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Original research paper

THERAPEUTIC PROPERTIES OF SOME SPECIFIC HONEY TYPES

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Abstract: This paper examines the physicochemical characteristics, as well as the antioxidant, antibacterial, and antiproliferative effects, of several honey types that are commercially available but not typical of Serbia. The analysis included moisture, pH, electrical conductivity, free acidity, and hydroxymethylfurfural (HMF). All tested honey samples met EU regulatory standards. The antioxidant activity was assessed by measuring total phenolic content (TPC) and scavenging activity on diphenylpicrylhydrazyl radicals ($DPPH^{\bullet}$). Forest honey exhibited the highest TPC level (30.6 ± 1.63 mg GAE/100 g), while buckwheat honey had the lowest (14.4 ± 0.75 mg GAE/100 g). This was consistent with the scavenging activity on $DPPH^{\bullet}$, which was the highest in manuka honey and lowest in buckwheat honey. Antibacterial activity was evaluated using microdilution test and minimal inhibitory concentration (MIC) measurements. Manuka honey demonstrated the strongest antibacterial effects against *Staphylococcus aureus* and *S. epidermidis*, with a MIC of 6.25% for both strains. Buckwheat honey also showed notable antibacterial activity against these strains. In terms of antiproliferative activity, manuka honey was the most effective among the tested honey types, with IC_{50} values of 21.9 ± 2.05 mg/mL for cervix cancer cells (HeLa) and 32.5 ± 3.69 mg/mL for MRC-5 cells derived from healthy lung tissue.

Key words: *honey, antioxidant activity, antibacterial activity, antiproliferative activity*

INTRODUCTION

Honey is a natural sweetener made by honeybees, and it has been used for both culinary and medicinal purposes throughout history. Its nutritional profile is influenced by its main components – carbohydrates and water, as well as a variety of minor compounds including organic acids, proteins, amino acids, minerals, vitamins, and others (da Silva, Gauche, Gonzaga & Costa, 2016). The specific content of these components can vary based on factors such as the type of nectar collected, the secretions from flowering plants or excretions of plant-sucking insects, and the conditions of the local climate and soil.

Honey is valued not only for its dietary benefits but primarily for its health-promoting properties, largely attributed to its antioxidant content. The antioxidant benefits of honey are mainly credited to its phenolic compounds

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rather than to ascorbic acid, carotenoids, organic acids, Maillard reaction products, amino acids, or proteins (Estevinho, Pereira, Moreira, Dias & Pereira, 2008). These antioxidant properties can vary depending on the botanical source of the nectar and the geographical origin of the honey (Sakač et al., 2019).

The main polyphenols in honey are phenolic acids and flavonoids, which have beneficial effects against some human degenerative diseases, as listed in the paper of Hossen et al. (2017). For example, buckwheat-specific flavonoids, such as hesperetin and rutin, contribute to the antibacterial activity of buckwheat honey (Dżugan et al., 2020). Also, the presence of some phytochemicals, i.e., flavonoids, phenolic acids, and 1,2-dicarbonyl compounds, is linked to Manuka honey health benefits, implying wound healing, anticancer, antioxidant, and anti-inflammatory effects (Alvarez-Suarez, Gasparrini, Forbes-Hernández, Mazzoni & Giampieri, 2014; El-Senduny, Hegazi, Abd Elghani & Farag, 2021).

Although honey's therapeutic properties significantly rely on its antioxidant nature, they also include other mechanisms and compounds that might be very effective in antibacterial, bacteriostatic, antifungal, antiinflammatory, antiglycemic, antimutagenic, and other honey activities (Alvarez-Suarez, Giampieri & Battino, 2013).

Honey's antibacterial agents destroy bacterial cells through different mechanisms and inhibit biofilm formation and quorum-sensing activities. Furthermore, honey's microflora secretes antibacterial agents to fight pathogenic microorganisms (Khataybeh, Jaradat & Ababneh, 2023). Antibacterial nature of honey is mostly attributed to 1) the high osmolarity and acidity of honey (Kwakman et al., 2010); 2) hydrogen peroxide, which is generated by glucose oxidase-mediated conversion of glucose in honey (Deng et al., 2018); 3) methylglyoxal (Schmidt, Eichelberger & Rohm, 2021); 4) bee defensin-1, a type of antimicrobial bee-derived peptide (Kwakman et al., 2010); and 5) phenolic compounds (Almasaudi, 2021).

Many studies highlight honey's anticancer potential, showing that honey has a chemopreventive effect against various cancer cell lines and tissues in both *in vitro* and *in vivo* models. This activity is attributed to several mechanisms, including cell cycle arrest, apoptosis induction, modulation of oxidative stress, and immunomodulation (Premratanachai & Chanchao, 2014). According to Fernandez-Cabezudo et al. (2013), Manuka honey exhibited several anti-tumour effects and inhibited the growth of several cancer cell lines by approximately 33%. Buckwheat honey also demonstrated an *in vitro* antiproliferative effect (Moskwa et al., 2014). In the study of Sakač et al. (2022), a range of honey types collected from the Western Balkans region were proven to exhibit antiproliferative activity towards breast (MCF7), cervix (HeLa), and colon (HT-29) cancer cells. The highest antiproliferative activity was obtained by linden honey from Fruška gora (Serbia). The mentioned study offered results that were reasonable to compare with other honey types not characteristic of Serbia but commercially available, and some of them are known as very therapeutically potent.

MATERIALS AND METHODS

Collection of honey samples

Six honey samples (mustard, manuka, forest, buckwheat, raspberry, and basil) were purchased from a local health food store, BioUna, in Novi Sad (Vojvodina, Serbia). The samples were stored at room temperature in a dark place until analyses.

Physicochemical parameters

The physicochemical parameters of honey samples (moisture, pH, electrical conductivity, and free acidity) were determined according to the methods of AOAC (2000) and the Harmonised Methods of the International Honey Commission (Bogdanov, 2009).

Hydroxymethylfurfural (HMF) analysis

The extraction procedure was described by Sakač et al. (2022) based on the method of Rufián-Henares and de la Cueva (2008) with some modifications (Petisca, Henriques, Pérez-Palacios, Pinho & Ferreira, 2014). HPLC analysis for determination of HMF was done using a liquid chromatograph (Agilent 1200 series, Agilent Technologies Santa Clara, CA, USA) with a DAD detector and an Eclipse XDB-C18, 1.8 μm, 4.6 × 50 mm column (Agilent) according to the method described by Ariffin, Ghazali and Kavousi (2014) and Tomasini et al. (2012). The column temperature was 30 °C and the injection volume was 2 μL. The mobile phase consisted of two eluents, H_2O (0.1%

HCOOH) (A) and methanol (B). The flow rate was 0.75 mL/min. The isocratic elution was applied with the ratio A:B (90:10, v/v). The total run time was 5 minutes.

Total phenolic content

The method described by Ferreira, Aires, Barreira and Estevinho (2009) was used to determine total phenolic content (TPC) with some modifications. Honey sample (1 g) was dissolved in 20 mL of distilled H_2O. The solution (8 mL) was mixed with 0.5 mL of Folin-Ciocalteu reagent. After 3 minutes, 1.5 mL of 25% sodium carbonate was added to the mixture. The solution was vortexed and left to stand in a dark place at 25 °C for 2 hours. The absorbance of the reaction mixture was mea-sured at a wavelength of 750 nm relative to the blank sample (Shimazu, UV-1800, Kyoto, Japan). Gallic acid (1.25–31.25 mg/mL) was used as the standard for constructing the cali-bration curve, and the TPC was expressed as gallic acid equivalents (GAE) (mg GAE/100 g of honey).

DPPH radical scavenging activity

The scavenging activity on 1,1-diphenyl-2-picrylhydrazyl radicals (DPPH$^{\bullet}$) was determined according to the method described by Hatano, Kagawa, Yasuhara and Okuda (1988). The honey sample (2 g) was dissolved in 10 mL of distilled water, then centrifuged at 3000 × g and filtered. Then, 0.1 mL of each honey solution at various concentrations (25.0, 50.0, 100, 200, 400, and 800 mg/mL) was diluted in 2.9 mL of methanol. To each mixture, 1 mL of a 90 μmol/L methanol solution of DPPH was added. The control was prepared with distilled water instead of honey solution. The reaction mixtures were vortexed and allowed to stand in the dark at 22 ± 1 °C for 1 hour. Absorbance was measured at 517 nm using a spectrophotometer (Shimazu, UV-1800, Kyoto, Japan). The scavenging activity was expressed as IC_{50} value (half maximal inhibitory concentration). The IC_{50} value (mg/mL) was determined as the concentration of the antioxidant needed to reduce 50% of the initial amount of DPPH$^{\bullet}$.

Antibacterial activity

Honey solutions were prepared by dissolving honey in sterile distilled water just before analysis in a series of dilutions (25.0%, 12.5%, 6.25%, 3.125%, 1.56%, and 0.75%). The antibacterial activity was assessed against various bacteria, including gram-negative strains (*Escherichia coli* ATCC 8739, *Escherichia coli* I (clinical strain), and *Proteus mirabilis* (clinical strain)) and gram-positive strains (*Staphylococcus aureus* ATCC 25923, *Staphylococcus epidermidis* (clinical strain), and *Enterococcus faecalis* ATCC 29212).

The minimal inhibitory concentration (MIC) was determined using a modified microdilution method (Szweda, 2017). Bacterial strains were subcultured on nutrient agar slants at 37 °C for 24 hours, and the suspensions of the tested strains were adjusted to the McFarland 0.5 standard (approximately 1.5×10^8 CFU/mL). MIC was assessed by adding 10 μL of a 1% solution of 2,3,5-triphenyl tetrazolium chloride to the wells and incubating at 37 °C for 2 hours. The MIC was identified as the lowest concentration of honey that prevented bacterial growth, indicated by the absence of red formazan colour development.

***In vitro* antiproliferative activity – MTT test**

Human solid tumor cell lines were used to evaluate the antiproliferative effects of tested honey types. The cell lines included estrogen receptor-positive (ER+) human breast adenocarcinoma cell line MCF-7 (ATCC HTB22), human colorectal adenocarcinoma cell line HT-29 (ATCC HTB38), human cervical carcinoma cell line HeLa (ATCC CCL2), and normal fetal lung fibroblasts MRC-5 (ATCC CCL 171).

Cells were cultured in Dulbecco's modified Eagle's medium (DMEM, PAA Laboratories GmbH, Pashing, Austria) with 4.5% glucose, supplemented with 10% fetal calf serum (FCS, Sigma), antibiotics, and antimycotics (Sigma). They were cultured in 25 mL flasks (Costar®) at 37 °C in a 100% humidified at-mosphere with 5% CO_2 (Heraeus). Exponen-tially growing viable cells were used for as-says.

Antiproliferative activity was assessed using the MTT (3-(4,5-dimethylthiazolyl-2)-2,5-diphenyltetrazolium bromide) assay in microwell plates (Mosmann, 1983). This assay measures mitochondrial dehydrogenase activity in viable cells by converting MTT into formazan. Cells were harvested, counted using trypan blue, and seeded into 96-well plates (Costar®) at a density of 5 × 103 cells per well to ensure logarithmic growth. After pre-incubation in com-

plete medium at 37 °C for 24 hours, honey samples were added at five concentrations ranging from 0.5 to 100 mg/mL (10 µL per well). The control was without samples. The incubation lasted 48 h. Three hours before the end of incubation, 10 µL of MTT solution were added to all wells. MTT solution was prepared by dissolving MTT in the medium to obtain 5 mg/mL. The solution was then filtered to sterilize it and to remove any insoluble residue present in some batches of MTT. Acid-isopropanol (100 µL of 0.04 mol/L HCl in isopropanol) was added to all wells and mixed thoroughly to dissolve the dark blue crystals. After a few minutes at room temperature, absorbance was read at 540/690 nm using a spectrophotometer (Multiskan Ascent, Thermo Labsystems, USA). Blank wells contained only com-plete medium and MTT.

Inhibition of growth was expressed as a percent of the control, and the cytotoxicity was calculated according to the formula:

$$(1 - A_{test}/A_{control}) \times 100$$

The IC_{50} value, defined as a dose of compound that inhibits the cell growth by 50% related to the control (untreated) cells, was determined for each tested compound by median effect analysis.

Statistical analyses

The data were processed statistically using the software package XLSTAT 2024 (Lumivero Denver, CO, USA). Results were expressed as mean ± standard deviation. Analysis of variance (ANOVA) and Tukey's HSD test ($\alpha = 0.05$) were used for comparison of samples means.

RESULTS AND DISCUSSION

Physicochemical parameters of honey

Aiming to compare the results of the antioxidant, antibacterial, and antiproliferative activities of different honey types characteristic of The Western Balkans region (Sakač et al., 2022) with honey types available in health food stores in Serbia that are either not characteristic of the region or are less frequently found in local markets, we examined six honey samples (mustard, manuka, forest, buckwheat, raspberry, and basil).

Several indicators of honey quality (moisture content, electrical conductivity, pH, free acidity, and HMF) were investigated, and the results are presented in Table 1.

All of the investigated honey samples fulfilled the criteria for honey quality defined by the Codex Alimentarius Commission (2019).

Table 1.
Physicochemical parameters of different honey types

Honey type	Moisture (%)	pH	Electrical conductivity (µS/cm)	Free acidity (meq/kg)	HMF (mg/kg)
Mustard	19.6 ± 0.14^{c}	4.51 ± 0.04^{c}	463 ± 15.6^{c}	22.7 ± 1.23^{bc}	5.12 ± 0.04^{b}
Manuka	17.0 ± 0.23^{a}	4.12 ± 0.02^{b}	614 ± 7.07^{d}	20.3 ± 0.30^{ab}	9.39 ± 0.11^{c}
Forest	19.0 ± 0.06^{bc}	5.23 ± 0.04^{d}	1008 ± 19.5^{e}	39.3 ± 0.46^{d}	8.12 ± 0.75^{c}
Buckwheat	17.9 ± 0.28^{ab}	3.50 ± 0.03^{a}	308 ± 2.12^{b}	17.3 ± 0.14^{a}	2.46 ± 0.21^{a}
Raspberry	18.2 ± 0.61^{ab}	3.63 ± 0.18^{a}	394 ± 8.49^{a}	39.2 ± 2.19^{d}	5.55 ± 0.23^{b}
Basil	15.0 ± 0.28^{d}	3.36 ± 0.03^{a}	400 ± 9.19^{a}	26.7 ± 1.41^{c}	3.22 ± 0.09^{a}

Means in the same column with different superscript are statistically different ($p \leq 0.05$)

Table 2.
Phenolic content and DPPH radical scavenging activity of different honey types

Honey type	Polyphenols (mg GAE/100 g)	DPPH, IC_{50} (mg/mL)
Mustard	23.8 ± 1.17^{a}	150 ± 3.03^{b}
Manuka	25.2 ± 0.87^{ab}	127 ± 2.02^{a}
Forest	30.6 ± 1.63^{b}	136 ± 2.42^{a}
Buckwheat	14.4 ± 0.75^{c}	331 ± 4.41^{d}
Raspberry	22.7 ± 1.16^{a}	175 ± 3.42^{c}
Basil	26.2 ± 2.23^{ab}	131 ± 2.40^{a}

Means in the same column with different superscript are statistically different ($p \leq 0.05$)
GAE – gallic acid equivalent

Forest honey exhibited a significantly higher value of electrical conductivity (1008 ± 19.5 μS/cm), as expected, due to its known high mineral content (Sakač et al., 2019). This honey belongs to the dark honey types, which are typically characterized by higher conductivity levels (Alqarni, Owayss & Mahmoud, 2016). Conversely, although buckwheat honey is also classified as dark honey, it had the lowest conductivity value (308 ± 2.12 μS/cm), suggesting the possibility that it may not be genuine buckwheat honey.

As HMF is a marker of honey freshness, whose amount of up to 10 mg/kg is naturally present in honey (Alqarni, Owayss & Mahmoud, 2016), it can be concluded that all investigated honey types were considered fresh (Table 1). HMF content was measured in the range of 2.46 ± 0.21 mg/kg (buckwheat honey) to 9.39 ± 0.11 mg/kg (Manuka honey).

Antioxidant potential of honey

Examined honey types significantly differed ($p \leq 0.05$) in TPCs (Table 2) as a consequence of different botanical and geographical origins (da Silva, Gauche, Gonzaga & Costa, 2016). Forest honey possessed the highest TPC level of 30.6 ± 1.63 mg GAE/100 g, comparable to previously measured TPCs in forest honey from the Serbian region (Marić et al., 2021). Forest honey turned out to be the most abundant in polyphenols among acacia, sunflower, forest, polyfloral, lime, and sea buckthorn honey from Romania (Cimpoiu, Hosu, Miclaus & Puscas, 2013).

Contrary, the lowest TPC was registered in the buckwheat honey (14.4 ± 0.75 mg GAE/100 g) (Table 2). The measured TPC in buckwheat honey was unexpected, as this honey type was recognised as rich in antioxidants (Deng et al., 2018; Dżugan et al., 2020), whose major antioxidant properties derive from its phenolic constituents, being effective in reducing reactive oxygen species (ROS) levels (van den Berg et al., 2013). Deng et al. (2018) reported a TPC of 1498 ± 37.3 mg GAE/kg in buckwheat honey, which is approximately ten times higher than the TPC measured in our sample. Dżugan et al. (2020) found approximately 250 mg GAE/100 g TPC in buckwheat honey. This substantial discrepancy raises concerns about the potential adulteration of the buckwheat honey we investigated, underscoring the need for reliable methods to detect such adulteration. Additionally, the study of Deng et al. (2018) documented a phenolic content of 561 ± 2.82 mg GAE/kg in Manuka honey, which is roughly twice the amount observed in our sample (Table 2).

Mustard honey was relatively rare in our market, but its total phenolic content (23.8 ± 1.17 mg GAE/100 g, Table 2) was comparable to the value reported by Trisha et al. (2023), which was 242.12 ± 2.6 mg GAE/kg.

In comparison to our previous study (Sakač et al., 2022), TPCs in the honey samples we investigated were lower than those found in linden, heather, phacelia, basil, sage, chestnut, and lavender honey, which are typical for The Western Balkans region.

Manuka honey is renowned for its high antioxidant capacity, often exceeding that of other nectar kinds of honey such as canola, acacia, or buckwheat honey (Schmidt, Eichelberger & Rohm, 2021). However, the manuka honey sample available in our market did not exhibit the high antioxidant activity reported in the literature (Deng et al., 2018).

The antioxidant activity of the honey samples was assessed using the DPPH test and expressed as IC_{50} values. The IC_{50} values ranged from 127 ± 2.02 mg/mL for Manuka honey to 331 ± 4.41 mg/mL for buckwheat honey (Table 2). The lower scavenging activity of buckwheat honey on $DPPH^{\bullet}$ corresponded with its TPC but was significantly lower than the findings of Ongalbek et al. (2024), who reported high antioxidant activity for buckwheat honey using both $DPPH^{\bullet}$ and 2,2'-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid) radical cation ($ABTS^{\bullet+}$) assays. The antioxidant activity of mustard honey on $DPPH^{\bullet}$ aligned with the results reported by Trisha et al. (2023).

The antioxidant activity exhibited a strong correlation with total phenolic content, with a high correlation coefficient of r = 0.907.

Antibacterial activity of honey

The antibacterial activity of honey is largely attributed to glucose oxidase, an enzyme that catalyses the conversion of glucose into gluconic acid and hydrogen peroxide (Deng et al., 2018). Phenolic compounds also play a role in honey's antibacterial properties (Almasaudi, 2021). Manuka honey stands out from other honey types due to its high antibacterial

capacity, which is primarily attributed to a high level of non-peroxidic compounds (Schmidt, Eichelberger & Rohm, 2021). Methylglyoxal, identified as the main compound responsible for manuka honey's antibacterial effects (Beitlich, Koelling-Speer, Oelschlaegel & Speer 2014), is formed through the non-enzymatic dehydration of dihydroxyacetone present in the nectar (Adams, Manley-Harris & Molan, 2009). Therefore, despite having a relatively low TPC of 25.2 ± 0.87 mg GAE/100 g (Table 2), Manuka honey demonstrated the strongest antibacterial activity among the investigated honey samples (Table 3).

Besides Manuka honey, other tested honey types also exhibited antibacterial activity. The following order of resistance was observed: *Enterococcus faecalis* > *Escherichia coli* = *Escherichia coli* ATCC 8739 > *Proteus mirabilis* > *Staphylococcus aureus* > *Staphylococcus epidermidis*. Gram-positive bacteria were more susceptible to the antibacterial effects of the honey samples. This higher inhibitory effect on gram-positive bacteria can be attributed to differences in their cell wall composition compared to gram-negative bacteria. Specifically, gram-positive bacteria lack an outer membrane, which facilitates easier penetration by antimicrobial agents (Matzen et al., 2018). The observed susceptibility of *S. aureus* and *S. epidermidis* to the examined honey samples can be attributed to the polyphenols in honey. The TPC shows a strong correlation with antibacterial activity against these strains, with correlation coefficients of r = −0.736 for *S. aureus* and r = −0.690 for *S. epidermidis*.

Buckwheat honey has been shown to exhibit relatively high antibacterial activity. The average MIC values were 6.25% against gram-positive bacteria, *S. aureus* and *S. epidermidis* (Table 3). This antibacterial activity is likely linked to its phenolic compounds, which are known to have efficacy against *Staphylococcus aureus* and *Pseudomonas aeruginosa* (Deng et al., 2018). The same authors stated that the antibacterial activity of buckwheat honey is comparable with that of Manuka honey. Both raspberry and basil honeys demonstrated the average MIC values of 12.5% against *Staphylococcus aureus* and *Staphylococcus epidermidis* (Table 3).

Table 3.
Minimum inhibitory concentrations (MIC) of different honey types against tested strains of *Escherichia coli, Escherichia coli* ATCC 8739, *Staphylococcus epidermidis*, *Proteus mirabilis*, *Staphylococcus aureus* ATCC 25923, and *Enterococcus faecalis* ATCC 29212

	MIC % against different strains of bacteria					
Honey type	*E. coli* ATCC 8739	*E. coli*	*Proteus mirabilis*	*S. aureus* ATCC 25923	*S. epidermidis*	*E. faecalis* ATCC 29212
Mustard	25.0	25.0	25.0	12.5	6.25	25.0
Manuka	12.5	12.5	12.5	6.25	6.25	25.0
Forest	25.0	25.0	25.0	25.0	25.0	25.0
Buckwheat	25.0	25.0	12.5	6.25	6.25	25.0
Raspberry	25.0	25.0	25.0	12.5	12.5	25.0
Basil	25.0	25.0	12.5	12.5	12.5	25.0

The determination of MIC was performed in triplicate.

Table 4.
Effects of honey samples on the growth of selected human cell lines

Honey type	**IC_{50} (mg/mL)***			
	HeLa	MCF7	HT-29	**MRC-5**
Mustard	46.5 ± 1.83^{c}	33.9 ± 3.03^{a}	31.7 ± 6.83^{a}	**32.7 ± 4.73^{a}**
Manuka	**21.9 ± 2.05^{a}**	35.7 ± 5.70^{ab}	34.6 ± 7.13^{a}	**32.5 ± 3.69^{a}**
Forest	$> 50^{c}$	$> 50^{b}$	$> 50^{b}$	$> 50^{b}$
Buckwheat	$> 50^{c}$	$> 50^{b}$	$> 50^{b}$	40.3 ± 7.35^{ab}
Raspberry	46.5 ± 1.24^{c}	32.6 ± 5.16^{a}	45.7 ± 1.56^{ab}	42.6 ± 2.26^{ab}
Basil	$> 50^{c}$	$> 50^{b}$	$> 50^{b}$	$> 50^{b}$
Standard glucose	40.0 ± 3.02^{b}	33.2 ± 5.57^{a}	34.0 ± 0.44^{a}	39.8 ± 1.07^{ab}

**Values represent means ± SD of four measurements (n = 4) obtained in 0.15–50 mg/mL concentration range.*
HeLa – HeLa cervical tumour cell line; MCF7 – MCF7 breast cancer cell line; HT-29 – HT-29 human colorectal adenocarcinoma cell line; MRC-5 – MRC-5 human lung tissue cell line

These bacteria were identified as the most sensitive to honey's antibacterial activity, according to Junie, Vică, Glevitzky and Matei (2016). Sakač et al. (2022) found that basil honey was less potent towards bacterial strains compared to the results presented in Table 3. Mustard honey was noted to have antibacterial activity against *S. aureus* (Khatun et al., 2022), and this is in line with our result (Table 3).

Antiproliferative activity of honey

The most potent honey sample in terms of antiproliferative activity was manuka honey (IC_{50}^{HeLa} = 21.9 ± 2.05 mg/mL and IC_{50}^{MRC-5} = 32.5 ± 3.69 mg/mL) towards cervix cancer cells (HeLa) and cells derived from healthy lung tissue (MRC-5) (Table 4). Mustard honey also affected the growth of MRC-5 cells with IC_{50}^{MRC-5} = 32.7 ± 4.73 mg/mL, while buckwheat honey had poor antiproliferative activity, although there is evidence of its potency (Deng et al., 2018). Breast cancer cell line (MCF-7) and human colorectal adenocarcinoma cell line (HT-29) expressed low sensitivity to the evaluated samples. Standard (glucose) had a lower and uniform cell growth effect with IC_{50} values ranging from 33–40 mg/mL towards all evaluated cell lines, indicating that active components in investigated honey types, other than sugars, contributed to cell growth activity.

The cellular antioxidant activity of manuka and buckwheat honey samples was assessed using a cell-based model involving HepG2 cells, which are an epithelial-like cell line derived from a 15-year-old male with hepatocellular carcinoma (Deng et al., 2018). According to Deng et al. (2018), buckwheat honey demonstrated stronger cellular antioxidant activity compared to Manuka honey when tested with HepG2 cells. Also, Moskwa et al. (2014) investigated an anticancer effect of different honeys from Poland (including buckwheat honey) on tumour cell line – glioblastoma multiforme U87MG and concluded that the examined honeys have a potent antiproliferative effect on the U87MG cell line in a time- and dose-dependent manner, being effective at concentrations as low as 0.5%. However, our results (Table 4) did not support the findings reported by the mentioned authors regarding the antitumor potency of buckwheat honey. Manuka honey is renowned not only for its antimicrobial properties (El-Senduny, Hegazi, Abd Elghani & Farag, 2021) but also for its anticancer effects through various metabolic mechanisms (Cianciosi et al., 2020).

It demonstrated anticancer activity against a variety of cancers, including colon cancer (Afrin et al., 2018), breast cancer (MCF-7 cell line) (Wong, Nigam & Owusu-Apenten, 2018), and both lung (A549) and breast (MDA-MB-231) cancers (Aryappalli et al., 2019).

However, our results did not align with these findings regarding the antiproliferative effects of Manuka honey on colon and breast cancer cell lines. Despite this, our manuka honey sample exhibited the strongest antiproliferative effect among the honey types tested, showing significant activity against HeLa and MRC-5 cells (Table 4).

Das et al. (2022) investigated the antiproliferative potential of various Indian honey samples, including mustard honey, on colon cancer cell growth and found that honey exhibited apoptotic activities. In our study, mustard honey purchased from the Serbian market demonstrated weak antiproliferative activity against HT-29 cells. However, it showed effecttiveness in suppressing the viability of MRC-5 cells (Table 4).

Among previously examined honey types collected from the region of the Western Balkans, linden honey from Fruška Gora, heather, anis, and lavender honey demonstrated significantly higher antiproliferative activity towards MCF-7, HeLa, and HT-29 cancer cells (Sakač et al., 2022) in comparison to the results obtained in this study (Table 4).

CONCLUSIONS

The physicochemical characterization and evaluation of antioxidant, antibacterial, and antiproliferative effects were conducted on six different honey types – mustard, manuka, forest, buckwheat, raspberry, and basil purchased from a local health food store in Novi Sad, Vojvodina, Serbia.

All samples met the physicochemical criteria established by EU regulations, including moisture content, pH, electrical conductivity, free acidity, and hydroxymethylfurfural (HMF) levels.

Forest honey possessed the highest TPC level of 30.6 ± 1.63 mg GAE/100 g, while buck-

wheat honey had the lowest (14.4 ± 0.75 mg GAE/100 g). The scavenging activity on DPPH$^{\bullet}$ was the highest for manuka honey (IC_{50} = 127 ± 2.02 mg/mL) and the lowest for buckwheat honey (IC_{50} = 331 ± 4.41 mg/mL).

In terms of antibacterial activity, manuka honey demonstrated the most significant effect against the examined bacterial strains, particularly *S. aureus* and *S. epidermidis*, with a MIC of 6.25% for both strains. Buckwheat honey followed in antibacterial efficacy.

Manuka honey exhibited the most potent antiproliferative activity among the tested honey types, with IC_{50}^{HeLa} = 21.9 ± 2.05 mg/mL for cervix cancer cells (HeLa) and IC_{50}^{MRC-5} = 32.5 ± 3.69 mg/mL for MRC-5 cells derived from healthy lung tissue. Mustard honey also demonstrated notable effects on MRC-5 cells, with an IC_{50}^{MRC-5} = 32.7 ± 4.73 mg/mL. The effectiveness in suppressing the viability of the cancer cell lines is attributed to active components other than sugars present in honey.

AUTHOR CONTRIBUTIONS

Conceptualization, M.B.S.; Methodology, M.B.S. and P.T.J.; Investigation, formal analysis, validation, A.Z.M., D.V.P., and D.S.J.; Writing-original draft preparation, M.B.S., B.B.Đ., and A.Z.M.; Writing-review and editing, M.B.S. and A.Z.M.; Supervision, P.T.J.

DATA AVAILABILITY STATEMENT

Data contained within the article.

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CONFLICT OF INTEREST

The authors declare no conflict of interest. The funders had no role in the design of the study; in the collection, analyses, or interpretation of data; in the writing of the manuscript, or in the decision to publish the results.

REFERENCES

Adams, C. J., Manley-Harris, M., & Molan P. C. (2009). The origin of methylglyoxal in New Zealand manuka (*Leptospermum scoparium*) honey. *Carbohydrate Research*, *344*, 1050–1053. https://doi.org/10.1016/j.carres.2009.03.020053

Afrin, S., Giampieri, F., Gasparrini, M., Forbes-Hernandez, T. Y., Cianciosi, D., Reboredo-Rodriguez, P., Manna, P. P., Zhang, J., Quiles, J. L., & Battino, M. (2018). The inhibitory effect of Manuka honey on human colon cancer HCT-116 and LoVo cell growth. Part 2: Induction of oxidative stress, alteration of mitochondrial respiration and glycolysis, and suppression of metastatic ability. *Food and Function*, *9*, 2158–2170. https://doi.org/10.1039/c8fo00165k

Almasaudi, S. (2021). The antibacterial activities of honey. *Saudi Journal of Biological Sciences*, *28*, 2188–2196. https://doi.org/10.1016/j.sjbs.2020.10.017

Alqarni, A. S., Owayss, A. A., & Mahmoud, A. A. (2016). Physicochemical characteristics, total phenols and pigments of national and international honeys in Saudi Arabia. *Arabian Journal of Chemistry*, *9*, 114–120. https://doi.org/10.1016/j.arabjc.2012.11.013

Alvarez-Suarez, J. M., Gasparrini, M., Forbes-Hernández, T. Y., Mazzoni, L., & Giampieri, F. (2014). The composition and biological activity of honey: A focus on manuka honey. *Foods*, *3*, 420–432. https://doi.org/10.3390/foods3030420

Alvarez-Suarez, J. M., Giampieri, F., & Battino, M. (2013). Honey as a source of dietary antioxidants: Structures, bioavailability and evidence of protective effects against human chronic diseases. *Current Medicinal Chemistry*, *20*, 621–638. https://doi.org/10.2174/092986713804999358

Ariffin, A. A., Ghazali, H. M., & Kavousi, P. (2014). Validation of a HPLC method for determination of hydroxymethylfurfural in crude palm oil. *Food Chemistry*, *154*, 102–107. https://doi.org/10.1016/j.foodchem.2013.12.082

Aryappalli, P., Shabbiri, K., Masad, R. J., Al-Marri, R. H., Haneefa, S. M., Mohamed, Y. A., Arafat, K., Attoub, S., Cabral-Marques, O., Ramadi, K. B., Fernandez-Cabezudo, M. J., & al-Ramadi, B. K. (2019). Inhibition of tyrosine-phosphorylated STAT3 in human breast and lung cancer cells by manuka honey is mediated by selective antagonism of the IL-6 receptor. *International Journal of Molecular Sciences*, *20*, 4340. https://doi.org/10.3390/ijms20184340

Association of Official Analytical Chemists (2000). *Official Methods of Analysis of the AOAC* (17th ed.). Gaithersburg, MD, USA: The Association.

Beitlich, N., Koelling-Speer, I., Oelschlaegel, S., & Speer, K. (2014). Differentiation of manuka honey from kanuka honey and from jelly bush honey using HS-SPME-GC/MS and UHPLC-PDA-MS/MS. *Journal of Agricultural and Food Chemistry*, *62*, 6435–6444. https://doi.org/10.1021/jf501818f

Bogdanov, S. (2009). *Harmonised Methods of the International Honey Commission*. Bern, Switzerland: International Honey Commission. http://www.ihc-platform.net/ihcmethods2009.pdf

Cianciosi, D., Forbes-Hernandez, Y. T., Afrin, S., Gasparrini, M., Quiles, J. L., Gil, E., Bompadre, S., Simal-Gandara, J., Battino, M., & Giampieri, F. (2020). The influence of *in vitro* gastrointestinal digestion on the anticancer activity of manuka honey. *Antioxidants*, *9*, 64.

https://doi.org/10.3390/antiox9010064

Cimpoiu, C., Hosu, A., Miclaus, V., & Puscas, A. (2013). Determination of the floral origin of some Romanian honeys on the basis of physical and biochemical properties. *Spectrochimica Acta Part A: Molecular and Biomolecular Spectroscopy*, *100*, 149–154. https://doi.org/10.1016/j.saa.2012.04.008

Codex Alimentarius Commission. (2019). *Revised Codex Standards for Honey*. Codex Standard 12-1981, Italy, Rome.

Das, N., Ray, N. Patil, A. R., Saini, S. S., Waghmode, B., Ghosh, C., Patil, S. B., Patil, S. B., Mote, C. S., Saini, S., Saraswat, B. L., Sircar, D., & Roy, P. (2022). Inhibitory effect of selected Indian honey on colon cancer cell growth by inducing apoptosis and targeting the β-catenin/Wnt pathway. *Food and Function*, *13*, 8283–8303. https://doi.org/10.1039/D1FO03727G

da Silva, P. M., Gauche, C., Gonzaga, L. V., & Costa, A. C. O. (2016). Honey: Chemical composition, stability and authenticity. *Food Chemistry*, *196*, 309–323. https://doi.org/10.1016/j.foodchem.2015.09.051

Deng, J., Liu, R., Lu, Q., Hao, P., Xu, A., Zhang, J., & Tan, J. (2018). Biochemical properties, antibacterial and cellular antioxidant activities of buckwheat honey in comparison to manuka honey. *Food Chemistry*, *252*, 243–249. https://doi.org/10.1016/j.foodchem.2018.01.115

Dżugan, M., Grabek-Lejko, D., Swacha, S., Tomczyk, M., Bednarska, S., & Kapusta, I. (2020). Physicochemical quality parameters, antibacterial properties and cellular antioxidant activity of Polish buckwheat honey. *Food Bioscience*, *34*, 100538. https://doi.org/10.1016/j.fbio.2020.100538

El-Senduny, F. F., Hegazi, N. M., Abd Elghani, G. E., & Farag, M. A. (2021). Manuka honey, a unique mono-floral honey. A comprehensive review of its bioactives, metabolism, action mechanisms, and therapeutic merits. *Food Bioscience*, *42*, 101038. https://doi.org/10.1016/j.fbio.2021.101038

Estevinho, L., Pereira, A. P., Moreira, L., Dias, L. G., & Pereira, E. (2008). Antioxidant and antimicrobial effects of phenolic compounds extracts of Northeast Portugal honey. *Food and Chemical Toxicology*, *46*, 3774–3779. https://doi.org/10.1016/j.fct.2008.09.062

Fernandez-Cabezudo, M. J., El-Kharrag, R., Torab, F., Bashir, G., George, J. A., El-Taji, H., & al-Ramadi, B. K. (2013). Intravenous administration of manuka honey inhibits tumor growth and improves host survival when used in combination with chemotherapy in a melanoma mouse model. *PLoS ONE*, *8*, e55993. https://doi.org/10.1371/journal.pone.0055993

Ferreira, I. C. F. R., Aires, E., Barreira, J. C. M., & Estevinho, L. M. (2009). Antioxidant activity of Portuguese honey samples: Different contributions of the entire honey and phenolic extract. *Food Chemistry*, *114*, 1438–1443. https://doi.org/10.1016/j.foodchem.2008.11.028

Hatano, T., Kagawa, H., Yasuhara, T., & Okuda, T. (1988). Two new flavonoids and other constituents in licorice root: Their relative astringency and radical scavenging effects. *Chemical and Pharmaceutical Bulletin*, *36*, 2090–2097. https://doi.org/10.1248/cpb.36.2090

Hossen, M. S., Ali, M. Y., Jahurul, M. H. A., Abdel-Daim, M. M., Hua Gan, S., & Khalil, M. I. (2017). Beneficial roles of honey polyphenols against some human degenerative diseases: A review. *Pharmacological Reports*, *69*, 1194–1205. https://doi.org/10.1016/j.pharep.2017.07.002

Junie, L. M., Vică, M. L., Glevitzky, M., & Matei, H. V. (2016). Physico-chemical characterization and antibacterial activity of different types of honey tested on strains isolated from hospitalized patients. *Journal of Apicultural Science*, *60*, 5–17. https://doi.org/10.1515/jas-2016-0013.

Khataybeh, B., Jaradat, Z., & Ababneh, Q. (2023). Antibacterial, anti-biofilm and anti-quorum sensing activities of honey: A review. *Journal of Ethnopharmacology*, *317*, 116830. https://doi.org/10.1016/j.jep.2023.116830

Khatun, M. A., Razzak, M., Hossain, M. A., Hossain, A., Islam, M., Shahjalal, M., Khan, R. A., & Huque, R. (2022). Gamma radiation processing of honey of Mustard, Black seed and Lychee flower: Measurement of antioxidant, antimicrobial, and Fourier transform infrared (FT-IR) spectra. *Measurement: Food*, *6*, 100026. https://doi.org/10.1016/j.meafoo.2022.100026

Kwakman, P. H. S., Velde, A. A. T., de Boer, L., Speijer, D., Vandenbroucke-Grauls, C. M. J. E., & Zaat, S. A. J. (2010). How honey kills bacteria. *FASEB Journal*, *24*, 2576–2582. https://doi.org/10.1096/fj.09-150789

Marić, A., Jovanov, P., Sakač, M., Novaković, A., Hadnađev, M., Pezo, L., Mandić, A., Milićević, N., Đurović, A., & Gadžurić, S. (2021). A comprehensive study of parameters correlated with honey health benefits. *RSC Advances*, *11*, 12434–12441. https://doi.org/10.1039/D0RA10887A

Matzen, R. D., Zinck Leth-Espensen, J., Jansson, T., Nielsen, D. S., Lund, M. N., & Matzen, S. (2018). The antibacterial effect *in vitro* of honey derived from various Danish flora. *Dermatology Research and Practice*, *2018*, 7021713. https://doi.org/10.1155/2018/7021713

Moskwa, J., Borawska, M. H., Markiewicz-Zukowska, R., Puscion-Jakubik, A., Naliwajko, S. K., Socha, K., & Soroczynska, J. (2014). Polish natural bee honeys are anti-proliferative and anti-metastatic agents in human glioblastoma multiforme U87MG cell line. *PLoS ONE*, *9*, e90533. https://doi.org/10.1371/journal.pone.0090533

Mosmann, T. (1983). Rapid colorimetric assay for cellular growth and survival: Application to proliferation and cytotoxicity assays. *Journal of Immunological Methods*, *65*, 55–63. https://doi.org/10.1016/0022-1759(83)90303-4

Ongalbek, D., Tokul-Ölmez, Ö., Şahin, B., Küçükaydın, S., Aydoğmuş-Öztürk, F., Sıcak, Y., Yeskaliyeva, B., & Öztürk, M. (2024). Classification of buckwheat honey produced in Kazakhstan according to their biochemical ingredients and bioactivities by chemometric approach. *Food Chemistry*, *451*, 139409. https://doi.org/10.1016/j.foodchem.2024.139409

Petisca, C., Henriques, A. R., Pérez-Palacios, T., Pinho, O., & Ferreira, I. M. P. L. V. O. (2014). Assessment of hydroxymethylfurfural and furfural

in commercial bakery products. *Journal of Food Composition and Analysis*, *33*, 20–25. https://doi.org/10.1016/j.jfca.2013.10.004

Premratanachai, P., & Chanchao, C. (2014). Review of anticancer activities of bee products. *Asian Pacific Journal of Tropical Biomedicine*, *4*, 337–344. https://doi.org/10.12980/APJTB.4.2014C1262

Rufián-Henares, J. A., & de la Cueva, S. P. (2008). Assessment of hydroxymethylfurfural intake in the Spanish diet. *Food Additive and Contaminants Part A*, *25*, 1306–1312. https://doi.org/10.1080/02652030802163406

Sakač, M., Jovanov, P., Marić, A., Četojević-Simin, D., Novaković, A., Plavšić, D., Škrobot, D., & Kovač, R. (2022). Antioxidative, antibacterial and antiproliferative properties of honey types from the Western Balkans. *Antioxidants*, *11*, 1120. https://doi.org/10.3390/antiox11061120

Sakač, M., Jovanov, P., Marić, A., Pezo, L., Kevrešan, Ž., Novaković, A., & Nedeljković, N. (2019). Physicochemical properties and mineral content of honey samples from Vojvodina (Republic of Serbia). *Food Chemistry*, *276*, 15−21. https://doi.org/10.1016/j.foodchem.2018.09.149

Schmidt, C., Eichelberger, K., & Rohm, H. (2021). New Zealand mānuka honey – A review on specific properties and possibilities to distinguish mānuka from kānuka honey. *LWT-Food Science and Technology, 136*, 110311. https://doi.org/10.1016/j.lwt.2020.110311

Szweda, P. (2017). Antimicrobial activity of honey. In V. de Alencar Arnaut de Toledo (Ed.), *Honey Analysis* (pp. 215–232). IntechOpen. https://doi.org/10.5772/67117

Tomasini, D., Sampaio, M. R. F., Caldas, S. S., Buffon, J. G., Duarte, F. A., & Primel, E. G. (2012). Simultaneous determination of pesticides and 5-hydroxymethylfurfural in honey by the modified QuEChERS method and liquid chromatography coupled to tandem mass spectrometry. *Talanta*, *99*, 380–386. https://doi.org/10.1016/j.talanta.2012.05.068

Trisha, S., Mortuza, M. G., Rana, J., Islam, K. H., Ferdoush, Z., Antora, R. A., Akash, S. I., Aziz, M. G., & Uddin, M. B. (2023). Evaluation of the physicochemical qualities and antioxidant properties of some Bangladeshi varieties of honey: A comparative study. *Journal of Agriculture and Food Research*, *14*, 100837. https://doi.org/10.1016/j.jafr.2023.100837

van den Berg, A. J. J., van den Worm, E., Quarles van Ufford, H. C., Halkes, S. B. A., Hoekstra, M. J., & Beukelman, C. J. (2013). An *in vitro* examination of the antioxidant and anti-inflammatory properties of buckwheat honey. *Journal of Wound Care*, *17*, 4. https://doi.org/10.12968/jowc.2008.17.4.28839

Wong, L. Y., Nigam, P. S., & Owusu-Apenten, R. (2018). Effect of iron and hydrogen peroxide supplementation on the total phenols content and cytotoxicity of honey for MCF-7 breast cancer cells. *Journal of Advances in Biology & Biotechnology*, *18*, 1–10. https://doi.org/10.9734/JABB/2018/42344

TERAPEUTSKA SVOJSTVA NEKIH SPECIFIČNIH VRSTA MEDA

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Sažetak: Ovaj rad ispituje fizičkohemijska svojstva, kao i antioksidativne, antibakterijske i antiproliferativne efekte nekoliko vrsta meda koji su komercijalno dostupni, ali nisu karakteristični za Srbiju. Analizirani su parametri kao što su vlaga, pH, električna provodljivost, slobodne kiseline i sadržaj hidroksimetilfurfurala (HMF). Svi ispitivani uzorci meda ispunili su standarde EU regulative. Antioksidativna aktivnost meda određena je merenjem sadržaja ukupnih fenola (TPC) i antiradikalske aktivnosti na difenilpikrilhidrazil radikale (DPPH$^{\bullet}$). Šumski med je imao najviši nivo TPC (30,6 ± 1,63 mg GAE/100 g), dok je med od heljde karakterisao najniži sadržaj (14,4 ± 0,75 mg GAE/100 g). Ovi rezultati su u korelaciji sa antiradikalskom aktivnošću na DPPH$^{\bullet}$, koja je bila najviša za manuka med, a najniža u slučaju meda od heljde. Antibakterijska aktivnost određena je korišćenjem mikrodilucionog testa i merenjem minimalne inhibitorne koncentracije (MIC). Manuka med je ispoljio najjaču antibakterijsku aktivnost protiv *Staphylococcus aureus* i *S. epidermidis*, sa MIC vrednošću od 6,25% za oba soja. Med od heljde je takođe pokazao značajnu antibakterijsku aktivnost protiv ovih sojeva. Pri određivanju antiproliferativne aktivnosti, manuka med je bio najefikasniji među testiranim vrstama meda, sa IC_{50} vrednostima od 21,9 ± 2,05 mg/ml za ćelije raka grlića materice (HeLa) i 32,5 ± 3,73 mg/ml za MRC-5 ćelije iz zdravog plućnog tkiva.

Ključne reči: *med, antioksidativna aktivnost, antibakterijska aktivnost, antiproliferativna aktivnost*

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