Honey is a complex natural product which has had a broad application for thousands of years (1, 2). Owing to its nourishing and curative properties, honey has been used in the treatment of postoperative infections, burns, skin diseases, and gastrointestinal disorders. What is more, honey has always been praised for its antioxidant, antimicrobial, antiviral, antiparasitic, anti-inflammatory and antimutagenic effects (3). Also, its active compounds could be used as antineoplastic agents with different mechanisms of action. Namely, honey phenolics have been marked as compounds with antiproliferative properties against several types of cancer (4).

The chemical composition of honey is responsible for its great dietary and prophylactic value. Phytochemicals from the nectar or honeydew of a plant can be transferred to honey and therefore honey could serve as a medium for transporting medicinal properties of plants. Previous studies have emphasized the fact that honey contains numerous antioxidants, including phenolic compounds, ascorbic acid, carotenoids, and enzymes such as glucose oxidase, catalase, and peroxidase (5, 6). The levels of phenolics depend on floral and geographical origins, climatic conditions and ways of processing (7). Further, phenolics express a wide range of biological effects and they are considered responsible for

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**NATURAL DRUGS**

**TOTAL PHENOLIC AND FLAVONOID CONTENTS, ANTIOXIDANT AND ANTIBACTERIAL ACTIVITIES OF SELECTED HONEYS AGAINST HUMAN PATHOGENIC BACTERIA**

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**Abstract:** The study analyzed color intensity, the total phenolic and flavonoid contents, as well as antioxidant and antibacterial activities of honeys from Serbia. All the tested honeys contained considerable levels of phenolics. The highest phenolic content was obtained in forest honey – 1389.71 mg gallic acid equivalents/kg. High levels of phenolics and flavonoids were also determined in honeydew, meadow and oregano honeys. The radical scavenging activity measured by DPPH method in linden and forest honey was 0.45 and 2.75 mmol Trolox equivalents/kg, respectively. The ferric reducing potential was the greatest in forest honey – 6.04 mmol Fe²⁺/kg. Darker honeys had greater phenolic content and antioxidant activity. The antimicrobial activity of honeys against six bacterial strains isolated from human material was studied using a microwell dilution assay. The minimal inhibitory concentration of honeys ranged from 6.25% w/w (for oregano honey against Enterococcus faecalis and honeydew honey against Streptococcus pneumoniae) to > 50% w/w (meadow honey against Staphylococcus aureus). The minimal bactericidal concentration ranged from 12.5% w/w (honeydew and bee pollen enriched honey against E. faecalis) to > 50% w/w. Higher values of antimicrobial activities were found in honeydew, oregano, and forest honeys, while meadow honey showed the lowest antimicrobial activity. Analyzed honeys exhibited a strong antioxidant and broad-spectrum antibacterial activity against multidrug-resistant pathogenic bacteria. Phenolic compounds, especially in dark amber forest and honeydew honey, as well as in amber oregano honey, may contribute to their efficacy in therapeutic administration.

**Keywords:** Antioxidant activity, antimicrobial activity, honey, color intensity, phenolic compounds

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the antioxidant effects of honey. Due to its significant antioxidant activity, honey could find its application in the management of oxidative stress-associated chronic diseases (8, 9).

The antimicrobial activity of honey has been attributed not only to its high osmolarity, antibacterial properties of hydrogen peroxide and acidity, but also to non-peroxide antibacterial substances such as lysozymes, and phytochemicals from the nectar of plants. Polyphenols like phenolic acids, their esters, and flavonoids increase the antimicrobial effect of honey (5, 10, 11). The growing overuse and misuse of antibiotics have led to the evolution of numerous forms of bacterial resistance. Being a natural antibacterial agent and an alternative to antibiotics, honey has attracted great attention since it could be successfully used against certain antibiotic-resistant bacterial strains (12, 13). Moreover, apart from the absence of antimicrobial resistance, other highly beneficial features of this natural preservative are nontoxicity, a practical application and low cost (14, 15). For instance, when manuka honey from New Zealand was first registered as medical-grade honey, the clinical use of honey in the topical treatment of wounds increased (16). This therapeutic honey expresses high antibacterial activity against various pathogens, especially against multi-drug resistant bacteria (17).

Blossom honey, which is made by honeybees mostly from the nectar of flowers, is the dominant type of honey. Honeydew honey is prepared from secretions of all living parts of plants other than the flower or excretions of sucker insects (18, 19). Therefore, the composition of honey is associated with the nectar source (botanical and geographical origin) and differs from one country to another (20). Serbia is ranked among countries with the highest floristic diversity in Europe. In addition, good natural conditions, moderate continental climate, many mountainous areas with low population density and long tradition of production make this country a perfect environment for beekeeping (21).

An abundance and diversity of plants in Serbia provide honey with numerous valuable nutritional, prophylactic and healing properties. The present study was undertaken to assess the antioxidant activity, color, total phenolic and flavonoid contents of Serbian honey. The antibacterial activity of these honey against multidrug-resistant human pathogenic bacterial isolates of wound and lungs infections was also examined. In addition, correlations between the antibacterial activity and the antioxidant activity, color, total phenolic and flavonoid contents were also sought. The obtained results will help in identifying types of honey with a high medicinal potential for future research.

MATERIALS AND METHODS

Honey samples
Eight honey samples were provided directly from experienced professional local apiarists who declared their botanical origin. Honey was collected from the two mountains in southeastern Serbia, the Suva Planina Mountain, and the Stara Planina Mountain. The types of honey were different, i.e. monofloral and multifloral honey. The samples were acquired between May and July 2016 and kept in sterilized polyethylene flasks at room temperature in a dark place until further analysis.

Chemicals and instruments
The free radical 2,2-Diphenyl-1-pircrylhydrazyl (DPPH), 2,4,6-tripyrdyl-S-triazine (TPTZ), and (+)-6-Hydroxy-2,5,7,8-tetramethylchromane-2-carboxylic acid (Syn. Trolox) were purchased from Sigma (Sigma-Aldrich GmbH, Germany). Folin-Ciocalteu reagent and (+)-catechin were obtained from Merck (Germany). Gallic acid was purchased from TCI Europe n.v. (Belgium). All other chemicals and reagents were of the highest analytical grade and obtained from commercial providers.

Color intensity
The honey samples were diluted (1 : 1, w/v) with ultrapure water and mixed for 5 min using a vortex mixer. The absorbance (Abs) was measured spectrophotometrically at 635 nm using glass cuvettes against a blank (water instead test samples). The results were calculated and expressed in millimeters on a Pfund scale (mm Pfund = -38.70 + 371.39 x Abs) (23).

Total phenolic and flavonoid contents determination
The free radical 2,2-Diphenyl-1-pircrylhydrazyl (DPPH), 2,4,6-tripyrdyl-S-triazine (TPTZ), and (+)-6-Hydroxy-2,5,7,8-tetramethylchromane-2-carboxylic acid (Syn. Trolox) were purchased from Sigma (Sigma-Aldrich GmbH, Germany). Folin-Ciocalteu reagent and (+)-catechin were obtained from Merck (Germany). Gallic acid was purchased from TCI Europe n.v. (Belgium). All other chemicals and reagents were of the highest analytical grade and obtained from commercial providers. Color intensity, total phenolic and flavonoid contents, and antioxidant activity were measured using Evolution 60 UV/Vis scanning spectrophotometer (Thermo Scientific, USA).

Prior to testing, honeys were first homogenized and sonicated at 45°C for 10 min until the dissolution of sugar crystals (22).

Colour intensity
The honey samples were diluted (1 : 1, w/v) with ultrapure water and mixed for 5 min using a vortex mixer. The absorbance (Abs) was measured spectrophotometrically at 635 nm using glass cuvettes against a blank (water instead test samples). The results were calculated and expressed in millimeters on a Pfund scale (mm Pfund = -38.70 + 371.39 x Abs) (23).

Total phenolic and flavonoid contents determination
The total phenolic content (TPC) was measured spectrophotometrically with a modified Folin-Ciocalteu method. This method based on the reduction of Folin-Ciocalteu reagent under basic conditions to form a blue chromophore which has an absorption maximum at 750 nm (2, 24, 25). After
homogenization, the samples were diluted (1 : 5, w/v) with ultrapure water and mixed using a vortex mixer for 5 min. An aliquot of 100 mL of a honey solution was shaken for 1 min with 0.5 mL of Folin-Ciocalteu reagent and 6 mL of distilled water. Further, 1.5 mL of 20% Na2CO3 was added and the mixture was shaken. Finally, the solution was brought up to 10 mL by adding water. After incubation at room temperature for 2 h in the dark, the absorbance was measured at 510 nm using glass cuvettes against a blank. A blank sample was prepared by replacing 100 µL of the sample with 100 µL of distilled water. The TPC was calculated using the standard calibration curve of gallic acid ranging between 15 and 200 mg/L. The equation for the gallic acid calibration curve was y = 1.0834.x + 0.0214 (where x – concentration of gallic acid equivalents (GAE) expressed as milligrams of GAE/mL; y – measured absorbance), and the squared correlation coefficient was R2 = 0.9975. After taking dilution factors into account, the results were calculated and expressed as milligrams of GAE per kg of honey.

The total flavonoid content (TFC) was estimated by the aluminum chloride colorimetric assay. The assay based on aluminum complex formation in the presence of sodium nitrate in alkaline medium with the development of a red solution which exhibits considerable absorbance at 510 nm (26, 27). Honeys were diluted (1 : 1, w/v) with ultrapure water and mixed vigorously using a vortex mixer for 5 min. An aliquot (1 mL) of solutions was added to a 10 mL volumetric flask containing 4 mL of H2O. A volume of 0.3 mL of 5% NaNO2 was added to the flask. After 5 min, 0.3 mL of 10% AlCl3 was added. After 6 min, the prepared solution was mixed with 2 mL of 1 mol/L NaOH and the total volume was brought up to 10 mL with H2O. The solution was mixed and the absorbance value was measured versus the prepared reagent blank at 510 nm. A blank sample was prepared by replacing 1 mL of the sample with 1 mL of distilled water. The total flavonoid content was calculated using the standard calibration curve of catechin (20-100 mg/L; y = 2.687.x + 0.007; R2 = 0.9982), where x – concentration of catechin equivalents (CE) expressed as milligrams of CE/mL; y – measured absorbance. After taking into account the sample dilution, the results were calculated and expressed as milligrams of CE per kg of honey.

DPPH radical scavenging activity

Spectrophotometric analysis of the antiradical activity was performed using a slightly modified DPPH method. DPPH radical is stable organic nitrogen-centered free radical with a dark purple color which when reduced to its nonradical form by antioxidants becomes yellow. The method measures the capacity of antioxidants to directly react with (scavenge) DPPH radicals by monitoring its absorbance at 517 nm with a spectrophotometer (2, 9). An aliquot of 100 mL of the honey solution (1 : 5, w/v, solvent ultrapure water) was added to 4 mL of 0.04 mmol/L DPPH in methanol and the mixture was shaken vigorously. After incubation at room temperature for 60 min in a dark place, the absorbance was measured with a spectrophotometer at 517 nm using glass cuvettes against a blank. Methanol served as a blank. The calibration curve was prepared with Trolox solution (0.05-1.0 mmol/L; y = -0.3919.x + 0.3517; R2 = 0.9737) where x – concentration of Trolox equivalents (TEAC) expressed as mmol TEAC/L; y – measured absorbance. After taking into account the sample dilution, the results were expressed as mmol TEAC per kg of honey.

Ferric reducing antioxidant potential assay (FRAP)

The measurement was carried out according to the method previously reported by Benzie and Strain. FRAP assay monitors the reaction of Fe2+ with TPTZ to form a violet-blue color Fe2+ complex with an absorbance maximum at 593 nm (28). The working FRAP reagent was prepared daily by mixing 10 volumes of 300 mmol/L acetate buffer pH 3.6 with 1 volume of 10 mmol/L TPTZ (in 40 mmol/L HCl) and with 1 volume of 20 mmol/L FeCl3. To determine the antioxidant activity, 100 mL of honey diluted with ultrapure water (1 : 5, w/v) were mixed with 3 mL of the FRAP reagent (2). The absorbance readings were started after 30 min and they were performed at 593 nm using a spectrophotometer against a blank. FRAP working solution with acetate buffer instead of a sample was used as a blank. The standard calibration curve was made using aqueous solutions of FeSO4 × 7 H2O (0.1-1 mmol/L; y = 0.722.x + 0.0594; R2 = 0.9730) where x – concentration of Fe2+ expressed as mmol Fe2+/L; y – measured absorbance). After taking into account the sample dilution, the results were calculated and expressed as millimoles of Fe2+ per kilogram of honey.

Antimicrobial activity test

The in vitro antimicrobial activity of honeys was tested against six bacterial strains isolated from human material: from nasal swabs – *Streptococcus pneumoniae*, from wound swabs – *Staphylococcus*
aureus, Enterococcus faecalis and Pseudomonas aeruginosa and from endotracheal aspirate – Escherichia coli and Acinetobacter baumannii. All the microorganisms were clinical isolates from the Laboratory of the Institute of Public Health in Niš, Serbia.

The antimicrobial activity of the honey samples, the minimum inhibitory concentration (MIC) and the minimum bactericidal concentration (MBC) were determined by the micro-well dilution method described by Clinical and Laboratory Standards Institute with some modifications (29). An overnight culture of the tested strains (incubated at 37°C for 24 h on Mueller-Hinton agar) was used for the preparation of Mueller-Hinton broth (MHB) suspension whose turbidity was adjusted to 0.5 McFarland using a densitometer. A 50% (w/w) stock solution of each type of honey was prepared by weighing 5 g of honey and bringing the volume up with 10 mL of water. A series of twelve dilutions of the stock solution of honey in water was prepared in a 96-well microtiter plate to obtain concentrations in the range of 0.02-50% (w/w). The final volume was 100 µL and the final concentration of bacterial suspensions was about 10^6 colony-forming units per mL (CFU/mL) in each well. Two controls were included – medium (negative control) and medium with erythromycin, ciprofloxacin, doxycycline, and gentamicin (positive control) (Sigma Aldrich, St Louis, MO, USA). Antibacterial drugs were prepared in the concentration range of 0.001 to 10 mg/mL. A broad-spectrum of antibacterial drugs were chosen because the selected bacterial strains previously expressed multiple antibiotic resistances. The plates were incubated at 37°C for 24 h. The bacterial growth was determined by adding 20 µL of 0.5% triphenyl tetrazolium chloride (TTC) aqueous solution in each well. The MIC was defined as the lowest concentration of honey or standard antibacterial drugs at which microorganisms showed no visible growth. To determine MBC broth was taken from each well without visible growth of microorganisms and inoculated in Mueller Hinton agar at 37°C for 24 h. The MBC was defined as the lowest concentration of the samples at which 99.9% of the inoculated bacteria were killed.

Statistical analysis
All the analyses for each sample were carried out in triplicate and the results were expressed as mean value ± standard deviation (SD). The one-way analysis of variance (ANOVA) was used to test significant differences between mean values followed by Tukey’s honest significant difference (HSD) post hoc comparison. Experimental data were also analyzed using the correlation analysis. The differences were accepted as significant for p < 0.05. The statistical software IBM Corp. SPSS 21.0 (30) was used.

RESULTS AND DISCUSSION

The color of honey can range from nearly colorless, through amber and dark reddish amber, to nearly black. This feature depends on the geographical

<table>
<thead>
<tr>
<th>Type of honey</th>
<th>Floral origin</th>
<th>Geographical origin in Serbia</th>
<th>Colour (mm Pfund)</th>
<th>Colour and consistency</th>
</tr>
</thead>
<tbody>
<tr>
<td>Acacia</td>
<td>Robinia spp.</td>
<td>The Stara Planina Mountain, Ljubatovica village</td>
<td>20</td>
<td>Pale-white, smooth, thick, jelly</td>
</tr>
<tr>
<td>Linden</td>
<td>Tilia spp.</td>
<td>The Stara Planina Mountain, Babin Kal village</td>
<td>70</td>
<td>Light amber, jelly-like, granulated</td>
</tr>
<tr>
<td>Oregano</td>
<td>Origanum spp.</td>
<td>The Stara Planina Mountain, Babin zub peak</td>
<td>90</td>
<td>Amber, solid, fine granulated</td>
</tr>
<tr>
<td>Lavender</td>
<td>Lavandula spp.</td>
<td>The Suva Planina Mountain, Sićevo village</td>
<td>115</td>
<td>Dark amber, solid, smooth</td>
</tr>
<tr>
<td>Honeydew</td>
<td></td>
<td>The Suva Planina Mountain, Sićevo village</td>
<td>135</td>
<td>Dark amber, solid, fine granulated</td>
</tr>
<tr>
<td>Meadow</td>
<td>Multifloral</td>
<td>The Stara Planina Mountain, Babin Kal village</td>
<td>98</td>
<td>Amber, fine granulated</td>
</tr>
<tr>
<td>Forest</td>
<td>Multifloral</td>
<td>The Stara Planina Mountain, Crni vrh village</td>
<td>146</td>
<td>Dark amber, solid, fine granulated</td>
</tr>
<tr>
<td>Bee pollen enriched</td>
<td>Multifloral</td>
<td>The Suva Planina Mountain, Divljana village</td>
<td>47</td>
<td>Extra light amber, solid, fine granulated</td>
</tr>
</tbody>
</table>
and floral origin of honey, and mineral, pollen and phenolic content (8, 23, 31). Being an important parameter for evaluating honey quality, color is in close relation with the type of flower nectar bees gather, and it has a great influence on consumers’ preferences (32).

Common names, floral origin, geographical origin in Serbia, color intensity, and appearance of analyzed honeys are presented in Table 1.

There were considerable differences in color intensity of honeys, with color variations from 20 mm Pfund for the pale-white acacia honey to 146 mm Pfund for the dark amber forest honey (Table 1). In addition to the forest honey, the dark amber honeydew and lavender honey were the darkest and their color intensity was higher than 100 mm Pfund. Moreover, the high color intensity (98 mm Pfund) was measured in the meadow honey as well. The color of most of the samples included dark amber (37.5%) and amber (25%), whereas other colors (white, light amber and extra light amber) were equally represented (12.5%). The results of the TPC, TFC, and antioxidant activity of honeys determined by using DPPH and FRAP assays are given in Table 2.

All the tested honeys contained significant levels of phenolic compounds. The total phenolic and flavonoid content of the various types of honey significantly differed statistically (p < 0.05) (Table 2). The TPC was between 379.34 ± 4.13 mg GAE/kg in the acacia honey and 1389.71 ± 9.84 mg GAE/kg in the forest honey. High phenolic content was also determined in honeydew and meadow honey, 1220.69 ± 8.10 and 1025.17 ± 11.03 mg GAE/kg, respectively. These results were consistent with published results for the same types of honey. According to Kaygusuz et al. (33), the phenolic content in monofloral honey from Anatolia varied from 98 mg GAE/kg in the acacia honey to 1326 mg GAE/kg in the heather honey, and the content measured in the lavender honey (567 mg GAE/kg) matched the TPC of this type of honey reported in Table 2.
the present study (543.75 mg GAE/kg) (Table 2). On the other hand, the phenolic content in Croatian honeys was slightly lower, and it ranged from 126.4 (special acacia) to 905.7 mg GAE/kg (forest honey) (34). In general, most studies show that the TPC in acacia honey is lower compared to the phenolic content in other analyzed honeys (2, 33-36), which is in agreement with our results.

The correlation between color intensity, TPC, TFC, and antioxidant activity tests is shown in Table 3. A high correlation between color intensity and the TPC (r = 0.815) was found (Table 3). The darker colored honeys are associated with a higher content of phenolic compounds and consequently have a higher antioxidant capacity (Table 1 and Table 2). These results are consistent with Isla et al. (37), who found that color intensity could be related to the pigment content (carotenoids, flavonoids) in honey. Also, these compounds are well-known for their antioxidant properties (9, 27).

A great dietary and prophylactic value of honey is a result of its chemical composition. The main phenolics in honey are flavonoids (flavonols, catechins) and phenolic acids including cinnamic acid derivatives (27, 38). Actually, natural honey is one of highly appreciated and valued flavonoid food sources. Flavonoids may greatly contribute to the total antioxidant activity of honey, bringing positive effects on human health (1). Likewise, the TPC, the lowest TFC (31.64 ± 0.55 mg CE/kg) was measured in acacia and the highest (162 ± 1.22 mg CE/kg) in forest honey. Our results were in accordance with the research of Moniruzzaman et al. (39), who found that the TFC ranged from 14.20 to 156.82 mg CE/kg in rubber tree and sourwood Malaysian honey, respectively. A high correlation between color intensity and the TFC (r = 0.771) was found, as well as a very high correlation between the TPC and TFC (r = 0.967) (Table 3). Moreover, a significant positive linear correlation between color and phenolic or flavonoid content was found in honey from Argentina (37).

The lowest TFC/TPC ratio of 0.08 was determined in acacia honey, whereas the highest, i.e. 0.13, was recorded in meadow and bee pollen enriched honey. Comparable to our results, Bueno-Costa et al. (22) reported that honeys from several regions in Brazil possessed the TFC approximately 10% of the average total phenolic content at the usage of gallic acid and quercetin as markers for the determination of the TPC and TFC, respectively. Additionally, flavonoids also expressed as quercetin equivalents represented 2-10% of the TPC of Turkish monofloral honeys (36).

The antioxidant activities of the tested honeys were analyzed by DPPH and FRAP assays. All the samples showed considerable antioxidant activity using both methods (Table 2). The DPPH free radical scavenging method is offered as the first approach for evaluating the overall hydrogen/electron donating activity of a compound, or other biological sources (40). The range of the DPPH activity was from 0.45 ± 0.09 to 2.75 ± 0.25 mmol TEAC/kg in linden and forest honey, respectively. Meadow and honeydew honey were also highly potent, 2.44 ± 0.23 mmol TEAC/kg and 1.27 ± 0.15 mmol TEAC/kg, respectively. A positive but not significant relationship (p > 0.05) between color intensity and the DPPH (r = 0.649) was determined (Table 3). Even though this correlation is not significant, it is considered a valuable result. Furthermore, a high correlation between the TPC and DPPH (r = 0.824) was found, and a very high correlation between the TFC and DPPH (r = 0.891) was recorded as well. In the same manner, a high correlation between the TPC and DPPH radical-scavenging activity in Spanish honeys was determined (41). The obtained results confirmed that phenolics, especially flavonoids, govern the antiradical potency of honey. The DPPH activity in Polish monofloral honeys varied from 0.2 to 1.2 mmol TEAC/kg, with buckwheat honey being the most active, whereas goldenrod honey was the least active (2). Strawberry tree honey from Italy expressed greater antioxidant potentials (2.0 mmol TEAC/kg) compared to New Zealand manuka honey (0.6 mmol TEAC/kg) (42). Cimpoiu et al. (35) found the highest DPPH antiradical activity in forest honey, whereas the lowest one was found in acacia honey, which is in close agreement with our results.

The FRAP assay determines the reduction of ferric iron (Fe³⁺) to ferrous iron (Fe²⁺) in the presence of antioxidants which are reductants with half-reaction reduction potential more negative (lower) than one of half-reaction Fe²⁺ + e⁻ = Fe³⁺ (43). The measuring of the ferric reducing power showed that the lowest reducing capacity was determined for acacia honey (0.83 ± 0.09 mmol Fe²⁺/kg) while the greatest was obtained for forest honey (6.04 ± 0.63 mmol Fe²⁺/kg). A high correlation between color intensity and FRAP (r = 0.762) was determined. A very high correlation between the TPC and FRAP, as well as the TFC and FRAP (r = 0.950 and 0.981, respectively), was found (Table 3). It indicated that the ferric reducing power of honey was due to phenolic/flavonoid compounds that can reduce Fe³⁺ to Fe²⁺, which is in accordance with the observations of other authors (2, 34). Also, DPPH and FRAP...
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methods were in a very high correlation with each other (r = 0.926). The results, which we obtained for the TPC and antioxidant activity of honey, are consistent with those of other authors who emphasized that they were in the same range as the results of some fruits and vegetables, meaning that the antioxidant capacity of honey could be comparable to theirs (1, 2, 41). The comparison between manuka and Scottish honey indicated that the greatest TPC (1248 mg GAE/kg) and FRAP activity (4.55 mmol Fe²⁺/kg) was reached in amber manuka honey. On the other hand, the lowest phenolic content and FRAP activity were determined in white Portobello Scottish honey, 183 mg GAE/kg and 1.91 mmol Fe²⁺/kg, respectively. A correlation between the TPC and FRAP assay was also found (44). The results of our study revealed that amber honeys possess stronger antioxidant activity than lighter honeys and that there was a significant correlation between honey color and the FRAP antioxidant activity. These findings are consistent with those of Alvarez-Suarez et al. (31) who confirmed a correlation between honey color and the antioxidant activity. There were marked differences in the DPPH and FRAP activity between honeys depending on their botanical origin. Using those methods, multifloral forest, multifloral meadow, followed by honeydew and oregano honey could be specified as those with greatest antioxidative potency, while the lowest activity was attributed to acacia honey. The most active forest honey has the DPPH antiradical potency six times greater, and the FRAP activity seven times greater than that of lowest ranking acacia honey (Table 2). Large differences in the FRAP antioxidant profile of honey was also observed by Beretta et al. (45), who reported acacia and other monofloral honeys to be least active. The FRAP assay was more appropriate for measuring the antioxidant activity of honey due to a higher value of the correlation coefficient between phenolic/flavonoid contents and the FRAP activity in comparison to the degree of correlation between phenolic/flavonoid contents and the DPPH radical scavenging activity (41). This statement is also valid for our results for corresponding correlation coefficients presented in Table 3.

The results of the antimicrobial activity of the selected honey samples against six bacterial isolates from human material examined by the broth microdilution assay are shown in Table 4.

Different types of honey showed notable differences in the antibacterial activity against the tested bacterial strains. Inhibitory effects ranged from 6.25% w/w (oregano honey against E. faecalis isolated from wound swabs and honeydew honey against S. pneumoniae isolated from nasal swab), to greater than 50% w/w (meadow honey against S. aureus isolated from wound swabs). With the exception of highly active honeydew and bee pollen enriched honey against E. faecalis isolated from wound swabs (MBC = 12.5% w/w), the bactericidal concentration of all other tested strains ranged from 25% w/w to >50% w/w. The highest antimicrobial activity was reached for oregano honey against E. faecalis (wound swabs) (MIC/MBC = 6.25/50% w/w), S. pneumoniae (nasal swabs) (MIC/MBC =

Table 4. Antimicrobial activity of honey samples against pathogenic bacterial isolates from human material.

<table>
<thead>
<tr>
<th>Type of honey</th>
<th>Gram-positive bacteria</th>
<th>Gram-negative bacteria</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>S. pneumoniae</td>
<td>S. aureus</td>
</tr>
<tr>
<td>Nasal swabs</td>
<td>Wound swabs</td>
<td>Aspirate</td>
</tr>
<tr>
<td>Acacia</td>
<td>25.0/50</td>
<td>25.0/50</td>
</tr>
<tr>
<td>Linden</td>
<td>25.0/50</td>
<td>50/50</td>
</tr>
<tr>
<td>Oregano</td>
<td>12.5/50</td>
<td>25.0/50</td>
</tr>
<tr>
<td>Lavender</td>
<td>50/50</td>
<td>25.0/50</td>
</tr>
<tr>
<td>Honeydew</td>
<td>6.25/50</td>
<td>25.0/50</td>
</tr>
<tr>
<td>Meadow</td>
<td>25.0/50</td>
<td>&gt;50/50</td>
</tr>
<tr>
<td>Forest</td>
<td>12.5/50</td>
<td>25.0/50</td>
</tr>
<tr>
<td>Bee pollen enriched</td>
<td>50/50</td>
<td>25.0/50</td>
</tr>
</tbody>
</table>

Superscripts ‘a’, ‘b’, ‘c’ and ‘d’ indicate that there is no significant difference (p > 0.05) in columns
12.50/50% (w/w) and *A. baumanii* (aspirate) (MIC/MBC = 12.50/50% (w/w)). Honeydew honey had very strong activity against *S. pneumoniae* (nasal swabs) (MIC/MBC = 6.25/50% w/w) and *E. faecalis* (wound swabs) (MIC/MBC = 12.50/12.50% w/w). Furthermore, honeydew honey exhibited strong activity against *P. aeruginosa* (wound swabs), *E. coli* (aspirate) and *A. baumanii* (aspirate) (MIC/MBC = 12.50/>50% w/w). In addition, bee pollen enriched honey expressed significant activity against *E. faecalis* (wound swabs) (MIC/MBC = 12.50/12.50% w/w). Similar significant antibacterial efficacy was obtained for acacia honey against *A. baumanii* (aspirate) and *E. faecalis* (wound swabs) (MIC/MBC = 12.50/50% w/w; 12.50/>50% w/w, respectively), followed by linden honey against *A. baumanii* (aspirate) (MIC/MBC = 12.50/50% w/w). In addition, *S. aureus* was the least sensitive gram-positive bacterium (wound swabs) (MIC ranged from 25.0% w/w to more than 50% w/w). A significant correlation between the antibacterial activity and other analyzed parameters (color intensity, TPC, TFC and antioxidant activity of honeys), as well as between the antimicrobial activity of particular bacterial strains themselves was not recorded. Table 5 shows the antibacterial activity (MIC/MBC) of the referent antibacterial drugs against pathogenic bacterial strains isolated from human material.

Table 5. Antibacterial activity of the referent antibacterial drugs against pathogenic bacterial strains isolated from human material.

<table>
<thead>
<tr>
<th>Antibacterial drugs</th>
<th>MIC/MBC (mg/mL)</th>
<th>Gram-positive bacteria</th>
<th>Gram-negative bacteria</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td><em>S. pneumoniae</em></td>
<td><em>S. aureus</em></td>
</tr>
<tr>
<td>Erythromycin</td>
<td>0.02&lt;sup&gt;a&lt;/sup&gt;</td>
<td>0.04&lt;sup&gt;b&lt;/sup&gt;</td>
<td>0.04&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>Ciprofloxacin</td>
<td>0.08&lt;sup&gt;c&lt;/sup&gt;</td>
<td>0.08&lt;sup&gt;c&lt;/sup&gt;</td>
<td>0.08&lt;sup&gt;c&lt;/sup&gt;</td>
</tr>
<tr>
<td>Doxycycline</td>
<td>0.08&lt;sup&gt;a&lt;/sup&gt;</td>
<td>0.04&lt;sup&gt;a&lt;/sup&gt;</td>
<td>0.02&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>Gentamicin</td>
<td>0.08&lt;sup&gt;a&lt;/sup&gt;</td>
<td>0.02&lt;sup&gt;a&lt;/sup&gt;</td>
<td>0.02&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
</tbody>
</table>

Superscripts ‘a’, ‘b’ and ‘c’ indicate that there is no significant difference (p > 0.05) in columns.
The most frequently isolated pathogens in health care facilities are coagulase-negative \textit{Staphylococci}, \textit{S. aureus}, \textit{Entercoccus} species, \textit{Candida} species, \textit{E. coli}, and \textit{P. aeruginosa} (50). \textit{S. aureus} is a common bacterium in the study of antimicrobial activity of honey. These bacteria can cause various infections, from the skin and soft tissue infections to pneumonia, meningitis, and endocarditis (13, 50). Most of the undiluted honeys inhibited the growth of \textit{S. aureus} and \textit{Staphylococcus epidermidis} (49). In our study, with the exception of linden (MIC/MBC = 50/50 w/w) and meadow honey (MIC/MBC = >50/> 50 w/w), all the types of honey expressed activity against \textit{S. aureus} (isolated from wound swabs) at 25% dilution (MIC/MBC = 25.0/> 50% w/w) (Table 4).

Honeydew honey expressed strong antibacterial activity and could be used as a potential agent to eradicate multi-drug resistant clinical isolates. Majtan et al. (51) compared the antibacterial activity of honeydew and manuka honey against 20 multi-drug resistant \textit{Stenotrophomonas maltophilia} isolates using the broth dilution method. The MICs for honeydew honey ranged from 6.25% to 17.5%, while those for manuka honey ranged from 7.5% to 22.5%. They also found that phenolic compounds of honeydew honey most likely participate in its strong antibacterial properties. Our findings revealed that honeydew honey expressed strong antibacterial activity analyzed by the broth dilution method, with the MIC value ranging from 6.25% w/w against \textit{S. pneumoniae} (nasal swabs) to 25.0% w/w against \textit{S. aureus} (wound swabs) (Table 4). Polish honeys at 75% dilution expressed antimicrobial activity against all the tested microorganisms (\textit{Bacillus subtilis}, \textit{M. luteus}, \textit{E. coli}, \textit{Proteus myxofaciens}, and \textit{Pseudomonas putida}) analyzed by the well diffusion assay. Moreover, they had the highest activity in concentrations of 5-50% against the most sensitive bacteria (\textit{E. coli} and \textit{P. myxofaciens}). Against \textit{E. coli} for acacia honey in concentrations of 5 and 50%, the inhibition zone diameter varied from 22.0 ± 1.0 to 31.6 ± 0.6 mm, respectively, while honeydew honey showed inhibition ranging from 20.6 ± 1.2 to 30.3 ± 1.5 mm (52). The present study (Table 4) showed that honeydew honey was twice more potent against \textit{E. coli} (MIC = 12.5% w/w) in comparison to acacia honey (MIC = 25.0% w/w).

Makarewicz et al. (53) studied the antimicrobial activity of Polish honeys using well diffusion assays and found that 75% of lavender honey expressed activity against all tested bacterial strains. For this type of honey in concentrations of 5 and 50%, the inhibition ranged from 15.3 ± 0.6 to 27.7 ± 0.6 mm for \textit{E. coli}. Our results showed a weak antibacterial activity of lavender honey against \textit{E. coli} (MIC/MBC = 50/> 50% w/w) (Table 4).

CONCLUSION

The results obtained in this study revealed that honeydew, oregano and forest honey exhibited excellent antibacterial activity against gram-positive and gram-negative bacteria. This observation could be associated with higher concentrations of phenolics and flavonoids in those types of honey. The antioxidant activity of honey is in strict correlation with the presence of efficient oxygen radical scavengers, such as phenolic compounds. This activity probably promotes strong antimicrobial effects of honey, particularly against used multidrug-resistant human pathogenic bacteria. The antimicrobial properties of honey as a topical agent have been well described and documented, and evidence supports its usefulness in wound healing. However, further research can be directed at \textit{in vitro} and clinical evaluation of the effects of honey, which is considered most active in treating skin infections caused by tested bacterial strains. Laboratory studies and/or clinical trials should be needed to determine the efficacy of the topical use of honey as a wound dressing, and honey skincare products in the treatment of wound and skin bacterial infections.

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Conflict of interest

The authors declare no conflict of interest.

REFERENCES


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