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ORIGINAL PAPER

Evaluation of the nutritional value of bee pollen by palynological, antioxidant, antimicrobial, and elemental characteristics

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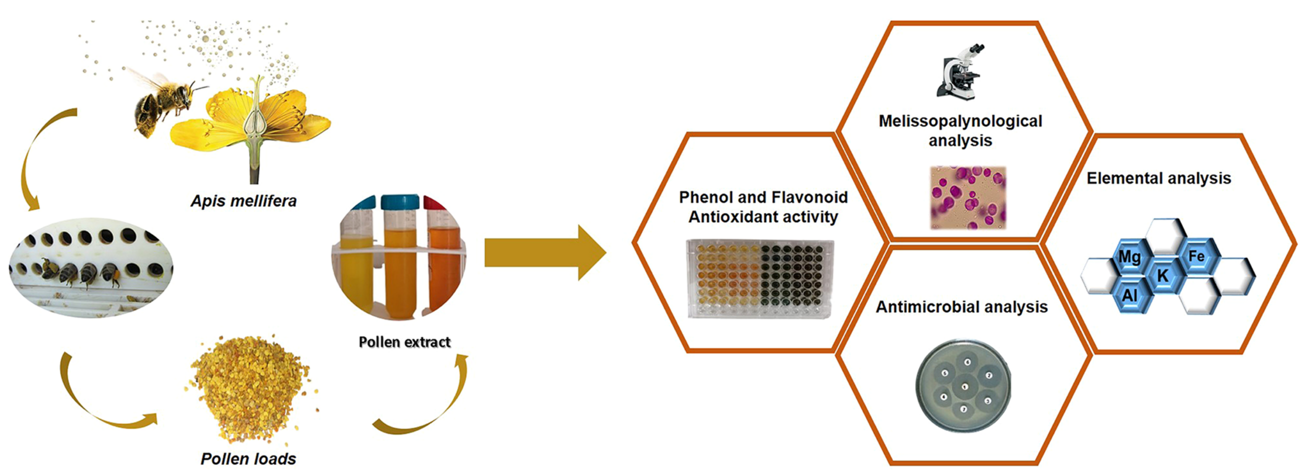
Abstract

The object of this study was to characterize bee pollen (BP) as a food supplement according to its palynological, antioxidant, antimicrobial properties, and elemental contents. Twelve plant families, 35 genera, and one species were determined by palynological analysis of BP. *Verbascum* spp., *Papaver* spp., and *Vicia* spp. were found the major floral sources of BP. Two samples were determined as monofloral *Verbascum* spp. bee pollen. Total flavonoid (TFC) and phenolic content (TPC) varied from 117.5 to 142.09 mg QE/100 g, and 386.59 to 743.73 mg GAE/100 g, respectively. According to 1,1-diphenyl-2-picrylhydrazyl (DPPH) radical and 2,2′-azino-bis3-ethylbenzothiazoline-6-sulfonic acid (ABTS) radical cation assays, the BP samples demonstrated high antioxidant activity. Result of ferric reducing antioxidant power (FRAP) and metal chelating activities (MCA) assays were ranging from 61.17 to 69.7% and 74.99 to 87.78%. Antimicrobial activities of the BP were determined by the agar well diffusion and microplate method. Obtained results indicated that BP showed appreciable antibacterial activity against *Escherichia coli, Bacillus cereus,* and *Staphylococcus aureus* strains by remarkably decreasing bacterial growth**.** Thirty-one elements were analyzed in BP samples by inductively coupled plasma-mass spectrometry (ICP-MS). Target hazard quotients (THQ), hazard index (HI), and estimated daily intake (EDI) values were calculated using selected elements' results. Considering these values, it was determined that the consumption of bee pollen was safe for adults and children. BP samples can be used as a food supplement because of their high antioxidant and antimicrobial capacity and elemental content.

Graphical abstract



Keywords Antimicrobial and antioxidant activity · Element · Palynological analysis · Pollen · Health risk assessment

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Introduction

Flower pollen is collected from the anthers, mixed with nectar and bee gland secretions by *Apis mellifera* (honey bee). They store this in pollen baskets on their hind legs as pellets and carry it to the hive [1, 2]. This product is called bee pollen (BP) and traps are placed at the entrance of the hive to collect the pollen loads from the legs of the bees.

About 250 different biologically active substances have been detected in bee pollen [3], including proteins, enzymes, amino acids, lipids, carbohydrates, vitamins, volatile compounds, phenolic acids, flavonoids, and minerals [4]. Among its constituents, phenolic acids and flavonoids act as potent free radical scavengers. BP has a strong antioxidant and antimicrobial activity due to the variety of primary and secondary metabolites that it contains [1, 5–7]. These components contribute to the therapeutic potential of bee pollen. Elements cannot be synthesized in the human body and, therefore, they must be taken in daily in appropriate amounts through the diet [8]. Bee pollen contains a variety of elements necessary for human nutrition, both macroelements (Ca, K, Mg, and Na) and microelements (Fe, I, Cu, Zn, Co, Cr, Mo, Se, Ni, Li V, and Mn) [9]. Bee pollen, which is preferred in various food and healthy products, has been used in traditional medicine, supplementary nutrition, and alternative diets [10, 11]. Unfortunately, the content of bee pollen is affected by environmental pollution [12, 13]. So, although bee pollen has a high nutritional value, it also contains toxic elements due to environmental pollution [14]. Therefore, BP is considered a bioindicator for chemical pollution of the environment [3]. The content of BP varies particularly depending on the plant sources, bee species, and environmental factors during pollen development, climatic conditions, soil type, and geographical origin [15]. The botanical origin of the BP must be investigated to obtain accurate data about difference between monofloral and multifloral bee pollen. Therefore, melissopalynological analysis is necessary to provide information on the botanical origin of BP [16].

Although beekeeping is very common and bee products are consumed on a widely international scale, very little data are accessible on the quality regarding the content of different monofloral bee pollens from Turkey. This report is on the evaluation of the quality of monofloral and multifloral bee pollen in the province of Bingöl. First, we applied a palynological analysis technique to determine the botanical origins of our BP samples. Then, their TPC and TFC values were investigated. To monitor the differentiation in the bioactivity profile of each BP samples, antioxidant activity assays (DPPH, ABTS, MCA, and FRAP), and antimicrobial activity tests were performed. Finally, the concentrations of thirty-one elements in the BP samples were determined, and the values of EDI, THQ, and HI were calculated. This study estimated a health risk assessment (non-carcinogenic) to identify the potential risk to consumers in terms of public health.

Materials and methods

Field study

A total of eight BP samples were obtained from different beehives of beekeepers from Bingöl in the autumn of 2019. Bingöl is a province in Turkey located in Eastern Anatolia (38° 27′ and 40° 27′E, 41° 20′ and 39° 54′N) (Fig. 1). BP was placed in foam boxes with ice and transported to the laboratory within two hours and frozen at 20 °C until analyses.

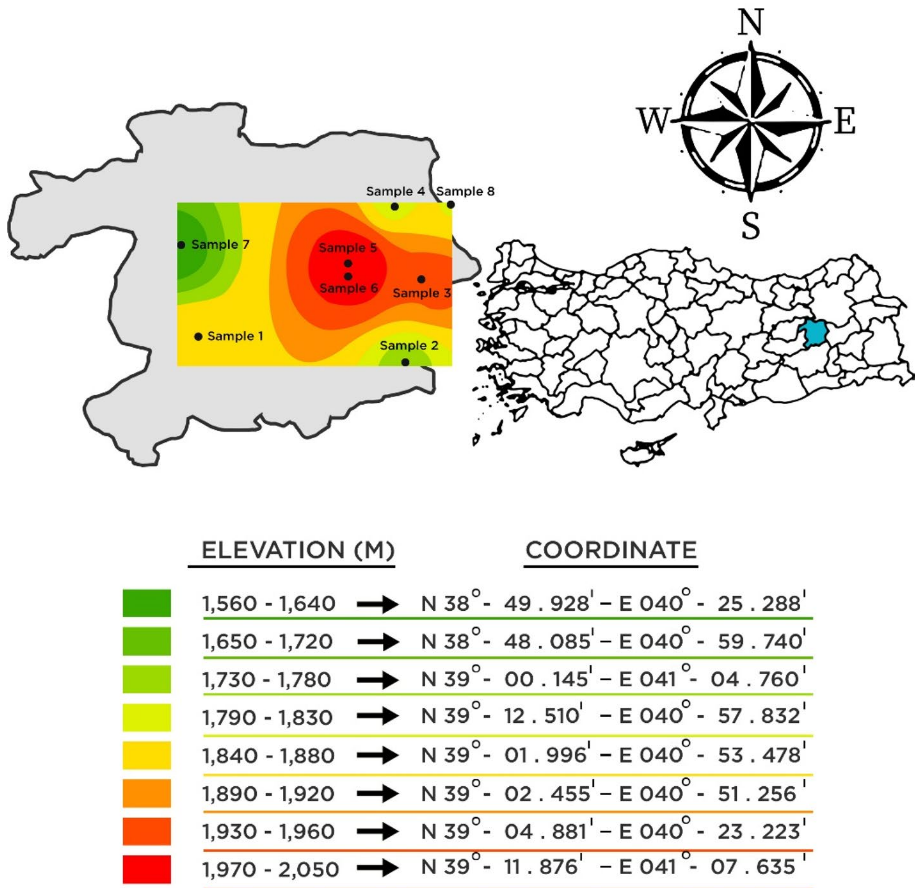
Reagents and chemicals

2,2-diphenyl-1-picrylhydrazyl (DPPH), Diammonium 2,2′-azino-bis (3-ethylbenzothiazoline-6-sulfonate) (ABTS), Iron(II) chloride, Ferrozine, Gallic acid, Trolox, Trichloroacetic acid, Butylated hydroxyanisole (BHA), 3,3′,4′,5,7-Pentahydroxyflavone 3-rutinoside (Rutin, RUT), Aluminum chloride ($AlCl_3$), Potassium phosphate dibasic (K_2HPO_4), Potassium dihydrogen phosphate monobasic (KH_2PO_4), Butylated hydroxyanisole (BHA)were purchased from Sigma-Aldrich Chemical Company (St. Louis, MO, USA). Basic Fuchsin was purchased from Pro-lab Diagnostics. Glycerol, Sodium carbonate (Na_2CO_3), Folin & Ciocalteu's phenol reagent, Nitric acid 65% were purchased from Merck KGaA, Darmstadt, Germany. Potassium persulfate, Potassium hexacyanoferrate (III), and Potassium acetate were purchased from Chemsolute. Ethyl alcohol was purchased from Alkolmed. All element standards used in the ICP-MS method were purchased from Sigma-Aldrich Chemical Company, Merck (St. Louis, MO, USA). Muller-Hinton Broth (MHB and Sabouraud Dextrose Broth (SDB) were purchased from Himedia.

Palynological analysis

BP samples were analyzed using the methodology of Barth et al. [17] with modifications. Two grams of mixed BP were homogenized with 13 mL 70% ethanol solution. This solution

Fig. 1 Geographical locations of BP samples



was kept in a sonicator (Wisd, Daihan Scientific, Korea) for 5 min to homogenize the BP. After centrifugation (Eppendorf SE, Hamburg, Germany) at 3500 rpm for 15 min, the final residue was diluted into a 1:1 water/glycerin mixture. The BP suspension of 10 µL was applied to the microscope slide and the suspension was fixed to the slides by glycerin gelatin with basic fuchsin [18]. The slides were examined by LED optical microscope (Leica DM 2500, Leica Microsystems, Germany) and pollen grains were analyzed at 600× and 1000× magnification. A minimum of 500 pollen grains were counted on each slide to evaluate the relative abundance of pollen type. All analyses were performed in triplicate for each specimen.

Preparation of extracts

Extracts of BP samples were prepared for use in total phenolic/flavonoid contents and antioxidant activity assays. 1 g of powdered BP sample was weighed into 15 mL falcon tubes and dissolved in 10 mL of 70% ethanol. 70% ethanol were used as a solvent because extracts prepared using this solvent have been reported to provide better antioxidant activity [19–21]. The solutions were incubated at 40 °C for 1 h in a sonicator. After incubation, the solutions were centrifuged at 3500 rpm for 10 min. The supernatant was filtered and stored at − 80 °C until analysis [22].

Total phenolic and total flavanoid content determination

The total phenolic content (TPC) of the samples was determined using the Folin–Ciocalteu method of Temizer et al. [6]. The aluminum chloride method was used to determine the TFC of samples [23]. The TPC values were expressed as miligrams of gallic acid equivalent 100 g of dry weight of BP (mg GAE/100 g dw). The TFC values were expressed as miligrams quercetin equivalent 100 g of dry weight of pollen (mg QE/100 g dw).

Antioxidant activity

1,1-diphenyl-2-picrylhydrazyl radical assay

The 1,1-diphenyl-2-picrylhydrazyl (DPPH) radical scavenging activity assays were carried out according to the method outlined by Gökçe et al. [24]. Absorbance values were measured at 517 nm using a UV spectrophotometer (Genesys 180, Thermo scientific, Waltham, Massachusetts, ABD). The radical scavenging activity results were calculated as IC_{50} (µg/mL) which represent the concentration required to scavenge 50% of the DPPH radical.

Metal chelating activities assay

The metal chelating activities (MCA) were examined using the method defined by Dinis et al.[25]. The absorbance values were recorded at 562 nm and the chelation effect on ferrous ion was determined as inhibition percentage of the formation of the Fe^{2+} and ferrozine complex (Eq. 1) as folows:

$$\mathrm{MCA}(\%) = \left[1 - \left(\frac{\mathrm{As}}{\mathrm{Ac}}\right)\right] \times 100 \quad (1)$$

where As refers to absorbance of samples and the ferrozine–Fe^{2+} mixture and Ac refers to absorbance of ferrozine–Fe^{2+} complex.

2,2′-azino-bis (3-ethylbenzothiazoline-6-sulfonic acid) radical cation assay

The 2,2′-azino-bis (3-ethylbenzothiazoline-6-sulfonic acid) (ABTS) radical cation scavenging assays were performed via the spectrophotometric method [24]. The activities were given as IC_{50} values (µg/mL) which represent the concentration required to scavenge 50% of the ABTS radical cation.

Ferric reducing antioxidant power assay

The ferric reducing antioxidant power (FRAP) method proposed by Gür et al. [26] was used with modifications. Absorbance value of the 2 mL [2 mL (50 µg mL^{-1} samples) + 2.0 mL (0.2 mol L^{-1}, pH 6.6 phosphate buffer solution (PBS)) + 2.0 mL (1.0% potassium ferricyanide) + trichloroacetic acid (TCA)] + 2 mL distilled water + 0.5 mL, 0.1% $FeCl_3$] solution was recorded at 700 nm. The Fe^{3+}/Fe^{2+} transformation was determined using Eq. 2:

$$\mathrm{FRAP}\ (\%) = \left(\frac{\mathrm{As}}{\mathrm{Ac}}\right) \times 100 \quad (2)$$

where As refers to absorbance of the extract and Ac refers to control's absorbance.

Antimicrobial activity

The antimicrobial activity of the BP samples was analysed with the agar well diffusion test method [27] and minimum inhibition concentration (MIC), which was determined using the microplate method [28]. In this study, the antimicrobial activity of the samples was determined using the following four different microorganisms: These microorganisms were gram-negative *Escherichia coli* ATCC 25922, gram-positive *Staphylococcus aureus* ATCC 29213, and *Bacillus cereus* ATCC 10876, and a yeast *Candida albicans* ATCC 10231. They were incubated in Muller-Hinton Broth (MHB) for bacteria and a Sabouraud Dextrose Broth (SDB) for yeast at 37 °C for 24 h. The minimum inhibition concentration (MIC) was determined according to the National Committee for Clinical Laboratory Standards. The extracts of the samples (8.33 g/mL) were diluted to 50% with MHB medium and a 50-µL of a microorganism culture solution was added to a 96-well microplate [28].

Elemental analysis

Concentrations of 31 elements, including sodium (Na), magnesium (Mg), aluminum (Al), potassium (K), calcium (Ca), vanadium (V), chromium (Cr), manganese (Mn), iron (Fe), cobalt (Co), nickel (Ni), copper (Cu), zinc (Zn), gallium (Ga), arsenic (As), selenium (Se), scandium (Sc), rubidium (Rb), strontium (Sr), yttrium (Y), rhodium (Rh), silver (Ag), cadmium (Cd), indium (In), cesium (Cs), barium (Ba), gold (Au), thallium (Tl), lead (Pb), bismuth(Bi), and uranium (U) were determined with inductively coupled plasma mass spectrometry (ICP-MS, NexION® 2000 C, Perkin Elmer, USA) using the method outlined by Altunatmaz et al. [14] with modifications. Approximately 1 g of each BP sample was transferred into a Teflon vessel with 10 mL of concentrated nitric acid for microwave digestion. The maximum temperature was raised to within 15 min and the sample was digested at 200 °C for 15 min by microwave (CEM brand Mars 6 One Touch USA). After cooling, the digested sample solutions were diluted to 50 mL with deionized water. 1 mL of this solution was diluted to 10 mL with 1:99 suprapurgrade nitric acid/deionized water.

Estimated dailyintake and human health risk assessment

This analysis was carried out to assess the chronic effects of the risks of the potentially toxic elements on human health. Trace elements in different environments can enter the human body through oral way, inhalation, and skin contact. Since bee pollen is an edible bee product, people take the elements found in bee pollen through the oral way. To assess the human health risk assessment associated with the regular use of bee pollen, we considered the oral way for adults and children.

The risk calculations for children and adults through pollen consumption were evaluated by calculating estimated daily intake (EDI), target hazard coefficient (THQ), and hazard index (HI) [29, 30].

Estimated daily intake (EDI) was calculated using the following equation (Eq. 3):

$$\mathrm{EDI} = \frac{\mathrm{DPC} \times \mathrm{C}}{\mathrm{BW}} \tag{3}$$

where EDI is the estimated daily intake (mg $kg^{-1}d^{-1}$); DPC; (Daily consumption, regarding pollen): (one tablespoon of dry BP ~ 10 g) 0.01 kg for an adult and (one teaspoon dry pollen ~ 2 g) 0.002 kg for a child per day [31]; C is the concentration of elements (mg/kg); BW (average body weight) values vary according to the nations and an average weight has been taken: 15 kg for children and 70 kg for adults [30–33].

The hazard quotient (THQ) was also calculated using Eq. 4 for potentially toxic elements in bee pollen via its consumption (Eq. 4.):

$$\mathrm{THQ} = \frac{\mathrm{EDI}}{\mathrm{RFD}} \tag{4}$$

where the reference oral dose (RFD) [34] for elements (mg kg^{-1} BW day). The RFD doses are given in Table 7.

Total health risk through exposure to different elements (Hazardous Index, HI) was calculated as follows:

$$\mathrm{HI} = \sum_{k=1}^{n} \mathrm{HQ1} + \mathrm{HQ2} + \mathrm{HQ3} + \cdots + \mathrm{HQ}n$$

Statistical analysis

Statistical analysis was performed based on the basis of different methodological perspectives to evaluate the BP samples. The Minitab 18 software was used for these analyses. The linear relationships between antioxidant activity and element assay results were measured, calculated, and interpreted for all samples by using Pearson's Product–Moment Correlation Coefficient (PPMCC). Principal component analysis (PCA) was also performed based on the antioxidant activity and elemental contents [35]. P value of < 0.05 was assumed as significant. Moreover, the antioxidant activity and element assay results were evaluated by performing the Kolmogorov–Smirnov normality test.

Results and discussion

Floral sources of BP samples by palynology

Palynological analysis of BP provides a powerful tool for determining the plants that the bees are visiting. BP samples with 80% or more of a pollen type were classified as mononofloral [36]; the other samples were classified as multifloral. In current study, 25% of the BP samples were classified as monofloral while %75 of them were classified as multifloral. In accordance with microscopic examination, Samples 6 and 8 were determined monofloral *Verbascum* spp. BP with 90.27% and 80% *Verbascum* spp. pollen grain, respectively. Other BP samples were classified as multifloral, according to frequency of pollen species (Table 1). We identified 38 plant taxa belonging to 12 families. Plant taxa belonging to Scrophulariaceae (31.9%), Papaveraceae (23.4%), Fabaceae (12%), and Asteraceae (10.1%) families were widespread and common in all BP samples. The pollen diagram has shown pollen taxons with a pollen frequency ≥ 10 (Fig. 2). The taxa of *Verbascum* spp., *Papaver* spp., *Vicia* spp., *Acantholimon calverti*, *Acantholimon* spp., and *Hypericum* spp. appeared with high incidence in all samples (Fig. 2).

In current study, it was detected that BP samples has similar botanical origin with previous studies conducted on the bee flora of Bingöl. Behçet et al. [37] determined that there are many taxa belonging to *Verbascum* spp. and it was concluded that those taxa could be sources of monofloral BP in this study. The melissopalynological analysis of the BP yielded different results from BP data collected in other countries such as Portugal, Brazil, Korea, and Italy [38–41]. Furthermore, the obtained data do not match the results of other BP palynological studies conducted in Turkey [42, 43]. Significant pollen types determined in this study, such as *Verbascum* spp., *Papaver* spp., and *Taraxacum* spp., have

Table 1 Botanical origin of pollen grains (%) in the BP samples

Family	Family/genus/species	%							
		Sample 1	Sample 2	Sample 3	Sample 4	Sample 5	Sample 6	Sample 7	Sample 8
Apiaceae[a]	*Bupleurum*[b]		0.43						
	Eryngium[b]				0.78				
	Heracleum[b]		17.46	1.71					
	Pimpinella[b]			9.59					11.8
Asteraceae	*Cichorium*[b]						0.88		
	Echinops[b]		0.65			0.96			
	Inula[b]		0.87					2.10	
	Senecio type[c]				1.56			4.20	
	Scorzonera[b]						1.77		
	Taraxacum type[c]	0.34*	1.08						
	Taraxacum[b]							41.26	
	Xeranthemum[b]		25.22						
Caryophyllaceae	Caryophyllaceae[a]	2.69						1.75	
Fabaceae	Fabaceae[a]	4.04		0.34		0.64		1.75	
	Astragalus[b]	4.38						6.99	
	Coronilla[b]			1.03	0.39				
	Genista[b]	0.67		0.34					
	Hedysarum[b]	6.06			0.39	1.92	2.21		
	Medicago[b]	0.34							
	Melilotus[b]	8.08						2.80	
	Onobrychis[b]							3.50	
	Vicia[b]		46.3	1.03	1.56	0.96			
Hypericaceae	*Hypericum*[b]		0.43	6.16	10.16	4.79	3.10	15.03	0.67
Lamiaceae	*Lamium*[b]							0.35	
	Phlomis[b]	8.42		1.03				6.99	
	Salvia[b]						0.44		
	Stachys[b]	5.72		0.34					
	Thymus[b]	4.71							
Onagraceae	*Epilobium*[b]	1.35				1.92			
Papaveraceae	*Papaver*[b]		1.08	72.9	70.7	36.42	0.44	0.70	4.69
Plantaginaceae	*Plantago*[b]	7.74	1.94				0.88	9.44	
Plumbaginaceae	*Acantholimon*[b]				0.78				
	Acantholimon type I[c]	20.54							
	Acantholimon calverti[d]	17.89						0.35	
Rosaceae	*Sanguisorba*[b]	3.03						1.05	
Scrophulariaceae	Scrophulariaceae[a]	4.04		5.48					
	Linaria[b]					6.39			
	Scrophularia[b]		4.53						2.67
	Verbascum[b]				13.70	46.00	90.26	1.75	80.00

*Unit of values:%. percentage of plant pollen grains in the samples

[a]Family

[b]Genus

[c]Type

[d]Species

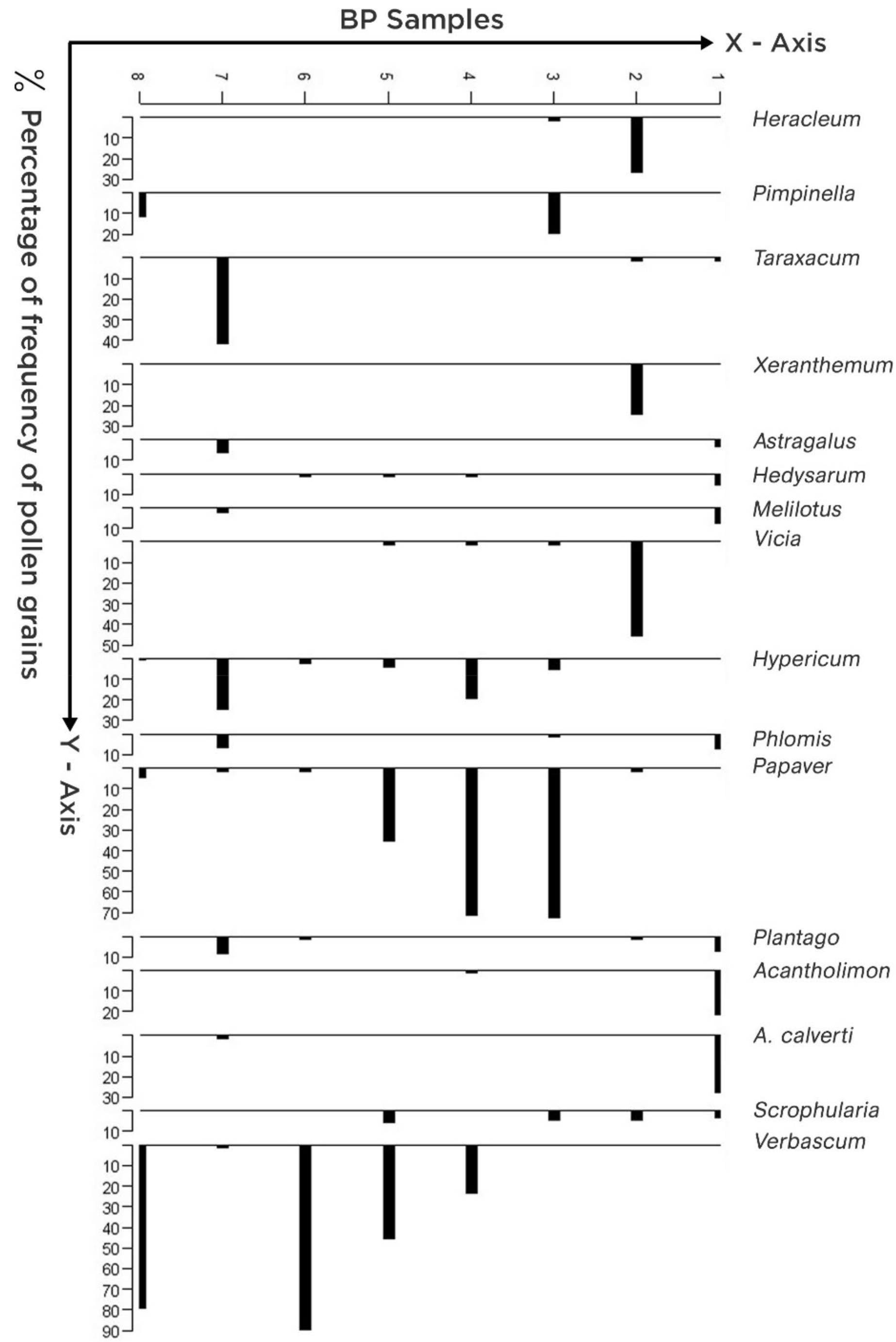


Fig. 2 Pollen diagram of BP samples

also been reported in BP samples from Bulgaria, Italy, Serbia, and Greece [44–47]. It was concluded that similarities as well as differences may be present about botanical origins of BP. These differences or similarities of botanical origin are related to factors such as floral biodiversity, geography, climate and environmental conditions.

TPC, TFC content and in vitro antioxidant activity

BP's antioxidant potential were evaluated with TPC, TFC and antioxidant activity assays (DPPH, MCA, ABTS and FRAP). The TPC and TFC contents ranged from 386.59 ± 0.344 to 743.73 ± 0.433 mg GAE/100 g and 117.05 ± 0.376 to 142.09 ± 0.754 mg QE/100 g. It was found

Table 2 Total flavonoid/phenol content and antioxidant activity assay results of BP samples and controls

Samples/controls	TFC	TPC	DPPH	ABTS	FRAP	MCA
Sample 1	134.14[a] ± 0.450[b]	743.73 ± 0.433	30.47 ± 0.050	37.23 ± 0.124	69.84 ± 0.235	87.65 ± 0.098
Sample 2	117.05 ± 0.376	386.59 ± 0.344	20.21 ± 0.045	24.45 ± 0.093	65.33 ± 0.123	85.82 ± 0.124
Sample 3	137.27 ± 0.170	524.86 ± 0.545	27.95 ± 0.055	27.3 ± 0.087	68.44 ± 0.097	86.81 ± 0.167
Sample 4	136.41 ± 0.364	433.64 ± 0.123	29.37 ± 0.075	25.95 ± 0.078	64.44 ± 0.099	85.5 ± 0.084
Sample 5	131.77 ± 0.233	505.64 ± 0.245	30.79 ± .0.095	28.83 ± 0.086	69.7 ± 0.135	74.99 ± 0.067
Sample 6	125.36 ± 0.125	501.55 ± 0.132	44.68 ± 0.034	30.7 ± 0.043	64.51 ± 0.073	85.6 ± 0.056
Sample 7	126.77 ± 0.738	622.64 ± 0.278	45.63 ± 0.235	32.03 ± 0.068	61.17 ± 0.068	87.78 ± 0.043
Sample 8	142.09 ± 0.754	442.23 ± 0.369	38.84 ± 0.046	30.73 ± 0.065	64.22 ± 0.079	85.62 ± 0.044
BHA[c,d]			8.08 ± 0.001	8.25 ± 0.003	70.53 ± 0.002	82.38 ± 0.0
RUT[c,e]			15.95 ± 0.002	15.24 ± 0.004	90.29 ± 0.001	84.48 ± 0.133
TRO[c,f]			25.52 ± 0.003	4.1 ± 0.006	51.87 ± 0.006	67.62 ± 0.075
	mg QE/100 g	mg GAE/100 g	IC_{50} (μg/mL)	IC_{50} (μg/mL)	%	%

[a]Mean values after three repetitions

[b]Standard deviation (Std)

[c]Controls

[d]Butylated hydroxyanisole (BHA)

[e]3,3′,4′,5,7-Pentahydroxyflavone 3-rutinoside (Rutin, RUT)

[f]Trolox (TRO)

that TPC and TFC levels of *Verbascum* spp., and multifloral pollen samples ranged from 442.23 to 501.55 and 386.59 to 743.73 mg GAE/100 g, 125.36–142.09 and 117.05–137.27 mg QE/100 g, respectively. While TPC values of multifloral BP samples were found to be higher than monofloral *Verbascum* spp. BP samples, TFC values of multifloral BP samples were detected lower than monofloral *Verbascum* spp. BP samples. TPC and TFC content of BP of Grecee [48], Italy [49], Portugal [50], Serbia [47], and Turkey [18, 42, 43] were determined to the range between 2.91–60.2 mg GAE/g and 0.84–57.6 mg QE/g. TPC and TFC contents of BP samples were consistent with previous studies in the literature. Bakchiche et al. [51] for Algerian BP and Temizer et al. [6] for Turkish BP found that the TPC amounts were higher than the samples examined in this study. It was found that the pollen samples contained significantly higher number of TFC than those in previous studies [47, 52, 53]. TFC value was obtained from the BP of the Eastern Black Sea region by Temizer et al. [6] and the results of our BP samples were close to each other. TPC and TFC of our monofloral *Verbascum* BP samples were much the same as the values determined by Kolarov et al.[54] with *Verbascum* flower.

In vitro, antioxidant activities of the BP extracts are given in Table 2. The radical scavenging activity of DPPH is used to indicate the antioxidant activity of BP [43, 55]. Sample 2 showed the highest DPPH scavenging ability with an IC_{50} value (20.21 μg/mL) compared with the other samples and the control (Trolox) TRO. ABTS radical cation assay is one of the most widely used in vitro antioxidant measuring methods. The positive controls' (BHA, TRO and RUT) values of the ABTS assay were found to be higher than those of the samples. Though all samples exhibited strong ABTS scavenging ability (Sample 2: 24.45 μg/mL; Sample 4: 25.95 μg/mL), the FRAP assay did not show significant differences in antioxidant activities among the samples. The results of FRAP assay of the BP samples were found to be lower than the controls except for TRO, and the activity of BHA was close to Samples 1, 3, and 5. All of the other BP samples and controls presented similar MCA values, except for Sample 5.

Regarding the DPPH, FRAP, and MCA assays of BP samples were comparable with the literature data [6, 51]. In the current study BP samples showed higher DPPH, and ABTS scavenging activities than Chapandinha's BP in Brazil [56]. DPPH radical scavenging activities of BP samples were consistent with Romanian BP [57].

These results showed that the examined samples have biochemical activity as much as the BP previously analyzed in literature in terms of both antioxidant contents and activity. The linear relationships between TPC, TFC, and all antioxidant assays were visually given in Fig. 3. As a result of

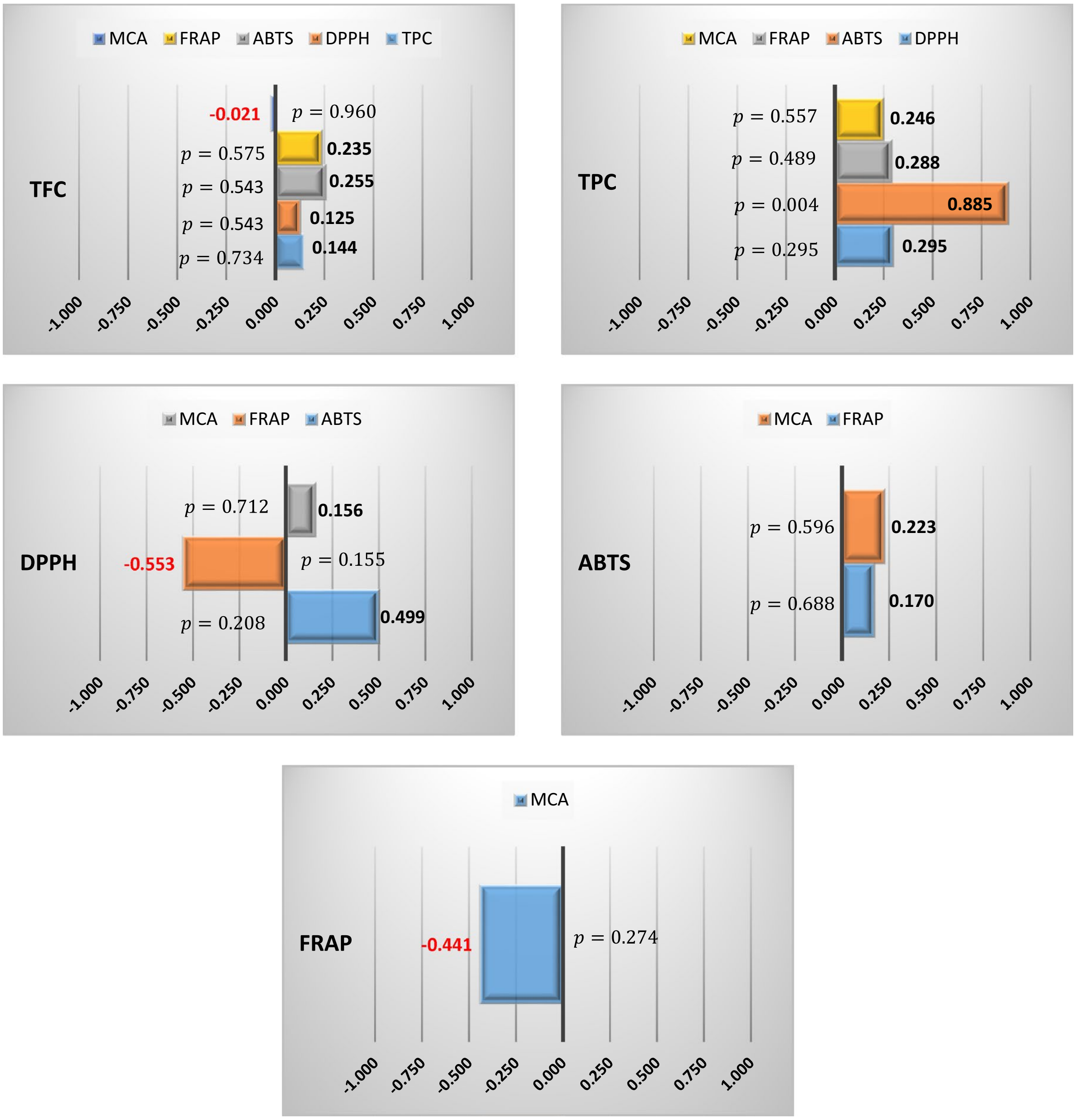
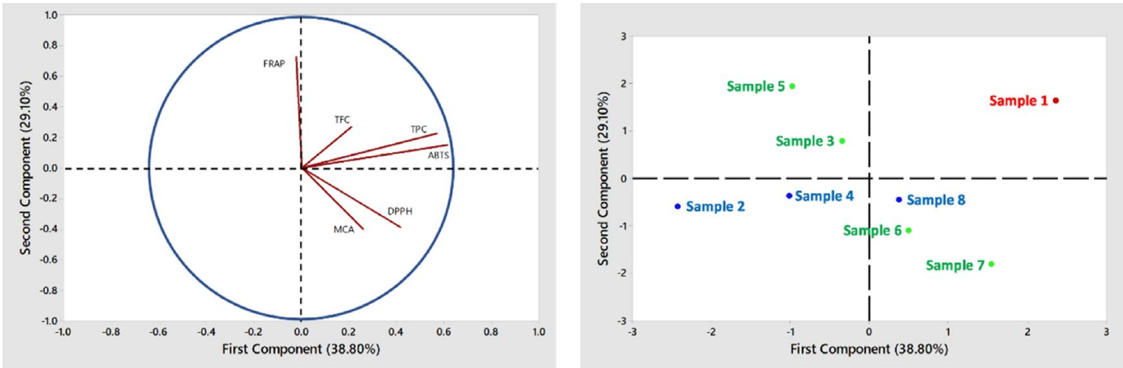


Fig. 3 The linear relationships analysis with PPMCC between antioxidant assays of BP samples

the linear relationships between TFC and other antioxidant assays were positive but not significant with a 95% confidence level ($p > 0.05$). Although TPC had positive linear relationships with all antioxidant assays, only the linear relationship with ABTS assay was statistically significant at a 5% significance level ($r = 0.885$). Also, in the normality tests of antioxidant assays, it was concluded that all assays except MCA did not show statistically significant deviations from the normal distribution ($p > \alpha = 0.05$). While, at a 5% significance level, FRAP assay had negative but statistically insignificant linear relationships with TFC and MCA assays ($r = -0.553$ and $r = -0.441$), it had positive and again statistically insignificant linear relationships with other antioxidants assays. The BP samples were subjected to PCA in terms of their antioxidant content. The first principal component (PC1) with an eigenvalue of 2.3296 explained 38.80%



a TPC; TFC and antioxidant assays PC1 and PC2 map

b The individual factor map for antioxidants in terms of PC1 and PC2

Fig. 4 Principal component analysis (PCA) of TPC, TFC, and antioxidant assays

of the total variance, while the second component (PC2) with an eigenvalue of 1.7476 explained 29.10%. Therefore, 68.10% of the total variance was explained by these two principal components. In Fig. 4a, TFC, TPC, DPPH, ABTS, and MCA assays were positive part of PC1 except FRAP. TFC, TPC and ABTS were positioned together on the same sides of the plot origin (on the same quadrants) and there were strong correlations between TPC and ABTS assays**.** With a similar interpretation, it could be said that all antioxidant assays except DPPH and MCA constitute the positive part of PC2. Moreover, a score plot of samples for PC1 and PC2 was given in Fig. 4b. Parallel to the clustering analysis results, it was seen that Sample 1 significantly dissociates from all others in terms of antioxidant activities because of it's TPC value was higher than other samples. It was concluded that this situation is due to high plant diversity of Sample 1.

Table 3 Antimicrobial activity of BP samples was determined by agar diffusion and MIC methods

	Microorganisms	Zone of inhibition[a] (mm)	MIC value[b] (g/mL)
BP samples			
Sample 1	*E.coli*	12	4.17
Sample 2	*B.cereus*	20	8.33
	E.coli	11	8.33
Sample 3	–	–	–
Sample 4	–	–	–
Sample 5	*B.cereus*	12	8.33
Sample 6	*B.cereus*	12	8.33
	S.aureus	12	8.33
Sample 7	*B.cereus*	12	8.33
Sample 8	*B.cereus*	12	8.33
Positive control			
Streptomycin	*E.coli*	30	0.15
	B.cereus	25	0.05
	S.aureus	20	0.125
Fluconazole	*C.albicans*	25	0.125
Negative control	Ethanol	–	–

[a]Zone of inhibition (mm)

[b]MIC (mg/mL). (SD ± 1.0. $n = 3$)

Antimicrobial activity

The antimicrobial activities of the investigated BP extracts were analyzed by agar diffusion and MIC methods against *E. coli, B. cereus, S. aureus,* and *C. albicans.* The results were evaluated according to the presence of inhibition zones and pollen concentration preventing the culture from growing on the microplate. According to the agar diffusion results, BP extracts (8.33 g/mL) demonstrated a high antimicrobial activity with inhibition zones between 11 and 20 mm in diameter. Sample 2 was found to have the most potent antibacterial effect on *B.cereus* by showing a 20mm inhibition zone. MIC was determined for only those organisms which showed a zone of inhibition and were sensitive to the pollen extract in the agar diffusion method. The antibacterial activity was determined in all BP samples except for Samples 3 and 4, where no activity was determined in *C. albicans* (Table 3). BP showed activity against *E. coli, B. cereus,* and *S. aureus*. The BP also showed significant MIC values against *E. coli* (4.17 g/mL). Antimicrobial resistance is a global concern that necessitates the development of new antimicrobial products. It is known that BP demonstrates antimicrobial activity [1, 7, 58]. However, studies on their antimicrobial properties are limited. The values obtained in our study were found to be higher than that for fermented BP with *Lactococcus lactis* and *Lactobacillus rhamnosus* bacteria which effects on its antimicrobial activity [59]. In another study while the activity in BP was very close to the

Table 4 The concentration of 31 elements of BP samples as mg/kg

Elements	Sample 1	%RSD[b]	Sample 2	%RSD	Sample 3	%RSD	Sample 4	%RSD	Sample 5	%RSD	Sample 6	%RSD	Sample 7	%RSD	Sample 8	%RSD
Na	126.67	0.01	165.43	0.02	109.88	0.01	120.87	0.01	141.68	0.01	138.07	0.01	102.69	0.01	122.30	0.02
Mg	571.97	0.02	683.26	0.03	643.79	0.03	648.01	0.03	930.69	0.03	1497.04	0.04	1416.00	0.05	638.63	0.04
Al	683.54	0.04	654.04	0.01	61.94	0.01	146.00	0.01	151.76	0.01	82.55	0.02	111.65	0.02	238.45	0.02
K	10,339.54	0.05	4237.86	0.05	5481.61	0.04	5516.84	0.06	6207.09	0.04	7205.91	0.03	7100.57	0.03	4189.86	0.06
Ca	277.14	0.02	332.89	0.03	84.45	0.01	87.55	0.01	147.90	0.01	220.43	0.02	218.03	0.02	176.12	0.02
V	1.50	0.01	1.18	0.01	Nd		Nd		0.03	0.01	Nd		Nd		0.32	0.01
Cr	1.13	0.01	1.35	0.01	Nd		Nd		0.10	0.01	Nd		Nd		0.35	0.01
Mn	27.67	0.01	22.63	0.02	27.67	0.02	31.35	0.01	33.32	0.01	36.39	0.04	38.55	0.04	20.80	0.02
Fe	734.99	0.02	619.71	0.01	72.00	0.02	118.27	0.04	147.82	0.02	70.94	0.03	97.21	0.03	276.20	0.03
Co	0.18	0.02	0.10	0.02	Nd		Nd		Nd		Nd		Nd		Nd	
Ni	1.15	0.01	2.13	0.01	Nd		0.24	0.01	0.85	0.02	0.11	0.01	0.32	0.01	2.18	0.01
Cu	8.00	0.03	8.94	0.03	6.61	0.02	7.35	0.02	8.52	0.01	8.93	0.01	10.97	0.01	7.50	0.02
Zn	22.00	0.02	23.90	0.04	31.38	0.03	30.88		82.26	0.02	38.33	0.02	46.32	0.02	23.00	0.02
Ga	0.51	0.02	0.63	0.01	Nd		0.01	0.01	0.04	0.01	0.02	0.01	0.09	0.01	0.24	0.01
As	Nd[a]		Nd		Nd		Nd		Nd		Nd		Nd		Nd	
Se	0.19	0.02	0.08	0.01	0.09	0.01	0.02	0.01	Nd		Nd		Nd		0.18	0.01
Sc	Nd		Nd		Nd		Nd		Nd		Nd		Nd		Nd	
Rb	13.35	0.03	13.47	0.01	5.57	0.01	5.50	0.01	12.95	0.01	14.56	0.02	12.65	0.01	5.31	0.01
Sr	4.10	0.04	4.40	0.03	0.93	0.01	1.36	0.01	1.65	0.01	2.35	0.01	2.65	0.01	2.06	0.01
Y	Nd		Nd		Nd		Nd		Nd		Nd		Nd		Nd	
Rh	Nd		Nd		Nd		Nd		Nd		Nd		Nd		Nd	
Ag	Nd		Nd		Nd		Nd		Nd		Nd		Nd		Nd	
Cd	Nd		Nd		Nd		Nd		Nd		Nd		Nd		Nd	
In	Nd		Nd		Nd		0.08	0.01	0.16	0.01	Nd		Nd		0.06	0.01
Cs	Nd		Nd		Nd		Nd		Nd		0.07	0.01	Nd		Nd	
Ba	3.51	0.04	3.44	0.04	0.51	0.01	1.12	0.01	1.29	0.01	1.16	0.01	1.56	0.01	2.31	0.02
Au	1.02	0.01	1.01	0.03	0.91	0.01	0.95	0.02	0.93	0.02	1.02	0.01	1.02	0.01	1.00	0.01
Tl	Nd		Nd		Nd		Nd		Nd		Nd		Nd		Nd	
Pb	0.40	0.02	0.16	0.01	Nd		Nd		Nd		0.20	0.01	Nd		Nd	
Bi	0.09	0.02	0.38	0.01	0.45	0.01	0.35	0.01	0.40	0.01	0.08	0.01	0.44	0.01	0.19	0.01
U	Nd		Nd		Nd		Nd		Nd		Nd		Nd		Nd	
Total	12,818.65		6777.01		6527.78		6716.74		7869.44		9318.15		9160.73		5707.05	

[a]Nd means not detected < 0.01

[b]RSD means relative standard deviation

Table 5 The linear relationships between the elemental contents of the BP samples [Pearson's Product–Moment Correlation Coefficient (PPMCC)]

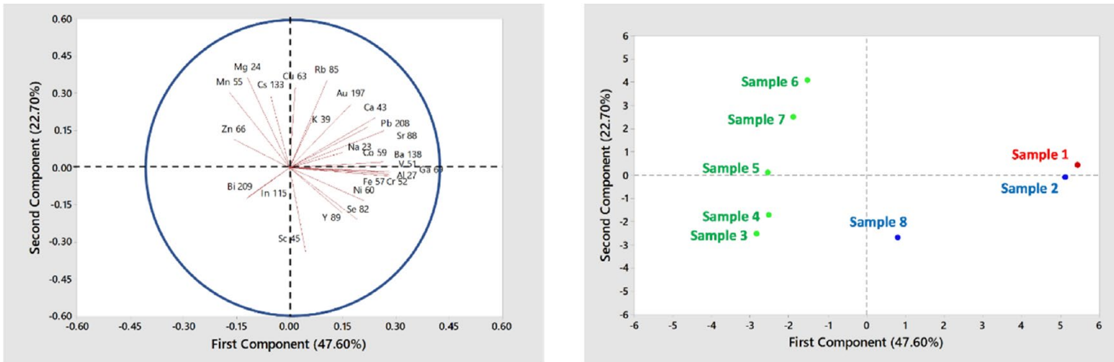
	Mg24	Al27	K39	Ca43	V51	Cr52	Mn55	Fe57	Co59	Ni60	Cu63	Zn66
Na23	0.143	0.524	− 0.226	0.576	0.583	0.660	− 0.337	0.429	0.452	0.540	0.042	− 0.095
	Mg24	− 0.500	0.262	0.119	− 0.558	− 0.457	**0.786***[a]	− 0.619	− 0.436	− 0.381	0.667	**0.810**[a]
		Al27	− 0.048	0.595	**0.939**[a]	**0.913**[a]	− 0.619	**0.976**[a]	**0.764**[a]	**0.881**[a]	0.167	− 0.643
			K39	0.253	− 0.076	− 0.228	0.438	− 0.143	0.218	− 0.339	0.215	0.214
				Ca43	0.609	0.634	− 0.188	0.476	**0.733**[a]	0.568	0.535	− 0.357
					V51	**0.973**[a]	− **0.710**[b, c]	**0.939**[a]	**0.814**[a]	**0.837**[a]	0.114	− 0.660
						Cr52	− **0.736**[b, c]	**0.913**[a]	**0.781**[a]	**0.862**[a]	0.190	− 0.609
							Mn55[c]	− 0.690	− 0.483	− **0.787**[b,c]	0.575	**0.833**[a]
								Fe57	**0.764**[a]	0.676	− 0.022	− 0.667
									Co59	0.426	− 0.003	− 0.655
										Ni60	− 0.027	− 0.524
											Cu63	0.333
												Zn66

	Ga69	Se82	Sc45	Rb85	Sr88	Y89	In115	Cs133	Ba138	Au197	Pb208	Bi209
Na23	0.476	− 0.049	− 0.306	0.643	0.530	0.384	0.136	0.247	0.497	0.223	0.518	− 0.176
	− 0.214	− **0.952**[b]	− **0.881**[b]	0.476	0.095	− 0.333	− 0.055	0.577	− 0.310	0.262	− 0.055	0.095
	0.857[a]	0.512	0.357	0.119	0.619	0.143	0.136	− 0.412	**0.881**[a]	0.238	0.409	− 0.381
	− 0.048	− 0.268	0.076	0.548	0.327	− 0.674	− 0.273	0.412	0.215	0.325	0.546	− 0.466
	0.833[a]	0.098	− 0.361	**0.714**[a]	**0.969**[a]	0.392	− 0.518	0.247	**0.846**[a]	**0.820**[a]	**0.791**[a]	− 0.357
	0.850[a]	0.624	0.330	0.216	0.609	0.190	− 0.029	− 0.351	**0.875**[a]	0.203	0.494	− 0.330
	0.875[a]	0.546	0.203	0.241	0.634	0.317	− 0.029	− 0.351	**0.850**[a]	0.152	0.436	− 0.254
	− 0.476	− **0.854**[b, c]	− 0.505	0.262	− 0.259	− **0.788**[b, c]	− 0.027	0.412	− 0.538	0.037	− 0.136	0.127
	0.810[a]	0.610	0.307	0.000	**0.869**[a]	0.417	0.136	− 0.577	**0.956**[a]	0.466	0.300	− 0.359
	0.733[a]	0.559	0.291	0.483	**0.838**	0.178	− 0.429	− 0.216	**0.858**[a]	0.438	**0.750**[a]	− 0.387
	0.881[a]	0.415	0.188	− 0.071	0.574	**0.773**[a]	0.191	− 0.412	**0.785**[a]	0.374	0.136	− 0.200
	0.548	− 0.537	− 0.653	0.619	0.428	− 0.085	− 0.327	0.247	0.170	0.618	0.300	0.116
	− 0.500	− **0.903**[b]	− 0.595	0.143	− 0.333	− 0.476	0.218	0.247	− 0.548	− 0.214	− 0.436	0.476
	Ga69	0.342	− 0.071	0.310	**0.881**[a]	0.333	− 0.218	− 0.247	**0.976**[a]	0.476	0.464	− 0.286
		Se82	**0.732**[a]	− 0.293	0.098	0.464	− 0.196	− 0.423	0.415	− 0.073	0.252	− 0.195
			Sc45	− 0.476	− 0.158	0.051	0.245	− 0.577	0.158	− 0.474	− 0.027	0.109
				Rb85	0.619	− 0.333	− 0.491	0.577	0.286	0.619	0.791	− 0.333
					Sr88	0.385	− 0.518	0.082	**0.921**[a]	**0.767**[a]	0.682	− 0.345
						Y89	− 0.327	− 0.412	0.506	0.210	− 0.191	0.142
							In115	− 0.283	− 0.218	− 0.546	− 0.563	0.027
								Cs133	− 0.247	0.577	0.472	− 0.577
									Ba138	0.644	0.518	− 0.386
										Au197	0.764	− 0.589
											Pb208	− 0.682

[*]Those highlighted in bold and underlined in the table represent the statistically significant correlation coefficient values at the 5% significance level

[a,b]Mg has statistically significant positive linear relationships between [a]Mn ($r = 0.786$) and Zn ($r = 0.810$); it has statistically significant negative linear relationships between [b]Se ($r = -0.952$) and Sc ($r = -0.881$). Similar interpretations can also be made for other elements

[c]However, there are statistically significant linear relationships between Mn and V ($r = -0.710$), Cr ($r = -0.736$), Ni ($r = -0.787$), Se ($r = -0.854$), and Y ($r = -0.788$)



a PC1 and PC2 map of element contents

b The individual factor map for element contents in terms of PC1 and PC2

Fig. 5 Principal component analysis (PCA) of element content of BP samples

values of our study, it was found to be at a very low level compared to fermented BP [60].

The present study, similar to Graikou et al. [61], Morais et al. [62], and Soares de Arruda et al.[7], gram-positive bacteria (*B.cereus*) were exhibited more sensitive to BP than gram-negative (*E.coli*). The reason for this effect is still unclear; some hypotheses suggest that they have a more chemically complex cell wall and higher lipid content compared to gram-positive bacteria [7, 62].

Elemental analysis

Elements are the most important catalysts for various chemical reactions that occur in the living body, and they are also essential and indispensable components in the growth and development process [63]. In the current study, macro (Na, Mg, K, Ca), trace elements (Fe, Cu, Co, Zn, Cr, Mn, Ga, Se, V, In, Au, Rb, Ag, Sr, and Ni), and toxic elements (Ba, Pb, Al, Cd, Sc, Bi, Tl, Y, Rh, U, and As) were quantified in bee pollen samples from Turkey as shown in Table 4 as mg/kg. In bee pollen, approximately 1.6% bio elements are present including macronutrients as well as micronutrients [11]. In the BP samples, the macro elements Ca, Mg, Na, and K were determined as 84.45–332.89, 571.97–1497.04, 102–69-165.43, 4189–10,339 mg/ kg, respectively. Essential trace elements play a crucial role in the body's growth, development, and human metabolism. Therefore, deficiency or excess of these essential elements can cause various metabolic disorders, acute growth defects, and even fatal diseases [10]. Fe, Cu, Zn, Mn, Ni, Co, Cr, Ga, V and Se which are essential trace elements, were found as 70.94–734.99, 6.61–10.97, 22–82.26, 20.8–36.39, 0–2.18, 0–0.28, 0–1.35, 0–0.63, 0–1.18 and 0–0.19 mg/ kg, respectively. The ranking of other trace elements' concentration was Rb > Sr > Au > In > Cs in bee pollen samples. While some element values was very close in the BP samples, there were differences between the Mg, Ca, K, Fe, and Mn values. It was detected in this study that differences and similarities may be present in the mineral levels of BP obtained from the same city [14].

While K constitutes 60% of the total mineral content of bee pollen, Mg, Na, and Ca constitutes 30% approximately [36]. K, Mg, Na, and Ca comprised 77.48% 10.83%, 1.58%, and 2.37% of the total element content in this study. According to Liolios et al. [64] K is the most abundant macroelement in BP, following Mg, Na, and Ca while P has significantly higher proportions. The results from current study, K and Mg was determined the highest macroelements in BP, while Na was found the lowest and these results were consistent with previously published by Liolios et al.[64]. It was detected that the BP samples had a higher K content than three BP samples from Eastern Anatolia evaluated by Altunatmaz et al.[14]. Our BP samples were characterized with higher K and Mg contents compared to Turkish bee pollen examined by Kalaycıoğlu et al. [55]. When comparing our results to other bee pollen from Turkey, Zn, Fe, Mg, and Mn values were found to be lower, while Ca, K, and Mn values were higher [14].

The mineral values found for monofloral *Verbascum* bee pollen samples (Sample 6, 8) are as follows: Zn: 38.33, 23; K: 7235, 4189.6; Ca: 220.43, 176.12; Mg: 1497.04, 638.63; Fe: 70.94, 276.2 mg/kg. In a study by Stanciu et al.[8] Ca and Zn, the content of *Verbascum* spp. bee pollen was found to be higher compared to our *Verbascum* spp. BP samples. Mg, K content of Sample 6 was found to be higher and the content of Fe lower, while Mg, K content of Sample 8 was found to be lower and Fe content higher.

Toxic elements, such as As, Cd, Tl, Y, Rh, and U were not detected in any samples. The ranking of other toxic element concentrations was Al > Ba > Bi > Pb. Toxic elements did not exceed the upper limit [36]. If we compare this study's findings for toxic elements with those of other neighboring countries' literature data, the amount of toxic elements was found to be mostly lower [14, 55, 65–68]. As a result of the normality tests of elements, it was concluded that Na, K, Ca, Mn, Ni, Cu, Sc, Sr, Y, Ba, Au, and Bi did not show statistically significant deviations from the normal distribution ($p > \alpha = 0.05$). The values of the linear relationships between elements are presented in Table 5. The element contents of the samples were examined by PCA. Figure 5a shows that all elements except Mg, Mn, Zn, In, Cs, and Bi constitute the positive part of PC1. With a similar evaluation, all elements except Al, V, Cr, Fe, Ni, Ga, Se, Sc, Y, In, and Bi constitute the positive part of PC2. Figure 5b presents a score plot of samples for PC1 and PC2. Parallel to the clustering analysis of samples according to elements, it was observed that Sample 1 significantly dissociates from all others in terms of element contents (Fig. 5b).

Estimation daily intake and health risk assessment

BP is suitable for taking orally both children and adults. But BP may constitute an important risk factor concerning the presence of contominants as toxic and potentially toxic elements (PTE) [69]. EDI is a value that estimates the daily exposure level of people to toxic and potentially toxic elements through food consumption [70]. In the study, PTE accumulation was estimated assuming 10 g DPC for 70 kg BW for adults and 2 g DPC for 15 kg BW for children [31]. EDI values of PTE such as Cu, Fe, Co, Zn, Cr, Mn, Se, V, Ni, Ba, Pb, Al, Bi, and Sr were determined (Table 6). Contrary to previous studies, for almost all elements, adults' EDI values were higher than that found for children in this study [70]. PTE exposure level varies depending on the amount of BP consumed daily and average body weight. Zafeiraki et al. [30] reported that Pb, Cd, Hg, Cr, Ni and As elements' EDI values are 0.601, 0.601, 0.029, 0.385, 1.982 and 0.777 for adults, and 1.403, 1.402, 0.068, 0.898, 4.626, and 1.812 for children as μg kg^{-1} day^{-1}, respectively. Zafeiraki et al.[30] found higher EDI values for Pb, Cd, Cr, As, and Ni elements than the values given in the current study (Table 6). Kostic et al. [71] determined EDI values for Al, Cr, Co, Cu, Mn, Ni, Pb, Se, Sr, and V element 0.019, 0.000043, 0.0000029, 0.0028, 0.0044, 0.00011, 0.000043, 0.0001, 0.0004, and 0.000043 as mg/kg bw for adults, respectively. EDI values of Cu, Mn, Pb, Se, and Sr were higher than those of Al, Cr, Co, Mn, Ni, and V were lower than the values in this study. The EDI levels of Cd, Cr, Ni, Pb, and Zn of Italian pollen [70] were lower compared to the current findings. THQ and HI values in foods are considered meaningful parameters for assessing health risks [3, 29, 30]. Both THQ and HI are less than 1, indicating that the exposed population is assumed to be safe [29]. THQ index between 1 and 5 indicates that the exposed population is at a relevant level [70]. In the current study, risk assessment for human health related to pollen consumption was evaluated. THQ values for the toxic and potentially toxic elements were calculated in each pollen sample for both adults and children (Table 7). The THQ values obtained in this study were less than 1 for each of the analysed elements (Table 7). In previous studies 6—12 toxic and potentially toxic elements were evaluated risk assessment [70–72]. In the current study, risk assessment for human health related to pollen consumption was evaluated with 13 elements. In addition, the HI value is lower than 1 in bee pollen samples for adults and children, suggesting that the exposed human population is supposed to be safe [70]. So, consumption of this bee pollen by adults and children was found to be suitable.

Conclusion

Bee pollen may be a good marker for environmental pollution. This means that this product should be strictly controlled in terms of quality and safety. In addition to monitoring the pollutant contaminations in bee pollen, public health risk assessments should also be conducted. Therefore, these products should be evaluated for health risk assessment before consumption. This work presents important data on the palynological analysis, antioxidant activities, antimicrobial activities, and elemental contents of bee pollen collected in Bingöl (Turkey).

The study demonstrated that bee pollen samples can provide a good source of antioxidant, antimicrobial activity, and elements for human consumption. It is demonstrated that the *Verbascum* spp. bee pollen sample also provides better bioactivity than the other bee pollen

Table 6 Estimated daily intake (EDI) rates of elements for adults and children

Elements	EDI[a] mg/kg BW[b]/day															
	Sample 1		Sample 2		Sample 3		Sample 4		Sample 5		Sample 6		Sample 7		Sample 8	
	Adults	Children	Adults	Children	Adults	Children	Adults	Children	Adults	Children	Adults	Children	Adults	Children	Adults	Children
Cu	0.001	0.001	0.001	0.001	0.001	0.001	0.001	0.001	0.001	0.001	0.001	0.001	0.002	0.001	0.001	0.001
Fe	0.105	0.098	0.089	0.083	0.010	0.010	0.017	0.016	0.021	0.020	0.010	0.009	0.014	0.013	0.039	0.037
Co	2.57E−05	0.000024	1.43E−05	1.33E−05	0	0	0	0	0	0	0	0	0	0	0	0
Zn	0.0031	0.0029	0.0034	0.0032	0.0045	0.0042	0.0044	0.0041	0.0118	0.0110	0.0055	0.0051	0.0066	0.0062	0.0033	0.0031
Cr	0.000161	0.000151	0.000193	0.00018	0	0	0	0	1.43E−05	1.33E−05	0	0	0	0	0.00005	4.67E−05
Mn	0.0040	0.0037	0.0032	0.0030	0.0040	0.0037	0.0045	0.0042	0.0048	0.0044	0.0052	0.0049	0.0055	0.0051	0.0030	0.0028
Se	2.71E−05	2.53E−05	1.14E−05	1.07E−05	1.29E−05	0.000012	2.86E−06	2.67E−06	0	0	0	0	0	0	2.57E−05	0.000024
V	0.000214	0.0002	0.000169	0.000157	0	0	0	0	4.29E−06	0.000004	0	0	0	0	4.57E−05	4.27E−05
Ni	0.000164	0.000153	0.000304	0.000284	0	0	3.43E−05	0.000032	0.000121	0.000113	1.57E−05	1.47E−05	4.57E−05	4.27E−05	0.000311	0.000291
Ba	0.000501	0.000468	0.000491	0.000459	7.29E−05	0.000068	0.00016	0.000149	0.000184	0.000172	0.000166	0.000155	0.000223	0.000208	0.00033	0.000308
Pb	5.71E−05	5.33E−05	2.29E−05	2.13E−05	0	0	0	0	0	0	2.86E−05	2.67E−05	0	0	0	0
Al	0.097649	0.091139	0.093434	0.087205	0.008849	0.008259	0.020857	0.019467	0.02168	0.020235	0.011793	0.011007	0.01595	0.014887	0.034064	0.031793
Bi	1.29E−05	0.000012	5.43E−05	5.07E−05	6.43E−05	0.00006	0.00005	4.67E−05	5.71E−05	5.33E−05	1.14E−05	1.07E−05	6.29E−05	5.87E−05	2.71E−05	2.53E-05
Sr	0.0006	0.0005	0.0006	0.0006	0.0001	0.0001	0.0002	0.0002	0.0002	0.0002	0.0003	0.0003	0.0004	0.0004	0.0003	0.0003

[a]EDI: Daily exposure level of adults and children to toxic and potentially toxic elements; (PTE) through pollen consumption

[b]Average body weight (15 kg for childrens; 70 kg for adults)

Table 7 Target Hazard Quotient (THQ) and Hazard Index (HI) of BP consumption to adults and children

	Elements	RFD_o [b]	THQ[a]															
			Sample 1		Sample 2		Sample 3		Sample 4		Sample 5		Sample 6		Sample 7		Sample 8	
			Adults	Children	Adults	Children	Adults	Children	Adults	Children	Adults	Children	Adults	Children	Adults	Children	Adults	Children
1	Al	1	0.09765	0.0911	0.0934	0.087205	0.008849	0.008259	0.020857	0.0195	0.0217	0.0202	0.012	0.011	0.016	0.0149	0.034	0.0318
2	Ba	20	2.5E−05	2E−05	2E−05	2.29E−05	3.64E−06	3.4E−06	0.000008	7E−06	9E−06	9E−06	8E−06	8E−06	1E−05	1E−05	2E−05	2E−05
3	Co	0.0003	0.08571	0.08	0.0476	0.044444	0	0	0	0	0	0	0	0	0	0	0	0
4	Cr	1.5	0.00011	0.0001	0.0001	0.00012	0	0	0	0	1E−05	9E−06	0	0	0	0	3E−05	3E−05
5	Cu	0.04	0.02857	0.0267	0.0319	0.0298	0.023607	0.022033	0.02625	0.0245	0.0304	0.0284	0.032	0.0298	0.039	0.0366	0.027	0.025
6	Fe	0.7	0.15	0.14	0.1265	0.11804	0.014694	0.013714	0.024137	0.0225	0.0302	0.0282	0.014	0.0135	0.02	0.0185	0.056	0.0526
7	Mn	0.024	0.1647	0.1537	0.1347	0.125722	0.164702	0.153722	0.186607	0.1742	0.1983	0.1851	0.217	0.2022	0.229	0.2142	0.124	0.1156
8	Ni	0.02	0.00821	0.0077	0.0152	0.0142	0	0	0.001714	0.0016	0.0061	0.0057	8E−04	0.0007	0.002	0.0021	0.016	0.0145
9	Pb	0.0035	0.01633	0.0152	0.0065	0.006095	0	0	0	0	0	0	0.008	0.0076	0	0	0	0
10	Se	0.0004	0.06786	0.0633	0.0286	0.026667	0.032143	0.03	0.007143	0.0067	0	0	0	0	0	0	0.064	0.06
11	Sr	0.6	0.00098	0.0009	0.001	0.000978	0.000221	0.000207	0.000324	0.0003	0.0004	0.0004	6E−04	0.0005	6E−04	0.0006	5E−04	0.0005
12	V	0.005	0.04286	0.04	0.0337	0.031467	0	0	0	0	0.0009	0.0008	0	0	0	0	0.009	0.0085
13	Zn	0.3	0.01048	0.0098	0.0114	0.010622	0.014943	0.013947	0.014705	0.0137	0.0392	0.0366	0.018	0.017	0.022	0.0206	0.011	0.0102
HI[c]			**0.67347**	**0.6286**	**0.5308**	**0.495384**	**0.259163**	**0.241885**	**0.281745**	**0.263**	**0.3271**	**0.3053**	**0.303**	**0.2824**	**0.329**	**0.3075**	**0.342**	**0.3188**

[a–c]THQ < 1 and HI < 1 indicates that the exposed population is assumed to be safe; [b]Rfd_o: Oral reference dose (mg/kg/bw/day) samples. It was also observed that samples with high antioxidant activities also demonstrated an increase in their antimicrobial activities. In the current study the non-carcinogenic health risk assessment showed that BP samples were suitable for consumption for both adults (10 g/day) and children (2 g/day). If these BP samples are consumed more than these rates on a daily basis, the accumulation of elements in the body increases and becomes harmful.

The results of such analyses contribute to the development of a content database that helps determine quality criteria, improving the marketing value of bee pollen, promoting its commercialization, and demonstrating its safety.

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Author's contributions DNÇ supplied the bee pollen samples. İKT and AG extracted all samples in the study. DNÇ and İKT conducted the palynological analyses, elemental analysis and calculated hazard risk assessment. AG performed the TPC, TFC, and antioxidant assays. EDC analyzed the samples for antimicrobial activity. UY performed the statistical evaluation. All authors wrote the manuscript and approved its final version.

Declarations

Conflict of interest The authors of this article declare that they have no known competing financial interests or personal relationships that could have appeared to affect this work.

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