24.82  $\pm$  1.48 mm for honey from the

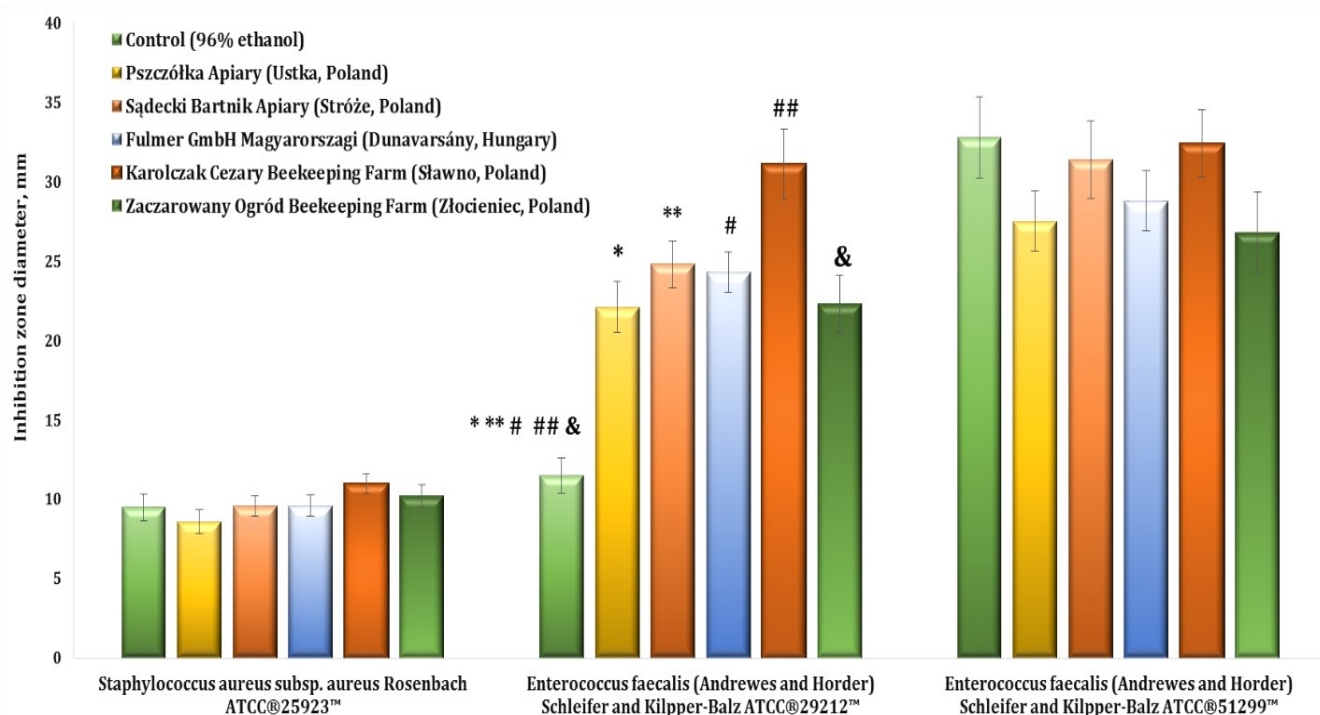
"Sądecki Bartnik" apiary (Stróże, Poland),  $24.28 \pm 1.33$  mm for honey from Fulmer Ltd. Magyarország (Dunavarsány, Hungary),  $31.07 \pm 2.17$  mm for honey from the "Karolczak Cezary" apiary (Sławno, Poland), and  $22.34 \pm 1.79$  mm for honey from the "Zaczarowany Ogród" apiary (Złocieniec, Poland). The respective percentage increases were 92.2% ( $p < 0.05$ ), 115.7% ( $p < 0.05$ ), 111.3% ( $p < 0.05$ ), 107.3% ( $p < 0.05$ ), and 93.9% ( $p < 0.05$ ) (Fig. 1).

Figure 2 summarises the results showing the average diameters of inhibition zones around the growth of gram-negative strains induced by different natural multifloral honeys produced by Polish and Hungarian manufacturers.

A similar trend was observed in the increase in the diameter of the inhibition zone following *in vitro* application of various natural multifloral honeys against the *E. coli* (Migula) Castellani and Chalmers ATCC®35218™ strains. The zone of growth inhibition increased significantly from the control ( $10.62 \pm 1.10$  mm) to  $22.14 \pm 2.21$  mm for honey from "Pszczółka" Apiary (Ustka),  $25.10 \pm 1.82$  mm for honey from

"Sądecki Bartnik" Apiary (Stróże, Poland),  $23.12 \pm 2.14$  mm for honey from Fulmer Ltd. Magyarország (Dunavarsány, Hungary),  $22.79 \pm 1.89$  mm for honey from the "Karolczak Cezary" apiary (Sławno, Poland) and  $29.78 \pm 1.56$  mm for honey from the "Zaczarowany Ogród" apiary (Złocieniec, Poland). The respective percentage increases were 108.5% ( $p < 0.05$ ), 136.8% ( $p < 0.05$ ), 117.9% ( $p < 0.05$ ), 115.1% ( $p < 0.05$ ) and 181.1% ( $p < 0.05$ ) (Fig. 2).

Honey samples from the "Pszczółka" (Ustka) and "Zaczarowany Ogród" (Złocieniec, Poland) apiaries showed greater efficacy against the *E. coli* (Migula) Castellani and Chalmers ATCC®25922™ strains. In addition, we observed a statistically non-significant increase in the growth inhibition zone from a control value of ( $7.10 \pm 0.56$  mm) to ( $7.81 \pm 0.87$  mm) for honey from the "Karolczak Cezary" apiary (Sławno, Poland) and ( $7.23 \pm 0.695$  mm) for honey from the "Sądecki Bartnik" apiary (Stróże, Poland). The percentage increases observed were 21.1% ( $p < 0.05$ ), 26.8% ( $p < 0.05$ ), 9.9% ( $p > 0.05$ ) and 1.4% ( $p > 0.05$ ), respectively (Fig. 2).



**Figure 1** The mean inhibition zone diameters induced by different natural multifloral honeys produced by Polish and Hungarian manufacturers against Gram-positive strains such as *Staphylococcus aureus* subsp. *aureus* Rosenbach ATCC@25923™, *Enterococcus faecalis* (Andrewes and Horder) Schleifer and Kilpper-Balz ATCC@29212™, vancomycin-susceptible *Enterococcus faecalis* (Andrewes and Horder) Schleifer and Kilpper-Balz ATCC@51299™ ( $M \pm m$ ,  $n = 8$ ).

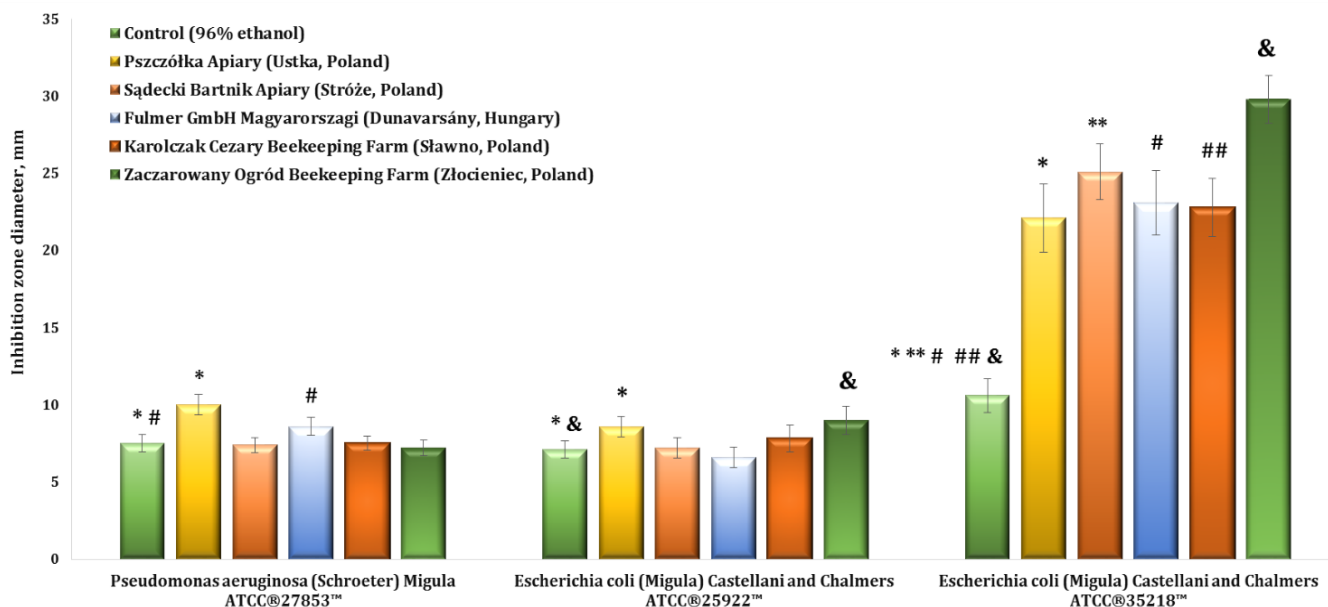
\* – changes were statistically significant when compared to 96% ethanol honey samples from the Pszczółka Apiary (Ustka, Poland);

\*\* – changes were statistically significant when compared to 96% ethanol honey samples from the "Sądecki Bartnik" apiary (Stróże, Poland);

# – changes were statistically significant when compared to 96% ethanol honey samples from the Fulmer Ltd. Magyarország (Dunavarsány, Hungary);

## – changes were statistically significant when compared to 96% ethanol honey samples from the "Karolczak Cezary" apiary (Sławno, Poland);

& – changes were statistically significant when compared to 96% ethanol honey samples from the "Zaczarowany Ogród" apiary (Złocieniec, Poland).



**Figure 2** The mean inhibition zone diameters induced by different natural multifloral honeys produced by Polish and Hungarian manufacturers against Gram-negative strains such as *Pseudomonas aeruginosa* (Schroeter) Migula ATCC®27853™, *Escherichia coli* (Migula) Castellani and Chalmers ATCC®25922™ and *Escherichia coli* (Migula) Castellani and Chalmers ATCC®35218™ (M ± m, n = 8).

\*- changes were statistically significant when compared to 96% ethanol honey samples from the Pszczółka Apiary (Ustka, Poland);

\*\* - changes were statistically significant when compared to 96% ethanol honey samples from the "Sąddecki Bartnik" apiary (Stróże, Poland);

# - changes were statistically significant when compared to 96% ethanol honey samples from the Fulmer Ltd. Magyarorszagi (Dunavarsány, Hungary);

## - changes were statistically significant when compared to 96% ethanol honey samples from the "Karolczak Cezary" apiary (Sławno, Poland);

& - changes were statistically significant when compared to 96% ethanol honey samples from the "Zaczarowany Ogród" apiary (Złocieniec, Poland).

The *P. aeruginosa* (Schroeter) Migula ATCC®27853™ strain showed resistance to several natural multifloral honeys. However, a statistically significant increase in the growth inhibition zone was observed following application of honey samples to dishes containing the *P. aeruginosa* ATCC®27853™ strain: 33.3% ( $p < 0.05$ ) for honey from "Pszczółka" Apiary (Ustka) and 14.7% ( $p < 0.05$ ) for honey from Fulmer Ltd. Magyarorszagi (Dunavarsány, Hungary) (Fig. 2).

The results of this study demonstrate the variable antimicrobial activity of natural multifloral honeys from different Polish and Hungarian sources against several bacterial strains, with notable differences in efficacy based on both honey origin and bacterial strain. The *S. aureus* subsp. *aureus* ATCC®25923™ strain showed resistance to the multifloral honeys tested, with only small, statistically non-significant increases in inhibition zone. This suggests that natural multifloral honeys may have limited efficacy against this strain of *S. aureus* (Fig. 1). While the *E. faecalis* ATCC®51299™ strain was resistant to all honeys with a decrease in inhibition zone compared to the control, the *E. faecalis* ATCC®29212™ strain was more susceptible. All honey samples tested significantly

increased the inhibition zone, indicating strain-specific responses within *E. faecalis* (Fig. 1). *E. coli* ATCC®35218™ and ATCC®25922™ strains were more susceptible to honey samples, with significant increases in inhibition zones observed (Fig. 2). In particular, honeys from the "Pszczółka" and "Zaczarowany Ogród" apiaries showed greater efficacy, highlighting the potential of these honeys against *E. coli* strains. The *P. aeruginosa* ATCC®27853™ strain generally showed resistance; however, honeys from the "Pszczółka" (Ustka) and Fulmer Ltd. (Hungary) apiaries showed significant increases in inhibition zones, suggesting that some honeys may still be effective against resistant *P. aeruginosa* strains under certain conditions (Fig. 2). Overall, these results suggest that while multifloral honeys have potential as antibacterial agents, their efficacy varies widely depending on bacterial strain and honey origin.

The results of this study provide a comprehensive evaluation of the antibacterial properties of various natural multifloral honeys against selected Gram-positive and Gram-negative bacterial strains. Our findings support the notion that multifloral honeys possess significant antibacterial activity, which varies

depending on both the botanical origin of the honey and the bacterial strain tested (Laallam et al., 2015; Cilia et al. 2020). In this study, several Gram-positive bacterial strains, including *Staphylococcus aureus* subsp. *aureus* Rosenbach ATCC®25923™, *Enterococcus faecalis* ATCC®29212™ and *Enterococcus faecalis* ATCC®51299™, were tested for susceptibility to multifloral honeys. Of the strains tested, *S. aureus* showed a limited response to the honey samples, with only small increases in inhibition zones observed. These results are consistent with studies by other researchers, such as Peng et al. (2022) and Wu et al. (2024), who reported that *S. aureus* may show some resistance to honey, possibly due to the strain's ability to form biofilms or other protective mechanisms. Despite this, some honeys from the "Pszczółka" and the "Karolczak Cezary" apiaries showed a trend towards higher antibacterial activity, suggesting that the specific floral composition or additional phytochemicals present in these honeys may enhance their efficacy against *S. aureus*.

Similarly, *E. faecalis* strains, including both vancomycin-resistant (ATCC®51299™) and susceptible (ATCC®29212™) variants, showed different susceptibility profiles. The increased resistance observed in the vancomycin-resistant strain (ATCC®51299™) could be attributed to its intrinsic resistance mechanisms, including the presence of efflux pumps or altered cell wall synthesis, which can reduce the effectiveness of natural antimicrobials such as those found in honey (Hollenbeck and Rice, 2012; Miller et al., 2014). Conversely, the more susceptible *E. faecalis* ATCC®29212™ strain showed a significant increase in inhibition zone diameter when exposed to several of the honeys tested. This observation is consistent with previous research indicating that the antimicrobial activity of honey is more pronounced against certain bacterial species, particularly those without acquired resistance mechanisms (Pimentel et al., 2013; Almasaudi, 2021; Luca et al., 2024).

Gram-negative bacteria such as *Pseudomonas aeruginosa* and *Escherichia coli* showed a more variable response to the multifloral honeys. In particular, *P. aeruginosa*, a common opportunistic pathogen, showed significant resistance to most honey samples, as evidenced by the smaller inhibition zones observed (Fig. 2). This is consistent with other studies reporting that *P. aeruginosa* tends to be less susceptible to the antibacterial effects of honey, possibly due to its outer membrane structure, which acts as a barrier to antimicrobial agents (Shenoy et al., 2012; Qin et al.,

2022). However, some honeys, especially those from the "Pszczółka" (Ustka) Apiary and Fulmer Ltd. Magyarországi (Dunavarsány, Hungary), showed statistically significant increases in the zone of inhibition, suggesting that specific components of these honeys, such as hydrogen peroxide, phenolic compounds or flavonoids, may contribute to overcoming bacterial resistance (Cianciosi et al., 2018; Combarros-Fuertes et al., 2020).

On the other hand, *E. coli* strains showed a more pronounced response to the multifloral honeys. A significant increase in the diameter of the inhibition zone was observed for most of the honey samples tested, especially from the "Pszczółka", "Sądecki Bartnik" and "Zaczarowany Ogród" apiaries. These results are in line with previous studies that demonstrated the broad-spectrum antibacterial properties of honey against gram-negative bacteria, attributed to its high osmolarity, acidity and the presence of bioactive compounds such as flavonoids and phenolic acids (Johnston et al., 2018; Al-Sayaghi et al., 2022). The significantly larger inhibition zones observed in our study, particularly for honey from the "Zaczarowany Ogród" apiary, may be related to specific types of polyphenols or other plant compounds that act synergistically to disrupt bacterial cell membranes or inhibit bacterial enzymes (Almasaudi, 2021; Romário-Silva et al., 2022).

The antibacterial activity of honey is multifactorial and several mechanisms of action have been proposed. Firstly, the high osmolarity of honey leads to dehydration of bacterial cells, which is particularly effective against Gram-positive bacteria due to the less complex structure of their cell walls (Mandal and Mandal, 2011; Almasaudi, 2021). In addition, the low pH of honey, generally between 3.2 and 4.5, further inhibits bacterial growth by altering enzyme activity and disrupting metabolic processes (Combarros-Fuertes et al., 2020; Almasaudi, 2021).

In addition, the presence of hydrogen peroxide in honey is well-documented as a potent antibacterial agent (Bizerra et al., 2012; Brudzynski, 2020). This compound is produced when honey comes into contact with moisture, catalysed by the enzyme glucose oxidase. Hydrogen peroxide exerts its antibacterial effects by generating reactive oxygen species (ROS) that damage bacterial proteins, lipids and DNA (Vatansever et al., 2013). However, some honeys, especially those with higher antioxidant content, may have antibacterial activity independent of hydrogen peroxide (Bizerra et al., 2012; Zhang et al., 2021).

Polyphenolic compounds such as flavonoids and phenolic acids are known to have antioxidant, anti-inflammatory and antimicrobial properties and may contribute to honey's ability to inhibit bacterial growth (Cianciosi et al., 2018; Becerril-Sánchez et al., 2021).

The variability in antibacterial activity observed between different honey samples in this study can be attributed to several factors, including botanical source, geographical origin and processing methods of the honeys. Differences in nectar composition, influenced by the types of plants available to bees, contribute to variations in the levels of bioactive compounds present in honey (Mandal and Mandal, 2011; Núñez-Gómez et al., 2024). In addition, the method of honey collection, storage and processing can influence its antibacterial activity. Raw, unprocessed honey tends to retain more of its natural antimicrobial properties than processed honey, which may lose some of its bioactive components through heating and filtration (Mandal and Mandal, 2011; Almasaudi, 2021).

Our results are consistent with previous studies that have demonstrated the antibacterial effects of honey against a variety of bacterial strains, including both gram-positive and gram-negative bacteria (Almasaudi, 2021; Wadi, 2022). The results are also consistent with the work of Voidarou et al. (2011) and Bucekova et al. (2019), who reported that honey from different regions showed different levels of antibacterial activity. However, there are some discrepancies between our study and others, especially regarding the antibacterial activity of honey against *P. aeruginosa* and *S. aureus* (Mandal and Mandal, 2011; Farkas et al., 2022). These differences could be due to variations in the types of honey tested, differences in the bacterial strains used or the methodologies employed (Mandal and Mandal, 2011; Almasaudi, 2021).

## Conclusions

In conclusion, our study shows that natural multifloral honeys have antibacterial activity with varying degrees of efficacy against different bacterial strains. While honeys showed more pronounced activity against *E. coli* and *E. faecalis* strains, the efficacy against *P. aeruginosa* and *S. aureus* was more limited. The antibacterial effects of honey are probably due to a combination of factors, including its osmolarity, acidity, hydrogen peroxide content and the presence of bioactive compounds. These findings support the potential of natural multifloral honeys as antimicrobial agents and highlight the need for further studies to

explore the mechanisms underlying their antibacterial properties. Furthermore, the variability in antibacterial activity among different honey samples highlights the importance of considering the botanical and geographical origin of honey when assessing its antimicrobial potential.

## Conflict of interest

The authors have no conflicts of interest to declare.

## Ethical statement

This article doesn't contain any studies that would require an ethical statement.

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