



DOI 10.32900/2312-8402-2024-131-105-115

UDC 579.64: 615.322

## **IN VITRO ANTIBACTERIAL PROPERTIES OF NATURAL ACACIA HONEY AGAINST SOME GRAM-POSITIVE AND GRAM-NEGATIVE BACTERIAL STRAINS**

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*Acacia honey has a number of different medicinal properties. Its antiseptic properties are used to treat conjunctivitis and other eye conditions, as well as neuro-dermatitis and eczema. Used regularly, acacia honey helps to lower blood pressure. It has a mild sedative effect and is beneficial for mental disorders and nervous breakdowns. The aim of the present study was to evaluate the in vitro antimicrobial activity of natural acacia honey produced by a Polish manufacturer against Gram-positive strains such as *Staphylococcus aureus* subsp. *aureus* Rosenbach (ATCC<sup>®</sup> 25923<sup>™</sup>), *Enterococcus faecalis* (Andrewes and Horder) Schleifer and Kilpper-Balz (ATCC<sup>®</sup> 51299<sup>™</sup>) (resistant to vancomycin; sensitive to teicoplanin) and *Enterococcus faecalis* (Andrewes and Horder) Schleifer and Kilpper-Balz (ATCC<sup>®</sup> 29212<sup>™</sup>) and Gram-negative strains such as *Pseudomonas aeruginosa* (Schroeter) Migula (ATCC<sup>®</sup> 27853<sup>™</sup>), *Escherichia coli* (Migula) Castellani and Chalmers (ATCC<sup>®</sup> 25922<sup>™</sup>), *Escherichia coli* (Migula) Castellani and Chalmers (ATCC<sup>®</sup> 35218<sup>™</sup>). The antibacterial activity of natural acacia honey was tested in vitro using the Kirby-Bauer disc diffusion technique. The results of the current study showed that acacia honey samples produced by a Polish manufacturer exhibited strong antibacterial activity against *Staphylococcus aureus* subsp. *aureus* Rosenbach (ATCC<sup>®</sup> 35218<sup>™</sup>), *E. faecalis* (Andrewes and Horder) Schleifer and Kilpper-Balz (ATCC<sup>®</sup> 51299<sup>™</sup>) and *E. faecalis* (Andrewes and Horder) Schleifer and Kilpper-Balz (ATCC<sup>®</sup> 29212<sup>™</sup>) strains, with the mean zone of growth inhibition ranging from 20.1 to 35.45 mm. *Pseudomonas aeruginosa* (Schroeter) Migula (ATCC<sup>®</sup> 27853<sup>™</sup>), *Escherichia coli* (Migula) Castellani and Chalmers (ATCC<sup>®</sup> 25922<sup>™</sup>) and *Staphylococcus aureus* subsp. *aureus* Rosenbach (ATCC<sup>®</sup> 25923<sup>™</sup>) strains were resistant to acacia honey samples. The antibacterial properties of acacia honey need to be validated using model food systems. Further research could indicate whether honey has potential as a preservative in minimally processed foods.*

**Key words:** *acacia honey, antibacterial activity, Staphylococcus aureus, Escherichia coli, Pseudomonas aeruginosa strains, Kirby-Bauer disc diffusion technique*



## **АНТИБАКТЕРІАЛЬНІ ВЛАСТИВОСТІ НАТУРАЛЬНОГО АКАЦІЄВОГО МЕДУ ЩОДО ДЕЯКИХ ГРАМПОЗИТИВНИХ І ГРАМНЕГАТИВНИХ ШТАМІВ**

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*Метою цього дослідження була оцінка in vitro антимікробної активності натурального акацієвого меду польського виробника щодо грампозитивних штамів, таких як *Staphylococcus aureus subsp. aureus* Rosenbach (ATCC® 25923™), *Enterococcus faecalis* (Andrewes and Horder) Schleifer and Kilpper-Balz (ATCC® 51299™) (резистентний до ванкоміцину; чутливий до тейкопланіну) та *Enterococcus faecalis* (Andrewes and Horder) Schleifer and Kilpper-Balz (ATCC® 29212™) і грамнегативних штамів, таких як *Pseudomonas aeruginosa* (Schroeter) Migula (ATCC® 27853™), *Escherichia coli* (Migula) Castellani and Chalmers (ATCC® 25922™), *Escherichia coli* (Migula) Castellani and Chalmers (ATCC® 35218™). Антибактеріальну активність in vitro натурального акацієвого меду досліджено за допомогою методу дискової дифузії Кірбі-Бауера. Результати цього дослідження показали, що зразки акацієвого меду польського виробника виявляють сильну антибактеріальну дію щодо *Staphylococcus aureus subsp. aureus* Rosenbach (ATCC® 35218™), штамів *Enterococcus faecalis* (Andrewes and Horder) Schleifer and Kilpper-Balz (ATCC® 51299™) і *Enterococcus faecalis* (Andrewes and Horder) Schleifer and Kilpper-Balz (ATCC® 29212™), із середніми значеннями зон пригнічення росту (від 20,1 до 35,45 мм). Штами *Pseudomonas aeruginosa* (Schroeter) Migula (ATCC® 27853™), *Escherichia coli* (Migula) Castellani and Chalmers (ATCC® 25922™) і *Staphylococcus aureus subsp. aureus* Rosenbach (ATCC® 25923™) були стійкі до зразків акацієвого меду. Антибактеріальні властивості акацієвого меду необхідно підтвердити за допомогою модельних харчових систем. Подальші дослідження можуть показати, чи є цей мед потенційним консервантом у продуктах з мінімальною обробкою.*

**Ключові слова:** акацієвий мед, антибактеріальна активність, штами *Staphylococcus aureus*, *Escherichia coli*, *Pseudomonas aeruginosa*, методика дискової дифузії Кірбі-Бауера

*Robinia pseudoacacia* L. commonly known as black locust and the false acacia. On the one hand, black locust is a tree species of high ecological and economic value (as a pioneer species, nurse species, ornamental species, fodder species, etc.). On the other hand, it is also a serious invasive species in some parts of the world (e.g. UK, Germany, France, Japan, etc.). Black locust is native to eastern North America and has already spread widely around the world through transplantation and cultivation over the last 300 years (Li G. et al., 2014). Research into the potential products that can be ob-



tained from this plant is a prerequisite for its practical use as a resource. One of these products is acacia honey.

Acacia honey comes from the flowers of the *Robinia pseudoacacia* and is extracted from the nectar of flowering acacia trees. Acacia honey is harvested in spring. Glucose and fructose make up the largest group of compounds in honey, but it also contains organic acids such as gluconic, malic and citric acids, which have the greatest influence on its flavour. The nectar contains over 50 aromatic compounds such as aldehydes, esters and polyphenolic compounds, as well as essential oils, which give honey its wonderful aroma (Khalil M.I. et al., 2010; Muhammad A. et al., 2016). The colour of acacia honey ranges from pale yellow to almost colourless (in the liquid state – pouring consistency), and crystallisation causes the colour to change to a creamy straw colour. Crystallised acacia honey does not lose its properties. The aroma of the honey comes from the acacia flowers (essential oils extracted from the nectar) and is similar to their scent, delicate and unobtrusive. True acacia honey has a distinctly sweet flavour (Khalil M.I. et al., 2010; Muhammad A. et al., 2016; Rao P.V. et al., 2016).

Acacia honey is composed of 40% fructose and 36% glucose, which gives it a delicate, non-sweet taste without bitterness. Acacia honey is rich in B vitamins and contains vitamins A and C. The nectar also contains important minerals such as manganese, chromium, copper, zinc, boron, sodium, iodine, iron, etc. This delicacy contains about 3% protein and 78% carbohydrates per 100 g. Calorie content - 337 kcal (Tumbas V.T. et al., 2012; Muhammad A. et al., 2016; Rao P.V. et al., 2016).

Over many centuries, people have studied the healing properties of honey and learned to use it to prevent and treat disease (Muhammad A. et al., 2016). Regular consumption of acacia honey (preferably in liquid form, diluted in lukewarm water) strengthens the body's immunity and at the same time has antibacterial properties, effectively limiting the development of bacterial infections (Muhammad A. et al., 2013-2015). Its supplementation in cases of upper respiratory tract diseases, sore throats and troublesome coughs effectively supports the treatment recommended by the doctor. Due to its high monosaccharide content, acacia honey is particularly useful in cases of physical and mental fatigue. Its consumption has a calming effect on the body, especially in conditions of increased stress, increased brain activity, fatigue, insomnia and general weakness of the body. It should be added that, thanks to their rapid absorption into the bloodstream, these simple sugars are responsible for nourishing muscles and other body cells (Samat S. et al., 2014; Muhammad A. et al., 2015).

Acacia honey has a wide range of uses in digestive disorders and is therefore successfully used in the treatment of gastric acidity, indigestion and other stomach problems. It accelerates the renewal of the mucous membranes, improves the granulation and secretion of mucus and thus helps in the fight against gastric and duodenal ulcers. It has a protective effect, protecting the gastrointestinal system from increased production of digestive acids, thereby reducing problems associated with reflux (Kim S. et al., 2017; Wadi M., 2022). Like other types of honey, black locust blossom honey is also good for skin conditions. It is highly effective in eliminating infections, especially purulent ones. It also speeds up the healing and closure of wounds (Iftikhar F. et al., 2010; Ker-Woon et al., 2014; Abd Ghafar N. et al., 2016). It is recommended for urinary tract disorders and kidney inflammation; it stimulates and improves liver function and aids detoxification (Muhammad A. et al., 2016). Acacia honey is often used in cosmetics, it intensely moisturises and strengthens the skin, preventing it from drying out and cracking (Burlando B. & Cornara L., 2013).

In the current study, *in vitro* antimicrobial profiling of natural acacia honey produced by a Polish manufacturer was performed, which showed inhibitory activity



against Gram-positive strains such as *Staphylococcus aureus* subsp. *aureus* Rosenbach ATCC<sup>®</sup>25923<sup>™</sup>, *Enterococcus faecalis* (Andrewes and Horder) Schleifer and Kilpper-Balz (ATCC<sup>®</sup> 51299<sup>™</sup>) (resistant to vancomycin; sensitive to teicoplanin) and *Enterococcus faecalis* (Andrewes and Horder) Schleifer and Kilpper-Balz (ATCC<sup>®</sup> 29212<sup>™</sup>) and Gram-negative strains such as *Pseudomonas aeruginosa* (Schroeter) Migula (ATCC<sup>®</sup> 27853<sup>™</sup>), *Escherichia coli* (Migula) Castellani and Chalmers (ATCC<sup>®</sup> 25922<sup>™</sup>), *Escherichia coli* (Migula) Castellani and Chalmers (ATCC<sup>®</sup> 35218<sup>™</sup>).

#### **Materials and methods.**

**Natural acacia honey.** Natural acacia honey. Natural acacia honey from the Polish producer Karolczak Cezary Apiary (Pasięka Karolczak Cezary, Sławno, Poland) was used in the present study. Samples were stored in resealable vials at 5°C in the dark, but allowed to reach room temperature before testing.

**Determination of antibacterial activity of honey samples by disc diffusion method.** The antibacterial activity of acacia honey was tested *in vitro* using the Kirby-Bauer disc diffusion technique (Bauer A. W. et al., 1966). In the present study, Gram-positive strains of such as *Staphylococcus aureus* subsp. *aureus* Rosenbach ATCC<sup>®</sup>25923<sup>™</sup>, *Enterococcus faecalis* (Andrewes and Horder) Schleifer and Kilpper-Balz (ATCC<sup>®</sup> 51299<sup>™</sup>) (resistant to vancomycin; sensitive to teicoplanin) and *Enterococcus faecalis* (Andrewes and Horder) Schleifer and Kilpper-Balz (ATCC<sup>®</sup> 29212<sup>™</sup>) and Gram-negative strains such as *Pseudomonas aeruginosa* (Schroeter) Migula (ATCC<sup>®</sup> 27853<sup>™</sup>), *Escherichia coli* (Migula) Castellani and Chalmers (ATCC<sup>®</sup> 25922<sup>™</sup>), *Escherichia coli* (Migula) Castellani and Chalmers (ATCC<sup>®</sup> 35218<sup>™</sup>) were used.

The strains were inoculated on Mueller-Hinton (MH) agar plates. Sterile filter paper discs impregnated with acacia honey samples were placed over each of the culture dishes. Isolates of bacteria with lime honey samples were then incubated at 37 °C for 24 hours. The Petri dishes were then observed for the zone of inhibition produced by the antibacterial activity of acacia 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 acacia honey was indicated by a clear zone of inhibition around the discs containing honey, and the diameter of the clear zone was taken as an indicator of susceptibility. The following zone diameter criteria were used to classify bacteria as susceptible or resistant to the phytochemicals tested: Susceptible (S)  $\geq$  15 mm, intermediate (I) = 10-15 mm and resistant (R)  $\leq$  10 mm (Okoth D.A. et al., 2013; Tkaczenko H. 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  $\pm$  standard error of the mean (S.E.M.). All variables were randomised according to the phytochemical activity of the acacia honey tested. All statistical calculations were performed on separate data from each strain. The data were analysed by one-way analysis of variance (ANOVA) using Statistica v. 13.3 software (TIBCO Software Inc., USA) (Zar J. H., 1999).

**Results.** Figure 1 summarises the results obtained from the mean diameters of the inhibition zone around the growth of Gram-positive strains such as *Staphylococcus aureus* subsp. *aureus* Rosenbach ATCC<sup>®</sup>25923<sup>™</sup>, *Enterococcus faecalis* (Andrewes and Horder) Schleifer and Kilpper-Balz (ATCC<sup>®</sup> 51299<sup>™</sup>) (resistant to vancomycin; sensitive to teicoplanin) and *Enterococcus faecalis* (Andrewes and Horder) Schleifer and Kilpper-Balz (ATCC<sup>®</sup> 29212<sup>™</sup>) and Gram-negative strains such as *Pseudomonas*



*aeruginosa* (Schroeter) Migula (ATCC® 27853™), *Escherichia coli* (Migula) Castellani and Chalmers (ATCC® 25922™), *Escherichia coli* (Migula) Castellani and Chalmers (ATCC® 35218™) induced by natural acacia honey.

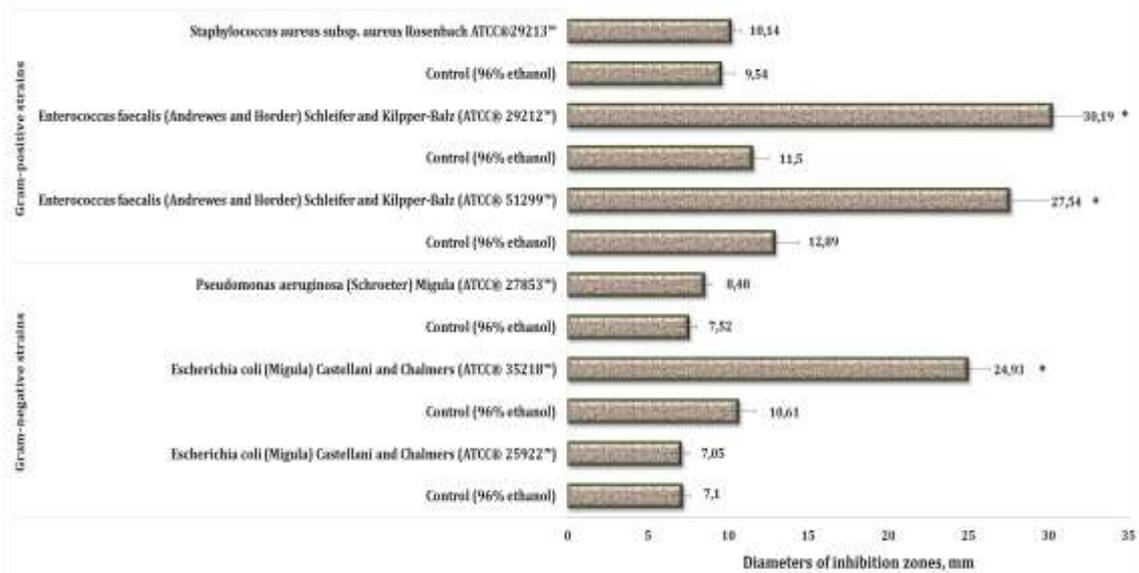


Fig. 1. The mean inhibition zone diameters induced by natural acacia honey against some Gram-positive and Gram-negative strains ( $M \pm m$ ,  $n = 8$ ).

\* – changes are statistically significant compared to the 96% ethanol.

After application of Acacia honey samples to *E. faecalis* (Andrewes and Horder) Schleifer and Kilpper-Balz (ATCC® 51299™) strain, we observed a statistically significant increase in the zone of growth inhibition of 113.7% ( $p < 0.05$ ) compared to the control samples ( $27.54 \pm 2.41$  mm vs.  $12.89 \pm 1.56$  mm). Similar trends were observed after *in vitro* application of Acacia honey samples against *E. faecalis* (Andrewes and Horder) Schleifer and Kilpper-Balz (ATCC® 29212™) strains, where we also observed a statistically significant increase in the zone of growth inhibition of 162.5% ( $p < 0.05$ ) compared to the control samples ( $30.19 \pm 1.92$  mm vs.  $11.50 \pm 1.10$  mm). The *Staphylococcus aureus* strain was resistant to Acacia honey samples. After application of Acacia honey samples to *Staphylococcus aureus* subsp. *aureus* Rosenbach (ATCC® 29213™) strain, a statistically non-significant increase in the zone of growth inhibition of 6.3% ( $p > 0.05$ ) was observed compared to the control samples ( $10.14 \pm 0.71$  mm vs.  $9.54 \pm 0.85$  mm) (Fig. 1).

Gram-negative strains such as *Pseudomonas aeruginosa* (Schroeter) Migula (ATCC® 27853™) and *Escherichia coli* (Migula) Castellani and Chalmers (ATCC® 25922™) were resistant to Acacia honey samples. The addition of Acacia honey samples to *Escherichia coli* (Migula) Castellani and Chalmers (ATCC® 25922™) strain resulted in statistically non-significant changes in the zone of growth inhibition (decrease of 0.7%,  $p > 0.05$ ) compared to control samples ( $7.05 \pm 0.54$  mm vs.  $7.10 \pm 0.56$  mm). Similarly, the *Pseudomonas aeruginosa* (Schroeter) Migula (ATCC® 27853™) strain was also resistant to the Acacia honey samples. The diameter of the zone of growth inhibition was ( $8.48 \pm 0.49$  mm) compared to the control ( $7.52 \pm 0.56$  mm). The increase in the zone of growth inhibition was 12.8% ( $p > 0.05$ ). After application of Acacia honey samples to *Escherichia coli* (Migula) Castellani and Chalmers (ATCC® 35218™) strains, we observed a statistically significant increase in the zone of growth inhibition



of 134.9% ( $p < 0.05$ ) compared to the control samples ( $24.93 \pm 1.41$  mm vs.  $10.61 \pm 1.10$  mm) (Fig. 1).

**Discussion.** The aim of the present study was to evaluate the *in vitro* antimicrobial activity of natural acacia honey produced by a Polish manufacturer against Gram-positive strains such as *Staphylococcus aureus* subsp. *aureus* Rosenbach (ATCC<sup>®</sup> 25923<sup>™</sup>), *Enterococcus faecalis* (Andrewes and Horder) Schleifer and Kilpper-Balz (ATCC<sup>®</sup> 51299<sup>™</sup>) (resistant to vancomycin; sensitive to teicoplanin) and *Enterococcus faecalis* (Andrewes and Horder) Schleifer and Kilpper-Balz (ATCC<sup>®</sup> 29212<sup>™</sup>) and Gram-negative strains such as *Pseudomonas aeruginosa* (Schroeter) Migula (ATCC<sup>®</sup> 27853<sup>™</sup>), *Escherichia coli* (Migula) Castellani and Chalmers (ATCC<sup>®</sup> 25922<sup>™</sup>), *Escherichia coli* (Migula) Castellani and Chalmers (ATCC<sup>®</sup> 35218<sup>™</sup>).

The results of the current study showed that acacia honey samples produced by a Polish manufacturer exhibited strong antibacterial activity against *Staphylococcus aureus* subsp. *aureus* Rosenbach (ATCC<sup>®</sup> 35218<sup>™</sup>), *E. faecalis* (Andrewes and Horder) Schleifer and Kilpper-Balz (ATCC<sup>®</sup> 51299<sup>™</sup>) and *E. faecalis* (Andrewes and Horder) Schleifer and Kilpper-Balz (ATCC<sup>®</sup> 29212<sup>™</sup>) strains, with the mean zone of growth inhibition ranging from 20.1 to 35.45 mm. *Pseudomonas aeruginosa* (Schroeter) Migula (ATCC<sup>®</sup> 27853<sup>™</sup>), *Escherichia coli* (Migula) Castellani and Chalmers (ATCC<sup>®</sup> 25922<sup>™</sup>) and *Staphylococcus aureus* subsp. *aureus* Rosenbach (ATCC<sup>®</sup> 25923<sup>™</sup>) strains were resistant to acacia honey samples (Fig. 1).

Acacia honey is highly nutritious with strong antioxidant and immunomodulatory potentials and can therefore be considered as a potential candidate for both cancer prevention and treatment. Neurologically, it can be considered as a viable therapeutic agent in the treatment of Alzheimer's disease (Muhammad A. et al., 2016). In addition, acacia honey from high altitudes had significantly more potent anticancer activity against HCT116 and MCF7 cells than honey from low altitudes (Mohammed M. E. A. et al., 2020). There were significant seasonal variations in the antioxidant potential of honey, and the time of honey collection affected its physicochemical properties. Moniruzzaman M. and co-workers (2013) investigated the changes in phenolic, flavonoid, antioxidant and other physicochemical properties of Malaysian acacia honey collected in different months over a two-year period. The DPPH (1,1-diphenyl-2-picrylhydrazyl) and FRAP (ferric reducing antioxidant power) methods were used to determine the total antioxidant activity of the honey samples. In general, honey samples collected at the beginning and middle of the year tended to have a higher sugar content due to their high acidity and low moisture content. There was a gradual increase in the phenolic content of the acacia honey samples collected between September and December. The honey sample collected at the beginning of the year (January) had the highest colour intensity and was dark amber in colour. It also contained the highest concentration of phenolic compounds ( $341.67 \pm 2.94$  mg gallic acid·kg<sup>-1</sup>), the highest flavonoid content ( $113.06 \pm 6.18$  mg catechin·kg<sup>-1</sup>) and the highest percentage of DPPH inhibition and FRAP value, confirming its high antioxidant potential. There was a positive correlation between DPPH and total phenolic content, suggesting that phenolic compounds are the major contributors to the radical scavenging activity of Malaysian acacia honey (Moniruzzaman M. et al., 2013).

Among the properties of acacia honey, its antibacterial, nematocidal, and anti-cancer properties should be highlighted. The study by Sateriale D. and co-workers (2019) provides relevant information on the *in vitro* anti-oomycete and antifungal activity of aqueous extracts of Italian acacia honey against two highly infectious and dangerous pathogenic species, *Aphanomyces astaci* and *Fusarium avenaceum*, responsible for important crayfish diseases. These researchers evaluated the *in vitro* inhibitory prop-



erties of Italian acacia honey extracts against pathogenic aquatic oomycete/fungal isolates that cause various diseases in crayfish, resulting in increased mortality. The antifungal activity of aqueous extracts of acacia honey was evaluated against strain UEF88662 of *Aphanomyces astaci* (oomycete) and strain SMM2 of *Fusarium avenaceum* (fungus). The anti-oomycete and antifungal activities were evaluated quantitatively by microbiological growth, survival and sporulation assays. The extracts showed a dose-dependent inhibitory effect on oomycete and fungal growth and survival, as well as on oomycete and fungal spore production (Sateriale D. et al., 2019).

In the study by Bilal B. and Azim M. K. (2018), the nematicidal activity of natural honey and its glycoproteins was characterised. Chromatographically separated honey glycoproteins showed potent anti-*C. elegans* activity ( $LD_{50} = 100$  ng protein/ $\mu$ L). Honey glycoproteins with molecular masses of  $\sim 260$  kD and  $\sim 160$  kD were composed of complexes with major royal jelly protein-1. In these complexes, MRJP1 was present in different glycosylation forms. Quantitative PCR-based gene expression assays described the molecular functions of *C. elegans* affected by honey and honey glycoproteins. The expression of 14 gene transcripts associated with key cellular and molecular functions, including energy metabolism, cytoskeleton, cell division, transcription and translation, was analysed. Acacia honey induced a concentration-dependent change in gene transcripts involved in the citric acid cycle (*mdh-1* and *idhg-1*) and cytoskeleton (*act-1*, *act-2* and *arp6*). Similarly, MRJP1-containing glycoproteins caused downregulation of *arp-6* and *idhg-1* and upregulation of *act-1* and *mdh-1* gene transcripts. The consistent downregulation of isocitrate dehydrogenase, encoded by the *idhg-1* gene, which is one of the rate-controlling enzymes of the citric acid cycle, was considered to be the major biochemical factor involved in the nematicidal activity of honey and MRJP1-containing glycoproteins. Acacia honey suppressed the expression of gene transcripts encoding *actin-2*, whereas honey glycoproteins did not. Thus, honey exerted anti-*C. elegans* activity in part by reducing the transcription of *actin-2* gene transcripts, as evidenced by a defect in locomotion and egg-laying. In addition, *arp-6* gene transcripts, encoding actin-related protein 6, were significantly and consistently downregulated by honey and honey proteins (Bilal B. & Azim M. K., 2018).

The phytochemical profiling and antimicrobial, antioxidant and anticancer properties of acacia honey (AH) from the Hail region of Saudi Arabia were carried out by Hamadou W. S. and co-workers (2022). Phytochemical profiling based on high-resolution liquid chromatography-mass spectrometry (HR-LCMS) revealed eight compounds and three small peptide-like proteins as constituents. The honey samples showed promising antioxidant activities (DPPH  $IC_{50} = 0.670$  mg/mL; ABTS  $IC_{50} = 1.056$  mg/mL;  $\beta$ -carotene  $IC_{50} > 5$  mg/mL). In the well diffusion assay, a high mean growth inhibition zone (mGIZ) was observed against *Staphylococcus aureus* ( $48.33 \pm 1.53$  mm), *Escherichia coli* ATCC<sup>®</sup> 10536<sup>™</sup> ( $38.33 \pm 1.53$  mm) and *Staphylococcus epidermidis* ATCC<sup>®</sup> 12228<sup>™</sup> ( $39.33 \pm 1.15$  mm). The microdilution assay showed that low concentrations of AH could inhibit the growth of almost all the bacterial and fungal strains tested, with minimal bactericidal concentrations (MBCs) ranging from 75 mg/mL to 300 mg/mL. On the contrary, high concentrations of AH were required to kill the tested microorganisms, with minimum bactericidal concentrations (MBCs) ranging from about 300 mg/mL to over 600 mg/mL and minimum fungicidal concentrations (MFCs) of about 600 mg/mL. The AH exhibited effective anticancer activity in a dose-dependent manner against breast (MCF-7), colon (HCT-116) and lung (A549) cancer cell lines with corresponding  $IC_{50}$  values of 5.053  $\mu$ g/mL, 5.382  $\mu$ g/mL and 6.728  $\mu$ g/mL, respectively. The *in silico* investigation revealed that the observed antimicrobial, antioxidant and anticancer activities of the constituents of AH are thermodynamical-



ly feasible, particularly those of the tripeptides (Asp-Trp-His and Trp-Arg-Ala) and the aminocyclitol glycoside (Hamadou W. S. et al., 2022).

The factors influencing the antibiotic activity of honey are quite complex. They can be divided into three groups: physical, chemical and biological. Physical factors include high osmotic pressure due to the high sugar content of honey and low pH due to the presence of organic acids. Chemical factors include primarily hydrogen peroxide produced by an enzymatic reaction (glucose oxidase), methylglyoxal found in Manuka honey and, in some varieties of honey, high levels of phenolic compounds including phenolic acids and flavonoid compounds. However, biological factors include peptides – lysozyme and defensin-1, probably the same substance, just named differently (Patton T. et al., 2006; Al-Waili N. S. et al., 2011; Kwakman P. H. & Zaat S. A., 2012).

Many studies have investigated the antibacterial activity of honeys from different botanical and geographical origins. Some types of honey (e.g. buckwheat, fir honeydew) were more effective than others (White J. W. et al., 1963). Bucekova M. and co-workers (2019) evaluated the antimicrobial activity of 57 Slovak flower honeys against *Staphylococcus aureus* and *Pseudomonas aeruginosa*, and investigated the role of several bioactive substances in the antimicrobial activity of honeys. The inhibitory and bactericidal activities of honeys were studied to determine the minimum inhibitory and bactericidal concentrations. The levels of glucose oxidase (GOX) enzyme, hydrogen peroxide (H<sub>2</sub>O<sub>2</sub>) and total polyphenols (TP) in honeys were determined. Honey samples showed different antibacterial efficacy against the tested bacteria as follows: wildflower honeys > acacia honeys > rapeseed honeys. The overall antibacterial activity of the honeys was statistically significantly correlated with the levels of H<sub>2</sub>O<sub>2</sub> and TP in the honeys. A strong correlation was found between H<sub>2</sub>O<sub>2</sub> and TP content. On the other hand, no correlation was found between GOX content and H<sub>2</sub>O<sub>2</sub> content. The antibacterial activity of 12 selected honeys was significantly reduced by treatment with catalase, but remained relatively stable after inactivation of GOX by proteinase K digestion (Bucekova M. et al., 2019).

In our previous study, we evaluated the *in vitro* antimicrobial profiling of different natural linden honeys produced by Polish manufacturers, which showed inhibitory activity against Gram-positive strains such as *Staphylococcus aureus* subsp. *aureus* Rosenbach (ATCC<sup>®</sup> 25923<sup>TM</sup>) and Gram-negative strains such as *Pseudomonas aeruginosa* (Schroeter) Migula (ATCC<sup>®</sup> 27853<sup>TM</sup>), *Escherichia coli* (Migula) Castellani and Chalmers (ATCC<sup>®</sup> 25922<sup>TM</sup>). Our study showed that all samples of natural linden honeys produced by Polish manufacturers showed mild antibacterial activity against *Staphylococcus aureus* subsp. *aureus* Rosenbach (ATCC<sup>®</sup> 25923<sup>TM</sup>), *Pseudomonas aeruginosa* (Schroeter) Migula (ATCC<sup>®</sup> 27853<sup>TM</sup>) and *Escherichia coli* (Migula) Castellani and Chalmers (ATCC<sup>®</sup> 25922<sup>TM</sup>) strains. *Staphylococcus aureus* subsp. *aureus* Rosenbach (ATCC<sup>®</sup> 25923<sup>TM</sup>) strain was more sensitive to all natural linden honey samples tested, followed by *Escherichia coli* (Migula) Castellani and Chalmers (ATCC<sup>®</sup> 25922<sup>TM</sup>) strain. *Pseudomonas aeruginosa* (Schroeter) Migula (ATCC<sup>®</sup> 27853<sup>TM</sup>) strain was resistant to several natural linden honeys (Tkaczenko H. et al., 2023).

**Conclusions.** The results of the current study showed that acacia honey samples produced by a Polish manufacturer exhibited strong antibacterial activity against *Staphylococcus aureus* subsp. *aureus* Rosenbach (ATCC<sup>®</sup> 35218<sup>TM</sup>), *E. faecalis* (Andrewes and Horder) Schleifer and Kilpper-Balz (ATCC<sup>®</sup> 51299<sup>TM</sup>) and *E. faecalis* (Andrewes and Horder) Schleifer and Kilpper-Balz (ATCC<sup>®</sup> 29212<sup>TM</sup>) strains, with the mean zone of growth inhibition ranging from 20.1 to 35.45 mm. *Pseudomonas aeruginosa* (Schroeter) Migula (ATCC<sup>®</sup> 27853<sup>TM</sup>), *Escherichia coli* (Migula) Castellani and Chalmers (ATCC<sup>®</sup> 25922<sup>TM</sup>) and *Staphylococcus aureus* subsp. *aureus* Rosenbach (ATCC<sup>®</sup>



25923™) strains were resistant to acacia honey samples. Further research could indicate whether honey has potential as a preservative in minimally processed foods.

**Acknowledgements.** *Our current scientific project was undertaken in the frame of the cooperation program between the Institute of Biology (Pomeranian University in Shupsk, Poland) and T.H. Shevchenko National University “Chernihiv Colehium”, Chernihiv, Ukraine. This work was supported by The International Visegrad Fund, and the authors are cordially grateful for this.*

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