Antimicrobial effectiveness of chestnut honey, pollen and propolis individually and in combination

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Abstract

The emergence of bacteria with antibiotic resistance and multiple resistance is characteristic of animal and human pathogens. It is wide known that bee products, which have been used in alternative medicine since ancient times, have antimicrobial potential. Application of bee products for therapeutic purposes is defined as apitherapy. The study aimed to evaluate the antimicrobial activity of commercial chestnut honey, pollen and propolis produced in western Bosnia and Herzegovina (Sanski Most) individually and in five combinations (apimixtures). The antimicrobial properties of samples were investigated using the agar well diffusion method against three Gram-positive bacteria (*Bacillus subtilis* subsp. *spizizenii* ATCC 6633, Methicillin-resistant *Staphylococcus aureus* ATCC 33591, *Enterococcus faecalis* ATCC 29212); three Gram-negative bacteria (ESBL producing *Escherichia coli* ATCC 35218, *Salmonella enterica* subsp. *enterica serovar* Enteritidis ATCC 13076, *Pseudomonas aeruginosa* ATCC 9027) and one fungal species (*Candida albicans* ATCC 10231). Pure bee pollen inhibited the growth of only Gram-negative bacteria, concentrated chestnut honey was active against all Gram-negative and Gram-positive bacteria, while 20% propolis extract and apimixtures A2 (80% honey and 20% propolis) and A3 (60% honey, 20% pollen and 20% propolis extract) inhibited the growth of all tested microorganisms. Chestnut honey and three apimixtures (A1, A2 and A3) showed the highest antibacterial action against all tested Gram-negative bacteria and MRSA compared to other investigated samples.

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In this study, examined honeybee products from Bosnia and Herzegovina and their mixtures had significant activity against tested bacteria, including strains with proven resistance to conventional antibiotics, MRSA and ESBL producing *E. coli*.

Key words: *bee products; apimixture; antibiotic; inhibition; resistance*

Introduction

The discovery of antibiotics and their use in treatment have saved numerous human lives. However, the use of antibiotics also led to the appearance of microorganisms that possess or develop resistance to them and currently, more than 20,000 potential resistant genes are known in sequenced bacterial genomes (Baloch et al., 2020). According to the World Health Organization (WHO, 2021), antimicrobial resistance is one of the most serious issues for global human health. The problem of antimicrobial resistance requires a different approach in the treatment of infectious diseases, where natural products can find their application. Bee products have been used in a traditional medicine for treating and preventing illnesses for centuries.

The concept of using bee products for medical purposes is known as apitherapy. Apitherapy involves the use of bee secretions (wax, venom, royal jelly) and bee products created by the modification of plant-derived materials (honey, propolis bee pollen, and bee bread – Perga) (Al Naggar et al., 2021). These natural products have numerous biological activities that are beneficial for human health, such as antioxidant, antimicrobial, antiviral, anti-inflammatory, and antitumour activity (Kolayli and Keskin, 2020). Since the first scientific report of the antimicrobial properties of honey by Van Ketel in 1892 (Molan, 1992), the antibacterial activity of honey has been described in numerous studies (Albaridi, 2019; McLoone et al., 2020; Almasaudi, 2021; Majtan et al., 2021). Honey is essentially a supersaturated sugar solution and its antimicrobial ability is a result of the synergy between different factors including acidity, high osmotic pressure, presence of phenolic acids, lysozymes, flavonoids, polyphenols, and methylglyoxal (Dumitru et al., 2022).

However, yeasts and moulds or some sporogenic bacteria can get into honey during the production process. Therefore, honey intended for medical use must be sterilised with gamma rays (Yupanqui Mieles et al., 2022), without any negative impact on its antimicrobial abilities. Bee pollen is a mixture of pollen grains collected from different plant species, nectar and bee saliva. Although there are about 250 substances that have been identified from different plant species (Komosinska-Vassev et al., 2015), the antimicrobial properties of bee pollen could be mainly attributed to the flavonoids and phenolic compounds in its composition (Illie et al., 2022). Like honey, pollen also can contain harmful components such as bacterial and fungal toxins, heavy metals, pesticides and allergens. Propolis is a resinous bee product that represents a mixture of plant exudates, bee secretions and wax. A systemic review that included research data on 600 different bacterial strains confirmed the antibacterial potential of propolis, which could primarily be explained by the content of flavonoids and phenolic compounds (Przybyłek and Karpiński, 2019). The antibacterial potential of honey, propolis and pollen are influenced by their geographical and floral origins.

The aim of this study was to determine whether chestnut honey, pollen and propolis, originating from the territory of Bosnia and Herzegovina, independently possess antimicrobial potential and whether the spectrum of their antimicrobial activity increases when they are combined.

Material and methods

Materials

The antimicrobial properties of chestnut honey, pollen and propolis (produced by BeeJapa, Sanski Most, Bosnia and Herzegovina) were tested against Gram-positive bacteria: *Bacillus subtilis subsp spizizenii* ATCC 6633, *Staphylococcus aureus* subsp. *Aureus* ATCC 33591 (MRSA strain), *Enterococcus faecalis* ATCC 29212, Gram-negative bacteria: ESBL producing *Escherichia coli* ATCC 35218, *Salmonella enterica* subsp. *Enterica serovar* Enteritidis ATCC 13076, *Pseudomonas aeruginosa* ATCC 9027, and one pathogenic fungus species: *Candida albicans* ATCC 10231. According to the manufacturer, propolis is a 20% ethanol extract while the pollen is multifloral.

Chemical analysis

Parameters determining the quality of honey (water, acidity, hydroxy methyl furfural-HMF, ash in honey, fructose content, glucose content, sucrose content and electrical conductivity) were tested using a methodology in accordance with the Ordinance on methods for the control of honey and other bee products (Official Gazette of BiH, 2019a).

Values of pesticides such as hexachlorocyclohexane (HCH) alpha isomer, hexachlorocyclohexane (HCH) beta isomer, lindane, heptachlor, endrin, endosulfan, dieldrin, DDT and methoxychlor were determined using a GC/ECD in house method UP-5-04.02/25 (Službeni glasnik BiH, 2019b). The AAS internal method was used to detect the presence of heavy metals, copper (Cu) and iron (Fe) (Službeni glasnik BiH, 2016). Propolis analyses include the determination of the percentage of dry matter and the presence of mechanical impurities.

Sample preparation

To investigate the antimicrobial potential of chestnut honey, pollen and propolis, the individual effect of each product was tested. Further, the antimicrobial potential of specially prepared combinations of these bee products was also analysed (Table 1). Honey and propolis were added with a pipette from the original packaging, while pollen grains were previously crushed in a sterile ceramic container with a pestle. The required amount of crushed pollen was measured on a technical scale and carefully added to numbered test tubes. The apimixtures in the test tubes were homogenised by vortexing and kept on a VWR DS 500 orbital shaker for 24 hours.

Table 1. Composition of the prepared apimixtures used in the study

Sample	Honey (%)	Pollen (%)	Propolis extract (%)
A1	80	20	0
A2	80	0	20
A3	60	20	20
A4	0	50	50
A5	0	30	70

Antimicrobial assays

The agar well diffusion method (Nigussie et al., 2021) was used to evaluate the potential antibacterial and antifungal activities of the tested bee products and apimixtures. The bacterial strains were cultured on Mueller Hinton (MH) medium, while the fungal strain was cultured in Sabouraund Dextrose Agar (Sigma-Aldrich, USA) overnight at 37°C. Afterward, following the recommendations of EUCAST (2022), each microbial strain was adjusted and prepared as inoculum at a concentration of 1–2 x 10^8 CFU/mL to obtain a uniform homogeneous turbidity corresponding to 0.5 McFarland. Inoculation was performed using a sterile cotton swab soaked in a suspension of the test microorganism. After inoculation, plates were drilled using a sterile borer and in every well 50 µL of the analysed sample was added. The plates were left for one hour at room temperature to achieve diffusion.

Petri dishes are lined with parafilm tape to prevent possible evaporation of active substances. The plates were incubated at 37°C for 24 hours. Antibiotics Colistin (10 µg), Streptomycin (10 µg), Ampicillin (10 µg) and Amoxicillin (25 µg), and the antifungal drug Nystatin (100 IU) (all by Oxoid™, Great Britain), were used as a positive control. Antimicrobial activity of selected bee products and apimixtures were evaluated based on the diameter of inhibition zones. All tests were done in triplicate and the mean value of the inhibition zones was calculated.

Statistical analysis

All experiments were repeated in triplicate and the results were expressed as mean ± SD (standard deviation) using Microsoft Office 2019 Excel (Microsoft Corporation, USA). One-way ANOVA ($P<0.05$ and $P<0.01$) and Tukey's multiple comparisons test were calculated using software STATISTICA 10 (StatSoft.Inc).

Results

Chemical analysis

Chemical analyses of chestnut honey included quality testing for the presence of heavy metals and pesticides in honey. The results of the analysis of chestnut honey quality are presented in Table 2.

Heavy metals copper (Cu) and iron (Fe) in honey were detected at concentrations of 0.25 mg/kg and 1.73 mg/kg, re-

Table 2. Results of chemical analysis for chestnut honey quality parameters

Parameter	Determined value	Reference val	Unit of measure
Water	18.45	Max 20	%
Acidity	10.50	Max 50	mmol/kg
Hydroxy methyl furfural – HMF	9.22	Max 40	mg/kg
Ash in honey	0.59	Max 0.6	%
Fructose content	42.21	Sum glucose and fructose Min 60	%
Glucose content	30.17	Sum glucose and fructose Min 60	%
Sucrose content	1.92	Max 8	%
Electrical conductivity	1.35	Min 0.8	mS/cm

Table 3. Results of chemical analyses of pollen quality parameters

Parameter	Determined value	Reference value	Unit of measure
Water	20.18	NP	%
Dry matter	79.82	Min. 60	%
Total protein	23.65	NP	%
Grease	5.20	NP	%
Mineral content (ash)	5.13	NP	%
Carbohydrates	45.74	NP	%
Sugars	38.64	NP	%
Fibre	7.00	NP	%
Salt content (NaCl)	0.10	NP	%
Energy value	1359/324	NP	kJ/Kcal

NP – Not prescribed by legislation

spectively. Regarding pesticide residues in honey, hexachlorocyclohexane (HCH) beta isomer, lindane, heptachlor, endrin, endosulfan and dieldrin were all detected at concentrations of <0.005 mg/kg, Hexachlorocyclohexane (HCH) alpha isomer and Metaxichlor <0.01 mg/kg, while DDT was detected at a concentration <0.025 mg/kg. In addition to honey, the quality of pollen was also examined by chemical analyses (Table 3).

Antimicrobial assays

The inhibitory effects of chestnut honey, pollen, propolis extract and specially prepared apimixtures were tested and the results, expressed as zone of inhibition (mm), are presented in Figures 1, 2 and 3, and in Table 4.

B. subtilis was most susceptible to the propolis extract and then to sample A5 (mixture of propolis and pollen). Of the positive controls, only the antibiotic ampicillin formed a slightly larger inhibition zone than the propolis extract. Compared to streptomycin, only propolis had a slightly larger zone of inhibition. In the case of *E. faecalis*, sample A5 showed the strongest antimicrobial activity, followed by the propolis extract, where the measured inhibition zones of these two samples were higher than the antibiotics tested. Colistin had no inhibitory effect against *E. faecalis* in contrast to the apimixtures and the inhibitory zone created by the action of streptomycin was smaller than the zone of action of propolis, and of samples A2, A3 and A5. Chestnut honey and apimixtures containing honey showed marked antimicrobial activity against methicillin-resistant *S.aureus* compared to other Gram-positive bacteria. The sizes of the inhibitory zones of honey and samples A1, A2 and A3 were much higher than the other examined samples. MRSA showed resistance to all three tested antibiotics. Pure pollen did not show an inhibitory effect against any of the tested Gram-positive bacteria. The largest inhibition zones among Gram-negative bacteria were determined for *S. enterica* caused by pure honey (Figure 1a), and apimixtures containing honey (A1, A2 and A3), which were significantly higher compared to the tested antibiotics.

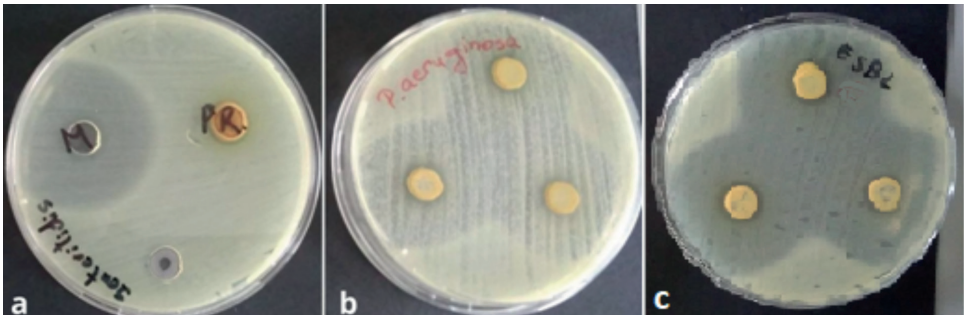

Figure 1. Inhibition zones of a) *S. enterica* formed by pure chestnut honey (M), propolis extract (PR) and pollen (•), b) *P. aeruginosa* caused by sample A1, c) ESBL-producing *E. coli* caused by sample A1

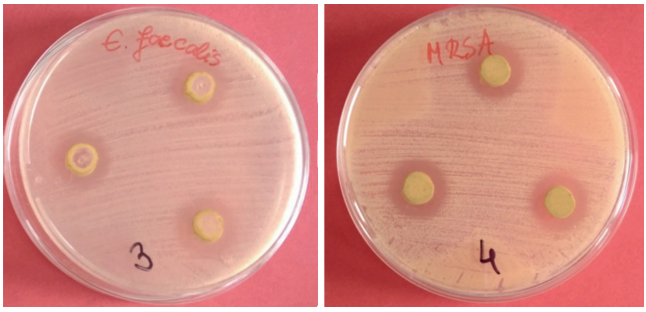

Figure 2 and 3. Inhibition zones of *E. fecalis* formed by sample A3 and inhibition zones of MRSA caused by sample A4

The antibacterial activity of chestnut honey on *S. enteric* was stronger than on MRSA.

Propolis extract showed the weakest bactericidal effect on this species, though it was stronger than the antibiotics ampicillin and colistin. The ESBL-producing strain of *E. coli* was the most sensitive to sample A1 (Figure 1c), followed by 100% honey and then samples A2 and A3. Pollen had the next largest inhibitory zone, and was more effective than propolis. This ESBL-producing strain of *E. coli* had the highest resistance to sample A4, and the antibiotic ampicillin and the apimixture A5 did not show any effect. Sample A4 formed an inhibitory zone equal to the zone of streptomycin. For all bee products, as well as other apimixtures that acted on ESBL *E. coli*, the inhibition zones were larger compared to the other two antibiotics. For the bacterium *P. aeruginosa*, sample A1 (Figure 1b), pure honey showed the greatest antibacterial effects, while samples A2 and A3 produced slightly smaller inhibitory zones. Streptomycin and colistin were more effective against this bacterium than bee pollen and samples A4 and A5, while only colistin was more effective than the propolis extract. Regarding the fungus *Candida albicans*, propolis extract and samples containing propolis exhibited the best antifungal activity. The inhibition zones caused by propolis and sample A5 were larger than those recorded for the antimycotic nystatin. *C. albicans* was

Table 4. Antimicrobial activity of used bee products and their combinations

		Microorganisms						
		B. subtilis	*E. faecalis*	MRSA	*S. enterica*	*P. aeruginosa*	ESBL *E. coli*	*C. albicans*
Bee products (mm)	**H**	11.50± 0.50 **b,c,d,e,f,h,i,j,k *g	13.00 **b,c,e,f,h,i,j,k	40.50±0.50 ** b,c,g,h,i,j	45.00 **b,c,d,e,f,g,h,i,j	37.00 ** b,c,e,f,g,h,i,j,k	39.00 ** b,c,d,e,f,g,h,i,j,k	NI **c,e,f,h,l
	BP	NI **a,c,d,e,f,g,h,i,k	NI **a,c,d,e,f,g,h,i,j	NI ** a,c,d,e,f,g,h	23.00 **a,c,d,e,f,g,h,i,j,k	13.00 **a,c,d,e,f,i,k *j	18.50±0.50 ** a,c,d,e,f,g,h,i,j,k	NI **c,e,f,h,l
	P	20.50±0.50 **a,b,d,e-f,g,h,i,k	22.50± 0.50 **a,b,d,e,f,g,h,i,j,k	23.50±0.50 **a,b,d,e,f,g,h,i,j	14.00 **a,b,d,e,f,g,h,i,j,k	15.00 **a,b,d,e,f,g,h,i,k *j	14.00 ** a,b,d,e,f,g,h,i,j,k	26.00 **a,b,d,e,f,g,l
Apimixtures (mm)	**A1**	12.83±0.29 **a,b,c,e,f,g,h,i,j,k	12.17± 0.29 **b,c,e,f,g,h,i,j,k	40.00 ** b,c,g,h,i,j	41.67±0.58 **a,b,c,e,f,g,h,i,j,k	37.33±0.58 ** b,c,e,f,g,h,i,j,k	44.00 ** a,b,c,e,f,g,h,i,j,k	NI **c,e,f,h,l
	A2	16.33±0.58 **a,b,c,d,g,h,i,j,k	17.33±0.58 **a,b,c,d,f,g,h,i,j,k	40.67±0.58 ** b,c,g,h,i,j	40.00 **a,b,c,d,g,h,i,j,k	35.67±0.58 ** a,b,c,d,g,h,i,j,k	34.33±0.58 ** a,b,c,d,g,h,i,j,k	18.67±0.58 **a,b,c,d,f,g,h,l
	A3	17.17±0.29 **a,b,c,d,g,h,i,j,k	15.67±0.58 **a,b,c,d,e,g,h,i,k	40.17±0.29 ** b,c,g,h,i,j	40.00 **a,b,c,d,g,h,i,j,k	35.00 ** a,b,c,d,g,h,i,j,k	34.00 ** a,b,c,d,g,h,i,j,k	17.00 **a,b,c,d,e,g,h,l
	A4	10.33±0.57 **b,c,d,e,f,h,i,j *a	13.50± 0.50 **a,b,c,d,e,f,h,i,j,k	11.50± 0.50 ** a,b,c,d,e,f,,h,i,j,k	16.33±0.58 **a,b,c,d,e,f,h,i,j,k	12.67±0.58 ** a,c,d,e,f,i,j,k	12.00 ** a,b,c,d,e,f,h,i,k	NI **c,e,f,h,l
	A5	19.00 **a,b,c,d,e,f,g,i,j,k	25.00 **a,b,c,d,e,f,g,i,j,k	22.00 **a,b,c,d,e,f,g,i,j,k	24.00 **a,b,c,d,e,f,g,i,j,k	12.83±0.29 ** a,c,d,e,f,i,j,k	NI ** a,b,c,d,e,f,g,j,k	25.67±0.58 **a,b,d,f,g,l
Antimicrobials (mm)	**Amp**	22.00 **a,b,c,d,e,f,g,h,k	9.00 **a,b,c,d,e,f,g,h,j,k	NI ** a, b,c,d,e,f,,h,g,	NI **a,b,c,d,e,f,g,h,j,k	NI ** a,b,c,d,e,f,g,h,j,k	NI ** a,b,c,d,e,f,g,j,k	–
	Str	20.00 **a,b,d,e,f,g,h,i,k	15.00 **a,b,c,d,e,g,h,i,k	NI ** a, b,c,d,e,f,g,h,	15.00 **a,b,c,d,e,f,g,h,i,k	12.00 ** a,d,e,f,g,h,i,j,k *b,c	14.00 ** a,b,c,d,e,f,h,i,j,k	–
	Col	10.00 **a,b,c,d,e,f,h,i,j	NI **a,c,d,f,g,h,i,j	NI ** a, b,c,d,e,f,g,h	11.00 **a,b,c,d,e,f,g,h,i,j	10.00 ** a,b,c,d,e,f,g,i,j,k	17.00 ** a,b,c,d,e,f,g,h,i,j	–
	Nys	–	–	–	–	–	–	20.67±0.58 **a,b,c,d,e,f,g,h

***Note:** The data are given as mean ± standard deviation (SD) of triplicate experiments. H- honey, BP-bee pollen, P, propolis extract, Amp- ampicillin, STR – streptomycin, Col – colistin, Nys – nystatin, NI = no inhibition. The statistical comparison between values from the different samples and the bacterial strains was done using One-way ANOVA (**$P<0.01$ and *$P<0.05$) and Tukey's multiple comparisons test. Statistically significant differences between rows (from a to k) are shown by different superscripts

not susceptible to chestnut honey, pollen, or samples A1 and A4. By comparing the effects of propolis and samples A2 and A3 (Table), it can be seen that apimixtures had a stronger action against MRSA, *S. enterica*, *P. aeruginosa* and ESBL *E. coli*, while propolis extract showed a stronger effect on the other tested microorganisms.

Discussion

The quality of bee products originating from the northwest Bosnia and Herzegovina for the all tested parameters correspond to the Ordinance on honey and other bee products (Official Gazette BiH, 2019a), the Ordinance on the maximum permitted amounts for certain contaminants in food (Official Gazette BiH, 2016) and the Ordinance on maximum levels of pesticide residues in food and animal feed of plant and animal origin (Official Gazette BiH, 2019b). Concentrated chestnut honey, bee pollen and 30% propolis ethanolic extract, as well as their combinations were examined for antimicrobial activity. Undiluted honey showed antibacterial potential that varied among different tested bacterial strains. Honey formed the largest zone of inhibition for the Gram-negative *Salmonella enteritidis*, followed by Gram-positive methicillin-resistant *S. aureus*. The literature indicates that chestnut honey shows pronounced activity against *S. enterica* (Karadal et al., 2018). The other two Gram-negative bacteria (*P. aeruginosa* and ESBL producing *E. coli*) had slightly smaller inhibition zones compared to MRSA, and significantly larger inhibition zones compared to other Gram-positive bacteria (*B. subtilis* and *E. faecalis*). The least pronounced effect of chestnut honey was on sporogenic bacteria *B. subtilis*, while the fungus *C. albicans* showed no susceptibility.

Other studies have also demonstrated the antibacterial capacity of chestnut honey (CH) from different geographical locations and at different concentrations against reference and clinical strains, although there is some debate about the effective concentration of honey. Küçük et al. (2007) investigated 50% methanol extracts of CH produced in Anatolia (Turkey) that showed moderate inhibition, where *Helicobacter pylori* and *S. aureus* were the most affected. Concerning Spanish CH (Combarros-Fuertes et al., 2018), *S. aureus* strains including MRSA (MIC= 0.05 g/mL) were the most sensitive, whereas *E. coli* strains (MIC=0.20 g/mL) were the most resistant, though significant differences between Gram-positive and Gram-negative bacteria were not observed for all samples. Also, CH produced on Mount Etna (Sicily, Italy) confirmed the strong antibacterial properties on *S. aureus*, *E. coli*, *P. aeruginosa* and *E. faecalis* (Ronsisvalle et al., 2019). Croatian CH diluted with sterile saline solution at 50% and 75% concentration showed inhibitory potential against *H. pylori* (Cviljević et al., 2020). Another tested CH produced in Bosnia and Herzegovina in Cazin (Sakač et al., 2022) diluted with distilled water exhibited stronger antibacterial activity against *S. aureus* and *S. epidermidis* (MIC=12.5%) compared to *Proteus mirabilis*, *E. faecalis* and *E. coli* strains (MIC=25%).

Honey also has an inhibitory effect on bacterial cell to cell communication. Truchado et al. (2009) investigated the anti-quorum sensing properties of 29 honey samples from 14 different floral origins against *Cromobacterium violaceum* and found that chestnut and linden honeys showed the best inhibition of quorum sensing. Portuguese CH demonstrated antibiofilm activity, when the *E. coli* and *P. aeruginosa* biofilms were treated with a 50% (w/v) honey (Oliveira et al., 2019). Chestnut honey produced in Hungary

was very potent against *S. epidermidis* and MRSA (MIC=10%), while *P. aeruginosa* required the higher concentration of honey (MIC=12.5%) to inhibit growth and the antibiofilm activity was the most remarkable, inhibiting the most sensitive *S. epidermidis* by 71.1% (Balázs at al., 2023).

In the present study, pure powdered bee pollen inhibited the growth of tested Gram-negative bacteria, but did not show any action against Gram-positive bacteria or *C. albicans*. In contrast, numerous reports have shown that Gram-negative bacteria are less sensitive to bee pollen (BP) extracts than Gram-positive ones (Abouda et al., 2011; Morais et al., 2011; Karadal et al., 2018; Bridi et al., 2019; Illie et al., 2022). However, a Turkish BP ethanol extract in tested concentrations from 6.25 to 100 mg/mL did not show detectable inhibition of *S. aureus*, *E. coli* and *P. aeruginosa* (Kahraman et al., 2022). Also, Slovenian BP diluted in saline solution was strongly active against *E. coli*, substantial against *C. jejuni*, and negligible against *L. monocytogenes* (Šimunović et al. 2019). The discrepancy between the data may be the result of differently prepared pollen samples. In other studies, antimicrobial activity of BP dissolved in ethanol, methanol or saline solution was examined and that could explain variable results. This is in agreement with the conclusion of Abdelsalam et al. (2018) that antimicrobial activity of BP differed according to the solvent used.

In the recent study, pollen mixed with honey and propolis showed inhibitory effects on Gram-positive bacteria and also on *C. albicans*, except samples A1 and A4. Antimicrobial effects also depend on the concentration and the type of pollen tested. According to Fatrcová-Šramková et al. (2013), the most sensitive bacteria of a poppy pollen ethanol extract was *S. aureus*, while the most sensitive bacteria of rapeseed pollen methanol extract and sunflower ethanol extract was *S. enterica*. Bridi et al. (2019) indicated that the evaluation of the bee pollen antimicrobial activity depends on the method applied and during MIC analysis, bioactive compounds in extracts come into contact more easily with microbial strains than in disc diffusion assays, but are not as reproducible. In Bosnia and Herzegovina, propolis is mainly obtained from the exudates of *Populus* buds. The examined 20% propolis extract showed a broad range of antimicrobial activity. The largest inhibition zone was recorded for the fungus *C. albicans*. The greater activity of propolis against Gram-positive compared to Gram-negative bacteria is in accordance with the literature data (Pobiega et al., 2019; Przybylek and Karpinski, 2019). Of all the tested bacteria, MRSA was the most sensitive to propolis extract. Regarding the propolis extracts, their antibacterial activity depends on extract concentration, type of propolis and type of bacteria tested.

In addition to propolis, samples A2 and A3 had a very broad spectrum of antimicrobial activity. Sample A2 had slightly better effects on MRSA compared to honey, as did sample A5 on *E. faecalis* compared to propolis extract. Although it did not show any effect against *C. albicans*, it is important to emphasise the antibacterial effects of sample A1. The most sensitive bacteria to the action of sample A1 was ESBL *E. coli*. This apimixture was effective against all tested bacteria, and its activity was stronger than honey on ESBL *E. coli* and *P. aeruginosa*. The significant antimicrobial potential of this apimixture should be emphasised in the context of the tested resistant bacterial strains, MRSA and ESBL *E. coli*.

Conclusions

All individually tested bee products from Bosnia and Herzegovina and their specially prepared combinations showed good antimicrobial potential, and this potential was increased in certain combinations and against certain microorganisms. All tested bacteria were sensitive to the action of pure chestnut honey and apimixtures A1, A2 and A3, and particularly high sensitivity are shown by the multi-resistant bacterial strains MRSA and ESBL producing *E. coli*. The combined application of both honey and propolis extract (4:1), and the combination of honey, bee pollen and propolis extract (6:2:2) increased the antimicrobial activity compared to honey alone on ESBL *E. coli* and *P. aeruginosa*, and on MRSA, respectively.

The obtained results represent another confirmation that bee products help to combat various microorganisms, especially when considering the growing number of human and animal pathogens resistant to antimicrobial drugs.

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Antimikrobna učinkovitost meda od kestena, polena i propolisa samostalno i u kombinaciji

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Pojava bakterija s rezistencijom na antibiotike ili višestrukom rezistencijom je karakteristika animalnih i humanih patogena. Dobro je poznato da pčelinji proizvodi, koji su u uporabi kao alternativno medicinsko sredstvo još od drevnih vremena, posjeduju antimikrobni potencijal. Aplikacija pčelinjih proizvoda u terapeutske svrhe naziva se apiterapija. Istraživanje je imalo cilj procijeniti antimikrobni potencijal komercijalnog meda od kestena, polena i propolisa proizvedenog u zapadnoj Bosni i Hercegovini (Sanski Most), kao i njihovih pet različitih mješavina. Antimikrobna svojstva uzoraka su istraživana uporabom agar-difuzione metode protiv tri Gram-pozitivne bakterije (*Bacillus subtilis* subsp. spizizenii ATCC 6633, Methicilin-rezistentni *Staphylococcus aureus* ATCC 33591 i *Enterococcus faecalis* ATCC 29212); zatim tri Gram - negativne bakterije (ESBL producirajuća *Escherichia coli* ATCC 35218, *Salmonella enterica* subsp. *enterica serovar Enteritidis* ATCC 13076 i *Pseudomonas aeruginosa* ATCC 9027) i jedne gljivice (*Candida albicans* ATCC 10231). Čisti pčelinji polen inhibirao je rast samo kod Gram - negativnih bakterija, zatim koncentrovani med od kestena je pokazao aktivnost protiv svih Gram - negativnih i Gram - pozitivnih bakterija, dok je 20 % ekstrakt propolisa i apimješavine A2 (80 % med i 20 % propolis) i A3 (60 % med, 20 % polen i 20 % ekstrakt propolisa) inhibirao rast svih testiranih mikroorganizama). Med od kestena i tri apimješavine (A1, A2 i A3) su pokazale najveće antimikrobno djelovanje protiv svih testiranih Gram - negativnih bakterija i MRSA u usporedbi s drugim ispitivanim uzorcima. U ovom istraživanju, ispitivani pčelinji proizvodi iz Bosne i Hercegovine i njihove mješavine su pokazale značajnu aktivnost protiv testiranih bakterija, uključujući sojeve s dokazanom rezistencijom na konvencionalne antibiotike, MRSA i ESBL producirajuću *E. coli*.

Ključne riječi: *pčelinji proizvodi, apimješavine, antibiotik, inhibicija, otpornost*