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ORIGINAL ARTICLE

Antioxidant properties and antimicrobial activity of manuka honey versus Polish honeys

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Abstract Originating from New Zealand manuka honey distinguishes itself from other honeys. The purpose of this study was to compare the antioxidant and antimicrobial properties of manuka honey and selected Polish honeys. Antioxidant capacity, total polyphenol and total flavonoid content were determined. Furthermore, the antimicrobial activity and the Minimum Inhibitory Concentration (MIC) were evaluated. Obtained results demonstrated that manuka honeys possessed relatively high antioxidant capacity compared to the other, Polish honeys. It was only honeydew honey that achieved comparable antioxidant properties to manuka honeys. The findings were supported by the results of microbial assays. Manuka MGO-250 and MGO-400, alongside honeydew honey, showed a stronger antimicrobial effect against Gram(+) than against Gram(−) bacteria. Moreover, the MIC-values, expressed as an inhibin number, proved the high antibiotic activity of manuka honey against the strains of *Staphylococcus aureus* and *Enterococcus faecalis*. Research concerning the influence of manuka honey on human health should be continued.

Keywords Honey · Antioxidant capacity · Polyphenols · Antimicrobial activity

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Introduction

Honey is a natural product which owes its health benefits to the content of numerous bioactive compounds that exert antibacterial, antioxidant and anti-inflammatory effects (Bogdanov et al. 2008). These properties depend on the type of honey, its origin, season and harvest conditions, processing and storage (Molan and Cooper 2000). Current studies show that dark honeys (buckwheat, heather, honeydew) are more healthful than light honeys, and multifloral honeys are more beneficial to health than monofloral ones.

Recent research has implicated that manuka honey, in comparison to other honeys, is characterized by a high content of polyphenols and relatively high antioxidant capacity. Manuka honey originates from New Zealand or Australia, where it is made by honey bees that collect nectar from Manuka myrtle (*Leptospermum scoparium*), a species in the *Myrtaceae* family. Manuka honey, like Revamil honey (Dutch origin), is a medicinal honey (Kwakman et al. 2011). It presents potent bactericidal and bacteriostatic features (Marshall et al. 2014; Alzahrani et al. 2012), which it owes to the presence of polyphenols, particularly flavonoids and phenolic acids (e.g. benzoic acid and its derivatives; cinnamic acid) and methylglyoxal (MGO), of which the latter occurs in other honeys in only trace amounts. The content of MGO in manuka honey can reach up to about 800 mg/kg, but is lower in commercial honeys, where it usually amounts to 100, 250, 400 and 550 mg/kg. An amount of MGO determines the antibacterial properties of honey. Methylglyoxal inhibits *Bacillus subtilis* and *Staphylococcus aureus*, while other cationic and non-cationic components of manuka honey, thus far unidentified, act against *Pseudomonas aeruginosa* and *Escherichia coli*. It has been found that manuka honey

maintains its bactericidal activity even after neutralization of methylglyoxal (Kwakman et al. 2011). Therefore, it is currently believed that the bactericidal effect of manuka honey depends on a high concentration of sugar, H_2O_2, methylglyoxal (MGO), low pH value, antimicrobial peptide bee defensin-1 and the presence of some other, unidentified compounds or factors (Bogdanov et al. 2008; Kwakman et al. 2011; Schneider et al. 2013). A new glycoside, MSYR (methyl syringate), has been recently detected in manuka honey and subsequently assigned the name leptosin. The concentration of this compound correlates with the antimicrobial activity and may represent a specific marker of manuka honey (Kato et al. 2012).

Polish honeys are high-quality products. Studies show that dark honeys (e.g. honeydew honey, buckwheat honey) have a higher content of bioactive compounds than light honeys (Bertoncelj et al. 2007; Wilczyńska 2010). Although these honeys do not contain methylglyoxal, they demonstrate a variety of antioxidant and antibiotic properties, with the differences between them arising from different composition of polyphenols and other bioactive compounds. More polyphenols were in dark heather, buckwheat and honeydew honeys, while the lowest total polyphenols were detected in rape, acacia, linden and multifloral honeys (Wilczyńska 2010). Aazza et al. (2014) and Attanzio et al. (2016) suggested that flavonoids are mainly responsible for the antioxidant properties of honeys. The literature confirms that dark honeys have better antioxidant properties than light honeys (Gheldof and Engeseth 2002; Can et al. 2015). Moreover, Vela et al. (2007) observed in their study that honeydew honeys had a nearly two-fold higher antioxidant activity and total polyphenols than determined in nectar honeys (including multifloral, rosemary, echium, lavender honeys).

In view of the above facts, the purpose of this study was to compare the antioxidant and antimicrobial properties of the most popular manuka honeys, containing 250 and 400 mg MGO per kg, with selected dark (buckwheat and honeydew) and light (linden and multifloral) Polish honeys.

Materials and methods

Material

The study comprised six honey samples, each tested in 3 replicates: buckwheat, honeydew, multifloral, linden and two manuka honey (MGO-250 and MGO-400; New Zealand). The honeys were purchased from a local health food store. All the honeys were produced in 2017 and analyzed in October the same year. Prior to analyses, the samples were stored at room temperature in the dark. Antioxidant properties were determined in honey solutions. A weighed amount of each honey (approx. 1 g) was diluted in distilled water, in a 1:10 (w/v) ratio.

Antioxidant capacity

The DPPH method is the most popular way to determine antioxidant capacity of food products, especially fruit, vegetables, juices (Apak et al. 2013; Samec and Piljac-Zegarac 2015; Nowak et al. 2016) and even honeys (Vela et al. 2007). The DPPH radical (1,1-diphenyl-2-picrylhydrazyl) has one unpaired electron located in the nitrogen atom. The DPPH alcoholic solution is dark purple in color. Radical scavenging is measured by the loss of absorbance at 517 nm as the yellow, non-radical form of the compound is produced (Molyneux 2004). The DPPH method is simple, rapid, sensitive and inexpensive (Apak et al. 2013; Benvenuti et al. 2004).

The antioxidant capacity (TAC) of the honey samples was determined by a modified Yen and Chen (1995) method. An amount of 0.1 ml of a sample was added to 2.9 ml of 0.1 mmol/l DPPH methanol solution (Sigma-Aldrich, Steinheim, Germany), mixed and left in the dark for 30 min. incubation at room temperature. The absorbance was measured on a Hitachi U-1900 spectrophotometer (Hitachi, Tokyo, Japan) at 517 nm. The antioxidant capacity was expressed as milligrams of Trolox equivalents (Sigma-Aldrich) per 100 g of a honey sample (mg Tx/100 g).

Total polyphenol content

Total polyphenols (TP) of the honey samples were determined by the Folin-Ciocalteu method (Sigma-Aldrich) (Singleton et al. 1999). An amount of 0.3 ml aliquot of a sample, 0.05 ml 2N Folin–Ciocalteu reagent, 0.5 ml 20% Na_2CO_3 and 4.15 ml distilled water were added to a 10 ml test tube and mixed, afterwards left at room temperature in the dark for 30-min incubation. Next, the absorbance was measured at 765 nm on a Hitachi U-1900 spectrophotometer. The results were expressed as milligrams of gallic acid equivalents per 100 g of honey sample (mg GAE/100 g).

Total flavonoid content was determined by the method described by Kapci et al. (2013); results were expressed as milligrams of catechin equivalents per 100 g of honey sample (mg CAE/100 g).

Antimicrobial activity

The antimicrobial activity in all the analyzed honeys was determined with the agar well diffusion method according to previous studies (Leszczyńska-Fik and Fik 1993). Diameters of the inhibition zones for the growth of test

Gram-positive and Gram-negative strains were identified (Tables 1, 2). The test strains originated from a collection of strains maintained at the Department of Industrial and Food Microbiology of the University of Warmia and Mazury in Olsztyn. Solutions of the analyzed honeys were prepared in sterile conical flasks. The following concentrations of the solutions were made: 5, 10, 20, 25, 30, 40, 50, 60, 70, 80 and 90%. Surface cultures (10^5 CFU/ml) of the test strains were started on sterile Petri plates filled with 20 ml nutrient agar growth medium (Merck). Next, wells of 10 mm size diameter were made with sterile borer into agar plates containing the bacterial inoculum and filled with the prepared solutions of the analyzed honeys, each in an amount of 0.7 ml. The plates were incubated at 37 °C for 24 h. After the incubation, the diameters of the inhibition zones for the growth of the test strains around the wells were determined. The experiment was replicated thrice.

Determination of MIC

The MIC (Minimum Inhibitory Concentration), i.e. the lowest concentration of honey inhibiting the growth of a test strain, was identified in the honeys which showed the highest antimicrobial activity (Balouiri et al. 2016). This analysis was carried out with the dilution method, and the results were expressed on a scale from 0 to 5, as the so-called inhibin number (Hołderna-Kędzia et al. 2008). The test was made by performing subsequent dilutions of honeys in a liquid stock medium (Antibiotic Medium Broth, Merck). 1 ml of the growth medium was transferred to each of the test tubes, which were then inoculated with a 24-h culture of the test strains (Table 3) with 10^4 cells/ml inoculum in an amount of 0.1 ml. The samples were incubated for 24 h at a temperature of 37 °C. The result, that is the inhibin number, was determined on the basis of the smallest honey concentration inhibiting the growth of a test strain (MIC, Minimum Inhibitory Concentration) as detected by the unaided eye. Results were expressed on a 0–5 scale (0 for honeys which inhibited the growth of the test strains at a concentration $> 25\%$, 1—at concentrations of 20–25%, 2—at concentrations of 15–20%, 3—at concentrations of 10–15%, 4—at concentrations of 5–10% and 5—for honeys which showed an antimicrobial activity at concentrations $< 5\%$).

Statistical analysis

The statistical analysis was performed with MS Excel 2010 Analysis ToolPak software (Microsoft). The results were presented as means and standard deviation. One-way analysis of variance (ANOVA) using the Tukey's as a post test was performed and different letters in the same row or column indicate statistical significance (at $p \leq 0.05$).

Results and discussion

The results of the antioxidant characteristics of the analyzed honeys showed that manuka honeys had a higher antioxidant capacity than the Polish honeys (Table 1). Although the highest antioxidant capacity was determined for honeydew honey (41.6 ± 0.2 mg Tx/100 g), manuka MGO-250 and MGO-400 honeys did not deviate from these results statistically significant (35.3 ± 0.1 and 40.0 ± 0.3 mg Tx/100 g, respectively). The lowest value was obtained for multifloral honey (9.9 ± 0.1 mg Tx/g). Chepulis and Francis (2012) determined a bit higher antioxidant activity in manuka MGO-250 than in manuka MGO-400.

In turn, the analysis of the total amount of polyphenolic compounds (TP) revealed that both manuka honeys were similar in TP to dark honeys (buckwheat and honeydew), all samples about 200 mg GAE/100 g. Whereas light

Table 1 Antioxidant properties of the analyzed honeys

Type of honey	DPPH mg Tx/100 g	TP mg GAE/100 g	TF mg CAE/100 g
Manuka MGO-250	35.3 ± 0.1[a]	217.0 ± 20.3[a]	115.7 ± 9.4[a]
Manuka MGO-400	40.0 ± 0.3[a]	203.5 ± 16.8[a]	118.9 ± 6.8[a]
Buckwheat	15.5 ± 0.1[b]	211.0 ± 11.4[a]	96.6 ± 8.7[b]
Honeydew	41.6 ± 0.2[a]	201.0 ± 9.9[a]	121.3 ± 7.6[a]
Multifloral	9.9 ± 0.1[c]	141.0 ± 7.3[b]	56.8 ± 4.1[c]
Linden	14.4 ± 0.1[b]	133.1 ± 5.4[b]	61.2 ± 2.8[c]

Data are a mean ± standard deviation (n = 3). Statistical analysis was performed by one-way ANOVA using the Tukey's post hoc test: different letters in the same column indicate statistical significance (at least $p \leq 0.05$)

DPPH 1,1-diphenyl-2-picrylhydrazyl, *Tx* Trolox equivalents, *TP* total polyphenol content, *GAE* gallic acid equivalents, *TF* total flavonoid content, *CAE* catechin equivalents

Table 2 Diameters of the growth inhibition zones for the Gram-negative strains created by the analyzed honeys

Test strain	Honey concentration (%)	Diameters of the growth inhibition zones [mm]					
		Manuka MGO-250	Manuka MGO-400	Buckwheat	Honeydew	Multifloral	Linden
Escherichia coli 22	90	18.0 ± 2.0	17.0 ± 1.0	13.0 ± 1.0	0	0	12.0 ± 0.0
	80	16.0 ± 1.0	15.0 ± 1.0	0	0	0	0
	70	15.0 ± 1.0	15.0 ± 1.0	0	0	0	0
	60	14.5 ± 0.5	14.0 ± 1.0	0	0	0	0
	50	13.5 ± 0.5	14.5 ± 0.0	0	0	0	0
	40	**12.0 ± 0.0[a]**	**13.0 ± 1.0[a]**	**0**	**0**	**0**	**0**
	30	0	12.0 ± 0.5	0	0	0	0
	< 25	0	0	0	0	0	0
Escherichia coli 26	90	17.0 ± 1.0	17.0 ± 2.0	0	0	0	0
	80	16.0 ± 1.0	15.0 ± 2.0	0	0	0	0
	70	15.0 ± 1.0	14.0 ± 1.0	0	0	0	0
	60	14.0 ± 1.0	13.0 ± 1.0	0	0	0	0
	50	**12.0 ± 0.0[a]**	**12.0 ± 0.0[a]**	**0**	**0**	**0**	**0**
	40	12.0 ± 0.0	0	0	0	0	0
	< 30	0	0	0	0	0	0
Escherichia coli 780	90	17.0 ± 1.0	16.0 ± 2.0	0	0	0	12.0 ± 1.0
	80	15.5 ± 0.5	17.0 ± 1.0	0	0	0	0
	70	14.0 ± 1.0	14.5 ± 0.5	0	0	0	0
	60	12.0 ± 1.0	12.0 ± 0.0	0	0	0	0
	50	**12.0 ± 0.0[a]**	**12.0 ± 0.0[a]**	**0**	**0**	**0**	**0**
	40	12.0 ± 0.0	0	0	0	0	0
	< 30	0	0	0	0	0	0
Salmonella Typhimurium 63 s	90	18.0 ± 2.0	22.0 ± 2.0	0	14.0 ± 1.0	0	12.0 ± 1.0
	80	16.0 ± 2.0	18.5 ± 1.5	0	13.5 ± 0.5	0	0
	70	15.0 ± 1.0	16.0 ± 1.0	0	12.0 ± 0.0	0	0
	60	**13.0 ± 1.0[b]**	**15.0 ± 1.0[a]**	**0**	**12.0 ± 0.0[b]**	**0**	**0**
	50	12.0 ± 1.0	14.0 ± 1.0	0	0	0	0
	40	12.0 ± 1.0	13.0 ± 1.0	0	0	0	0
	< 30	0	0	0	0	0	0
Salmonella Typhimurium 235	90	18.0 ± 2.0	19.0 ± 1.0	0	20.0 ± 2.0	0	0
	80	16.0 ± 1.0	17.0 ± 1.0	0	16.0 ± 1.0	0	0
	70	14.0 ± 1.0	17.0 ± 1.0	0	14.0 ± 1.0	0	0
	60	13.0 ± 1.0	14.4 ± 1.5	0	13.0 ± 0.0	0	0
	50	**12.0 ± 0.0[a]**	**12.0 ± 1.0[a]**	**0**	**12.0 ± 0.0[a]**	**0**	**0**
	40	0	12.0 ± 0.0	0	0	0	0
	< 30	0	0	0	0	0	0
Salmonella Eteritidis 61 s	90	20.0 ± 1.0	19.0 ± 2.0	0	20.0 ± 2.0	0	0
	80	18.0 ± 1.0	18.0 ± 1.0	0	17.0 ± 1.0	0	0
	70	16.0 ± 0.0	16.0 ± 1.0	0	13.0 ± 1.0	0	0
	60	13.0 ± 1.0	16.5 ± 0.5	0	13.0 ± 1.0	0	0
	50	**13.0 ± 1.0[a,b]**	**14.0 ± 1.0[a]**	**0**	**12.0 ± 0.0[b]**	**0**	**0**
	40	0	12.0 ± 0.0	0	0	0	0
	< 30	0	0	0	0	0	0

Table 2 continued

Test strain	Honey concentration (%)	Diameters of the growth inhibition zones [mm]					
		Manuka MGO-250	Manuka MGO-400	Buckwheat	Honeydew	Multifloral	Linden
Salmonella Arizona 34	90	20.0 ± 2.0	15.5 ± 1.5	0	14.0 ± 1.0	13.0 ± 1.0	0
	80	17.0 ± 1.0	14.0 ± 1.0	0	13.5 ± 0.5	0	0
	70	16.5 ± 0.5	14.0 ± 1.0	0	13.0 ± 1.0	0	0
	60	**13.5 ± 0.5[a]**	**12.0 ± 2.0[a,b]**	**0**	**12.0 ± 0.0[b]**	**0**	**0**
	50	12.0 ± 0.0	13.0 ± 1.0	0	0	0	0
	40	0	13.0 ± 0.0	0	0	0	0
	< 30	0	0	0	0	0	0
Pseudomonas aeruginosa $W_2 3$	90	13.0 ± 1.0	15.0 ± 2.0	0	13.0 ± 1.0	14.0 ± 1.0	14.0 ± 1.0
	80	12.0 ± 1.0	13.5 ± 1.5	0	12.0 ± 0.0	12.0 ± 0.0	12.0 ± 1.0
	70	**12.0 ± 0.0[a]**	**13.0 ± 1.0[a]**	**0**	**12.0 ± 0.0[a]**	**0**	**0**
	60	11.5 ± 1.5	12.0 ± 0.0	0	0	0	0
	50	12.0 ± 1.0	12.0 ± 0.0	0	0	0	0
	<40	0	0	0	0	0	0

Data are a mean ± standard deviation (n = 3). Statistical analysis was performed for honey concentration at which at least three samples demonstrated antimicrobial activity (except *Escherichia coli*). Bold values indicate the statistical significance (different concentration for each test strain). Different letters in the same row indicate statistical significance (at least $p \leq 0.05$)

honeys (multifloral and linden) contained a statistically significantly lower total polyphenols (about 130–140 mg GAE/100 g). Marshall et al. (2014) reported that manuka honeys had the highest total phenolic content (averaging 96.1 mg GAE/100 g) among 20 analyzed honey (average 77.8 mg GAE/100 g). The other honeys contained a total of polyphenols between 28.6 and 104.0 mg GAE/100 g. Wilczyńska (2010) analyzed antioxidant characteristics of 32 different types of honey, whose total polyphenols (TP) ranged from 17.6 (rape honey) to 189.5 mg GAE/100 g (heather honey).

In our research, honeydew honey had a comparable total content of polyphenols to the manuka honeys mentioned above, but it was much higher than detected in multifloral and linden honeys. Likewise, Can et al. (2015) determined that multifloral and linden honey had relatively low values of total polyphenols (29.5 and 41.2 mg GAE/100 g, respectively).

Our study showed that honeydew honey had a higher antioxidant capacity than buckwheat honey, reversely to Socha et al. (2011), who reported that the antioxidant activity of buckwheat honey was over twofold higher than for honeydew honey. The same researchers reported that the total polyphenols in buckwheat honey were also twofold higher than for honeydew honey (Socha et al. 2011).

Analyzed honeys were also a valuable source of flavonoids (Table 1). In our studies honeydew and manuka honeys had the highest total flavonoid content (121.3 and about 116–119 mg CAE/100 g, respectively). Other honeys had significantly lower ($p \leq 0.05$) total flavonoid content. These compounds were within the range reported by other authors (Escuredo et al. 2011; Aazza et al. 2014). Flavonoids usually accounted for about 25–70% of total polyphenols, depending on the honey origin (Khalil et al. 2011; Attanzio et al. 2016). In our study, these ratio of flavonoids was about 40–50% of TP.

In addition to the above comparison of manuka honeys with the Polish honeys in terms of their antioxidant properties, all the analyzed honeys were tested for their antimicrobial activity.

At the first stage, all the honeys were analyzed for their antimicrobial efficacy according to the well diffusion method, and the results were presented in Tables 2 and 3.

While analyzing the results, we can conclude that only three of the tested honeys (manuka MGO-250, manuka MGO-400 and honeydew honey) demonstrated antimicrobial activity. It was slightly higher towards Gram-positive (Table 3) than Gram-negative bacteria (Table 2). It is worth noticing that manuka honeys had an antimicrobial effect on all the test strains at a concentration of 30–40% at the least. Among the other types of honeys submitted to analyses, only honeydew honey showed antimicrobial activity on most of the bacterial strains except for *Escherichia coli*, although slightly higher concentrations were needed, i.e. 50% for G(−) and 40% for G(+) bacteria. Linden, buckwheat and multifloral honeys inhibited only some of the test strains and only at concentrations of 80–90%.

The honeys we investigated showed a stronger antimicrobial effect on Gram-positive strains. Other researchers have also determined that Gram-positive bacteria are more sensitive than Gram-negative microorganisms to the

Table 3 Diameters of the growth inhibition zones for the Gram-positive strains created by the analyzed honeys

Test strain	Honey concentration (%)	Diameters of the growth inhibition zones (mm)					
		Manuka MGO-250	Manuka MGO-400	Buckwheat	Honeydew	Multifloral	Linden
Staphylococcus aureus G3	90	18.0 ± 1.0	17.0 ± 1.0	0	22.0 ± 2.0	0	11.5 ± 0.5
	80	16.0 ± 1.0	16.0 ± 2.0	0	20.0 ± 1.0	0	0
	70	14.0 ± 1.0	15.0 ± 1.0	0	17.5 ± 0.5	0	0
	60	12.0 ± 0.0	15.0 ± 1.0	0	14.0 ± 1.0	0	0
	50	**12.0 ± 0.0^{b}**	**15.0 ± 1.0^{a}**	**0**	**13.0 ± 1.0^{b}**	**0**	**0**
	40	0	12.0 ± 0.0	0	12.0 ± 0.0	0	0
	< 30	0	0	0	0	0	0
Staphylococcus aureus 5.1	90	19.0 ± 1.0	23.0 ± 2.0	0	24.0 ± 1.0	0	0
	80	17.5 ± 0.5	20.0 ± 2.0	0	22.0 ± 2.0	0	0
	70	17.0 ± 1.0	19.0 ± 1.0	0	20.0 ± 1.0	0	0
	60	15.0 ± 1.0	18.5 ± 0.5	0	17.0 ± 1.0	0	0
	50	13.0 ± 1.0	15.0 ± 1.0	0	15.0 ± 0.0	0	0
	40	**12.0 ± 0.0^{b}**	**14.0 ± 1.0^{a}**	**0**	**13.0 ± 1.0^{a,b}**	**0**	**0**
	< 30	0	0	0	0	0	0
Staphylococcus aureus 629G	90	22.0 ± 2.0	23.0 ± 2.0	0	30.0 ± 1.0	0	0
	80	19.0 ± 1.0	20.0 ± 0.0	0	26.0 ± 2.0	0	0
	70	17.0 ± 1.0	18.0 ± 1.0	0	24.0 ± 1.0	0	0
	60	15.5 ± 0.5	16.0 ± 1.0	0	20.0 ± 1.0	0	0
	50	15.5 ± 0.5	13.0 ± 1.0	0	17.0 ± 1.0	0	0
	40	**12.0 ± 1.0^{a}**	**12.0 ± 1.0^{a}**	**0**	**13.0 ± 1.0^{a}**	**0**	**0**
	30	0	0	0	12.0 ± 0.0	0	0
	< 25	0	0	0	0	0	0
Enterococcus faecalis 8a	90	21.0 ± 2.0	19.0 ± 1.0	12 ± 0.0	18.0 ± 2.0	0	12.5 ± 0.5
	80	19.0 ± 1.0	17.0 ± 2.0	0	17.0 ± 1.0	0	0
	70	18.5 ± 0.5	16.0 ± 1.0	0	17.5 ± 0.5	0	0
	60	17.0 ± 1.0	15.0 ± 1.0	0	16.0 ± 1.0	0	0
	50	14.0 ± 1.0	15.0 ± 1.0	0	13.0 ± 0.0	0	0
	40	**12.0 ± 0.0^{b}**	**14.5 ± 0.5^{a}**	**0**	**11.5 ± 0.5^{b}**	**0**	**0**
	< 30	0	0	0	0	0	0
Enterococcus faecalis 842	90	28.5 ± 0.5	19 ± 1.0	0	19.0 ± 1.0	0	0
	80	27.0 ± 2.0	17 ± 1.0	0	16.0 ± 1.0	0	0
	70	23.0 ± 2.0	16 ± 1.0	0	14.0 ± 1.0	0	0
	60	20.0 ± 0.0	16 ± 1.0	0	12.0 ± 1.0	0	0
	50	**18.5 ± 0.5^{a}**	**15 ± 1.0^{b}**	**0**	**12.0 ± 0.0^{c}**	**0**	**0**
	40	14.0 ± 1.0	12 ± 0.0	0	0	0	0
	30	12.0 ± 0.0	0	0	0	0	0
	< 25	0	0	0	0	0	0
Enterococcus faecium Ent 2	90	14.0 ± 2.0	16.0 ± 1.0	0	15.0 ± 1.0	0	0
	80	13.0 ± 1.0	13.0 ± 2.0	0	14.0 ± 1.0	0	0
	70	**12.0 ± 1.0^{a}**	**12.0 ± 1.0^{a}**	**0**	**13.0 ± 1.0^{a}**	**0**	**0**
	60	0	12.0 ± 0.0	0	13.5 ± 0.5	0	0
	50	0	12.0 ± 0.0	0	12.0 ± 0.0	0	0
	< 40	0	0	0	0	0	0

Data are a mean ± standard deviation (n = 3). Statistical analysis was performed for honey concentration at which at least three samples demonstrated antimicrobial activity. Bold values indicate the statistical significance (different concentration for each test strain). Different letters in the same row indicate statistical significance (at least $p \leq 0.05$)

bactericidal activity of honeys (Alvarez-Suarez et al. 2010; Escuredo et al. 2012). Same as in our study, Schneider et al. (2013) demonstrated high bactericidal efficacy of manuka honey, although at slightly higher concentrations. Manuka honey at concentrations of 50 and 75% successfully inhibited the growth of the strains *Escherichia coli, Pseudomonas aeruginosa* and *Staphylococcus aureus* (Schneider et al. 2013). Also Alzahrani et al. (2012) showed a more potent microbial action of manuka honeys towards strains of *S. aureus* than other light honeys (e.g. acacia, lavender). As mentioned above honeydew honey analyzed in our study showed antimicrobial activity. Poli et al. (2018) in their study on Corsican honeydew honey, suggested that the antimicrobial activity of honeys was connected to the synergistic action of hydrogen peroxide and phenolic compounds. Likewise, Alzahrani et al. (2012) concluded that phenolic compounds played a major role in the antimicrobial activity of honey.

In the second stage of our study, the honeys with the highest antimicrobial properties, i.e. manuka MGO-250, manuka MGO-400 and honeydew honey, were also analyzed to determine their MIC, and the results were expressed as an inhibin number (Table 4). The analyzed honeys showed the highest antibiotic activity towards the strains *Staphylococcus aureus* and *Enterococcus faecalis* (the inhibin numbers 3–4, i.e. MIC 5–15%). The lowest results were scored by all the honeys with respect to the strain *Pseudomonas aeruginosa* (MIC > 25%). It is worth noticing that manuka honey MGO-400 was the only honey which showed an antibiotic activity towards all the strains of *Salmonella* (MIC 20–25%).

Table 4 Inhibin numbers of the analyzed honeys (on a 0–5 scale)

Test strain	Honey sample		
	Manuka MGO-250	Manuka MGO-400	Honeydew
Escherichia coli 22	1	2	Not tested
Escherichia coli 26	1	0	Not tested
Escherichia coli 780	1	0	Not tested
Salmonella Typhimurium 63 s	1	1	0
Salmonella Typhimurium 235	0	1	0
Salmonella Eteritidis 61 s	0	1	0
Salmonella Arizona 34	0	1	0
Pseudomonas aeruginosa $W_2 3$	0	0	0
Staphylococcus aureus G3	2	3	3
Staphylococcus aureus 5.1	3	3	3
Staphylococcus aureus 629G	3	3	4
Enterococcus faecalis 8a	3	3	3
Enterococcus faecalis 842	4	3	1
Enterococcus faecium Ent 2	0	2	2

0 for the honeys which inhibited the growth of the test strains at a concentration > 25%; 1 at concentrations 20–25%; 2 at concentrations 15–20%; 3 at concentrations 10–15%; 4 at concentrations 5–10%; 5 at concentrations < 5%

Copper et al. (2002) also reported a high antibiotic activity of manuka honey towards various strains of *Staphylococcus aureus* and *Enterococcus faecalis* (MIC 3% and 5%, respectively). Alzahrani et al. (2012), provided evidence showing a higher antibiotic activity of manuka honey against strains of *Staphylococcus aureus* (MIC 6–7%), compared to the other honeys these researchers tested (MIC 10–25%). Regarding the strain *Pseudomonas aeruginosa* manuka honey showed a similar antibiotic activity as that exerted by the other honeys (Alzahrani et al. 2012).

Furthermore, we observed than antibiotic activity of manuka honey varied depending the methylglyoxal content. Manuka MGO-250 was more effective against the strains of *Escherichia coli* (except *E. coli 22*), while manuka MGO-400 against all strains of *Salmonella* (Table 4). Both types of manuka honey had similar inhibin number for *Staphylococcus aureus* (MIC 10–15%), although honeydew honey was a bit more effective (especially against *Staphylococcus aureus 629G*; MIC 5–10%).

Other researchers also reported a high antibiotic activity manuka honey. A study by Tan et al. (2009) compared the manuka honey and the Malaysian Tualang honey. The results achieved for both honeys were comparable (MIC 8.75–25.0%), although manuka honey was a more potent bactericidal against the strains of *Staphylococcus aureus* and *Escherichia coli* (Tan et al. 2009). Another study was conducted to compare the antibiotic activity of manuka honey with some Polish honeys. In it, manuka honey obtained similar results as the Polish honeydew and heather honeys, but higher ones than multifloral and acacia honeys (Kędzia et al. 2014).

Conclusion

The obtained results showed that manuka honeys MGO-250 and MGO-400 were characterized by relatively high antioxidant properties in comparison with the Polish honeys. It was only honeydew honey that presented the antioxidant capacity, total polyphenols and total flavonoids comparable to the ones identified in both manuka honeys. Phenols and flavonoids were mainly responsible for the antioxidant properties of honeys. The relatively high antioxidant properties of manuka honey have been confirmed in the subsequent microbiological analysis. Manuka honey showed antimicrobial effect on all test strains at a concentration of 30–40% at the lowest. Manuka honeys MGO-250 and MGO-400, alongside honeydew honey, had a stronger antimicrobial activity against strains of Gram-positive bacteria such as *Staphylococcus aureus* and

Enterococcus faecalis. The MIC assay, showing the lowest honey concentration that inhibited the growth of a test strain and expressed with an inhibin number, confirmed the high antibiotic activity of manuka honey against the strains of *Staphylococcus aureus* and *Enterococcus faecalis* while being less bactericidal towards the strain *Pseudomonas aeruginosa*. Antimicrobial activity of manuka honey depends on the content of methylglyoxal and polyphenols. A strong bactericidal effect of manuka honey allows to use it for medical purposes, e.g. acceleration of wound healing (skin burn, ulceration) or reduction of inflammation of gastrointestinal mucosa (reduction gastroenteritis). Furthermore, it would be a possibility to use manuka honey as a preservation agent for food and beverages (e.g. health snacks or nutritional drinks). Research on antioxidant properties of honeys and their antimicrobial characteristics should be continued.

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