

**Research Article** 

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## *In vitro* Antibacterial Efficacy of Various Natural Rapeseed Honey Against Some Gram-positive and Gram-negative Bacterial Strains

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With the ongoing threat of antibiotic resistance and the limitations of conventional antimicrobial therapies, honey represents a valuable natural alternative with the potential to complement existing treatment strategies and reduce the spread of resistant bacterial strains. Known to be rich in bioactive compounds, rapeseed honey is a promising alternative to traditional antibiotics amid growing concerns about antibiotic resistance. In the current study, in vitro antimicrobial profiling was performed on different natural rapeseed honeys produced by Polish manufacturers, which showed inhibitory activity against Gram-positive strains such as Staphylococcus aureus subsp. aureus Rosenbach ATCC®25923™, Enterococcus faecalis (Andrewes and Horder) Schleifer and Kilpper-Balz ATCC®29212<sup>™</sup>, 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<sup>™</sup>. The antibacterial activity of rapeseed honeys was tested *in vitro* using the Kirby-Bauer disc diffusion technique. The results of our study showed that *S. aureus* subsp. aureus Rosenbach ATCC®25923™, P. aeruginosa (Schroeter) Migula ATCC®27853™ and E. faecalis (Andrewes and Horder) Schleifer and Kilpper-Balz ATCC®51299<sup>™</sup> strains were resistant to the different natural rapeseed honeys produced by Polish manufacturers. On the other hand, the *E. faecalis* (Andrewes and Horder) Schleifer and Kilpper-Balz ATCC®29212™ strain was susceptible to some of the rapeseed honey samples tested. Similar trends were observed in the increase in the diameter of the inhibition zone after in vitro application of different natural rapeseed honeys against E. coli

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Arciszewski 22b, 76-200 Słupsk, Poland halina.tkaczenko@upsl.edu.pl (Migula) Castellani and Chalmers ATCC®35218<sup>™</sup> strain, where we also observed a statistically significant increase in the zone of growth inhibition. Thus, the research highlights the potential of rapeseed honey as a valuable natural antimicrobial agent with broad-spectrum activity, offering a promising alternative to conventional antibiotics in the face of increasing antibiotic resistance.

**Keywords:** rapeseed honey, antibacterial activity, *Staphylococcus aureus, Escherichia coli, Pseudomonas aeruginosa, Enterococcus faecalis* strains, Kirby-Bauer disc diffusion technique

#### Introduction

Honey has been revered for its medicinal properties for centuries, with documented use dating back to ancient civilisations (Mandal and Mandal, 2011). Among its many purported benefits, its antimicrobial properties have been of particular interest (Romário-Silva et al., 2022; Hulea et al., 2022). With the rise of antibiotic resistance posing a significant global health threat, there has been a renewed focus on research into alternative antimicrobials, including natural products such as honey (Kwakman et al., 2010; Kwakman and Zaat, 2012; Morroni et al., 2018).

Rapeseed honey, obtained from the nectar of the plant *Brassica napus* L., is one such natural product that has attracted attention for its potential therapeutic properties (Na et al., 2024). Oilseed rape is widely grown in Central and Eastern Europe for the production of seed oil. Rising temperatures associated with climate change may pose significant challenges to *B. napus* production in different countries and regions of the world. In particular, areas that are currently climatically unsuitable for the crop may become suitable as temperatures rise. This could have a significant impact on *B. napus* cultivation due to its high sensitivity to thermal parameters (Borges et al., 2023).

*B. napus* is one of the most important spring sources of nectar and pollen for bees, producing large quantities of very pure unifloral honey (Kędzierska-Matysek et al., 2016). Rape honey granulates relatively quickly (often in crystallised form with very small crystals) due to its high glucose concentration, which averages 40.5%, a glucose/fructose ratio of about 1.1, and a reducing sugar content of about 80% (Kędzierska-Matysek et al., 2016). Rich in bioactive compounds such as phenolic acids, flavonoids, and enzymes, rapeseed honey has inherent antimicrobial properties that have been increasingly studied in recent years (Cianciosi et al., 2018; Almasaudi, 2021; Mackin et al., 2023).

*In vitro* studies play a crucial role in assessing the antibacterial efficacy of natural products such as rapeseed honey (Romário-Silva et al., 2022). These studies provide valuable insights into the ability of honey to inhibit the growth of pathogenic bacteria,

including both Gram-positive and Gram-negative strains. Understanding the spectrum of antibacterial activity of rapeseed honey against different bacterial species is essential for its potential application in clinical settings.

This study aims to analyse *in vitro* antibacterial efficacy of different natural rapeseed honey produced by Polish producers against a range of Gram-positive and Gramnegative bacterial strains. By synthesising existing research findings, we aim to elucidate the potential of rapeseed honey as a natural antimicrobial agent and contribute to ongoing efforts to combat antibiotic resistance.

#### Materials and methodology

#### Natural rapeseed honey

The various natural rapeseed honey from Polish producers such as the Pszczółka Apiary (Ustka, Poland; 54° 34' 43" N 16° 52' 09" E), the Mazurskie Miody Bogdan Piasecki Apiary (Tomaszkowo, Poland; 53° 43′ 4″ N 20° 24′ 37″ E) and "Nadbużańska Pasieka Wędrowna" (The Bug River Migratory Apiary, Lublin region, Poland; 51° 25′ 36″ N 23° 34′ 38″ E) were used in the current study. Honey from the Bug River Migratory Apiary is mainly produced in the Lublin region (Poland). A large part of it is in the vicinity of the Sobibór Landscape Park, which is covered by the Natura 2020 program. Samples were stored in resealable bottles at 5 °C in the dark, allowed to reach room temperature before analysis. Nutritional values of rapeseed honey: Energy value (300-340 kcal), carbohydrates (78-83 g), including sugars (70-80 g), proteins (0.2–0.6 g).

# Determination of antibacterial activity of honey samples by disc diffusion method

The antibacterial activity of rapeseed honey was tested *in vitro* using the Kirby-Bauer disc diffusion technique (Bauer et al., 1966). In the present study, Grampositive strains such as *Staphylococcus aureus* subsp. *aureus* Rosenbach ATCC®25923<sup>™</sup>, *Enterococcus faecalis* (Andrewes and Horder) Schleifer and Kilpper-Balz ATCC®29212<sup>™</sup>, vancomycin-susceptible

*Enterococcus faecalis* (Andrewes and Horder) Schleifer and Kilpper-Balz ATCC®51299<sup>™</sup>, and Gramnegative strains including *Pseudomonas aeruginosa* (Schroeter) Migula ATCC®27853<sup>™</sup>, *Escherichia coli* (Migula) Castellani and Chalmers ATCC®25922<sup>™</sup> and *Escherichia coli* (Migula) Castellani and Chalmers ATCC®35218<sup>™</sup> were used.

The strains were inoculated onto Mueller-Hinton (MH) agar dishes. Sterile filter paper discs impregnated with rapeseed honey samples were placed over each culture dish. Bacterial isolates with rapeseed honey samples were then incubated at 37 °C for 24 h. The Petri dishes were then observed for the zone of inhibition produced by the antibacterial activity of rapeseed honey. A control Petri dish impregnated with 96% ethanol was used in each experiment. At the end of the 24 hours, the inhibition zones formed were measured in millimetres using a vernier. Eight replicates were tested for each strain (n = 8). The Petri dishes were observed and photographed. The susceptibility of the test organisms to the rapeseed honey was indicated by a clear zone of inhibition around the discs containing the rapeseed honey, and the diameter of the clear zone was used as an indicator of susceptibility. The following zone diameter criteria were used to classify bacteria as susceptible or resistant to the tested phytochemicals: susceptible (S)  $\geq$ 15 mm, intermediate (I) = 10-15 mm, and resistant (R)  $\leq 10$  mm (Okoth et al., 2013; Tkachenko et al., 2023).

#### Statistical analysis

The diameters of the zones were determined and averaged. The statistical analysis of the data obtained was carried out using the mean ± standard error of the mean (S.E.M.). All variables were randomised according to the phytochemical activity of the rapeseed honey tested. All statistical calculations were performed on separate data from each variety. The data were analysed by one-way analysis of variance (ANOVA) using Statistica v. 13.3 software (TIBCO Software Inc., USA) (Zar, 1999).

#### **Results and discussion**

Figure 1 and 2 summarise the results obtained by the mean diameters of the inhibition zone around the growth of Gram-positive and Gram-negative strains induced by different natural rapeseed honeys produced by Polish manufacturers.

The results of our study showed that *S. aureus* subsp. *aureus* Rosenbach ATCC $@25923^{\text{m}}$  strain was resistant to the different natural rapeseed honey produced by

Polish manufacturers. After applying different natural rapeseed honey to *S. aureus* subsp. *aureus* Rosenbach ATCC®25923<sup>TM</sup> strain, we observed a statistically nonsignificant increase in the zone of growth inhibition by 21.1% (p >0.05) for rapeseed honey from Pszczółka Apiary (Ustka), by 1.1% (p >0.05), for rapeseed honey from "Mazurskie Miody" (Bogdan Piasecki Apiary, Tomaszkowo) and a statistically significant decrease of 31.6% (p <0.05) for rapeseed honey from "Bug River Migratory Apiary" (Lublin region) compared to the control samples (9.54 ±0.85 mm) (Figure 1).

The *E. faecalis* (Andrewes and Horder) Schleifer and Kilpper-Balz ATCC®51299<sup>TM</sup> strain was also resistant to the different natural rapeseed honey tested. All honey samples resulted in a reduction of the zone of growth inhibition from 32.82 ±2.56 mm as a control to 25.80 ±2.31 mm for rapeseed honey from Pszczółka Apiary (Ustka), 30.04 ±2.65 mm for rapeseed honey from "Mazurskie Miody" (Bogdan Piasecki Apiary, Tomaszkowo) and 31.5 ±2.56 mm for rapeseed honey from "Bug River Migratory Apiary" (Lublin region). The percentages of decrease were 21.4% (p <0.05), 8.5% (p >0.05) and 4% (p >0.05), respectively (Figure 1).

On the other hand, the *E. faecalis* (Andrewes and Horder) Schleifer and Kilpper-Balz ATCC®29212<sup>TM</sup> strain was susceptible to some of the rapeseed honey samples tested. All honey samples showed an increase in the zone of growth inhibition from 11.51 ±1.12 mm (as a control) to 23.64 ±1.22 mm for rapeseed honey from Pszczółka Apiary (Ustka), 24.60 ±1.63 mm for rapeseed honey from "Mazurskie Miody" (Bogdan Piasecki Apiary, Tomaszkowo) d to 24.0 ±1.55 mm for rapeseed honey from "Bug River Migratory Apiary" (Lublin region). The percentage increases were 105% (p <0.05), 113.7% (p <0.05), and 108.5% (p >0.05), respectively (Figure 1).

Figure 2 summarises the results obtained by the mean diameters of the inhibition zone around the growth of Gram-negative strains induced by different natural rapeseed honey produced by Polish manufacturers.

Similar trends were observed in the increase in the diameter of the inhibition zone after *in vitro* application of different natural rapeseed honeys against *E. coli* (Migula) Castellani and Chalmers ATCC®35218<sup>TM</sup> strain, where we also observed a statistically significant increase in the zone of growth inhibition from 10.62 ±1.10 mm (as a control) to 20.0 ±1.45 mm for rapeseed honey from Pszczółka Apiary (Ustka), 30.93 ±1.74 mm for rapeseed honey from "Mazurskie Miody" (Bogdan Piasecki Apiary, Tomaszkowo) to 22.25 ±1.95 mm for rapeseed honey from "Bug River

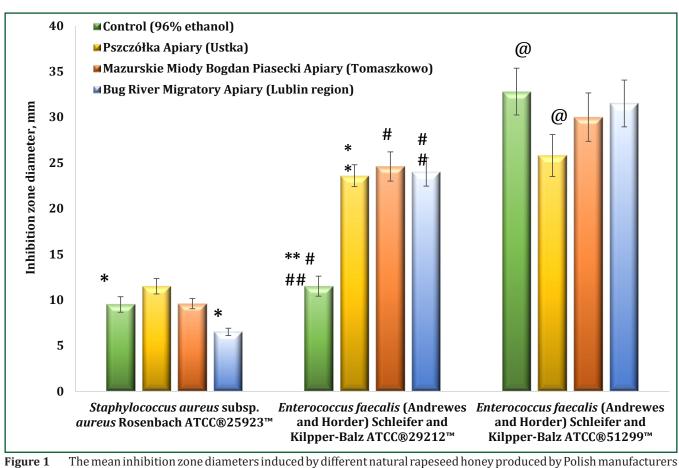


Figure 1 The mean inhibition zone diameters induced by different natural rapeseed honey produced by Polish manufacturers against Gram-positive strains such as *Staphylococcus aureus* subsp. *aureus* Rosenbach ATCC®25923<sup>™</sup>, *Enterococcus faecalis* (Andrewes and Horder) Schleifer and Kilpper-Balz ATCC®29212<sup>™</sup>, vancomycin-susceptible *Enterococcus faecalis* (Andrewes and Horder) Schleifer and Kilpper-Balz ATCC®51299<sup>™</sup> (M ±m, n = 8) \*- changes were statistically significant when compared to 96% ethanol using the *S. aureus* subsp. *aureus* Rosenbach ATCC®25923<sup>™</sup> strain; \*\*, #, ## – changes were statistically significant when compared to 96% ethanol using the *E. faecalis* (Andrewes and Horder) Schleifer and Kilpper-Balz ATCC®29212<sup>™</sup> strain; @ – changes were statistically significant when compared to 96% ethanol using the *E. faecalis* (Andrewes and Horder) Schleifer and Kilpper-Balz ATCC®29212<sup>™</sup> strain; @ – changes were statistically significant when compared to 96% ethanol using the *E. faecalis* (Andrewes and Horder) Schleifer and Kilpper-Balz ATCC®29212<sup>™</sup> strain; @ – changes were statistically significant when compared to 96% ethanol using the *E. faecalis* (Andrewes and Horder) Schleifer and Kilpper-Balz ATCC®29212<sup>™</sup> strain; @ – changes were statistically significant when compared to 96% ethanol using the *E. faecalis* (Andrewes and Horder) Schleifer and Kilpper-Balz ATCC®29212<sup>™</sup> strain; @ – changes were statistically significant when compared to 96% ethanol using the *E. faecalis* (Andrewes and Horder) Schleifer and Kilpper-Balz ATCC®51299<sup>™</sup> strain

Migratory Apiary" (Lublin region). The percentage increases were 88.7% (p <0.05), 173.7% (p <0.05) and 109.9% (p >0.05), respectively (Figure 2).

Honey samples such as "Bug River Migratory Apiary" (Lublin region) and "Mazurskie Miody" (Bogdan Piasecki Apiary, Tomaszkowo) were more effective against the *E. coli* (Migula) Castellani and tChalmers ATCC®35218<sup>TM</sup> strain than Pszczółka Apiary (Ustka). We also observed a statistically significant increase in the zone of growth inhibition from 7.10 ±0.56 mm as a control to 8.0 ±0.65 mm for rapeseed honey from Pszczółka Apiary (Ustka), 12.0 ±0.95 mm for rapeseed honey from "Mazurskie Miody" (Bogdan Piasecki Apiary, Tomaszkowo) and to 26.10 ±0.78 mm for rapeseed honey from "Bug River Migratory Apiary" (Lublin region). The percentage increases were 12.7% (p >0.05), 53.2% (p <0.05), and 267.6% (p <0.05), respectively (Figure 2).

*P. aeruginosa* (Schroeter) Migula ATCC®27853<sup>™</sup> strain was resistant to several natural rapeseed honeys. After application of honey samples to *Pseudomonas aeruginosa* (Schroeter) Migula ATCC®27853<sup>™</sup> strain, a statistically significant increase in the zone of growth inhibition was observed, i.e. by 33.3% (p <0.05) for rapeseed honey from "Pszczółka" Apiary (Ustka), by 34.7% (p <0.05) for rapeseed honey from "Bug River Migratory Apiary" (Lublin region). The rapeseed honey from "Mazurskie Miody" (Bogdan Piasecki Apiary, Tomaszkowo) increased the zone of growth inhibition by 9.3% (p >0.05), which was not statistically significant compared to the control (Figure 2).

The results of the *in vitro* studies reviewed in this article underscore the potential of natural rapeseed honey as an effective antibacterial agent against a variety of Gram-positive and Gram-negative bacterial strains. The results showed that rapeseed honey has a broad spectrum of antibacterial activity, inhibiting

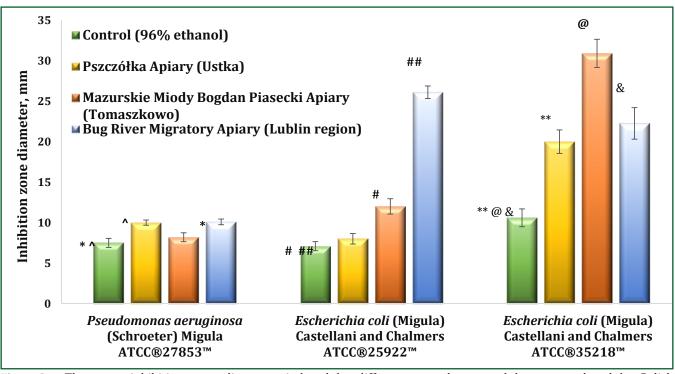


Figure 2 The mean inhibition zone diameters induced by different natural rapeseed honeys produced by Polish manufacturers against Gram-negative strains such as *Pseudomonas aeruginosa* (Schroeter) Migula ATCC®27853<sup>™</sup>, *Escherichia coli* (Migula) Castellani and Chalmers ATCC®25922<sup>™</sup>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

\*, ^ – changes were statistically significant when compared to 96% ethanol using the *P. aeruginosa* (Schroeter) Migula ATCC®27853<sup>™</sup> strain; #, ## – changes were statistically significant when compared to 96% ethanol using the *E. coli* (Migula) Castellani and Chalmers ATCC®25922<sup>™</sup> strain; @ – changes were statistically significant when compared to 96% ethanol using the *E. coli* (Migula) Castellani and Chalmers ATCC®35218<sup>™</sup> strain;

the growth of both Gram-positive and Gram-negative bacteria. Different rapeseed honey samples were more effective against Gram-positive strains such as *E. faecalis* (Andrewes and Horder) Schleifer and Kilpper-Balz ATCC®29212<sup>TM</sup>, vancomycin-susceptible *E. faecalis* (Andrewes and Horder) Schleifer and Kilpper-Balz ATCC®51299<sup>TM</sup> and Gram-negative *E. coli* (Migula) Castellani and Chalmers ATCC®35218<sup>TM</sup>. This broad activity suggests that rapeseed honey could potentially be used as an alternative or adjunctive therapy in the treatment of bacterial infections caused by these bacterial strains.

Although the exact mechanisms underlying the antimicrobial activity of rapeseed honey are not fully understood, it is likely to be multifactorial. The presence of various bioactive compounds, including phenolic acids and flavonoids, may contribute to its antimicrobial properties (Cianciosi et al., 2018; Al-Kafaween et al., 2023). The antibiotic effect of honey can be influenced by several factors, both intrinsic to the honey itself and external to the environment. Different types of honey are derived from the nectar of different plant species, each containing unique phytochemicals

and floral sources (Bouali et al., 2022; Al-Kafaween et al., 2023). The plant source can significantly affect the antimicrobial activity of honey due to the variation in the concentration and composition of bioactive compounds (Nolan et al., 2019; Almasaudi, 2021). It is important to note that the composition of rapeseed honey may vary depending on factors such as geographical location, botanical source, and processing methods. These variations may influence the antibacterial efficacy of the honey (Pauliuc et al., 2020; Becerril-Sánchez et al., 2021; Vîjan et al., 2023).

Environmental factors, soil composition, climate, and seasonal variations may influence the floral sources available to bees and thus the chemical composition of honey (Papp et al., 2022). Honey produced in different geographical regions may have different levels of antimicrobial activity due to differences in floral diversity and environmental conditions (Pita-Calvo and Vázquez 2018; Warui et al., 2019; Raweh et al., 2023). In addition, the chemical composition of honey, including its sugar content, pH, moisture content, and the presence of bioactive compounds such as phenolic acids, flavonoids, and enzymes, may influence its antimicrobial efficacy (Kunat-Budzyńska et al., 2023). The production of hydrogen peroxide  $(H_2O_2)$ , bee defensin-1, high osmolarity, and low pH seem to be important for the antibacterial activity of honey. Other phytochemicals, especially phenolic compounds, have been reported to be essential elements in the antibacterial activity of honey (Wang and Li, 2011; Almasaudi, 2021; Al-Kafaween et al., 2023). Honey processing methods such as filtration, heating, and pasteurisation can affect its antimicrobial properties by altering the concentration of bioactive compounds and enzymes. In addition, exposure to light, heat, and air during storage can degrade these compounds and reduce the antibiotic activity of honey over time (Mandal and Mandal, 2011; Chen et al., 2012).

The susceptibility of bacterial pathogens to honey varies depending on their species, strain, and inherent resistance mechanisms. Some bacterial strains may be more sensitive to the antibacterial compounds present in honey, while others may exhibit resistance or reduced susceptibility (Mohapatra et al., 2011; Ng et al., 2020). Gram-positive target strains were most sensitive to honey samples (Figure 1). In contrast, Gram-negative microbes were less susceptible to all honey samples, in line with previous observations (Kwakman et al., 2011; Mandal and Mandal, 2011). The difference in susceptibility to honey and other antibacterial agents between Gram-positive and Gram-negative microbes may be due to cell wall composition. Unlike Gramnegative bacteria, Gram-positive bacteria do not have an outer membrane to protect the peptidoglycan layer, making it easier for antimicrobial agents to penetrate and cause damage (Matzen et al., 2018). The acidic pH and high osmolarity of honey create unfavourable conditions for bacterial growth by inhibiting microbial metabolism and osmotically dehydrating bacterial cells. The pH and osmolarity of honey can vary depending on factors such as floral source, processing methods, and storage conditions, thereby influencing its antibiotic effect (Almasaudi, 2021; Yupanqui Mieles et al., 2022).

Studies have also suggested potential synergistic effects between rapeseed honey and conventional antibiotics, enhancing their antimicrobial activity against resistant bacterial strains. This highlights the possibility of using rapeseed honey as part of a combination therapy to effectively combat antibiotic resistance (Combarros-Fuertes et al., 2020; Almasaudi, 2021). For example, the use of tetracycline with Manuka honey resulted in better antimicrobial potential against *S. aureus* and *P. aeruginosa* than either treatment alone. This finding suggests that such a combination is a potential treatment strategy for wound healing (Jenkins and Cooper, 2012). In another study, the combination of rifampicin with sub-inhibitory concentrations of Medihoney reversed rifampicin resistance in clinical isolates of S. aureus, including methicillin-resistant S. aureus (MRSA) (Müller et al., 2013). Other data also support the use of honey with antibiotics to modulate antibiotic resistance. For example, Jenkins and Cooper (2012) reported that MRSA became susceptible to oxacillin after the application of subinhibitory concentrations of honey. In addition, synergistic effects against biofilms have been observed using combinations of honey and antibiotics (Almasaudi, 2021). This has been shown for the use of Manuka honey with vancomycin against S. aureus and the combination of Manuka honey with gentamicin against P. aeruginosa (Campeau and Patel, 2014). In addition, a synergism reported between Portuguese honey and phage therapy showed that 25% (w/v) honey caused synergism with phage and was also effective in eradicating E. coli biofilms compared to 50% (w/v) honey alone (Oliveira et al., 2017). Further research is warranted to investigate the specific mechanisms of action and to identify the key bioactive components responsible for the observed antibacterial effects (Almasaudi, 2021).

A result of the synergy between the active ingredients of honey and medicinal plants was also revealed. Miłek et al. (2023) evaluated the effect of the addition of selected fruits and herbs belonging to the category of "superfoods" on the bioactivity of a rapeseed honey matrix. Flavoured cream honey were prepared with nine types of different additives (2 and 4% of the content). The highest enrichment was obtained with the addition of powdered sea buckthorn leaves and black raspberry fruits. Honey with the addition of sea buckthorn leaves inhibited the growth of P. aeruginosa, *S. aureus*, and *K. pneumonia*, whereas honey with black raspberry and blackcurrant fruits only showed activity only against the latter two strains. More interestingly, honey supplemented with sea buckthorn leaves and black raspberry fruits inhibited S. aureus biofilm formation at sub-minimal inhibitory concentrations (sub-MICs), showing a dose-dependent anti-biofilm effect (Miłek et al., 2023).

The antioxidant profile and antimicrobial activity of four different types of monofloral honey (manuka, brassica, acacia, and linden) against some bacterial/fungal ATCC strains and some multi-drug resistant strains isolated from chronic otitis in dogs was investigated by Hulea and co-workers (2022). Brassica rapeseed honey was most effective against *Shigella flexneri* (ATCC 12022), *Pseudomonas aeruginosa* (ATCC 27853), *Escherichia coli* (ATCC 25922), *Haemophilus influenzae* type B (ATCC 10211) and Candida albicans (ATCC 10231), with bacterial inhibition rates (BIR) ranging from 18.65% to 25.42%, with values obtained at the 20% concentration tested. There was also a difference between the inhibitory activity on the four strains mentioned. While for S. flexneri and P. aeruginosa the inhibitory activity developed in parallel with the concentration, for E. coli, H. influenzae, and C. albicans an increase in the concentration led to a decrease in the inhibitory capacity, demonstrating a strain-enhancing effect. For the other strains tested (Streptococcus pyogenes ATCC 19615 and Staphylococcus aureus ATCC 25923), Brassica rapeseed honey had almost no detectable effect, with BIR% ranging from-4.85 to 8.47%. For Candida parapsilopsis (ATCC 22019), Brassica rapeseed honey was shown to have a growth-promoting effect, with a mycelial inhibition rate (MIR) compared to the control (%) ranging from 0.43% to negative values of 31.32% compared to the positive control (Hulea et al., 2022).

Overall, the results of this study add to the growing body of evidence supporting the research and use of natural products such as rapeseed honey as alternative antimicrobial agents. By harnessing the therapeutic potential of rapeseed honey and furthering our understanding of its antibacterial effects, we can contribute to the development of novel strategies to combat antimicrobial resistance and improve patient outcomes in the management of infectious diseases.

### Conclusions

The results of this study highlight the significant in vitro antibacterial efficacy of various natural rapeseed honeys against a selection of gram-positive and gram-negative bacterial strains. The results of our study showed that *S. aureus* subsp. *aureus* Rosenbach ATCC®25923™, P. aeruginosa (Schroeter) Migula ATCC®27853<sup>™</sup> and *E. faecalis* (Andrewes and Horder) Schleifer and Kilpper-Balz ATCC®51299<sup>™</sup> strains were resistant to the different natural rapeseed honeys produced by Polish manufacturers. On the other hand, the E. faecalis (Andrewes and Horder) Schleifer and Kilpper-Balz ATCC®29212<sup>™</sup> strain was susceptible to some of the rapeseed honey samples tested. Similar trends were observed in the increase in the diameter of the inhibition zone after in vitro application of different natural rapeseed honeys against *E. coli* (Migula) Castellani and Chalmers ATCC®35218<sup>™</sup> strain, where we also observed a statistically significant increase in the zone of growth inhibition. The antibacterial activity of honey samples produced by "Bug River Migratory Apiary" (Lublin region) and "Mazurskie

Miody" (Bogdan Piasecki Apiary, Tomaszkowo) was more effective against of "Pszczółka Apiary" (Ustka). The research thus highlights the potential of rapeseed honey as a valuable natural antimicrobial agent with broad-spectrum activity, offering a promising alternative to conventional antibiotics in the face of increasing antibiotic resistance. The observed antibacterial activity of rapeseed honey against both Gram-positive and Gram-negative bacteria suggests its potential applicability in the treatment and prevention of a wide range of bacterial infections. In addition, the diversity of bioactive compounds presents in rapeseed honey, including phenolic acids, flavonoids, and enzymes, are likely to contribute to its antimicrobial properties, making it a versatile therapeutic agent. While the *in vitro* studies provide valuable insights into the antibacterial efficacy of rapeseed honey, further research is warranted to elucidate its mechanisms of action, optimise its therapeutic use and evaluate its clinical efficacy. Clinical trials are needed to assess the safety and efficacy of rapeseed honey in human populations, paving the way for its potential incorporation into clinical practice.

#### **Conflicts of interest**

The authors have no competing interests to declare.

#### Ethical statement

This article does not include any studies that would require an ethical statement.

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