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# Biologically active antimicrobial and antioxidant substances in the *Helianthus annuus* L. bee pollen

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**ABSTRACT**

The objective of this study was to measure the content of flavonoids, polyphenols, and carotenoids in the *Helianthus annuus* L. bee pollen. It was also to evaluate the ability of the dried, frozen, and freeze-dried extracts of sunflower (*H. annuus*) pollen, its scavenged free radicals and reducing action. Another aim of this study was to investigate the antimicrobial *in vitro* action of the *H. annuus* pollen extracts against the Gram-positive and Gram-negative bacteria and fungi. All pollen extracts showed medium antiradical activity and reductive ability. The most effective was the freeze-dried extract in both evaluation systems. The evaluation of the protective effects of DNA using a biosensor showed an opposite trending—frozen > dried > freeze-dried pollen. For the evaluation of antiradical activity, the DPPH method was used, and reductive ability was assessed by means of phosphomolybdic complex formation. The comparison of the polyphenols content shows higher values in freeze-dried bee pollen than in the dried and frozen pollen. The highest content of flavonoids was found in the frozen samples and the most carotenoids were present in the dried samples. In our study, the best antibacterial effects of the dried sunflower bee pollen extracts were found against *Paenibacillus larvae, Pseudomonas aeruginosa*, and *Enterococcus raffinosus*. The best inhibitory properties of the frozen sunflower bee pollen extracts were found against *Escherichia coli*, *Pseudomonas aeruginosa*, and *Paenibacillus larvae*. Very good inhibitory effects of freeze-dried sunflower bee pollen were found against *Paenibacillus larvae, Brochotrix thermosphacta*, and *Enterococcus raffinosus*. The best antifungal activity of the sunflower bee pollen was found in the frozen bee pollen extracts against *Aspergillus ochraceus* and freeze-dried bee pollen extracts against *Aspergillus niger*.

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**KEYWORDS**
Bee pollen; antioxidant activity; flavonoids; antimicrobial action; *Helianthus annuus* L



## Introduction

Pollen is a fine, powder-like material produced by the flowering plants and gathered by the bees. Pollen is the male reproductive cells of flowers. Pollen, or the "bees primary food source", contains concentrations of phytochemicals and nutrients and is rich in carotenoids, flavonoids and phytosterols.[1–4] In fact, bee pollen is referred to as the "only perfectly complete food", as it contains all the essential amino acids needed by the human body. However, the composition of bee pollen depends strongly on plant source and other factors such as climatic conditions, soil type, and beekeeper activities.[5] Bee pollen is considered a healthy food with a wide range of therapeutic properties such as antimicrobial, antifungal, antioxidant, anti-radiation, hepatoprotective, chemoprotective and/or chemopreventive and anti-inflammatory benefits.[6–8]

Bee pollen contains mainly the following biologically active components: polyphenols, carotenoids, proteins, lipids, vitamins, minerals.[1–4] Bee pollen is interesting not only because of its composition, but also because of its effects. Numerous studies have demonstrated the antimicrobial and antioxidant properties,[3,5,8–14] antifungal properties,[15,16] and anti-cancer properties[17–20] of bee pollen in the *in vitro* and *in vivo* systems. Pollen also prevents the oxidation of lipids.[9]

The antioxidant properties of pollen are mainly influenced by the content of substances from the group of polyphenols (anthocyanins, flavonoids, phenolic acids, and ketones, stilbenes, tannins), carotenoids, ascorbic acid, presence of fats, proteins, and other substances.

Carotenoids and flavonoids are an important group of natural antioxidants. The mechanisms of their antioxidant action are different. Carotenoids can react with the free radicals in several ways—electron transfer, addition reactions, and elimination of hydrogen. The antioxidant effect of individual carotenoids is affected by the presence of functional groups in the $\beta$-ion circle and the number of conjugated double bonds.[21]

Flavonoids inhibit the action of the free radicals by direct elimination of the radicals, interaction with enzymes or chelatically binding the metal cations. The high efficiency of flavonoids as radical quenchers is explained by their ability to donate a hydrogen atom from the hydroxyl group, resulting in the emergence of a flavonoid phenoxyl radical and a stable molecule. The studies focusing on the relation between the

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structure and activity have confirmed the effect of the number and location of the hydroxyl groups on the antiradical activity.[22]

Bee pollen diet supplements in the amount of 500 mg.kg$^{-1}$ significantly ($P < 0.05$) increased the albumin content and total antioxidant status in rats. Bee pollen additives in diets may be a source of antioxidants in humans and animals.[23] The consumption of bee pollen affects ovarian functions in rats.[24]

The antioxidant activity and total flavonoid and phenol content of malt beverages enriched with different types of bee pollen was evidently improved. The results indicate that bee pollen can be used as a very interesting raw material for brewing industry. Malt beverages enriched with bee pollen had higher phenolic and flavonoid content and antioxidant potential than the control sample—pure wort.[25]

The aim of this work was to determine the levels of flavonoids, carotenoids and antioxidant activity and to monitor the impact of bee sunflower pollen (drying, freezing, lyophilization) on these parameters. Another aim of this study was to investigate the antimicrobial *in vitro* action of the *Helianthus annuus* L. pollen extract against the Gram-positive and Gram-negative bacteria and fungi.

## Materials and methods

### Pollen samples and their preparation

The samples of the monofloral bee pollen from sunflower (*Helianthus annuus* L.) collected in Western Slovakia were prepared by freezing, drying and lyophilization. The frozen samples were stored at a temperature of −18°C (the humidity of the sample was 20%) for a period of approximately 6 months. The samples were dried approximately 8 hours at a maximum temperature of 35°C until the target humidity of 9–11% was reached. The lyophilization of the samples was carried out on a laboratory lyophysicator LYOVAC GT 2 by Amsco/Finn-Aqua (Hürth, Germany) for 80 hours without heating until the target sample moisture of 2% was reached.

To determine the polyphenols, antioxidant and antiradical activities, ethanolic extracts were used, prepared by dissolving 5 g of homogenized pollen extract in 50 mL of 90% ethanol in a water bath at 70°C for 30 minutes. After the filtration, the samples were used for the individual analyses and stored at 5°C. The extracts were subject to spectral analysis. The spectra of the extracts in the visible field were measured directly, and for the UV measurements, the extracts were diluted by the factor of 100.

### Determination of antiradical activity (DPPH method)

The DPPH method was used to evaluate the ability to deactivate the free radical (antiradical activity), which is based on measuring the color change of the stable radical 2,2-diphenyl-1-picrylhydrazyl (DPPH$^{\bullet}$) from dark purple to yellow at 515 nm, caused by the antioxidant providing the hydrogen atom. We used a modified Brand–Williams[26] method. This method is based on the reduction of the stable radical 2,2-diphenyl-1-picrylhydrazyl (DPPH$^{\bullet}$) in a methanol solution in the presence of antioxidants, which is manifested by the decrease in absorbance at 515 nm. The decrease is recorded in the predefined time intervals until the reaction equilibrium is reached at the Shimadzu 1601 UV-VIS spectrophotometer. The effectiveness of the extracts is calculated on the basis of the relation (Equation 1):

$$\text{inhibition } \% = [(A_{CO} - A_{At})/A_{CO}] \times 100, \quad (1)$$

where $A_{CO}$ is the absorbance of the control sample at time $t = 0$ min (DPPH$^{\bullet}$ solution),

$A_{At}$ is the absorbance in the presence of an antioxidant at $t$ min.

### Determination of reducing ability

The work procedure was modified according to Prieto et al.[27] The method to create the phosphomolybdate complex to assess the ability of an antioxidant to provide an electron is based on the reduction of Mo(VI) to Mo(V) with the effect of reducing compounds in an acid medium in the presence of phosphorus to form the green phosphomolybdate complex. The absorbance of the solution was measured at 705 nm (Shimadzu 1601 UV-VIS, Tokyo, Japan). The reducing ability of compounds ($RP_{AA}$) is expressed by the amount of ascorbic acid necessary to achieve the same effect in the $\mu$g.mL$^{-1}$ by the Equation 2:

$$RP_{AA} = (A_{705nm} - 0.0011)/0.00236 \quad (2)$$

### Determination of antioxidant activity using a DNA biosensor

The activity against the damage of DNA was determined by a biosensor. The antioxidant activity was evaluated on the basis of the degree of damage to the deoxyribonucleic acid (DNA) immobilized on the surface of a screen-printed electrode (SPE) (Fach, Prešov, Slovakia).[28] The damage to DNA, or the effect of the antioxidants, is expressed through the value of the relative signal I/I0 where I–DPV indicates the indicator current on the electrode after the solution with the fission mixture and the antioxidant and I0–DPV indicates the indicator current on the electrode in the solution before fission. The higher the ratio, the higher the antioxidant activity of the sample.

### Determination of flavonoids with HPLC

To determine the flavonoids in the pollen samples, we used the HPLC system consisting of LC 10ADvp (Shimadzu, Japan), UV/VIS detector SPD 10AV/VP (Shimadzu, Japan), and the Purospher Star RP-18e column (Merck), 250 × 4 mm. I.D., 5 $\mu$m with the Purospher Star (Merck) precolumn, 4 × 4 mm, 5 $\mu$m. The detection was carried out at 360 nm and a dose of 20 mL, mobile phase flow rate of 1 mL.min.−1, mobile phase A—0.05% TFA (trifluoroacetic acid) in water: methanol (95: 5, V/V), B—methanol: 0.05% TFA in water (95:5; V/V), gradient: 0–15 min. 40% B; 15–30 min. 40–55% B; 30–35 min. 55–70% B.

The ethanolic extracts of pollen and standard solutions (quercetin, luteolin, kaempferol and apigenin) were analyzed in the above conditions.

### Determination of carotenoids

Carotenoids were determined spectrophotometrically[29] after the extraction with acetone and preextraction into petroleum ether. After appropriate dilution, the absorbance of the petroleum ether extract is measured at 450 nm (Shimadzu 1601 UV-VIS, Tokyo, Japan).

The content of colorants in $mg.kg^{-1}$ is expressed as $\beta$-carotene and calculated from the relation (Equation 3):

$$C_{kar} = (A.r.V.10).\left(n.E^{1\%}{}_{1cm}\right)^{-1} \quad (3)$$

where: $A$—absorbance, $r$—dilution, $V$—volume of extract ($cm^{-3}$), $n$—sample (g),

$E^{1\%}{}_{1cm}$—absorption coefficient for $\beta$-carotene = 2520.

### Determination of polyphenol content

To determine the total phenol content, we used colorimetry and the Folin–Ciocalteu reagent.[30] After the phenolic compounds reacted with the Folin–Ciocalteu reagent, the coloring turned blue. In the analysis, 0.5 mL of the sample, 0.5 mL of the Folin–Ciocalteu reagent, and 5 mL of 20% $Na_2CO_3$ was applied with a pipette. The samples in this manner were diluted 20 times. Absorbance was measured at 700 nm after 30 minutes. The total content of phenolic compounds was expressed in mg of tannin per 1 kg of pollen.

### Antimicrobial activity

10 g of pollen collected by the bees was added to 100 mL of EtOH and it was let set for 2 weeks at room temperature in the dark. The bee-collected pollen solution was then filtered with a 6 mm filter paper (Whatman, UK). After the filtration, the bee-collected pollen filtrate was evaporated using the RE300DB vacuum rotary evaporator (Stuart, UK). The yield of crude pollen was dissolved in DMSO and it was prepared into a 70% pollen solution. The bacterial strains were purchased from the Czech Collection of Microorganisms (CCM) (Brno, Czech Republic). The microscopic filamentous fungi were provided by the Slovak University of Agriculture in Nitra, Department of Microbiology. The antimicrobial effects of the extracts were tested using the agar well diffusion method in the Mueller–Hinton agar (MHA) for bacteria and the Sabouraud agar (SA) for microscopic filamentous fungi. After 30 min of initial drying, the agar plates were inoculated with 200 $\mu$L of microorganism suspension at a density of $10^7$ CFU $mL^{-1}$ in a saline solution and spread on the surface. Subsequently, four equidistant wells, each 9 mm in diameter, were punched into the inoculated medium with sterile glass. The bacteria were incubated at 37°C and fungi at 25°C. The inhibition zones in mm around the disks were measured after 24 h of cultivation. Chloramphenicol was used as a positive control for the bacteria, a 40% phenol solvent was used for fungi and 99.9% methanol was evaluated for comparison. Five different strains of bacteria; *Escherichia coli* CCM 3988, *Enterococcus raffinosus* CCM 4216, *Paenibacillus larvae* CCM 4483, *Brochotrix thermosphacta* CCM 4769, *Pseudomonas aeruginosa* CCM 1960, and five different strains of microscopic filamentous fungi *Aspergillus flavus, Aspergillus fumigatus, Aspergillus niger*, *Aspergillus ochraceus*, and *Aspergillus versicolor* were tested in sets of plates, and simultaneously processed for each strain. All the experiments were repeated twice, including the chloramphenicol control. After the incubation period, the zones of inhibited growth of the bacteria and microscopic filamentous fungi around the disks were measured. The mean values of the three trials and standard deviations were calculated.

### Statistical analysis

The results were statistically processed using one-factorial variance analysis (ANOVA) of SAS. Means were separated using Fischer LSD multiple range test. Confidence limits were added at $P < 0.05$; $P < 0.01$; $P < 0.001$.

## Results and discussion

As mentioned in the introduction, the mechanism of action of the antioxidant substances is different. Apart from their own structure, it is also determined by the type of radical against which they have to operate. The action of antioxidants happens either by means of hydrogen transfer or an electron transfer from the antioxidant to the oxidant (free radical).

Thanks to its biological properties, bee pollen has become a frequent subject of interest to the scientists. Many scientific papers mention its high antioxidant activity assessed by several methods with different mechanisms of action.[5,9–10,12–14,31] Our assessments of pollen extracts confirm this fact. The extracts were active in all three systems (Table 1).

It follows from Table 1 that the efficiency of extracts is the same for the antiradical and reduction method: freeze-dried > dried > frozen pollen. The trending is inversed when evaluating the protective effect of the ethanol extract on DNA. In the assessed extracts, the extract of freeze-dried pollen proves to be

**Table 1.** Composition and antioxidant activity of bee pollen.

| Bee pollen | Polyphenols ($mg.kg^{-1}$) | Carotenoids ($mg.kg^{-1}$) | DPPH (% of inhibition) | $RP_{AA}$ ($\mu g.mL^{-1}$) | Biosensor $I/I_0$ |
|---|---|---|---|---|---|
| Frozen | $691.67 \pm 7.76^{a}$ | $235.17 \pm 1.51^{a*}$ | $47.97 \pm 0.29^{a*}$ | $2778.67 \pm 3.30^{a*}$ | $0.37 \pm 0.04^{a*}$ |
| Dried | $763.67 \pm 5.56^{b}$ | $261.33 \pm 1.36^{b}$ | $48.43 \pm 0.29^{a**}$ | $3150.33 \pm 3.09^{b}$ | $0.28 \pm 0.02^{b}$ |
| Freeze-dried | $803.33 \pm 3.30^{c}$ | $223.10 \pm 1.24^{c}$ | $50.46 \pm 0.43^{b}$ | $3154.67 \pm 3.86^{b}$ | $0.20 \pm 0.02^{c}$ |

The results are expressed as Mean ± SD; Polyphenols – total polyphenols; DPPH - 2,2-diphenyl-1-picrylhydrazyl; $RP_{AA}$—Reduction power of compounds; the differences between bee pollen types were analyzed by *t*-test and different letters mean that differences are significant ($P \leq 0.05$).
Sources: *Fatrcová-Šramková et al.[8], **Fatrcová-Šramková et al.[32]

the most effective anti-radical and reducing agent, although the content of carotenoids in the pollen decreases in the following order: dried > frozen > freeze-dried pollen. The effects of carotenoids on the overall efficiency are smaller than the effects of polyphenols. It is generally stated that the greater the content of polyphenols, the higher the antioxidant action.[12,31,33] The values of antiradical activity of pollen extracts are in the group with medium ability to eliminate the DPPH radicals.[14]

The comparison of the content of individual flavonoids defined in the HPLC method shows that the highest content of quercetin and luteolin is in the frozen pollen. Higher content of apigenin was identified in freeze-dried and the dried pollen (Table 2). The amount of kaempferol in sunflower pollen was below the detection limit. The amount of selected and monitored flavonoids decreased in the following order: dried > frozen > freeze-dried bee pollen.

Since the antioxidant activity of flavonoids depends on their chemical structure, the determination of content of individual flavonoids may explain why some pollen with a higher content of total flavonoids has lower antioxidant activity. The antioxidant activity of the monitored flavonoids decreases in the following order: kaempferol > quercetin > luteolin > apigenin.[22] The absence of kaempferol as the most effective of the monitored flavonoids is a potential explanation of the lower antioxidant activity of sunflower pollen compared to other monofloral pollen.[8,9,14]

The representation of the individual active antioxidants in pollen is also reflected by their spectral characteristics. Carotenoids have their absorption maxima in the range of 420-480 nm, flavonoids have two absorption maxima in the range of 330–380 nm and 240–280 nm. However, the 270 nm range also includes the absorption of phenolic acids. Figs. 1 and 2 shows the comparison of the spectral characteristics of sunflower pollen extracts and extracts from *B. napus* and *P. somniferum*. The representation of active substances of this pollen is summarized in our previous studies.[11,13,34]

Carotenoids are found only in the sunflower pollen, which is reflected in the VID spectrum by the presence of peaks in the range of 440–480 nm.

According to Brindza et al.[35] and Synytsya et al.[36] spectrophotometry shows that the sunflower pollen contains a high amount of carotenoids and the HPLC indicates the presence of different quercetin, gallic and chlorogenic acids derivatives and other unidentified components.

The analysis of the UV spectra shows that the highest polyphenol content in rape pollen is also reflected by the highest peak in the 310–330 nm range.

**Table 2.** Content of flavonoids in sunflower bee pollen (mg.kg$^{-1}$).

| | Bee pollen | | |
|---|---|---|---|
| Flavonoid (mg.kg$^{-1}$) | Frozen | Dried | Freeze-dried |
| Quercetin | 14.30 ± 0.37$^{a}$ | 10.19 ± 0.22$^{b}$ | 12.04 ± 0.20$^{c}$ |
| Luteolin | 66.39 ± 0.15$^{a}$ | 63.62 ± 0.32$^{b}$ | 46.96 ± 0.44$^{c}$ |
| Kaempferol | udl | udl | udl |
| Apigenin | 23.99 ± 0.83$^{a}$ | 32.01 ± 1.73$^{b}$ | 34.40 ± 1.23$^{b}$ |
| Sum | 104.68 ± 1.35 | 105.82 ± 2.27 | 93.40 ± 1.86 |

The results are expressed as Mean ± SD; udl—under detection limit; the differences between bee pollen types were analyzed by *t*-test and different letters mean that differences are significant ($P \leq 0.05$).

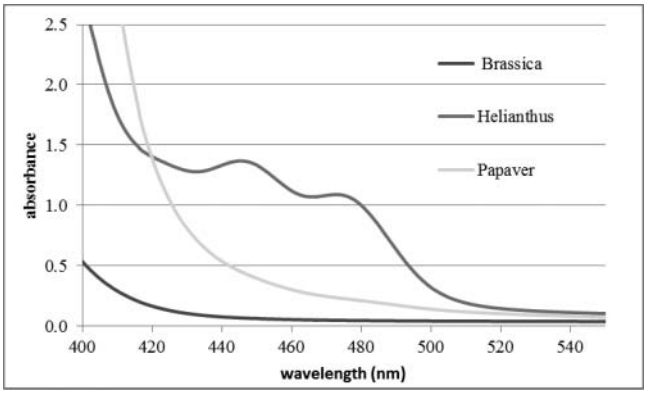


**Figure 1.** Spectral characteristics of monofloral bee pollen—VID spectrum (dilution = 1).

All monitored antioxidant properties of pollen extracts decreases in the following order: *Brassica napus* > *Papaver somniferum* > *Helianthus annuus*. The content of polyphenols decreases, respectively.[8] The stronger antioxidant activity of poppy pollen in comparison with sunflower pollen is most likely caused by the presence of phenolic acids and characterized by increased absorbance in the 270 nm range.

The antimicrobial activities of the dried, frozen, and freeze-dried sunflower bee-pollen extracts in the *in vitro* test against the different Gram positive, Gram negative bacteria and the microscopic filamentous fungi are shown in Table 3.

In our study, the best antibacterial effects of the dried sunflower bee pollen extracts were found against *Paenibacillus larvae* (2.60 ± 0.20), *Pseudomonas aeruginosa* (2.23 ± 0.15), and *Enterococcus raffinosus* (2.00 ± 0.10). The best inhibitory properties of the frozen sunflower bee pollen extracts were found against *Escherichia coli* (2.70 ± 0.56), *Pseudomonas aeroginosa* (2.27 ± 0.15), and *Paenibacillus larvae* (2.23 ± 0.38). Very good inhibitory effects of freeze-dried sunflower bee pollen were found against *Paenibacillus larvae* (2.73 ± 0.21), *Brochotrix thermosphacta* (2.64 ± 0.15), and *Enterococcus raffinosus* (2.57 ± 0.21). The best antifungal activity of sunflower bee pollen was found in the frozen bee pollen extract against *Aspergillus ochraceus* (1.77 ± 0.25) and freeze-dried bee pollen extract against *Aspergillus niger* (1.77 ± 0.15). Statistically significant differences ($P < 0.05$) of pollen samples were not found

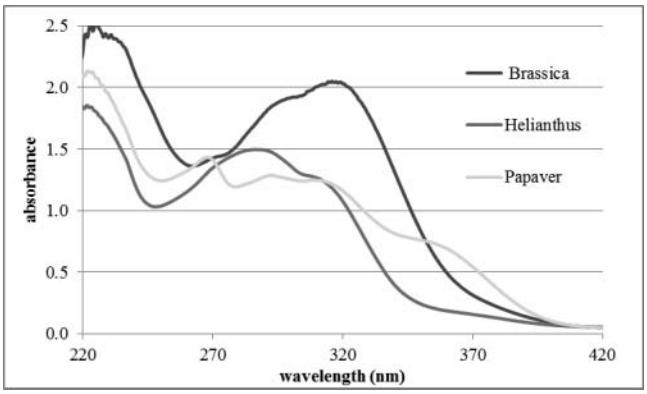


**Figure 2.** Spectral characteristics of monofloral bee pollen—UV spectrum (dilution = 100).

**Table 3.** Inhibitory effects of dried, frozen and freeze-dried pollen (inhibition zone diameter in mm).

| Microorganism | Dried pollen | Frozen pollen | Freeze-dried |
|---|---|---|---|
| *Escherichia coli* CCM 3988 | 1.83 ± 0.29 | 2.70 ± 0.56 | 2.47 ± 0.35 |
| *Enterococcus raffinosus* CCM 4216 | 2.00 ± 0.10 | 2.20 ± 0.36 | 2.57 ± 0.21 |
| *Brochotrix thermosphacta* CCM 4769 | 1.97 ± 0.38 | 2.20 ± 0.27 | 2.64 ± 0.15 |
| *Paenibacillus larvae* CCM 4483 | 2.60 ± 0.20 | 2.23 ± 0.38 | 2.73 ± 0.21 |
| *Pseudomonas aeruginosa* CCM 1960 | 2.23 ± 0.15 | 2.27 ± 0.15 | 2.47 ± 0.15 |
| *Aspergillus flavus* | 1.43 ± 0.12 | 1.63 ± 0.15 | 1.70 ± 0.17 |
| *Aspergillus fumigatus* | 1.50 ± 0.30 | 1.63 ± 0.15 | 1.33 ± 0.15 |
| *Aspergillus niger* | 1.40 ± 0.10 | 1.47 ± 0.25 | 1.77 ± 0.15 |
| *Aspergillus ochraceus* | 1.50 ± 0.30 | 1.77 ± 0.25 | 1.70 ± 0.17 |
| *Aspergillus versicolor* | 1.47 ± 0.15 | 1.63 ± 0.15 | 1.97 ± 0.12 |

between all tested microorganisms. Statistically significant differences ($P < 0.05$) of tested microorganisms were found only in dried bee pollen among *Escherichia coli* and *Aspergillus flavus*, *A. fumigatus*, *A. niger*, *A. ochraceus*, and *A. versicolor*, between *Enterococcus rhamnosus* and *A. fumigatus*, *Brochotrix thermosphacta* and *A. fumigatus*, *Paenobacillus larvae* and *A. fumigatus*, *Pseudomonas aeruginosa* and *A. fumigatus*.

The antimicrobial effects of the bee-collected pollen extract have not been studied by many researchers, however, its activity with some selected bacteria was studied by Morais et al.[5] In their study, they tested more bacteria against the bee-collected pollen extract and they determined that the strongest effects of the bee-collected pollen extract were against *E. coli* and *S. enterica* serovar *typhi*, *Enterobacteriaceae*, respectively. Also, they determined the lower antimicrobial activity of the bee-collected pollen extract against *Bacillus cereus* and *Staphylococcus aureus*. Fatrcová-Šramková et al.[8] came with very similar results when studying the antimicrobial effects of the bee-collected monofloral pollen extract against some pathogenic bacteria. They determined that the bee-collected pollen extract had antimicrobial effects to *E. coli* CCM 3988 and to *Salmonella enterica* CCM 4420. They used the same ethanolic extract (70%) as in our experiment. Their results were as follows: the diameter of the inhibition zone was (3 ± 1) mm after the application of bee-collected poppy pollen extract to *E. coli* CCM 6988 and (2 ± 1) mm to *S. enterica* CCM 4420. The bee-collected rape pollen extract had similar effects in their experiment. Also, Kačániová et al.[37] researched the antimicrobial properties of bee-collected pollen in their study. They determined that the bee-collected pollen extract had antimicrobial effects on some selected pathogenic bacteria, yeasts and fungi. Similar to our study, they used the 70% ethanolic bee pollen extract and they determined the following values. The diameter of the inhibition zones was: (1.83 ± 0.29) mm in the *E. coli* CCM 3988 strain and (2.17 ± 0.76) mm in the *S. enterica* CCM 4420 strain. Similar results were achieved by Carpes et al.[38] who tested *Pseudomonas aeruginosa*. These bacteria were inhibited by the extracts of pollen in the 80 and 90% ethanol solution and the *Staphylococcus aureus* bacteria were inhibited at the 50, 60, 70 and 80% ethanol solution. Different results were reported by Almeida–Muradian et al. (2005) We found very good antibacterial effects of the ethanolic pollen extracts of *Pseudomonas aeruginosa,* similar to the results presented by Carpes et al.[38] and Abouda et al.[39] The same non-inhibitory effects of the bee pollen extracts to the *Staphylococcus aureus* bacteria were found in Parana pollen[38] in all extraction conditions, which were also applied in our experiment.

## Conclusion

Bee pollen contains many biologically important agents that may degrade by environmental effects. Therefore, it is vital to provide optimum conditions during storage. Given the polyphenol content, antioxidant activity and reducing ability of sunflower bee pollen extracts, our results show that lyophilization is the most effective treatment method.

The best antibacterial effects of the dried sunflower bee pollen extracts were found against *Paenibacillus larvae, Pseudomonas aeroginosa*, and *Enterococcus raffinosus*. The best inhibitory properties of the frozen sunflower bee pollen extract were found against *Escherichia coli*, *Pseudomonas aeroginosa* and *Paenibacillus larvae*. Very good inhibitory effects of the freeze-dried sunflower bee pollen were found against *Paenibacillus larvae, Brochotrix thermosphacta* and *Enterococcus raffinosus*. The best antifungal activity of the sunflower bee pollen was found in the frozen bee pollen extract against *Aspergillus ochraceus* and freeze-dried bee pollen extract against *Aspergillus niger*. The antimicrobial activity of bee pollen against bacteria was better than the activity against the microscopic filamentous fungi.

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