



Research Article



Comparative Evaluation of the Antioxidant Potential of Natural Multifloral Honeys from Poland and Hungary

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Honey is a complex natural product with a variety of biological properties, which are attributed to its rich phytochemical composition, particularly the presence of phenolic acids and flavonoids. This study compared the antioxidant potential of multifloral honeys from Poland and Hungary with artificial honey using four analytical methods: ferric reducing antioxidant power (FRAP), total phenolic content (TPC), antioxidant content (AC), and DPPH radical scavenging activity. Natural honeys demonstrated markedly higher antioxidant capacities than artificial honey in all assays. FRAP values ranged from 1,875.65 to 2,569.12 $\mu\text{mol TE}\cdot\text{kg}^{-1}$, indicating a 25.7-fold increase compared to artificial honey (99.85 $\mu\text{mol TE}\cdot\text{kg}^{-1}$). Similarly, TPC values ranged from 612.74 to 725.85 $\text{mg GAE}\cdot\text{kg}^{-1}$, approximately five to six times higher than the control. AC values (18.54–20.14 $\text{mg AA}\cdot 100\text{ g}^{-1}$) and DPPH radical inhibition levels (39.05–44.29%) further confirmed the superior antioxidant capacity of natural honeys, corresponding to a 225–269% increase relative to artificial honey. A strong positive correlation was observed among the results for FRAP, TPC, DPPH, and AC, confirming that phenolic compounds are the primary determinants of honey's antioxidant activity. Samples from Poland ("Zaczarowany Ogród" and "Karolczak Cezary") and Hungary (Fulmer GmbH Magyarországi) exhibited the highest antioxidant capacities, reflecting their diverse floral origins and rich polyphenolic profiles. These findings provide compelling evidence that natural multifloral honeys are valuable dietary sources of antioxidants. The results emphasise the functional superiority of natural honey, its potential to reduce oxidative stress, and the importance of botanical and geographical origin in determining its biochemical quality.

Keywords: multifloral honey, antioxidant capacity, phenolic compounds, FRAP, DPPH, total phenolic content, bioactive composition

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Introduction

Honey is one of the most complex natural products of plant origin. It is synthesised by *Apis mellifera* bees from floral nectar, honeydew, or plant secretions (Durazzo et al., 2021). As well as being used traditionally as a natural sweetener, honey is recognised as a biologically active substance with well-documented antioxidant, antimicrobial, anti-inflammatory, and wound-healing properties (Anjos and Miguel, 2025; Tlak Gajger et al., 2025). Interest in honey as a functional food is growing due to its rich phytochemical composition, particularly of phenolic acids and flavonoids, which contribute to its capacity to neutralise reactive oxygen species (ROS) and prevent oxidative damage in biological systems (Cianciosi et al., 2018; Saad, 2025; Sharaf El-Din et al., 2025).

The antioxidant properties of honey are determined by its botanical and geographical origins, which influence the types and concentrations of bioactive compounds present (Al-Kafaween et al., 2023; Bereksi-Reguig et al., 2024). Generally, darker honeys derived from multifloral or forest sources have a higher total phenolic content and stronger antioxidant capacity than lighter monofloral honeys (Gheldof and Engeseth, 2002). The phenolic profile of honey, which includes compounds such as quercetin, chrysin, gallic acid and caffeic acid, plays a vital role in maintaining redox homeostasis via hydrogen atom transfer and single-electron transfer mechanisms (Cianciosi et al., 2018; Tlak Gajger et al., 2025). By contrast, artificial honey consists primarily of a mixture of sugars (glucose, fructose, and sucrose) and lacks enzymatic and phytochemical components. While it mimics the sweetness and texture of natural honey, artificial honey contains negligible amounts of antioxidants or phenolic compounds, rendering it biologically inactive (Hu et al., 2024). Therefore, direct comparative analyses of natural and artificial honeys provide valuable insight into the biochemical significance of naturally derived compounds.

In Europe, particularly in Central and Eastern regions such as Poland and Hungary, beekeeping traditions are deeply rooted in local biodiversity and cultural heritage. These countries are characterised by diverse landscapes comprising meadows, forests, and agricultural fields that provide a wide variety of nectar sources for honeybees. Such ecological diversity directly influences the chemical composition and biological activity of honeys, leading to strong regional variation in their functional properties (Gośliński et al., 2020). Polish honeys are widely recognised as high-quality, natural products valued for their rich chemical composition and unique sensory characteristics.

The Polish market offers a wide range of native honeys, including lime, rape, dandelion, heather, acacia, phacelia, and goldenrod. Each type of honey exhibits a distinct botanical and biochemical profile determined by the plant origin and environmental conditions during nectar collection (Majewska et al., 2024). These differences arise primarily from variations in polyphenolic composition and the presence of other bioactive compounds such as organic acids, enzymes, and volatile components (Wilczyńska, 2010). It is these compounds that are responsible for honey's diverse antioxidant and antimicrobial properties, with phenolics and flavonoids playing a pivotal role in its biological activity (Aazza et al., 2014; Attanzio et al., 2016). Flavonoids, phenolic acids, and Maillard reaction products contribute synergistically to the radical-scavenging potential of natural honeys, which is further modulated by their floral and geographical origin (Gheldof and Engeseth, 2002; Majewska et al., 2024).

Multifloral honeys produced in Central Europe, including Poland and Hungary, are typically derived from mixed plant sources, resulting in a complex matrix of phenolic compounds and antioxidant constituents (Džugan et al., 2018; Pentoś et al., 2020; Majewska et al., 2024). Such honeys often demonstrate intermediate or enhanced antioxidant activity compared to unifloral varieties, reflecting the additive or synergistic effects of diverse phytochemicals. Despite these valuable insights, systematic comparative studies evaluating the antioxidant potential and bioactivity of Polish and Hungarian honeys using complementary analytical approaches remain scarce (Cziple et al., 2022).

The present study evaluated and compared the antioxidant properties of multifloral honeys from various beekeepers in Poland and Hungary with those of artificial honey. This multi-assay approach enables the quantitative estimation of antioxidant activity and the qualitative differentiation of natural honeys based on their bioactive composition. The findings provide insight into the relationship between phenolic content and antioxidant capacity, emphasizing the superior functionality of natural multifloral honey compared to artificial honey as a dietary antioxidant source.

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.

The artificial honey used as a control sample in this study was a commercial product produced by HUZAR Sp. z o.o. (Nowy Sącz, Poland) and sold under the Auchan brand in Poland. According to the label, it contained 79% sucrose, water, starch syrup, citric acid (used as an acidity regulator), and natural honey flavouring. The product contained no proteins or lipids (less than 0.5 g of protein and 0 g of fat per 100 g) and provided an energy value of 1,409 kJ (332 kcal) per 100 g. The artificial honey was used to simulate the physicochemical properties of natural honey without its bioactive components and served as a negative control in the analyses.

Ferric Reducing Antioxidant Power (FRAP)

Assay

The ferric reducing antioxidant power (FRAP) assay was performed according to the method described by Benzie and Strain (1996), with slight modifications. The FRAP reagent was freshly prepared by mixing 2.5 ml of 10 mM TPTZ (2,4,6-tripyridyl-s-triazine) dissolved in 40 mM HCl, 2.5 ml of 20 mM FeCl₃, and 25 ml of 0.3 M acetate buffer (pH 3.6). A 0.2 ml aliquot of the honey solution (1 g·10 ml⁻¹) was combined with 1.8 ml of the FRAP reagent, and the reaction mixture was incubated at 37 °C for 10 minutes. The absorbance was then measured spectrophotometrically at 593 nm using a SPEKOL 11 (Analytik Jena, Jena, Germany), against a reagent blank. A calibration curve was prepared using Trolox standard solutions in ethanol over the concentration range of 0–300 nmol·ml⁻¹. The antioxidant capacity of honey samples was expressed as micromoles of Trolox equivalents per kilogram of honey (μmol TE·kg⁻¹).

Estimation of Total Phenolic Contents

The total phenolic content (TPC) of the honey samples was assessed using a modified Folin-Ciocalteu colorimetric method described by Singleton et al.

(1999), with slight modifications. Briefly, 5 g of each honey sample was dissolved in distilled water and diluted to a final volume of 50 ml, followed by filtration through Whatman No. 1 filter paper. An aliquot of 0.5 ml of the prepared solution was combined with 2.5 ml of 0.2 N Folin-Ciocalteu reagent (Sigma-Aldrich Chemie, Germany) and allowed to react for 5 minutes. Subsequently, 2 ml of sodium carbonate solution (75 g·l⁻¹; POCH, Gliwice, Poland) was added, and the mixture was incubated for 2 hours at room temperature. After incubation, the absorbance was recorded at 760 nm using a spectrophotometer (SPEKOL 11 Analytik Jena, Jena, Germany), with methanol serving as the blank. Gallic acid (0–200 mg·l⁻¹; Sigma-Aldrich Chemie, Germany) was employed to construct the calibration curve. All measurements were performed in triplicate, and the total phenolic content was expressed as milligrams of gallic acid equivalents (mg GAE) per 1 kg of honey.

Estimation of Antioxidant Contents (AC)

The antioxidant content of the honey samples was assessed using the method of Chen et al. (2000), with slight modifications. Honey samples were dissolved in methanol at concentrations of 0.02 or 0.04 g·ml⁻¹. Each sample (0.75 ml) was combined with 1.5 ml of a DPPH solution (0.02 mg·ml⁻¹ in methanol) and incubated for 15 minutes at room temperature. Absorbance was then measured spectrophotometrically at 517 nm. A blank was prepared using 0.75 ml of the honey solution mixed with 1.5 ml of methanol. Standard calibration curves were generated using ascorbic acid (0–10 μg·ml⁻¹). The results were expressed as milligrams of ascorbic acid equivalent antioxidant content per 100 g of honey, based on the mean of three replicate determinations.

Determination of Antioxidant Activity Against the DPPH Radical

The free radical scavenging capacity of the honey samples was determined using the 2,2-diphenyl-1-picrylhydrazyl (DPPH) assay, following the procedure described by Velázquez et al. (2003), with slight modifications. Briefly, each honey sample was dissolved in methanol to obtain concentrations ranging from 2.65 to 170 mg·ml⁻¹. A 0.75 ml aliquot of the honey solution was mixed with 1.5 ml of DPPH reagent (0.02 mg·ml⁻¹) prepared in methanol, while methanol alone served as the blank.

The reaction mixtures were incubated at room temperature for 15 minutes, after which the absorbance was measured at 517 nm using a spectrophotometer.

Ascorbic acid (0–40 mg·l⁻¹) was included as a reference antioxidant. The percentage of DPPH radical inhibition was calculated using the formula:

$$\% \text{ Inhibition} = [(A_{\text{blank}} - A_{\text{sample}}) / A_{\text{blank}}] \times 100.$$

All measurements were performed in triplicate, and the mean inhibitory concentration (IC₅₀), representing the concentration of honey required to scavenge 50% of DPPH radicals, was determined graphically from the dose-response curve.

Statistical Analysis

The results are presented as the mean ± standard error of the mean (SEM). Statistical analyses were performed using Statistica v. 13.3 software (TIBCO Software Inc., Palo Alto, CA, USA). The normality of the data distribution was verified using the Shapiro-Wilk test, and homogeneity of variances was assessed using Levene's test. Differences among honey samples were evaluated using one-way analysis of variance (ANOVA), followed by Tukey's post hoc test to determine significant pairwise differences. The level of statistical significance was set at $p < 0.05$ (Zar, 1999).

Correlation analyses were performed between total phenolic content (TPC), ferric reducing antioxidant power (FRAP), and radical scavenging activity (DPPH and AC) using Pearson's correlation coefficient (r). Graphical visualisation of the results was carried out using Microsoft Excel.

Results and Discussion

The study compared the antioxidant properties and total phenolic content (TPC) of multifloral honeys obtained from various producers in Poland and Hungary with those of an artificial honey sample used as a control. The results clearly showed that the natural honeys had a much higher biological potential than the artificial product. The antioxidant properties and total phenolic content of multifloral honeys produced by various beekeepers in Poland and Hungary are shown in Figure 1 and 2.

For the control sample, the ferric reducing antioxidant power (FRAP) value was only 99.85 $\mu\text{mol TE} \cdot \text{kg}^{-1}$, while the TPC was 121.54 mg GAE·kg⁻¹, confirming the absence of significant antioxidant activity in artificial honey. By contrast, all natural honeys displayed multiple-fold

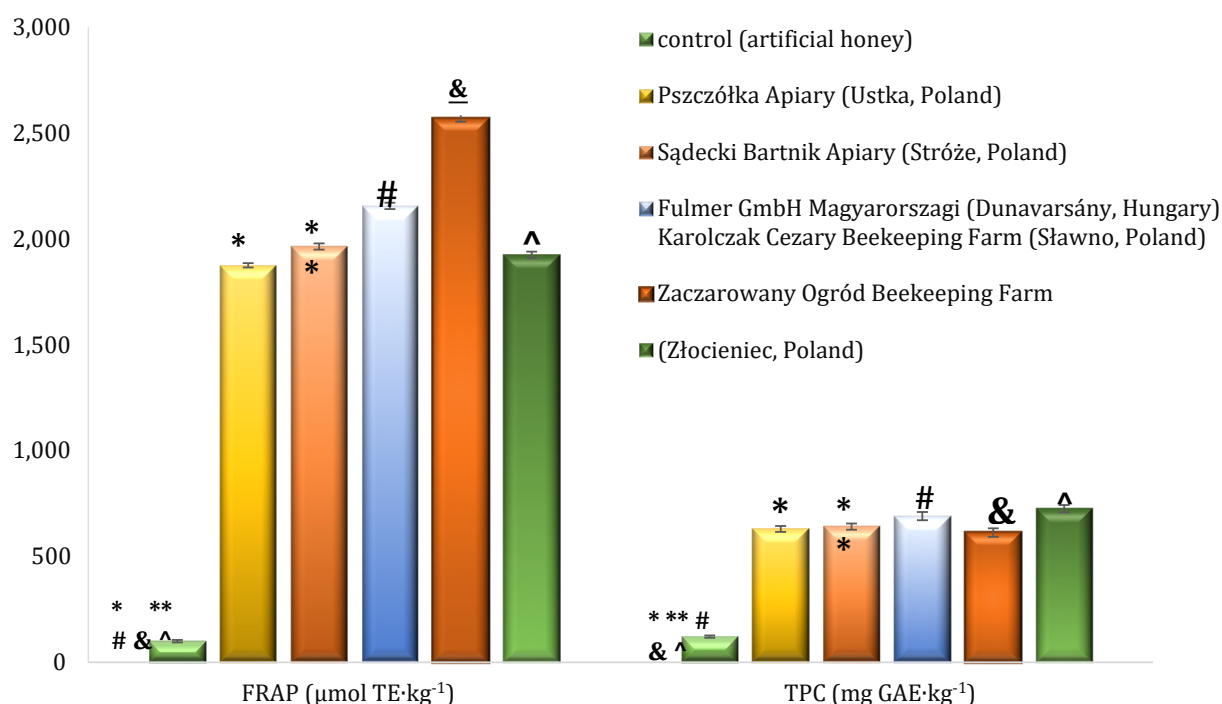


Figure 1 Analysis of the antioxidant properties and total phenolic content of multifloral honeys obtained from various producers in Poland and Hungary ($M \pm m$, $n = 8$)

* – statistically significant differences compared with honey samples from the “Pszczółka” apiary (Ustka, Poland) and artificial honey samples ($p < 0.05$); ** – statistically significant differences compared to honey samples from the “Sądecki Bartnik” apiary (Stróże, Poland) and artificial honey samples ($p < 0.05$); # – statistically significant differences compared to honey samples from Fulmer Ltd. Magyarországi producer (Dunavarsány, Hungary), and artificial honey samples ($p < 0.05$); & – statistically significant differences compared to honey samples from the “Karolczak Cezary” apiary (Sławno, Poland) and artificial honey samples ($p < 0.05$); ^ – statistically significant differences ($p < 0.05$) compared to honey samples from the “Zaczarowany Ogród” apiary (Złocieniec, Poland) and artificial honey samples

higher reducing capacities, with FRAP values ranging from 1,875.65 to 2,569.12 $\mu\text{mol TE}\cdot\text{kg}^{-1}$, corresponding to an increase of approximately 1,778 to 2,472% relative to the control. The highest antioxidant activity was observed in multifloral honey from the “Karolczak Cezary” Beekeeping Farm in Sławno, which reached 2,569.12 $\mu\text{mol TE}\cdot\text{kg}^{-1}$, representing a 25.7-fold increase compared with artificial honey. Similarly, the TPC values of all natural honeys were substantially higher than the control value, ranging from 612.74 to 725.85 $\text{mg GAE}\cdot\text{kg}^{-1}$ – approximately 5.0 to 6.0 times greater than artificial honey. The sample from “Zaczarowany Ogród” Beekeeping Farm (Złocieniec, Poland) had the highest phenolic content, with a TPC of 725.85 $\text{mg GAE}\cdot\text{kg}^{-1}$, indicating a 497% increase compared with the control (Figure 1).

Of the analysed honeys, those from Poland – particularly the samples from “Karolczak Cezary” and “Zaczarowany Ogród” – exhibited the greatest antioxidant and phenolic potential. However, the Hungarian honey from Fulmer GmbH Magyarországi in Dunavarsány also showed very high FRAP and TPC values (2,156.03 $\mu\text{mol TE}\cdot\text{kg}^{-1}$ and 690.74 $\text{mg GAE}\cdot\text{kg}^{-1}$, respectively), confirming its high biological quality (Figure 1).

The antioxidant properties of multifloral honeys produced by various beekeepers in Poland and Hungary were assessed using two complementary analytical methods: AC was used to determine total antioxidant content, and the DPPH radical scavenging assay was used to measure free radical inhibition efficiency. As demonstrated in Figure 2, the antioxidant properties of multifloral honeys obtained from different producers in Poland and Hungary were determined using the DPPH method to measure free radical scavenging capacity and the AC method to measure antioxidant content.

In the AC assay, all natural honeys demonstrated significantly higher antioxidant capacity than artificial honey, which recorded a value of only 3.52 $\text{mg AA}\cdot 100\text{ g}^{-1}$. Among the natural samples, the AC values ranged from 18.54 to 20.14 $\text{mg AA}\cdot 100\text{ g}^{-1}$, representing a five- to six-fold increase relative to the control. The honey with the highest antioxidant capacity was from Fulmer GmbH Magyarországi (Dunavarsány, Hungary), with a value of $20.14 \pm 0.25\text{ mg AA}\cdot 100\text{ g}^{-1}$, closely followed by samples from “Zaczarowany Ogród” Beekeeping Farm (Złocieniec, Poland) and “Karolczak Cezary” Beekeeping Farm (Sławno, Poland), with values of

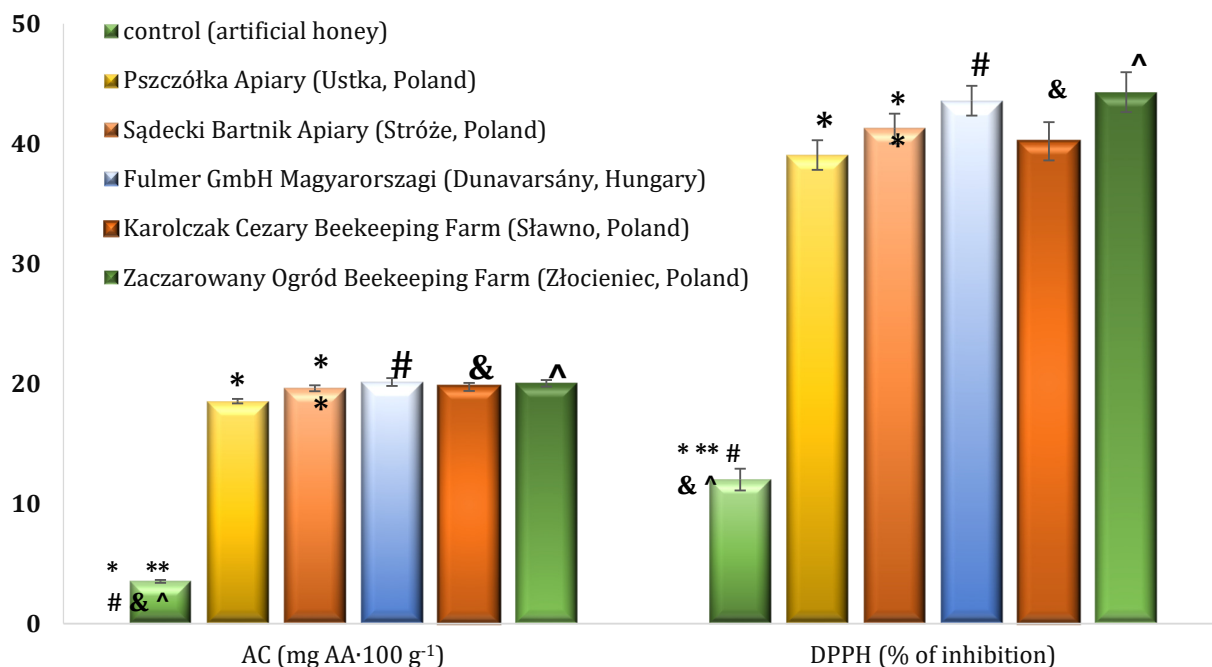


Figure 2 Analysis of antioxidant content and free radical scavenging capacity in multifloral honeys from different producers in Poland and Hungary ($M \pm m$, $n = 8$)

* – statistically significant differences compared with honey samples from the “Pszczółka” apiary (Ustka, Poland) and artificial honey samples ($p < 0.05$); ** – statistically significant differences compared to honey samples from the “Sądecki Bartnik” apiary (Stróże, Poland) and artificial honey samples ($p < 0.05$); # – statistically significant differences compared to honey samples from Fulmer Ltd. Magyarországi producer (Dunavarsány, Hungary), and artificial honey samples ($p < 0.05$); & – statistically significant differences compared to honey samples from the “Karolczak Cezary” apiary (Sławno, Poland) and artificial honey samples ($p < 0.05$); ^ – statistically significant differences ($p < 0.05$) compared to honey samples from the “Zaczarowany Ogród” apiary (Złocieniec, Poland) and artificial honey samples

20.03 ± 0.27 and 19.73 ± 0.33 mg AA · 100 g⁻¹, respectively. These results indicate that the antioxidant potential of natural honeys is approximately 5.5 times greater than that of artificial honey, confirming the presence of bioactive compounds capable of scavenging reactive oxygen species (Figure 2).

Similarly, the DPPH radical scavenging activity was significantly higher in all natural honeys than in the control. The artificial honey exhibited only 12.01% inhibition, while the natural samples displayed inhibition levels between 39.05 and 44.29%, corresponding to an increase of about 225–269% relative to the control. The strongest free radical scavenging activity was recorded for honey from “Zaczarowany Ogród” Beekeeping Farm (Złocieniec, Poland) (44.29% ± 1.66), followed by Hungarian honey from Fulmer GmbH (43.58% ± 1.25) and a sample from “Sądecki Bartnik Apiary” (Stróże, Poland) (41.25% ± 1.23).

The results obtained using these assays showed a consistent trend: natural honeys, regardless of their geographical origin, exhibited markedly higher antioxidant potential than artificial honey. The observed differences likely reflect the presence of phenolic acids, flavonoids, and other reducing substances inherent to natural floral sources. These findings confirm that multifloral honeys are valuable natural antioxidants, and consuming them may help reduce oxidative stress in biological systems.

The present study provides compelling evidence that natural multifloral honeys from Poland and Hungary have a markedly superior antioxidant potential to artificial honey. Using various analytical methods, including ferric reducing antioxidant power (FRAP), total phenolic content (TPC), antioxidant content (AC), and DPPH radical scavenging assays, it was found that natural honeys had multiple times higher antioxidant activity. This confirms that the biological value of honey primarily depends on its natural origin and chemical composition.

The FRAP assay, which reflects the electron-donating capacity of antioxidants, revealed that the reducing power of natural honeys ranged from 1,875.65 to 2,569.12 μmol TE · kg⁻¹, which is an increase of up to 25.7-fold compared to artificial honey (99.85 μmol TE · kg⁻¹). This enhancement highlights the rich redox-active matrix of natural honeys, primarily due to the presence of phenolic acids and flavonoids (Socha et al., 2011; Ahmed et al., 2018). The highest FRAP value was obtained in honey produced by the “Karolczak Cezary” Beekeeping Farm (Sławno, Poland), suggesting a diverse range of floral nectar sources and favourable

environmental conditions that promote polyphenol synthesis. This is consistent with the findings of Álvarez-Suárez et al. (2010) and Mărgăoan et al. (2021), who demonstrated that multifloral honeys derived from diverse botanical sources tend to exhibit higher reducing power than monofloral varieties.

Similarly, the total phenolic content (TPC) was markedly higher in natural honeys, ranging from 612.74 to 725.85 mg GAE · kg⁻¹, approximately 5–6 times higher than in the control sample. Honey from the “Zaczarowany Ogród” beekeeping farm (Złocieniec, Poland) exhibited the highest TPC (725.85 mg GAE · kg⁻¹), indicating a 497% increase compared to artificial honey. The strong correlation between TPC and FRAP values supports the well-established idea that phenolic compounds mainly contribute to honey’s antioxidant capacity (Cianciosi et al., 2018; Becerril-Sánchez et al., 2021; Bratosin et al., 2025). Phenolic acids, such as caffeic, ferulic, and gallic acids, and flavonoids, such as quercetin and chrysin, are known to donate hydrogen atoms or electrons, thereby stabilising free radicals and inhibiting oxidative reactions (Mathew et al., 2015; Kumar and Goel, 2019).

The AC assay, which assesses total water-soluble antioxidant content, further confirmed these trends. Artificial honey displayed negligible antioxidant activity (3.52 mM AA · kg⁻¹), whereas natural honeys ranged from 18.54 to 20.14 mg AA · 100 g⁻¹ – approximately 5.5 times higher. This substantial increase reflects the contribution of both enzymatic (e.g. catalase, peroxidase, glucose oxidase) and non-enzymatic (e.g. ascorbic acid, amino acids, phenolic compounds) antioxidants (Khalil et al., 2012; Al-Farsi et al., 2018). Notably, Hungarian honey from Fulmer GmbH Magyarországi (Dunavarsány) exhibited the highest total antioxidant capacity, surpassing Polish samples by a small margin. This suggests that, while geographic origin influences honey composition, the decisive factor in determining antioxidant richness is the multifloral character, encompassing a wide range of nectar sources (Becerril-Sánchez et al., 2021; Vîjan et al., 2023).

The DPPH radical scavenging assay, a widely used method for assessing free radical neutralisation, revealed a similar pattern. Artificial honey achieved only 12.01% inhibition, whereas natural honeys displayed inhibition levels of 39.05–44.29%, representing a 225–269% increase relative to the control. The strongest scavenging activity was found in the “Zaczarowany Ogród” sample (44.29% ± 1.66), followed by the “Sądecki Bartnik” sample (41.25% ± 1.23) and Hungarian honey (43.58% ± 1.25). These values are consistent with reports indicating

that darker, polyphenol-rich honeys possess higher radical scavenging activity (Džugan et al., 2018; Majewska et al., 2024). The DPPH results complement the findings of the FRAP and AC assays, suggesting that the antioxidant system in honey acts through both hydrogen atom transfer and single-electron transfer mechanisms.

Numerous studies have demonstrated that dark honeys, such as buckwheat, honeydew, and heather honeys, contain considerably higher concentrations of phenolic compounds and exhibit stronger antioxidant capacity than lighter varieties, such as rape, acacia, linden, and multifloral honeys (Bertoncelj et al., 2007; Wilczyńska, 2010). Comparative analyses have further confirmed this trend, showing that honeydew honeys have nearly twice the antioxidant activity and total phenolic content of nectar honeys, including multifloral, rosemary, echium, and lavender honeys (Gheldof and Engeseth, 2002; Vela et al., 2007; Can et al., 2015). The higher levels of phenolic acids and flavonoids in dark honeys increase their ability to scavenge free radicals, making them particularly valuable from a nutraceutical standpoint.

A strong positive correlation ($r > 0.85$, $p < 0.05$) between FRAP, TPC, DPPH, and AC values indicates that phenolic compounds largely account for the overall antioxidant potential of natural honeys. These compounds, together with enzymatic antioxidants, create a synergistic defence system that stabilises reactive oxygen species (ROS), thereby reducing oxidative stress (Olas, 2020; Tlak Gajger et al., 2025). Consistent results across all methods confirm that honey's antioxidant activity is multifactorial, reflecting both its composition and environmental factors such as floral diversity, soil quality, and seasonal variation (Bratosin et al., 2025; Sharaf El-Din et al., 2025).

Comparing Polish and Hungarian samples also provides valuable insight into the influence of geographical and botanical origin. The high antioxidant capacity of Polish honeys, particularly those from Sławno and Złocieniec, may reflect the abundance of wildflower species in these regions. Meanwhile, the comparable performance of Hungarian honey indicates a similar level of floral biodiversity. These findings are consistent with previous European studies, which demonstrate that geographic origin, rather than national boundaries, primarily dictates honey quality through its effect on nectar plant composition (Scholz et al., 2020; Schiassi et al., 2021).

The results of our previous study confirm the antibacterial potential of natural multifloral honeys, particularly against Gram-negative bacteria (Tkaczenko

et al., 2023, 2024). While the effectiveness varied across the tested strains, all honey samples demonstrated significant inhibitory effects against *Escherichia coli* and *Enterococcus faecalis*. This suggests that honey could be used as a complementary treatment alongside conventional antimicrobial therapies. However, *Pseudomonas aeruginosa* and *Staphylococcus aureus* exhibited resistance, highlighting the need for further research into the mechanisms of action and the specific bioactive compounds responsible for honey's antibacterial properties (Tkaczenko et al., 2024). Such resistance patterns may reflect differences in bacterial cell wall permeability, enzymatic detoxification mechanisms, or limited diffusion of honey-derived compounds. This underlines the importance of identifying phenolic and non-phenolic components that contribute to antimicrobial efficacy.

Studies have demonstrated that Polish honeys possess comparable antioxidant and antibacterial activities when their total phenolic and flavonoid content is considered (Kuś et al., 2014; Puścion-Jakubik et al., 2020). Comparative analyses have emphasised that the bioactivity of Polish honeys is largely determined by their phenolic composition and colour intensity, both of which are reliable indicators of antioxidant strength. For instance, buckwheat honey demonstrated the highest total phenolic content ($185.76 \text{ mg GAE} \cdot 100 \text{ g}^{-1}$) and phenolic acid content ($18.83 \text{ mg GAE} \cdot 100 \text{ g}^{-1}$), both of which were strongly correlated with antioxidant activity (Majewska et al., 2024). Similarly, darker honeys, such as buckwheat, heather, and forest types, typically demonstrate higher ferric reducing capacity and radical scavenging potential than lighter varieties, confirming that colour can serve as a simple proxy for antioxidant potency.

There is a strong positive correlation between colour, phenolic content and antioxidant activity, as confirmed by numerous reports (Moniruzzaman et al., 2014; Pontis et al., 2014; Bouhlali et al., 2016; Al-Farsi et al., 2018). Budzyński and Miotto (2018) proposed that phenolic compounds could be incorporated into melanoidin structures, thereby affecting the formation and function of these polymers. Similarly, Imtara et al. (2019) demonstrated that the melanoidin and polyphenol content of honey contribute jointly to its antioxidant potential. This synergistic effect between polyphenols and Maillard reaction products may significantly enhance honey's overall reducing capacity, stability, and biological activity during storage. Taken together, these observations support the idea that phenolic compounds are the main determinants of honey's antioxidant properties and play a direct role

in its ability to neutralise reactive oxygen species and prevent oxidative damage in biological systems (Gheldof and Engeseth, 2002; Aazza et al., 2014; Imtara et al., 2019).

These results demonstrate that natural multifloral honeys are a rich source of bioactive antioxidants, whereas artificial honey lacks measurable reducing or radical scavenging capacity. Thanks to their high phenolic content and reducing power, natural honeys can be categorized as functional foods that have the potential to promote health by protecting against oxidative damage, inflammation, and chronic metabolic disorders (Samarghandian et al., 2017; Sharaf El-Din et al., 2025). In this context, evaluating the antioxidant activity of multifloral honeys from different Central European regions using multiple complementary assays (e.g., FRAP, DPPH, and AC) provides valuable insight into the relationship between botanical origin, chemical composition, and bioactivity. Such comparative studies are essential for substantiating the nutritional and therapeutic value of local honeys, as well as supporting their inclusion in evidence-based functional food formulations.

Conclusions

The present study demonstrated that natural multifloral honeys from Poland and Hungary have a significantly higher antioxidant potential than artificial honey, as shown by all the analytical methods used (FRAP, TPC, AC, and DPPH). The natural samples exhibited a 25.7-fold increase in reducing power (FRAP) and a five- to six-fold increase in total phenolic content (TPC) relative to artificial honey. The highest antioxidant capacity was observed in samples from 'Karolczak Cezary' (Sławno, Poland) and 'Zaczarowany Ogród' (Złocieniec, Poland), while the Hungarian sample from Fulmer GmbH Magyarországi also demonstrated comparably strong antioxidant properties. These results highlight the functional superiority of natural multifloral honeys as rich sources of bioactive compounds capable of scavenging reactive oxygen species and reducing oxidative stress. Artificial honey, devoid of these phytochemicals, lacks biological activity and cannot substitute for natural honey in terms of health benefits. The consumption of natural multifloral honey may contribute to enhanced antioxidant defence and overall health promotion, particularly in mitigating the oxidative processes associated with chronic diseases. The findings also emphasise the importance of the botanical and geographical origin of honey in determining its quality, and encourage further research into regional variations in the bioactive composition of European honeys.

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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Not applicable.

References

- Aazza, S., Lyoussi, B., Antunes, D., & Miguel, M. G. (2014). Physicochemical characterization and antioxidant activity of 17 commercial Moroccan honeys. *International Journal of Food Sciences and Nutrition*, 65(4), 449–457. <https://doi.org/10.3109/09637486.2013.873888>
- Ahmed, S., Sulaiman, S. A., Baig, A. A., Ibrahim, M., Liaqat, S., Fatima, S., Jabeen, S., Shamim, N., & Othman, N. H. (2018). Honey as a potential natural antioxidant medicine: An insight into its molecular mechanisms of action. *Oxidative Medicine and Cellular Longevity*, 2018, 8367846. <https://doi.org/10.1155/2018/8367846>
- Al-Farsi, M., Al-Amri, A., Al-Hadhrani, A., & Al-Belushi, S. (2018). Color, flavonoids, phenolics and antioxidants of Omani honey. *Heliyon*, 4(10), e00874. <https://doi.org/10.1016/j.heliyon.2018.e00874>
- Al-Kafaween, M. A., Alwahsh, M., Mohd Hilmi, A. B., & Abulebdah, D. H. (2023). Physicochemical characteristics and bioactive compounds of different types of honey and their biological and therapeutic properties: A comprehensive review. *Antibiotics* (Basel, Switzerland), 12(2), 337. <https://doi.org/10.3390/antibiotics12020337>
- Alvarez-Suarez, J. M., Tulipani, S., Díaz, D., Estevez, Y., Romandini, S., Giampieri, F., Damiani, E., Astolfi, P., Bompadre, S., & Battino, M. (2010). Antioxidant and antimicrobial capacity of several monofloral Cuban honeys and their correlation with color, polyphenol content and other chemical compounds. *Food and Chemical Toxicology: An International Journal Published for the British Industrial Biological Research Association*, 48(8-9), 2490–2499. <https://doi.org/10.1016/j.fct.2010.06.021>
- Anjos, O., & Miguel, M. D. G. (2025). Unveiling the chemistry and bioactivity of bee products and their derivatives. *Foods* (Basel, Switzerland), 14(17), 3058. <https://doi.org/10.3390/foods14173058>

- Attanzio, A., Tesoriere, L., Allegra, M., & Livrea, M. A. (2016). Monofloral honeys by Sicilian black honeybee (*Apis mellifera* ssp. *sicula*) have high reducing power and antioxidant capacity. *Heliyon*, 2(11), e00193. <https://doi.org/10.1016/j.heliyon.2016.e00193>
- Becerril-Sánchez, A. L., Quintero-Salazar, B., Dublán-García, O., & Escalona-Buendía, H. B. (2021). Phenolic compounds in honey and their relationship with antioxidant activity, botanical origin, and color. *Antioxidants* (Basel, Switzerland), 10(11), 1700. <https://doi.org/10.3390/antiox10111700>
- Benzie, I. F., & Strain, J. J. (1996). The ferric reducing ability of plasma (FRAP) as a measure of “antioxidant power”: the FRAP assay. *Analytical Biochemistry*, 239(1), 70–76. <https://doi.org/10.1006/abio.1996.0292>
- Bereksi-Reguig, D., Allali, H., Taib, N., Aissaoui, N., Wlodarczyk-Stasiak, M., & Kowalski, R. (2024). Bioactive compounds, antioxidant properties, and antimicrobial profiling of a range of west algerian honeys: *in vitro* comparative screening prior to therapeutic purpose. *Foods* (Basel, Switzerland), 13(24), 4120. <https://doi.org/10.3390/foods13244120>
- Bertoncelj, J., Dobersek, U., Jamnik, M., & Golob, T. (2007). Evaluation of the phenolic content, antioxidant activity and colour of Slovenian honey. *Food Chemistry*, 105(2), 822–828. <https://doi.org/10.1016/j.foodchem.2007.01.060>
- Bouhlali, E., Bammou, M., Sellam, K., Ramchoun, M., Benlyas, M., Alem, C., & Filali-Zegzouti, Y. (2016). Evaluation of antioxidants, antibacterial and antifungal activities of eleven monofloral honey samples collected from Morocco. *Journal of Chemical and Pharmaceutical Research*, 8, 299–306.
- Bratosin, E. D., Tit, D. M., Pasca, M. B., Purza, A. L., Bungau, G., Marin, R. C., Radu, A. F., & Gitea, D. (2025). Physicochemical and sensory evaluation of romanian monofloral honeys from different supply chains. *Foods*, 14(13), 2372. <https://doi.org/10.3390/foods14132372>
- Bratosin, E. D., Tit, D. M., Purza, A. L., Pasca, M. B., Bungau, G. S., Marin, R. C., Radu, A. F., & Gitea, D. (2025). Exploratory analysis of phenolic profiles and antioxidant capacity in selected romanian monofloral honeys: influence of botanical origin and acquisition source. *Antioxidants*, 14(10), 1248. <https://doi.org/10.3390/antiox14101248>
- Brudzynski, K., & Miotto, D. (2011). Honey melanoidins: Analysis of the compositions of the high molecular weight melanoidins exhibiting radical-scavenging activity. *Food Chemistry*, 127(3), 1023–1030. <https://doi.org/10.1016/j.foodchem.2011.01.075>
- Can, Z., Yildiz, O., Sahin, H., Akyuz Turumtay, E., Silici, S., & Kolayli, S. (2015). An investigation of Turkish honeys: their physico-chemical properties, antioxidant capacities and phenolic profiles. *Food Chemistry*, 180, 133–141. <https://doi.org/10.1016/j.foodchem.2015.02.024>
- Chen, L., Mehta, A., Berenbaum, M., Zangerl, A. R., & Engeseth, N. J. (2000). Honeys from different floral sources as inhibitors of enzymatic browning in fruit and vegetable homogenates. *Journal of Agricultural and Food Chemistry*, 48(10), 4997–5000. <https://doi.org/10.1021/jf000373j>
- Cianciosi, D., Forbes-Hernández, T. Y., Afrin, S., Gasparrini, M., Reboredo-Rodríguez, P., Manna, P. P., Zhang, J., Bravo Lamas, L., Martínez Flórez, S., Agudo Toyos, P., Quiles, J. L., Giampieri, F., & Battino, M. (2018). Phenolic compounds in honey and their associated health benefits: A review. *Molecules* (Basel, Switzerland), 23(9), 2322. <https://doi.org/10.3390/molecules23092322>
- Czige, S., Filep, R., Balažová, E., Szentgyörgyi, H., Balázs, V. L., Kocsis, M., Purger, D., Papp, N., & Farkas, Á. (2022). Antioxidant capacity determination of hungarian-, slovak-, and polish-origin goldenrod honeys. *Plants* (Basel, Switzerland), 11(6), 792. <https://doi.org/10.3390/plants11060792>
- Durazzo, A., Lucarini, M., Plutino, M., Lucini, L., Aromolo, R., Martinelli, E., Souto, E. B., Santini, A., & Pignatti, G. (2021). Bee products: A representation of biodiversity, sustainability, and health. *Life* (Basel, Switzerland), 11(9), 970. <https://doi.org/10.3390/life11090970>
- Džugan, M., Tomczyk, M., Sowa, P., & Grabek-Lejko, D. (2018). Antioxidant activity as biomarker of honey variety. *Molecules* (Basel, Switzerland), 23(8), 2069. <https://doi.org/10.3390/molecules23082069>
- Gheldof, N., & Engeseth, N. J. (2002). Antioxidant capacity of honeys from various floral sources based on the determination of oxygen radical absorbance capacity and inhibition of *in vitro* lipoprotein oxidation in human serum samples. *Journal of Agricultural and Food Chemistry*, 50(10), 3050–3055. <https://doi.org/10.1021/jf0114637>
- Gośliński, M., Nowak, D., & Kłębukowska, L. (2020). Antioxidant properties and antimicrobial activity of manuka honey versus Polish honeys. *Journal of Food Science and Technology*, 57(4), 1269–1277. <https://doi.org/10.1007/s13197-019-04159-w>
- Hu, Y., Liu, J., Pan, Q., Shi, X., & Wu, X. (2024). Effects of artificial sugar supplementation on the composition and nutritional potency of honey from *Apis cerana*. *Insects*, 15(5), 344. <https://doi.org/10.3390/insects15050344>
- Imtara, H., Elamine, Y., & Lyoussi, B. (2018). Physicochemical characterization and antioxidant activity of Palestinian honey samples. *Food Science & Nutrition*, 6(8), 2056–2065. <https://doi.org/10.1002/fsn3.754>
- Khalil, M. I., Moniruzzaman, M., Boukraâ, L., Benhanifia, M., Islam, M. A., Islam, M. N., Sulaiman, S. A., & Gan, S. H. (2012). Physicochemical and antioxidant properties of algerian honey. *Molecules*, 17(9), 11199–11215. <https://doi.org/10.3390/molecules170911199>
- Kumar, N., & Goel, N. (2019). Phenolic acids: Natural versatile molecules with promising therapeutic applications. *Biotechnology Reports*, 24, e00370. <https://doi.org/10.1016/j.btre.2019.e00370>
- Majewska, E., Drużyńska, B., Derewiaka, D., Ciecierska, M., & Pakosz, P. (2024). Comparison of antioxidant properties and color of selected polish honeys and manuka

- honey. *Foods* (Basel, Switzerland), 13(17), 2666. <https://doi.org/10.3390/foods13172666>
- Mărgăoan, R., Topal, E., Balkanska, R., Yücel, B., Oravec, T., Cornea-Cipcigan, M., & Vodnar, D. C. (2021). Monofloral honeys as a potential source of natural antioxidants, minerals and medicine. *Antioxidants* (Basel, Switzerland), 10(7), 1023. <https://doi.org/10.3390/antiox10071023>
- Mathew, S., Abraham, T. E., & Zakaria, Z. A. (2015). Reactivity of phenolic compounds towards free radicals under *in vitro* conditions. *Journal of Food Science and Technology*, 52(9), 5790–5798. <https://doi.org/10.1007/s13197-014-1704-0>
- Moniruzzaman, M., Khalil, M. I., Sulaiman, S. A., & Gan, S. H. (2013). Physicochemical and antioxidant properties of Malaysian honeys produced by *Apis cerana*, *Apis dorsata* and *Apis mellifera*. *BMC Complementary and Alternative Medicine*, 13, 43. <https://doi.org/10.1186/1472-6882-13-43>
- Olas B. (2020). Honey and Its phenolic compounds as an effective natural medicine for cardiovascular diseases in humans? *Nutrients*, 12(2), 283. <https://doi.org/10.3390/nu12020283>
- Pentoś, K., Łuczycka, D., Oszmiański, J., Lachowicz, S., & Pasternak, G. (2020). Polish honey as a source of antioxidants – A comparison with Manuka honey. *Journal of Apicultural Research*, 59(5), 939–945. <https://doi.org/10.1080/00218839.2020.1723837>
- Pontis, J. A., Costa, L. A. A., Silva, S. J. R., & Flach, A. (2014). Color, phenolic and flavonoid content, and antioxidant activity of honey from Roraima, Brazil. *Food Science and Technology (Campinas)*, 34, 69–73. <https://doi.org/10.1590/S0101-20612014005000015>
- Saad, B. (2025). Immunomodulatory and anti-inflammatory properties of honey and bee products. *Immuno*, 5(2), 19. <https://doi.org/10.3390/immuno5020019>
- Samarghandian, S., Farkhondeh, T., & Samini, F. (2017). Honey and health: A review of recent clinical research. *Pharmacognosy Research*, 9(2), 121–127. <https://doi.org/10.4103/0974-8490.204647>
- Schiassi, M. C. E. V., de Souza, V. R., Lago, A. M. T., Carvalho, G. R., Curi, P. N., Guimarães, A. S., & Queiroz, F. (2021). Quality of honeys from different botanical origins. *Journal of Food Science and Technology*, 58(11), 4167–4177. <https://doi.org/10.1007/s13197-020-04884-7>
- Scholz, M. B. D. S., Quinhone Júnior, A., Delamuta, B. H., Nakamura, J. M., Baudraz, M. C., Reis, M. O., Kato, T., Pedrão, M. R., Dias, L. F., Dos Santos, D. T. R., Kitzberger, C. S. G., & Bianchini, F. P. (2020). Indication of the geographical origin of honey using its physicochemical characteristics and multivariate analysis. *Journal of Food Science and Technology*, 57(5), 1896–1903. <https://doi.org/10.1007/s13197-019-04225-3>
- Sharaf El-Din, M. G., Farrag, A. F. S., Wu, L., Huang, Y., & Wang, K. (2025). Health benefits of honey: A critical review on the homology of medicine and food in traditional and modern contexts. *Journal of Traditional Chinese Medical Sciences*, 12(2), 147–164. <https://doi.org/10.1016/j.jtcms.2025.03.015>
- Singleton, V. L., Orthofer, R., & Lamuela-Raventós, R. M. (1999). Analysis of total phenols and other oxidation substrates and antioxidants by means of Folin-Ciocalteu reagent. In: *Methods in Enzymology* (vol. 299, pp. 152–178). Academic Press. [https://doi.org/10.1016/S0076-6879\(99\)99017-1](https://doi.org/10.1016/S0076-6879(99)99017-1)
- Socha, R., Juszczak, L., Pietrzyk, S., Gałkowska, D., Fortuna, T., & Witczak, T. (2011). Phenolic profile and antioxidant properties of Polish honeys. *International Journal of Food Science and Technology*, 46(3), 528–534. <https://doi.org/10.1111/j.1365-2621.2010.02517.x>
- Tkaczenco, H., Kurhaluk, N., Lukash, O., Yakovenko, O., Opryshko, M., Maryniuk, M., Gyrenko, O., Buyun, L. (2023). *In vitro* antibacterial efficacy of different natural linden honey against some gram-positive and gram-negative strains. *Agrobiodiversity for Improving Nutrition, Health and Life Quality*, 7(2), 173–180. <https://doi.org/10.15414/ainhql.2023.0018>
- Tkaczenco, H., Lukash, O., Yakovenko, O., Opryshko, M., Maryniuk, M., Gyrenko, O., Buyun, L., & Kurhaluk, N. (2024). Evaluation of the *in vitro* antibacterial activity of natural multifloral honeys against selected gram-positive and gram-negative bacterial strains. *Agrobiodiversity for Improving Nutrition, Health and Life Quality*, 8(2), 170–179. <https://doi.org/10.15414/ainhql.2024.0019>
- Tkaczenco, H., Lukash, O., Yakovenko, O., Opryshko, M., Maryniuk, M., Gyrenko, O., Buyun, L., Kurhaluk, N. (2024). *In vitro* antibacterial efficacy of various natural rapeseed honey against some Gram-positive and Gram-negative bacterial strains. *Agrobiodiversity for Improving Nutrition, Health and Life Quality*, 8(1), 17–25. <https://doi.org/10.15414/ainhql.2024.0003>
- Tlak Gajger, I., Dar, S. A., Ahmed, M. M. M., Aly, M. M., & Vlaineć, J. (2025). Antioxidant capacity and therapeutic applications of honey: health benefits, antimicrobial activity and food processing roles. *Antioxidants* (Basel, Switzerland), 14(8), 959. <https://doi.org/10.3390/antiox14080959>
- Velázquez, E., Tournier, H. A., Mordujovich de Buschiazzo, P., Saavedra, G., & Schinella, G. R. (2003). Antioxidant activity of Paraguayan plant extracts. *Fitoterapia*, 74(1–2), 91–97. [https://doi.org/10.1016/S0367-326X\(02\)00293-9](https://doi.org/10.1016/S0367-326X(02)00293-9)
- Vîjan, L. E., Mazilu, I. C., Enache, C., Enache, S., & Topală, C. M. (2023). Botanical Origin Influence on Some Honey Physicochemical Characteristics and Antioxidant Properties. *Foods* (Basel, Switzerland), 12(11), 2134. <https://doi.org/10.3390/foods12112134>
- Wilczyńska, A. (2010). Phenolic content and antioxidant activity of different types of Polish honey – A short report. *Polish Journal of Food and Nutrition Sciences*, 60, 309–313
- Zar, J.H. (1999). *Biostatistical Analysis*. 4th ed., Prentice Hall Inc., New Jersey.