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## Ethanollic extract of Turkish bee pollen and propolis: phenolic composition, antiradical, antiproliferative and antibacterial activities

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### ABSTRACT

Bee pollen and propolis are considered as health-promoting foods with many therapeutic (antibacterial, antifungal and antioxidant) activities. This study analyzed the phenolic profile and the antioxidant properties of Turkish bee pollen and propolis ethanollic extracts and assayed their antiproliferative effect on myeloma cells and *in vitro* antibacterial activity against *Staphylococcus aureus*, *Escherichia coli* and *Pseudomonas aeruginosa*. The antibacterial activity assays included agar well diffusion and microdilution methods. The phenolic profile and several aromatic compounds of the extracts were determined by high-performance liquid chromatography with diode-array detection (HPLC-DAD). The antiproliferative activity on myeloma cells was determined by MTT test. The propolis extract had higher total phenolic content (TPC), free-radical scavenging activity (DPPH) and half-maximal inhibitory concentration (IC<sub>50</sub>) than the pollen ethanollic extract. Benzoic and cinnamic acid were the most abundant aromatic substances in the pollen and propolis extracts, respectively. The IC<sub>50</sub> values of pollen and propolis extracts on myeloma cells were 1.49% and 2.88%, respectively. The propolis extract was active against *S. aureus* and *E. coli*, but not *P. aeruginosa*. The pollen extract presented no detectable inhibition zone against the three bacterial strains. The minimum inhibitory concentration (MIC) of both extracts for *S. aureus* and *E. coli* was 0.63% (*w/v*). The minimum bactericidal concentration (MBC) of the propolis extract was 1.25% for *S. aureus* and *E. coli*. MIC could not be determined for the pollen extract in the tested bacteria. The pollen and propolis extracts did not exert antimicrobial activity against *P. aeruginosa* up to 2.5% concentration.

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### KEYWORDS



Antiradical; antibacterial; antiproliferative effect; bee pollen; propolis; total phenolic content

## Introduction

Bee pollen and propolis contain a wide range of phenolic compounds, proteins, amino acids, carbohydrates, lipids and fatty acids, enzymes, coenzymes, vitamins and bio-elements [1, 2]. Propolis is a sticky, resinous mixture produced by honeybees from plant exudates, beeswax and bee secretions. Honeybees use it for various purposes, including nest construction and as a defense against pathogens, which results in sustainable colony health [3]. Bee pollen, a honeybee derivative product, is the main essential nutrient source used in the feeding of immature (larval) bees [4]. They are considered as health-promoting foods with many therapeutic activities like antibacterial, antifungal, antioxidant, hepatoprotective, anti-inflammatory benefits due

to the presence of biologically active compounds: polyphenols, carotenoids, proteins, lipids, vitamins and minerals. The chemical composition of these compounds depends on the local flora where they were collected, the period of beekeeping season, and the genetics of the bees [5–10].

Plant phenolics, including flavonoids and phenolic acids, can be found in varying concentrations in bee pollen and propolis and exhibit antioxidant activity [1, 11]. Studies have reported that intake of these natural antioxidants reduces the risk of some diseases such as cancer, diabetes, cardiovascular and neurodegenerative diseases [12–14]. Phenolics also show antimicrobial activity on pathogenic bacteria and fungi through disrupting their cytoplasmic membranes [15–17]. In

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addition to antioxidant and antimicrobial activities, the phenolics significantly inhibit the cell growth and reduce the differentiation and proliferation of tumor cells [1, 18]. Apart from phenolics, pollen and propolis contain many compounds such as coumarins and isocoumarins, xanthenes, naphthoquinones, stilbenes, anthraquinones, flavonoids and lignin, which produce the mentioned effects. The content of bee products varies according to various factors such as climates and regions; therefore, they differ in their effects [11, 19]. The chemical composition and biological properties of propolis and pollen have been examined extensively in many countries [16, 19–21]. The aim of this study was to determine the phenolic profile and the antioxidant properties of Turkish bee pollen and propolis ethanolic extracts as well as to measure the anti-proliferative effect on myeloma cells and *in vitro* antibacterial activity against *Staphylococcus aureus*, *Escherichia coli* and *Pseudomonas aeruginosa*.

## Materials and methods

### Sampling

Fresh bee pollen and propolis samples were collected from apiaries in Muğla province of Turkey. The samples were kept in a dry place and stored at 4°C until tests. The ethanolic extracts were prepared by extracting 50 grams of each homogenized sample in 200 mL of ethanol (>96%, Merck, Germany) in the dark at room temperature for 7 days. Then the samples were filtered, and the collected filtrates were subjected to a rotary evaporator (RV10, IKA®, Germany) at 40°C to remove the entire solvent. After the evaporation, the concentrated extracts were lyophilized with a freeze dryer (Martin Christ, Alpha 1-2LD plus, Germany) and kept in tubes in the dark at 4°C for further analysis.

### Total phenolic content

The total phenolic content (TPC) of pollen and propolis ethanolic extracts was determined by using the Folin–Ciocalteu method described by [22]. Gallic acid (Sigma, USA) was used as the standard. The results are expressed as microgram Gallic Acid Equivalents (GAE) per milligram of crude extract materials. Briefly, the solutions of gallic acid at a concentration ranging from 3.12 to 200 µg/mL (two-serial dilutions) were prepared in ethanol (Merck, Germany). Two hundred microliters of filtered extract (1 mg/mL in ethanol) or the standard solution (gallic acid) was mixed with 400 µL of distilled water in a tube and 200 µL of 10% Follin–Ciocalteu's (F–C) phenol reagent (diluted in distilled water) was

added to the tube. After 5 min, 200 µL of 1 mol/L sodium carbonate solution were added to the tube. The mixture was incubated for 30 min at room temperature and protected from light. After incubation, the mixture (300 µL) was added to a 96-well plate. Ethanol was used as blank. The absorbance was measured at 750 nm with a microplate spectrophotometer (Multiskan Go, Thermo Scientific).

### Phenolic compounds

Analysis of phenolic acid compounds from ethanolic extracts of pollen and propolis was carried out by high performance liquid chromatography (HPLC) using a chromatograph equipped with an Agilent Eclipse XDB-C18 (250×4.60 mm) 5-micron column and a diode array detector (SPD-M10A, Shimadzu). The column was eluted using a linear gradient of 3% acetic acid (solvent A) and methanol (solvent B) with a solvent flow rate of 0.8 mL/min. Chromatograms were recorded at 278 nm with the photodiode array detector. The gradient program and HPLC conditions are given at Tables 1 and 2. The standard solutions were prepared using methanol to dissolve the chemicals to reach concentrations ranging from 0.7 to 500.0 µg/mL for gallic acid, protocatechuic acid, catechin, *p*-hydroxybenzoic acid, chlorogenic acid, caffeic acid, syringic acid, *p*-coumaric acid, ferulic acid, sinapinic acid, benzoic acid, *o*-coumaric acid, rutin, hesperidin, rosmarinic acid, eriodictyol, cinnamic acid, quercetin, luteolin, kaempferol, apigenin. These standards are phenolic compounds, except for benzoic and cinnamic acid which are aromatic acids. We added these aromatic compounds to the standard pool since there is little research analyzing these compounds in bee products. The dried extract of propolis and pollen, 20 mg of each sample, were dissolved into 1 mL of ethanol. The mixtures were filtered with a polytetrafluoroethylene (PTFE) (0.45 µm) filter, and 20 µL of the sample filtrate was injected into the HPLC system.

### Antiradical activity

The measurement of DPPH (2,2-diphenyl-1-picrylhydrazyl) was conducted according to the method of [23] with slight modifications. Briefly, 50 µL of ethanol crude extract dissolved in ethanol (10, 100 and 1000 µg/mL) was mixed with 150 µL of 200 µmol/L methanolic DPPH solution in a 96-well plate. The mixture was incubated for 30 min in a dark condition at 25°C. Absolute methanol was used as blank. Absorbance was measured at 517 nm by using a microplate reader (Multiskan Go, Thermo Scientific). The DPPH radical scavenging activity (%) was calculated as follows: *DPPH scavenging activity*

**Table 1.** Gradient program of HPLC.

	Time	Module	Action	Value
1	0.01	Controller	Start	
2	0.10	Pumps	Pump B Conc.	7
3	20.00	Pumps	Pump B Conc.	28
4	28.00	Pumps	Pump B Conc.	25
5	35.00	Pumps	Pump B Conc.	30
6	50.00	Pumps	Pump B Conc.	30
7	60.00	Pumps	Pump B Conc.	33
8	62.00	Pumps	Pump B Conc.	42
9	70.00	Pumps	Pump B Conc.	50
10	73.00	Pumps	Pump B Conc.	70
11	75.00	Pumps	Pump B Conc.	80
12	80.00	Pumps	Pump B Conc.	100
13	81.00	Pumps	Pump B Conc.	7
14	90.00	Controller	Stop	

**Table 2.** HPLC conditions and settings.

Conditions	Settings
Instrument	Shimadzu HPLC-DAD system
Detector	SPD-M 10A vp DAD detector (Max = 278nm)
System Controller	SCL-10Avp
Auto Sampler	SIL-10AD vp
Pump	LC-10ADvp
Degasser	DGU-14A
Column Oven	CTO-10A VP
Column	Agilent Eclipse XDB-C18 (250×4.60 mm) 5 micron
Column Temperature	30°C
Mobile Phase	A: 3% Acetic Acid, B: Methanol
Injection Volume	20 µL
Flow Rate	0.8 mL/min

(%) =  $[(Ac - As)/Ac] \times 100$ , where  $Ac$  is the absorbance of the control [DPPH+Methanol without sample] and  $As$  is the absorbance of the sample [DPPH+Sample (extract)].

### Antiproliferative activity

MTT test was used to calculate the half-maximal inhibitory concentration ( $IC_{50}$ ), which is the drug concentration that causes a 50% decrease in the cell viability;  $IC_{50}$  is used to evaluate a drug in inhibiting a specific biological function *in vitro* [24, 25]. F0 (ATCC CRL-1646) Mouse Myeloma cell line was used in the antiproliferative activity assay (MTT test) of bee pollen and propolis samples in this study. The cells were cultured in Dulbecco's Modified Eagle's Medium (DMEM) containing 10% FBS (Fetal bovine serum), 0.1% gentamicin, 1% sodium pyruvate and 2% L-glutamine and maintained at 37°C, in 5%  $CO_2$  and 95% relative humidity in a humidified incubator (Steri-Cycle i160, Thermo Scientific). The cells were seeded in a 96-well flat-bottom microtiter plate at a density of  $1 \times 10^4$  cells/well and allowed to adhere overnight at 37°C in the incubator. After overnight incubation, the culture medium was replaced with fresh medium. The cells were then treated with various concentrations (50, 25, 12.5, 6.25, 3.12, and 1.56 mg/mL) of the extracts for

24 h at 37°C in the incubator. The medium only and 0.1% Triton X-100 served as negative and positive controls, respectively. After treatment, the MTT solution at a final concentration of 0.5 mg/mL was added to each well for about 3 h in the incubator at 37°C. The medium containing MTT was removed from the wells and 250 µL of dimethylsulfoxide (DMSO) was added to each well to solubilize the formazan crystals. Absorbance was measured at 540 nm using a microplate spectrophotometer (Multiskan Go, Thermo Scientific). Cell viability was expressed as the percentage of MTT reduction, assigning the 100% value to the absorbance of the control cells. All experiments were performed in triplicate and expressed as mean values with standard deviation ( $\pm$ SD).

### Antimicrobial activity

The antimicrobial activity of pollen and propolis extract was assessed by agar well diffusion [26] and microdilution method [27].

### Bacterial strains

*Staphylococcus aureus* (ATCC 25923), *Escherichia coli* (ATCC 29998) and *Pseudomonas aeruginosa* (ATCC 27853) strains were obtained from the stock culture collection of the Department of Food Hygiene and Technology Laboratory, Burdur Mehmet Akif Ersoy University. The bacterial strains were transferred on Tryptic Soy Agar (TSA, BK047HA, BİOKAR) and incubated for 18-24 h at 37°C. Each bacterial cell was transferred into 0.9% sterile saline buffer and adjusted to 0.5 McFarland scale ( $1-1.5 \times 10^8$  CFU/mL).

### Agar well diffusion

The serial dilutions of the samples (100, 50, 25, 12.50, and 6.25 mg/mL) were prepared in ethanol. Each microbial inoculum was streaked on Mueller Hinton Agar (BK048HA, BİOKAR) using sterile cotton swabs. Wells of 6 mm size and 4 mm depth were prepared on each plate with sterile borer and 100 µL volume of each dilution was dispensed into the wells. Enrofloxacin (64 µg/mL) and absolute ethanol were used as positive and negative controls, respectively. The plates were incubated at 37°C for 24 h. The plates were observed for presence of inhibition zones around the wells and the diameter of inhibition zones was measured with a digital caliper.

### Microdilution method

Minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) values of bee pollen

and propolis extracts were determined by using microdilution method in 96-well microplates according to the CLSI guidelines [27]. The bacterial strains were grown in TSA (BK047HA, BLOKAR) and incubated for 18 h at 37°C. Bacterial inoculums were adjusted to 0.5 McFarland (approximately  $1.5 \times 10^8$  CFU/mL) in 0.9% sterile saline buffer. The serial dilutions of the samples (25, 12.50, 6.25, 3.12, 1.56, 0.78, and 0.39 mg/mL) were prepared in Mueller Hinton broth (BK048HA, BLOKAR) and transferred to the wells of microplates. Twenty microliters of each bacterial inoculum were inoculated into each well and plates were incubated at 37°C for 24 h. Following the incubation, microbial growth was determined at 600 nm using a microplate reader (Epoch, BioTek, USA). The lowest concentration of each extract inhibiting the visible growth of the bacteria was recorded as the MIC. The MBC value was determined by removing 10 µL of the suspension from each well and inoculating on Mueller Hinton agar plates. The plates were incubated at 37°C for 24 h. The MBC was identified by determining the lowest concentration of extracts that completely inhibited the growth of bacteria [28].

### Data analysis

All experiments were replicated three times. The results of the agar well diffusion and MTT were expressed as mean values with standard deviation ( $\pm$ SD).

## Results and discussion

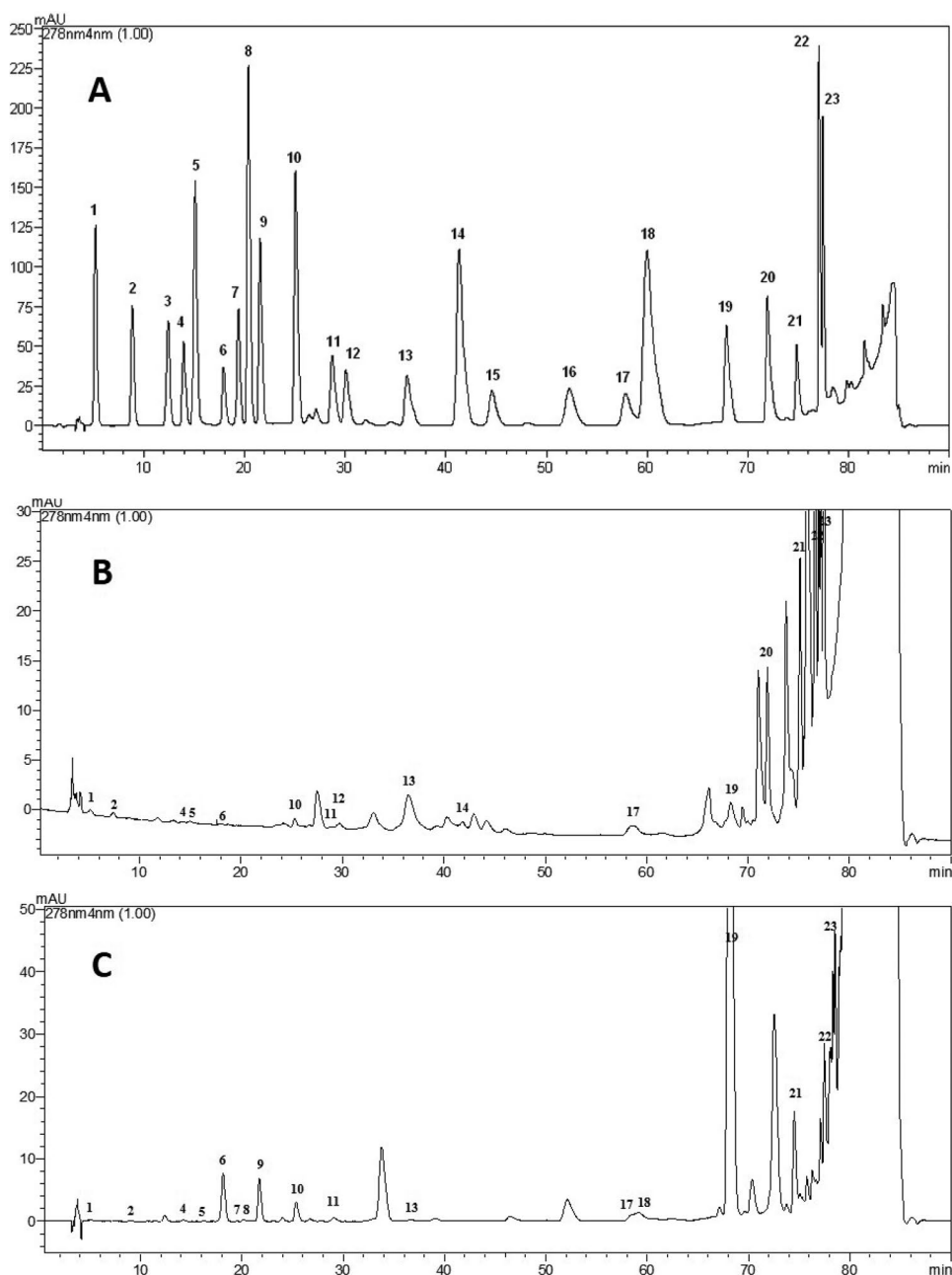
### Phenolic compounds and total phenolic content

Nowadays, bee products, especially propolis and pollen, are considered to be multifunctional, an excellent natural source of a broad spectrum of vitamins, minerals and proteins and interesting alternatives to synthetic antioxidants and antimicrobials [8, 11, 15]. Bee products exhibit antioxidant, antibacterial, anti-inflammatory and anticancer properties due to their active ingredients, especially, phenolic acids and flavonoids [1, 15, 29]. In the present study, the phenolic compounds in pollen and propolis ethanolic extracts were investigated by using HPLC-DAD. Figures (1A), (1B) and (1C) show chromatograms corresponding to a mixture of phenolic standards, the phenolic profile of bee pollen and propolis ethanolic extracts, respectively. Table 3 shows the phenolic compounds ( $\mu$ g/g) in pollen and propolis ethanolic extracts. The pollen extract did not contain catechin, epicatechin, syringic acid, vanillin, rutin, hesperidin, eriodictiol, but had higher amounts of benzoic acid (8900.6  $\mu$ g/g) and apigenin (6477.2  $\mu$ g/g) and had lower amounts of protocatechuic acid (19.1  $\mu$ g/g) and

*p*-hydroxy benzoic acid (19.6  $\mu$ g/g). The propolis extract did not contain catechin, sinapinic acid, *o*-coumaric acid, rutin, hesperidin, quercetin, but had higher amounts of cinnamic acid (8256.1  $\mu$ g/g) and apigenin (3965.9  $\mu$ g/g) and had lower amounts of chlorogenic acid (24.3  $\mu$ g/g) and syringic acid (18.4 mg/g). Benzoic acid was the most abundant compound in bee pollen, whereas cinnamic acid was the most abundant compound in propolis. Apigenin was the second most abundant phenolic compound in both pollen and propolis extracts.

In the literature, the phenolic profile in bee products has been generally examined. Therefore, in this study, we assessed the concentrations of cinnamic acid and benzoic acid, which have important antioxidant and antimicrobial activities [30–32]. Aliyazıcıoğlu *et al.* [33] reported that propolis methanolic extracts have benzoic acid in the range of 242.7–7262.7  $\mu$ g/g. Ulusoy and Kolaylı [34] reported that methanolic bee pollen extracts have benzoic acid in the range of 0–10.7  $\mu$ g/g. Ozdal *et al.* [35] reported that propolis ethanolic extracts have benzoic acid and *t*-cinnamic acid in the range of 0–95.6 and 0–1149.0  $\mu$ g/g, respectively. Another study reported that ethanolic propolis extracts have cinnamic acid in the range of 50.0–4000.0  $\mu$ g/g [36]. In the present study, bee pollen ethanolic extracts had benzoic acid and cinnamic acid with mean concentrations of 8900.6 and 273.8  $\mu$ g/g, respectively, while propolis ethanolic extracts had benzoic acid and cinnamic acid with a mean concentration of 349.7 and 8256.1  $\mu$ g/g, respectively. The higher antibacterial activity of pollen and propolis extracts in this study compared to other studies may be related to these aromatic compounds found in high concentrations in the extracts.

Authors have used solvents containing ethanol, methanol, water, dichloromethane and ethyl acetate alone or together (such as ethanol/water) to prepare pollen and propolis extract. In the literature, differences were observed in the phenolic profile according to the solvent used in the preparation of the effect and the flora from which the bee product was obtained. Karkar *et al.* [37] did not find gallic acid in the ethanolic extract of chestnut bee pollen, but found a small amount of syringic acid. In addition, they found lesser amount of kaempferol than our results. These results are not similar to our study results. Alimoglu *et al.* [38] found that luteolin and kaempferol in dichloromethane extract of bee pollen were 310 and 260  $\mu$ g/g, respectively. In addition, they reported that the ethyl acetate extract contains chlorogenic acid, caffeic acid and rutin at concentrations of 1370, 280 and 3390  $\mu$ g/g, respectively. Compared to the study of [39], the levels of chlorogenic acid, caffeic acid and



**Figure 1.** HPLC analysis. **(A)** Standard chromatogram. 1: Gallic Acid, 2: Protocatechic Acid, 3: Catechin, 4: P-Hydroxy Benzoic Acid, 5: Chlorogenic Acid, 6: Caffeic Acid, 7: Epicatechin, 8: Syringic Acid, 9: Vanilin, 10: P-Coumaric Acid, 11: Ferulic Acid, 12: Sinapinic Acid, 13: Benzoic Acid, 14: O-Coumaric Acid, 15: Rutin, 16: Hesperidin, 17: Rosmarinic Acid, 18: Eriodictiol, 19: Cinnamic Acid, 20: Quercetin, 21: Luteolin, 22: Kaempferol, 23: Apigenin. **(B)** Chromatogram of the bee pollen ethanolic extract. **(C)** Chromatogram of the propolis ethanolic extract.

rutin were higher in the ethanolic extract obtained in our study, while the levels of luteolin, quercetin and kaempferol were lower. These results demonstrate that the solvent used in the extraction changes the phenolic profile.

The compound that is present in the largest quantity in ethanolic extract of bee pollen from Poland was found to be *p*-coumaric acid, ranging from 3754 to 116950  $\mu\text{g/g}$  [39]. Coelho *et al.* [40] reported that

ethanolic extract of sixteen propolis samples from different areas of Brazil had *p*-coumaric acid at concentrations of 1000–27200  $\mu\text{g/g}$ . Kumazawa *et al.* [41] and Ahn *et al.* [42] found *p*-coumaric acid at concentrations of 900–27400 and 2300–52200  $\mu\text{g/g}$  in propolis extracts, respectively. In the present study, *p*-coumaric acid in propolis extract was quite low compared to the studies mentioned above. In addition to *p*-coumaric acid, these differences are also

**Table 3.** Phenolic compounds of bee pollen and propolis ethanolic extracts.

Classification	Phenolic compounds	Bee pollen ( $\mu\text{g/g}$ )	Propolis ( $\mu\text{g/g}$ )
Phenolic	Gallic Acid	82.9	41.2
	Protocatechic Acid	19.1	65.9
	p-Hydroxy Benzoic Acid	19.6	69.6
	Chlorogenic Acid	86.1	24.3
	Caffeic Acid	24.0	1212.4
	Epicatechin	ND	35.8
	Syringic Acid	ND	18.4
	Vanilin	ND	429.8
	p-Coumaric Acid	53.5	150.9
	Ferulic Acid	78.6	147.3
	Sinapinic Acid	94.8	ND
	o-Coumaric Acid	107.5	ND
	Rutin	ND	ND
	Rosmarinic Acid	902.6	556.1
	Eriodictiol	ND	106.4
	Flavanone	Hesperidin	ND
Flavanol	Quercetin	3575.1	ND
	Catechin	ND	ND
Flavones	Kaempferol	3264.5	1899.4
	Luteolin	3930.2	567.0
	Apigenin	6477.2	3965.9
Aromatic	Benzoic Acid	8900.6	349.7
	Cinnamic Acid	273.8	8256.1

ND: Not detected.

noticeable in other phenolic compounds. There are great differences in phenolic profiles among ethanolic extracts of propolis collected from different regions. However, bee pollen and propolis extracts exhibited different phenolics' profiles agreeing with literature [34, 43–45].

In the present study, the phenolic contents of the ethanolic pollen and propolis extracts were  $45.24 \pm 5.75$  and  $86.90 \pm 11.15 \mu\text{g}$  of GAE/mg of dry extracts, respectively. However, different results were found in the literature. The TPC value of the pollen extract in this study was similar to those in the range of 9.15 to 462.02 mg GAE/g pollen [38, 46–50]. Also, the TPC value of the propolis extract was similar to those reported in various propolis samples from different regions with TPCs ranging from 31.00 to 302.00 mg GAE/g propolis [42, 51–54]. Numerous studies on TPC analyzes in bee products describe various ranges for total phenolics of pollen and propolis from different geographical origins, depending on the standard (gallic acid, pinocembrin, chlorogenic acid) and solvent (methanol, ethanol) used [55–58].

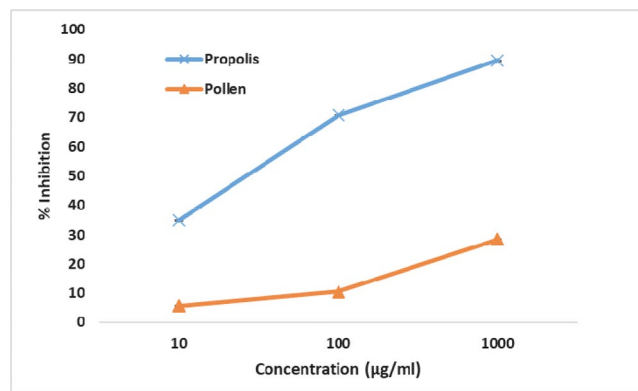
### Antiradical activity

DPPH method has been widely used to determine the antiradical activity of various samples [59]. In the present study, we used the DPPH method to predict the antioxidant activities of the pollen and propolis ethanolic extracts *in vitro* (Figure 2). The DPPH scavenging activities of the pollen extract at the concentrations of 10, 100 and 1000  $\mu\text{g/mL}$  were  $5.75 \pm 0.01$ ,  $10.51 \pm 0.01$

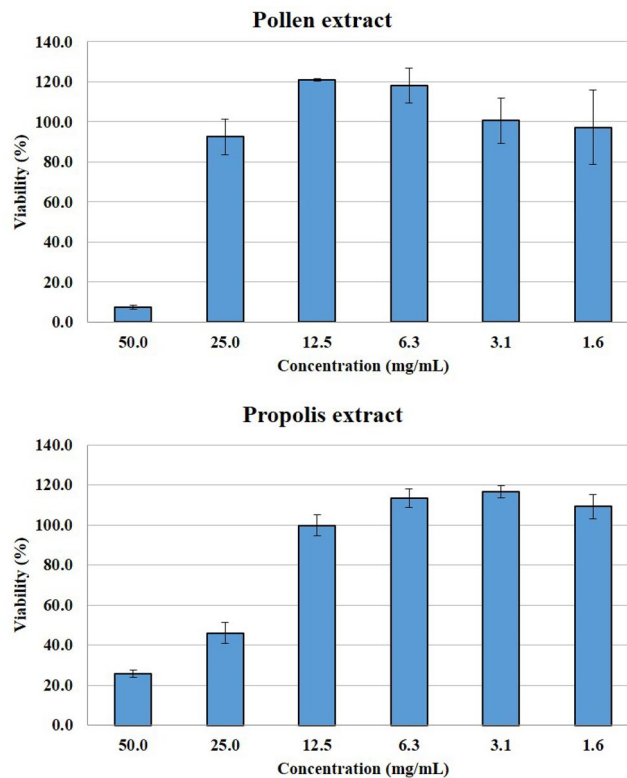
and  $28.42 \pm 0.02\%$ , respectively. The DPPH scavenging activities of the propolis extract at the concentrations of 10, 100 and 1000  $\mu\text{g/mL}$  were  $34.92 \pm 0.01$ ,  $70.80 \pm 0.02$  and  $89.57 \pm 0.01\%$ , respectively. The scavenging activity was approximately 34.9% at a concentration of 10  $\mu\text{g/mL}$  of propolis extract; it was about 5.8% at the same concentration of pollen extract. In the present study, the propolis extract with higher TPC value ( $86.90 \pm 11.15 \mu\text{g}$  of GAE/mg) exhibited higher antiradical activity against DPPH compared to the pollen extract ( $45.24 \pm 5.75 \mu\text{g}$  of GAE/mg). Mohdaly *et al.* [16] observed that the scavenging activity of propolis extract was superior to that of pollen extract and the DPPH scavenging had a linear relation with TPC concentration. The results of the present study supported the suggestion that the antioxidant activity of the extracts mainly depends on the concentration of the phenolic compounds present.

### Antiproliferative activity

In the present study, the antiproliferative activities of pollen and propolis ethanolic extracts were tested on Myeloma cells (Figure 3). It has been shown in the literature that pollen and propolis have antiproliferative activity against various cell lines. In a previous study, propolis ethanolic extract exhibited good antiproliferative activity against MCF7 (human breast cancer), HGC27 (human gastric carcinoma) and A549 (human lung adenocarcinoma) cancer cell lines with  $\text{IC}_{50}$  values in the range of 58.6–90.7  $\mu\text{g/mL}$  in MTS test [60]. In another study, water-soluble propolis and bee pollen



**Figure 2.** Scavenging activity of propolis and pollen extracts against DPPH radicals at different concentrations.



**Figure 3.** Effects of pollen and propolis extracts on viability of Myeloma cells. MTT assay. Values are means from 3 independent experiments, with standard deviation ( $\pm$ S.D.)

showed antiproliferative activity in MCF-7 cells, with  $IC_{50}$  values of  $10.8 \pm 0.06$  and  $18.6 \pm 0.03$  mg/mL, respectively [61]. Choudhari *et al.* [18] reported that ethanolic extract of propolis at a concentration of 250  $\mu$ g/mL exhibited  $\geq 50\%$  lethality in MCF-7, HT-29 (human colon adenocarcinoma), Caco-2 (human epithelial colorectal adenocarcinoma) and B16F1 (murine melanoma) cells. In the present study,  $IC_{50}$  values of pollen and propolis extracts on myeloma cells were founded to be 1.49% and 2.88%, respectively. Compared with studies examining the antiproliferative activity of bee pollen and propolis on other cancer lines, the results of this study

may indicate that myeloma cells are less sensitive to pollen and propolis ethanolic extract than other cell lines.

### Antibacterial activity

In the present study, the antibacterial activities of bee pollen and propolis ethanolic extracts were evaluated against *S. aureus*, *E. coli* and *P. aeruginosa* (Tables 4 and 5). According to the agar well diffusion test, the propolis ethanolic extract in the tested concentrations (from 6.25 mg/mL to 100 mg/mL) showed antibacterial



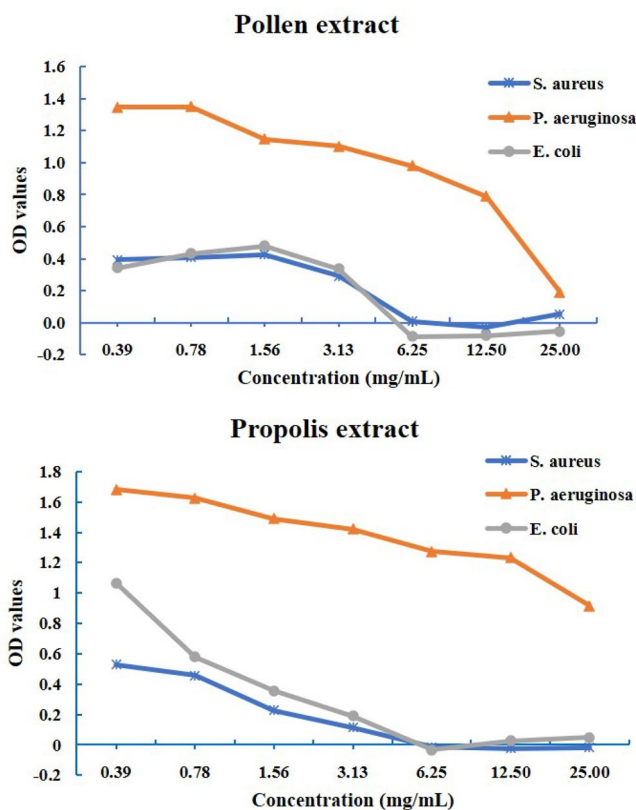
**Table 4.** Antimicrobial activity of pollen and propolis extracts against selected pathogens using agar well diffusion method (mm).

Concentrations (mg/mL)	Zone of inhibition (mm)					
	<i>S. aureus</i>		<i>E. coli</i>		<i>P. aeruginosa</i>	
	Propolis	Pollen	Propolis	Pollen	Propolis	Pollen
100	19.9±0.6	ND	17.3±1.1	ND	ND	ND
50	18.9±0.2	ND	15.4±0.1	ND	ND	ND
25	17.8±0.6	ND	13.9±0.2	ND	ND	ND
12.5	15.2±0.9	ND	12.9±0.2	ND	ND	ND
6.25	12.6±1.0	ND	11.8±0.5	ND	ND	ND
Enrofloxacin	32.6±0.1	34.05±0.2	30.0±0.6	31.55±0.2	17.7±0.4	17.45±0.2

Each value represents the mean ± standard deviation (SD) derived from three repeats. The diameter of each well was 6 mm. ND: Not detected.

**Table 5.** Minimum inhibitory and bactericidal concentrations of bee pollen and propolis extracts against selected pathogens (mg/mL).

	<i>S. aureus</i>		<i>E. coli</i>		<i>P. aeruginosa</i>	
	Propolis	Pollen	Propolis	Pollen	Propolis	Pollen
MIC	6.25	6.25	6.25	6.25	>25	>25
MBC	12.5	>25	12.5	>25	>25	>25

**Figure 4.** MIC values of the extracts for all tested bacteria.

activity against *S. aureus* and *E. coli* with an inhibition zone from  $12.6 \pm 1.0$  to  $19.9 \pm 0.6$  mm and from  $11.8 \pm 0.5$  to  $17.3 \pm 1.1$  mm, respectively. The bee pollen ethanolic extract presented no detectable antibacterial activity against *S. aureus*, *E. coli* and *P. aeruginosa* in the concentrations used. There was no visible inhibition zone against *P. aeruginosa* for both pollen and

propolis ethanolic extracts. Propolis extract was more potent in inhibiting *S. aureus* than *E. coli*. Similar to the present study, several studies reported that gram-positive bacteria are more sensitive to ethanolic extract of bee pollen and propolis than gram-negative bacteria [62–66]. However, some papers reported that ethanolic extract of bee pollen has no antibacterial

activity against *E. coli* O157:H7 and *S. aureus* [62, 63]. Similarly, there are different results on the antibacterial activity of pollen and propolis on *P. aeruginosa* [66, 67]. The differences in the antibacterial properties of bee pollen and propolis may be related to their chemical composition depending strongly on botanical and geographic origin, climate and soil type, extraction methods and solvents used in extractions [68].

The microdilution method showed that all extracts did not exhibit antibacterial activity against *P. aeruginosa* (Figure 4 and Table 5) like the agar well diffusion test. Both extracts showed high antimicrobial activity against *S. aureus* and *E. coli* with a MIC value of 6.25 mg/mL. Although the MBC values of the propolis extract against *S. aureus* and *E. coli* were 12.5 mg/mL, the MBC value could not be determined for the propolis extract, which had no antibacterial effect against *P. aeruginosa*. The pollen extract had no antibacterial activity against all tested bacteria at the applied concentrations (0.39–25 mg/mL).

Several studies have reported that bee pollen and propolis extracts exhibited higher antibacterial effect against gram-positive cocci (*S. aureus*) than gram-negative bacteria (*E. coli* and *P. aeruginosa*) [16, 69–71]. The reported values of MICs are widely divergent for pollen and propolis ethanolic extracts. Previous studies have reported that the MICs of propolis ethanolic extracts ranged between 0.008–3.1 mg/mL, 0.016–5.0 mg/mL, 0.31–7.9 mg/mL, against *S. aureus*, *E. coli* and *P. aeruginosa*, respectively [20, 72–75]. Similarly, the MICs of bee pollen ethanolic extracts are widely divergent. Several studies have reported that pollen ethanolic extracts show antibacterial activity against *S. aureus*, *E. coli* and *P. aeruginosa* in the range of 0.78–>20 mg/mL, 1.25–>20 mg/mL, 10–>20 mg/mL, respectively [16, 21, 76, 78]. The lower activity of propolis and pollen on gram-negative bacteria can be attributed to the structure of the outer membrane of the bacteria and their ability to produce hydrolytic enzymes that degrade the active components of propolis [78]. Gram-negative bacteria, which have a double cell wall of lipopolysaccharides and proteins, are resistant to the antibacterial effect of bee pollen [71]. In the present study, propolis extracts demonstrated lower efficiency than previous studies, while pollen extracts showed their effect in a line with other studies [16, 76]. Propolis ethanolic extract demonstrated higher antioxidant and antibacterial activities than pollen ethanolic extract, which can be attributed to the presence of higher phenolic and flavonoid contents of the former [16, 21].

The antibacterial activities of bee pollen and propolis extracts obtained using different solvents were

evaluated in several reports. The MIC values of the hydroethanolic extract of bee pollen for *S. aureus*, *E. coli* and *P. aeruginosa* ranged from 0.064 to 0.512 mg/mL [38]. The MIC values of the methanolic extract of propolis against *S. aureus*, *E. coli* and *P. aeruginosa* were in the range of  $\geq 1.25$ –>5 mg/mL, >1.25–>5 mg/mL, and  $\leq 1.25$  mg/mL, respectively [33]. Variations between the antimicrobial effects of the extracts probably depend not only on the floral origin of pollen and propolis, but also on the extraction method and solvents used.

## Conclusions

This study showed that ethanolic extracts of bee pollen and propolis had antimicrobial, free-radical scavenging and antiproliferative properties. Due to presence of bioactive compounds responsible for these activities, Turkish bee pollen and propolis could be a natural source of antiradical and antibacterial ingredients as a dietary supplement and could be used as an agent for preventing food for spoilage in the food industry.

## Conflict of interest

The authors declare that there is no conflict of interest.

## Data availability statement

The data that support the findings reported in this study are available from the corresponding author upon reasonable request.

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