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Phenolic Profile and Antioxidant Potential of Propolis from Azerbaijan

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ABSTRACT

This study analyzed the antioxidant properties and phenolic compositions of propolis samples collected from 15 different locations in Azerbaijan. Antioxidant activities were measured using three different methods, i) total phenolic contents (TPC), ii) ferric reducing/antioxidant power (FRAP) and iii) 2,2-diphenyl-l-picrylhydrazyl (DPPH) radical scavenging activity. TPC of the propolis samples were between 10.94 and 79.23 mg GAE/g, FRAP values were between 170 and 438 μM Trolox/g and DPPH radical scavenging activities (SC_{50} value) were between 18 and 128 μg/mL. Phenolic acids and flavonoids were identified using HPLC-DAD in comparison with standards. Of the 15 samples investigated, propolis samples from the Ismayilli, Zerdap and Qax regions exhibited higher antioxidant capacity compared to the other regions of Azerbaijan. The total antioxidant potential of the samples varied depending on the flora of the different collection regions; however, there were also significant differences among samples collected within the same region. The study findings reveal that the antioxidant potential of propolis samples does not depend only on the flora of the region, but that the condition and age of the beehive, as well as the strength of the colony and method of collection of the samples are also important dynamics.

Key words: Propolis, antioxidant activity, phenolic compounds, FRAP, DPPH.

Introduction

Propolis is a natural resinous substance collected by honeybees from the buds and barks of various plants for storage inside the hive [1]. Chestnut, beech, birch and some conifer trees in particular are well known sources of propolis [2]. Honeybees use propolis for various purposes, such as to protect the hive from microbial infection, to provide thermal insulation, to fill cracks or apertures within the hive and to embalm dead organisms [3,4]. Propolis has been extensively used in traditional medicine to treat various diseases since ancient times [2,5]. During the last decade, propolis from different regions of the world was extensively studied to reveal its major bio-active properties, such as antimicrobial [5,6] antifungal [7], antioxidant [4,8,9] anti-inflammatory [8,9] antitumoral [10,11,12,13] immunomodulatory [14], immunoregulatory [15], antidiabetic [16], antiulcerative [17,18] and antidepressant [19]

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activities. Due to these various critical biological properties, propolis is used in apitherapy to treat numerous diseases and also in the food industry as an additive for a range of purposes.

Many researchers have reported a positive correlation between total phenolic contents (TPC) and antioxidant capacities in honey as well as in propolis [1,6,20,21,22]. Phenolic compounds are synthesized by plants as secondary metabolites, such as phenolic acids, flavones, flavonoids, and anthocyanins, which serve as defense mechanisms in plants to counteract reactive oxygen, a process essential for survival [23,24]. There are numerous phenolic constituents in plants, each of which has different antioxidant capacities [25]. The antioxidant and radical scavenging activity of phenolic acids depends on the number and position of hydroxyl (–OH) groups, ortho-dihydroxy groups and methoxy ($-OCH_3$) substituent in the molecules [20,22].

The chemical composition, physical structure and bioactivity properties of propolis largely depend on its botanical and geographical characteristics. Raw propolis contains nearly 50% resin, made up of phenolic compounds, 30% wax, 10% essential oils, 5% pollen and 5% various organic compounds [2]. TPC and antioxidant activities of propolis samples have been shown to vary when collected from different parts of the hive. In our previous study, propolis samples collected from the entrance of the hives exhibited higher antioxidant properties than those obtained from inside the hive [8]. In addition, there are studies showing that the composition and bioactivity of propolis differ due to seasonal effects [26] and floral changes [1]. In recent years, several studies have investigated different propolis species from various regions of the world, such as red Brazilian propolis, [27] Korean propolis, [28] Turkish propolis [6,8] and Iranian propolis [25], which exhibit different physical, chemical and bioactive properties. Interestingly, propolis from Azerbaijan has not been studied to date.

In this study, we characterized the phenolic composition and bioactivity properties of propolis samples collected from 15 different locations in Azerbaijan. Phenolic composition was determined using HPLC, and antioxidant capacity was measured using TPC, FRAP assay and DPPH radical scavenging activities. Antioxidant values were compared with BHT and Trolox, used as positive controls. The TPC and bioactivity potential of the propolis samples varied depending on the flora of the region of collection; however, there were also significant differences among samples collected from the same region.

Materials and Methods

Chemicals and samples

2,4,6-tripyridyl-s-triazine (TPTZ), Folin-Ciocalteu's phenol reagent, 2, 2-diphenyl-1-picrylhydrazyl (DPPH), Trolox (6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid) were purchased from Sigma Chemical Co. (St Louis, MO, USA). All chemicals used for HPLC–DAD were of analytical grade. Standards of common phenolic compounds, gallic, protocatechuic acid, p-OH benzoic acid, catechin, chlorogenic acid, vanillic acid, caffeic acid, p-coumaric acid, ferulic acid, rutin, fisetin, quercetin, apigenin, kaempferol, isorhamnetin and propylparaben were obtained from Sigma-Aldrich (Steinheim, Germany). Of the HPLC grade organic reagents, acetonitrile was supplied by Sigma-Aldrich Co. (St. Louis, MO, USA) and methanol was obtained from Merck (KGaA, Darmstandt, Germany). HPLC syringe filters (RC-membrane, 0.2 μm) were Sartorius Minisart RC 15, Sartorius (Germany).

Propolis samples were collected as crude materials from the beehives of local beekeepers in 15 areas of Azerbaijan during the 2012 harvest season. Figure 1 shows the collection sites of each sample (Aktaş, Zerdap, Ismayıllı, Astara, Şemkır, Qax, Nahcıvan, Mingeçevir, Şeki, Qusar and Quba).

Sample preparation

Raw propolis samples were kept in a freezer at –20°C until use. Samples were ground to powder; 5.0 g was placed in a falcon tube (50 mL) and 95% ethanol was added to make up a volume of 30 mL. The suspensions were stirred continuously with a shaker (Heidolph Promax 2020, Schwabach, Germany) at room temperature for 24 h and then sonicated for 3 h with a sonicator (ElmaÒ Transsonic Digital, Germany). The suspensions were filtered with filter paper (Whatson) and concentrated in a rotary evaporator (IKA-Werke, Staufen-Germany) under reduced pressure at 40°C. The residue was then resuspended with a minimal volume of ethanol and kept at 4°C until use.

HPLC measurements

HPLC-UV analyses were performed using a Thermo Finnigan Surveyor HPLC equipped with a UV-Vis detector supplying

Figure 1. Sample collection regions in Azerbaijan

a simultaneous double wavelength. HPLC-UV analyses were performed on a reverse phase C18 column (150 mm × 4.6 mm id, 5 mm particle; Fortis). Benzoic acid derivatives (such as gallic acid, protocatechuic acid, *p*-hydroxybenzoic acid and vanillic acid) and flavanols (such as catechin) were analyzed at 280 nm; cinnamic acid derivatives such as chlorogenic acid, caffeic acid, *p*-coumaric acid, ferulic acid, and flavonols such as rutin, fisetin, quercetin, apigenin, kaempherol, and isorhamnetin were analyzed at 315 nm. Propylparaben (IS) was analyzed at 280 nm, a normalization calibration method being used. Gradient elution was used for HPLC analyses, modifying the method [29]. Mobile phase (A) 2% acetic acid in water and (B), 70:30 acetonitrile/water mixtures: the following gradient was used; 0–3 min 5% B; 3–8 min 5–15% B; 8–10 min 15–20% B; 10–12 min 20–25% B; 12–20 min 25–40% B; 20–30 min 40–80% B, before returning to the initial conditions. Injection volume was 25 mL, column temperature was 30°C and flow rate was 1.2 mL/min.

Limit of detection (LOD) was calculated according to the EPA method as an S/N level of 3, and limit of quantification (LOQ) was calculated as an S/N level of 10 (Table 4). Calculated amounts per compound were prepared as follows; 0.5 mg/L for gallic acid, protocatechuic acid, *p*-OH benzoic acid, chlorogenic acid, vanillic acid, caffeic acid, *p*- coumaric acid, rutin and propylparaben, 1 mg/L for ferulic acid, fisetin, apigenin kaempherol, and isorhamnetin and 2 mg/L for catechin and Quercetin. Each mixture was injected seven times to verify the LOD and LOQ of each compound and then calculated as the percentage relative standard deviation of peak area and retention time. The result was calculated as mg/100 g raw propolis.

Antioxidant activity assays

Total phenolic contents (TPC) of the ethanol extracts were determined using Folin-Ciocalteu assay [30]. All phenolic compounds, including phenolic acids, flavonoids and anthocyanins, in the ethanolic extracts of propolis resulted in the formation of a blue color complex with Folin reagents whose maximum absorbance can be read at 740 nm. Gallic acid was used as the reference standard compound, and the results were expressed as mg gallic acid per g propolis. Subsequently, 680 µL distilled water, 20 µL ethanol propolis extracts and 400 µL of 0.2 N Folin-Ciocalteu regents were mixed in a tube, vortexed for 2 min. Next, 400 µL of Na_2CO_3 (7.5%) was added and the mixture incubated for 2 h at room temperature. Absorbance was measured at 760 nm at the end of the incubation period. All the measurements were performed in triplicate**.**

FRAP assay

The reducing power ability of ferric tripyridyltriazine (Fe-III-TPTZ) complex (FRAP) from the ethanolic extracts of the propolis samples was measured using reported methods [31] with some modifications. The test involved the reduction of ferric tripyridyltriazine (Fe-III-TPTZ) complex to a blue-colored Fe (II) TPTZ by antioxidant agents of samples. Working FRAP reagent was prepared by mixing 25 mL of 300 mM acetate buffer, pH 3.6, with 2.5 mL of 10 mM TPTZ solution in 40 mM HCl and 2.5 mL of 20 mM $FeCl_3 \cdot 6H_2O$ solution. Next, 3 mL freshly prepared FRAP reagent and 100 µL of the samples were mixed and incubated for 4 min at 37°C, and the absorbance was read at 595 nm against reagent blank containing distilled water. Trolox was used as a positive control to construct a reference curve (62.5–1000 µM). FRAP values were expressed as µM Trolox equivalent of

Table 1. Total phenolic content and antioxidant activities of propolis samples from Azerbaijan

Sample region	Total phenolic content (mg GAE/g)	FRAP (μM Trolox/g)	DPPH SC_{50} (mg/mL)
Aktaş	(71.677±0.64)^{i}	(400.23±4.97)^{i}	(22±1.10)^{c}
Zerdap	(74.86±1.76)^{j}	(436.43±2.65)^{l}	(15±1.00)^{a}
İsmayıllı-I	(69.73±2.02)^{i}	(437.90±8.00)^{l}	(18±1.20)^{abc}
İsmayıllı-2	(23.31±1.62)^{d}	(190.45±0.95)^{c}	(109±3.20)^{g}
Quba-1	(17.50±0.89)^{c}	(195.45±1.77)^{c}	(128±6.10)^{h}
Quba-2	(61.72±0.65)^{g}	(414.13±3.12)^{j}	(16±2.00)^{ab}
Quba-3	(66.02±0.49)^{h}	(370.89±1.48)^{g}	(65±3.40)^{f}
Nahcivan-I	(54.88±1.79)^{g}	(330.34±2.88)^{f}	(58±3.10)^{e}
Nahcivan-2	(40.17±1.103)^{f}	(300.41±2.37)^{e}	(31±1.20)^{d}
Qusar	(69.66±0.97)^{i}	(380.45±2.21)^{h}	(20±2.30)^{bc}
Astara	(10.94±0.15)^{a}	(170.27±0.38)^{a}	(198±3.40)^{i}
Şeki	(13.41±1.31)^{b}	(178.21±0.89)^{b}	(58±3.40)^{e}
Qax	(79.23±2.06)^{k}	(429.95±1.09)^{k}	(30±2.10)^{d}
Şemkır	(30.21±0.88)^{e}	(250.63±7.97)^{d}	(108±2.30)^{g}
Mingeçevir	(31.79±1.62)^{e}	255.22±3.25^{d}	67±2.0^{f}

a,b,c,d,e,h,f,g,l,i,j,k Each values are significantly different from others ($p < 0.05$).

g propolis. The higher the FRAP value, the higher the antioxidant capacity of the samples.

DPPH assay

The scavenging of 2,2-diphenyl-1-picrylhydrazyl (DPPH) radicals was assayed using reported method [32]. The method is based on the radical having a purple color which decays after interaction with antioxidant agents, turning yellow. The change in absorbance due to colors can be spectrophotometrically monitored at 517 nm. Briefly, 1.5 mL of the ethanolic extract solution was mixed with 1.5 mL of 0.1 mM DPPH (dissolved in methanol), vortexed and incubated for 50 min in the dark at room temperature until stable absorbance values were obtained. After the incubation period, the absorbance was recorded at 517 nm against a blank and control. The control solution contained DPPH solution without sample.

Table 2. Statistical difference between total phenolic contents and antioxidant parameters

	Correlations (r^2)		
	TPC	**FRAP**	**DPPH**
TPC	1	0.98	–0.79
FRAP	0.98	1	–0.81
DPPH	–0.79	–0.812	1

** **Correlation is significant at the 0.**05 level (2-tailed).

The results were expressed as SC_{50} (mg/mL), calculated from the curves by plotting absorbance values. The SC_{50} values represent the concentration of the extract (mg/mL) required to inhibit 50% of the radicals.

Statistical analysis

The results were presented as mean values and standard deviations. Data and regression analysis were performed on Microsoft Office Excel 2013 (Microsoft Corporation, Redmond, WA, USA). Data were tested using SPSS (version 9.0 for Windows 98, SPSS Inc.). Statistical analyses of the results were based on the Kruskal-Wallis test and Pearson correlation analysis, a nonparametric test. Significance was set at $p < 0.05$.

Results and Discussion

Total phenolic contents (TPC)

The TPC values of the 15 propolis samples collected from different areas of Azerbaijan (Figure 1) ranged between 10.94 and 79.86 mg GAE per gram of raw propolis (Table 1). Significant differences in TPC were determined among the propolis samples ($p<0.05$). Propolis from the Qax region exhibited the highest amount of TPC (79.86 mg/g GAE), while the sample from the Astara and Şeki regions contained the lowest (10.94 mg/g GAE; 13.41 mg/g GAE). In addition to the Qax region, propolis from Zerdap, Aktaş, Ismayıllı-I and Qusar also had high phenolic contents. In terms of the differences between propolis samples from the same regions, significant differences were determined in TPC levels between Quaba I, II and III, between Nahcivan I and II, and between Ismayıllı I and II ($p<0.05$).

FRAP values

The ability of the Fe (III)-TPTZ complex reflects the total antioxidant capacity of plants as well as of propolis and other honeybee products. Under this method, it is assumed that the higher the FRAP value, the higher the antioxidant activity. The FRAP values calculated for the propolis samples are shown in Table 1. These ranged from 170 to 438 µM Trolox/g raw propolis. The highest FRAP values were detected in the samples from the Ismayılli (I), Zerdap, Qax, and Aktaş regions, and were higher than 400 µM Trolox equivalent of g propolis. The lowest FRAP values were detected in the samples from the Astara, Şeki, and Ismayılli (II) regions, at below 200 µM Trolox equivalent of g propolis. The reducing power measurement for the samples showed a concentration-dependent pattern.

A positive correlation was detected between the samples' FRAP values and TPC (r^2: 0.98), which suggests that total antioxidant activity of the propolis samples originates from the phenolic substances within the propolis (Table 2).

DPPH-scavenging activity

The antioxidant agents present in the propolis samples enhanced the decay of the purple color of the DPPH as they interacted with the radicals and scavenged these. The change in absorbance was monitored spectrophotometrically at 517 nm. The propolis samples from Zerdap, Quba (II), Aktaş, Ismayıllı (I) and Qusar exhibited higher DPPH radical-scavenging activity with lower SC_{50} values, within the range of 15–22 mg/ml (Table 1). As with TPC and FRAP values, significant differences were also determined in DPPH radical scavenging activities in propolis specimens from the same regions ($p<0.05$). DPPH radical scavenging activities in the propolis samples used in the study were correlated with amounts of TPC (r^2: –0,79) and FRAP values (r^2: –0,81).

Table 3. HPLC analysis of phenolic composition of ethanolic propolis extracts

Sample	Phenolic acids (mg/100 g)								Flavonoids (mg/100 g)						
	Gallic	Protocathecuic	p-hydroxy –benzoic	Chlorogenic	Vanillic	Caffeic	p-coumaric	Ferulic	Catechin	Rutin	Fisetin	Quercetin	Apigenin	Kaempherol	Isorhamnetin
Aktaş	0.10	1.80	1.30	–	–	271.10	93.70	24.30	–	–	–	93.93	–	–	92.42
Zerdap	–	3.30	4.20	–	9.04	532.00	237.40	48.10	–	–	–	16.27	120.50	38.80	11.51
İsmayıllı-I	–	1.70	4.44	–	3.93	391.72	185.50	117.7	–	–		48.80	70.80	57.50	13.30
İsmayıllı-II	–	–	9.91	–	41.80	35.30	279.60	367.50	–	30.40	–	33.26	–	12.40	–
Quba-I	–	0.30	–	–	–	41.12	9.20	0.80	–	–	–	62.36	53.05	94.03	–
Quba-II	–	–	–	–	–	288.44	108.20	20.60	–	683.00	–	89.64	118.70	99.00	15.30
Quba-III	–	918.00	4.92	–	–	166.02	41.64	26.03	–	154.40	–	39.24	55.95	99.04	19.67
Nahcivan-I	–	0.028	4.24	–	–	102.60	11.80	1.40	–	–	–	78.13	108.93	75.06	9.60
Nahcivan-II	–	–	–	–	–	81.50	20.54	33.00	–	6.70	–	129.60	148.10	215.90	7.50
Qusar	–	–	–	–	–	3.00	121.04	67.10		7.80	–	86.42	180.72	117.01	15.45
Astara	8.80	3.30	–	–	–	81.52	1.50	0.50	–	–	–	22.70	17.70	49.70	11.83
Şeki	–	–	–	–	–	57.60	23.54	13.23	–	–	–	90.20	76.30	64.80	11.20
Qax	–	–	22.50	–	19.40	195.04	288.80	145.80	–	–	–	697.6	179.90	81.90	59.30
Şemkır	–	–	–	–	–	4.64	1.90	0.94	–	–	15.10	–	27.15	33.84	9.82
Mingeçevir	–	0.30	–	–	–	163.30	61.10	21.00	–	–	–	74.10	113.90	79.30	17.20

Phenolic compounds of Azerbaijan propolis

Fifteen reference standards, including gallic, protocatechuic acid, p-OH benzoic acid, catechin, chlorogenic acid, vanillic acid, caffeic acid, epicatechin, p-coumaric acid, ferulic acid, rutin, myricetin, quercetin, apigenin, kaempferol and isorhamnetin were used to determine and quantify amounts of phenolics. All of the phenolic reference standards used was present in ethanolic extracts at different amount and selected samples. Only chlorogenic and catechin were absent from all samples. Caffeic acid was the most abundant phenolic acid among all propolis samples, followed by coumaric acid and ferulic acid. Protocathechuic, *p*- hydroxy benzoic acid, vanillic acid and gallic acid were found in some of the samples (Table 3).

Apigenin was the major flavonoid present in the samples, while quercetin, isorhamnetin and kaempferol were present at lower

concentrations among the studied flavonoids, although fisetin was detected only in one sample, from the Şemkır region. Rutin was detected in a sample from Quba-2 (Table 3).

Propolis is a multifunctional honeybee product consisting of exudates from plants mixed with beeswax. It is used by bees for various purposes, such as temperature insulation and beehive repairs, embalming dead organisms, etc. It has been used since as early as 3000 BC [15]. During the last two decades researchers have become interested in its pharmacological agents and biological activities [33,34]. The bioactivity capacity of propolis largely depends on its phenolic contents, including phenolic acids, flavonoids, anthocyanins, and several aromatic acids and esters [35]. As stated in the introduction, the physical properties and chemical composition, as well as the bioactive potential, of propolis are highly dependent on their region of origin. Such factors therefore need to be investigated by collecting samples from different regions. Several studies have been performed on propolis samples from different regions of the world in order to study their biological activity potentials [1,6,28,32,36].

In this study, we evaluated the TPC, antioxidant activity and phenolic profile of Azerbaijan propolis. TPC in the ethanolic propolis extracts ranged from nearly 10 to 80 mg GAE/g propolis, revealing significant differences among the propolis samples from the studied regions (Table 1). This difference in TPC levels may be attributed to the geographical origin of the samples; however, some propolis samples collected from different hives in the same area exhibited a wide variation in TPC. The three samples collected from Quba province, Quba I, II and III (Figure 1), had TPC values of 17.5, 61.72 and 66.02 mg GAE/g propolis, respectively. Additionally, the sample from Qusar city, which is adjacent to Quba, had a TPC value of 69.66 mg GAE/g propolis. Similarly, the two samples from Ismayıllı city, Ismayıllı I and II, also exhibited variation within the same region, with TPC values of 69.73 and 23.31 mg GAE/g propolis, respectively. Our TPC results for the Azerbaijani propolis samples were similar to those for Iranian propolis [21], which may be due to similarity of geography and vegetation. [37] studied propolis samples collected from 16 different areas of the world and reported TPC between 31 mg GAE/g and 299 mg GAE/g propolis; Thailand propolis had the lowest TPC (31 mg GAE/g) and Hubei-China the highest (299 mg GAE/g) [1]. studied TPC in 20 propolis samples from 12 different regions of China and reported levels between 42 mg GAE/g and 302 mg GAE/g propolis, with Yunan region propolis having the lowest TPC (42 mg GAE/g) [21]. studied propolis samples from three regions of Iran and reported TPC levels ranging from 30.80 mg GAE/g to 84.60 mg GAE/g propolis [36]. determined a TPC level in aquatic extract of propolis from the province of Erzurum in Turkey of 124 mg GAE/g propolis. In our previous study, we determined TPC levels in methanolic extracts of Turkish propolis within a range of 115 to 210 mg GAE/g propolis [6]. These results suggest that propolis samples collected from the same region and in the same season may exhibit differences in terms of TPC, which may be due to some other factors, such as condition and age of the beehive, as well as the strength of the honeybee colony.

Although there are various assays for measuring the antioxidant capacity of natural products, the reducing power of Fe (III) test has been used as a significant indicator of total antioxidant activity [4]. Propolis extracts from Azerbaijan exhibited a wide range

Table 4. The standard chromatogram values of fifteen individual phenolic substances and an internal standard using HPLC.

No	RT_{Mean} [a]	Compound	m[b]	c[c]	R^2	% RSD (RT)	% RSD (AREA)	LOD[d]	LOQ[d]
1	2.78	Gallic acid	0.137	0.011	0.9979	0.241	4.479	0.135	0.449
2	4.90	Protocatechuic Acid	0.098	0.341	0.9974	0.239	2.506	0.086	0.285
3	8.26	*p*-OH Benzoic acid	0.092	0.047	0.9953	0.657	3.164	0.124	0.414
4	9.34	Catechin	0.023	0.008	0.9982	0.580	3.966	0.325	1.083
5	10.09	Chlorogenic acid	61.187	1.323	0.9977	0.087	1.516	0.089	0.267
6	10.21	Vanillic acid	0.109	0.050	0.9970	0.354	1.808	0.483	1.609
7	10.98	Caffeic acid	120.930	7.158	0.9998	0.079	2.312	0.211	0.632
8	13.90	*p*-Coumaric acid	172.550	21.797	0.9997	0.077	1.342	0.299	0.896
9	15.05	Ferulic acid	115.110	11.597	0.9997	0.081	1.628	0.331	0.994
10	15.70	Rutin	39.478	3.915	0.9996	0.085	2.152	0.282	0.847
11	18.65	Fisetin	99.511	-79.950	0.9973	0.104	3.147	0.193	0.579
12	19.07	Quercetin	77.745	-22.523	0.9997	0.137	2.607	0.315	0.946
13	22.24	Apigenin	61.404	-14.844	0.9998	0.086	0.858	0.190	0.570
14	25.25	Kaempferol	33.222	31.621	0.9991	0.083	2.752	0.259	0.776
15	27.78	Isorhamnetin	73.751	-21.476	0.9992	0.078	3.959	0.209	0.627
	26.49	Propylparaben (IS)				0.069	4.107	0.179	0.596

[a] Time is expressed as minute. [b] Slope. [c] Intercept. [d] Values are expressed as mg/L

of FRAP values, from 170 to 437.90 Trolox/g. Ismayılli-I, Zerdap, Aktaş and Quba-II samples had FRAP values greater than 400 μM, while Astara, Şeki, Quba-II and Ismayılli samples had the lowest FRAP values, at less than 200 μM Trolox/g. These FRAP value findings are in accordance with our previous study of propolis samples collected from Turkey, which ranged from 182 to 325 μM Trolox/g propolis. Furthermore, the FRAP values of the samples were highly correlated with their TPC values (r^2: 0.98)**.** This positive correlation has also been reported in several previous propolis studies [6,21,36]. Overall, our results confirm that phenolic contents are the major determinants of the antioxidant potential of propolis on the basis of FRAP assay.

All propolis samples exhibited free radical (DPPH) scavenging activity to some extent; however, considerable differences were observed in SC_{50} values among the samples. In agreement with the FRAP results, the Zerdap, Quba-II, Ismayılli-I and Qusar samples exhibited higher radical scavenging capacities, and the Astara, Quba-I and Ismayılli-II samples lower DPPH-scavenging activity. We thus

determined positive and negative correlations between FRAP and DPPH values (r^2 = –0,81, p<0.05), TPC and DPPH values (r^2: –0.79, p<0.05) (Table 2). Relationships among the TPC, FRAP and DPPH activities of honeybee products have been investigated in several studies [1,6,8,24,33].

[36] demonstrated that the phenolic compounds present in honeybee products are responsible for their antioxidant and free radical-scavenging potential. More detailed analyses of phenolic compounds were therefore necessary to evaluate the antioxidant capacity of the propolis samples. In our study, samples were analyzed using RP-HPLC for eight individual phenolic acids and seven individual flavonoids to quantify and evaluate the compositions of the ethanolic propolis extracts (Table 3). All propolis samples contained caffeic, p-coumaric and ferulic acids as the major phenolic acids and apigenin, quercetin, kaempherol and isoramnetin as the main flavonoids, in parallel with other propolis studies from across the world [33,36,38,39]. The phenolic protocatechuic, p- hydroxybenzoic, vanillic and gallic acids were detected in a limited number of the propolis samples, while chlorogenic acid was not detected in any sample. Protocatechuic acid was detected in eight samples (out of 15), and Quba-III sample contained the highest level (918.8 mg/100 g) of propolis. In [6] study, benzoic, ferulic, caffeic and p-coumaric acid were identified as the main phenolic acids of Turkish propolis, which is highly compatible with our findings for Azerbaijan propolis.

Quercetin, apigenin, kaempherol and isoramnetin were the most abundant flavonoids in the samples, at levels ranging from 16.20 to 697.60 mg/100g. Although rutin was detected in a limited number of samples (four out of 15), Quba-II propolis contained a significantly higher amount (683.00 mg/100 g) than other samples. Rutin (quercetin-3-rhamnosyl glucoside), a natural lipophilic flavone derivative, possesses numerous biological activities, such as antioxidant, antibacterial, cytoprotective and anticarcinogenic properties [40,41]. Rutin is commonly found in vegetables and fruits and is a major dietary component of buckwheat [42]. [27] studied the phenolic and flavonoid compositions of red Brazilian propolis and detected rutin at 70 mg/100 g in their samples [6]. reported that the quercetin was the main flavonoid in Turkish propolis, while rutin was barely detected in their propolis samples. Our findings suggest that Azerbaijan propolis from the Quba region is rich in rutin, an important flavonoid with potent biological activity.

In the study, TPC was the highest amount of the Qax region propolis, and likewise the propolis sample rich in quercetin, p-coumaric acid, caffeic acid, apigenin, kaempherol, isorhamnetin, vanillic acid and *p*-OH benzoic acid.

Conclusions

In this study, we analyzed 15 propolis samples collected from different regions of Azerbaijan in order to determine their phenolic composition and antioxidant capacity. We found that the TPC of the Azerbaijan propolis are similar to those of neighboring countries. The antioxidant activity potential of the propolis samples was largely dependent on their phenolic and flavonoid composition. However, TPC and FRAP values, as well as DPPH-scavenging activity, varied significantly among samples collected from the same regions. Our results suggest that the antioxidant capacity of propolis samples depend their phenolic contents and the phenolic contents may also depend not only on the flora of the region, but

also on many other factors, such as the age and the conditions of the beehives, as well as the strength of the colony and method used to collect the samples.

In summary, considering the area from which samples of Azerbaijan propolis were collected as a model region, in additional to antioxidant capacities, the bioactive characteristics of propolis samples also vary in terms of the physical conditions of the hive, the floral characteristics of the region of collection, the colony species in the hive and the strength of the colony.

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Azerbaycan Propolislerinin Antioksidan Potansiyeli ve Fenolik Profili

Öz: Çalışmada Azerbaycan'ın 15 farklı bölgesinden toplanan propolis örneklerinin fenolik kompozisyonu ve antioksidan özellikleri analiz edildi. Antioksidan aktiviteleri üç farklı metod kullanılarak belirlendi; I) toplam fenolik içerik, ii) Demir indirgeme antioksidan kapasite (FRAP testi), iii) DPPH radikali süpürme aktivitesi. Propolis örneklerinin toplam fenolik içeriği 10.94–79.23 mg GAE/g arasında, FRAP değerleri 170–438 µM Trolox/g arasında, DPPH radikal süpürme aktivitesi (SC_{50} değeri) 18–128 µg/mL arasında bulundu.Fenolik asitler ve flavonoidler HPLC-DAD kullanılarak, standartlarla karşılaştırılarak tanımlandı. Araştırılan 15 örnekten, Ismayilli, Zerdap ve Qax bölgesinden toplanan propolislerin kapasitesi diğer bölgelerde toplananlardan yüksek bulundu. Örneklerin toplam antioksidan kapasiteleri propolislerin toplandığı bölgenin florasına bağlı olarak değişmektedir ancak bununla birlikte aynı bölge içerisin de toplanan örnekler arasında da anlamlı farklılıklar tespit edildi. Çalışma bulguları propolis örneklerinin antioksidan potansiyelinin sadece bölgenin florasına bağlı olmadığını, kovanın konumuna, kovanda bulunan propolisin ne kadar süredir kovanda biriktiğine, koloninin gücüne ve numunelerin toplanma yeri ve şekline de bağlı olduğunu gösterdi.

Anahtar kelimeler: Propolis, antioksidan aktivite, fenolik bileşikler, FRAP, DPPH

REFERENCES

[1] AHN, MR., KUMAZAWA S; USUI, Y; NAKAMURA, J; MATSUKA, M; ZHU, F; NAKAYAMA, T (2007) Antioxidant activity and constituents of propolis collected in various areas of China. Food Chemistry, 101, 1383-1392.

[2] PIETTA, P G; GARDANA, C; PIETTA, A M (2002) Analytical methods for quality control of propolis. Fitoterapia, 73 Suppl 1, S7-20.

[3] LASKAR, R A; SK, I; ROY, N; BEGUM, N A (2010) Antioxidant activity of Indian propolis and its chemical constituents. Food Chemistry, 122, 233-237.

[4] MOREIRA, L; DIAS, L G; PEREIRA, J A; ESTEVINHO, L (2008) Antioxidant properties, total phenols and pollen analysis of propolis samples from Portugal. Food and chemical toxicology, 46, 3482-3485.

[5] SANTOS, F A; BASTOS, E M; UZEDA, M; CARVALHO, M A; FARIAS, L M; MOREIRA, E S; BRAGA, F C (2002)Antibacterial activity of Brazilian propolis and fractions against oral anaerobic bacteria. Journal of ethnopharmacology, 80, 1-7.

[6] ALIYAZICIOGLU, R; SAHIN, H; ERTURK, O; ULUSOY, E; KOLAYLI, S (2011) Properties of Phenolic Composition and Biological Activity of Propolis from Turkey. International Journal of Food Properties,16, 277-287.

[7] HEGAZI, A G; EL MINIAWY, H F; EL MINIAWY, F

A (1995) Effect of some honey bee products on immune response of chicken infected with virulent Newcastle disease virus (NDV). The Egyptian journal of Immunology, 2, 79-86.

[8] SARIKAYA, A O; ULUSOY, E; OZTURK, N; TUNCEL, M; KOLAYLI, S (2009) Antioxidant Activity and Phenolic Acid Constituents of Chestnut (Castania Sativa Mill.) Honey and Propolis. Journal of Food Biochemistry, 33, 470-481.

[9] HEGAZI, A G (2012) Medical importance of bee products. Uludag Bee Journal,12, 136-146.

[10] WATANABE, M A; AMARANTE, M K; CONTI, B J; SFORCIN, J M (2011) Cytotoxic constituents of propolis inducing anticancer effects: a review. The Journal of pharmacy and pharmacology, 63, 1378-1386.

[11] AKYOL, S; GINIS, Z; ARMUTCU, F; OZTURK, G; YIGITOGLU, M R; AKYOL, O (2012) The potential usage of caffeic acid phenethyl ester (CAPE) against chemotherapy-induced and radiotherapy-induced toxicity. Cell biochemistry and function, 30, 438-443.

[12] OZTURK, G; GINIS, Z; AKYOL, S; ERDEN, G; GUREL, A; AKYOL, O (2012) The anticancer mechanism of caffeic acid phenethyl ester (CAPE): review of melanomas, lung and prostate cancers. European review for medical and pharmacological sciences, 16, 2064-2068.

[13] CINEGAGLIA, N C; BERSANO, P R; ARAUJO, M J; BUFALO, M C; SFORCIN, J M (2013) Anticancer effects of geopropolis produced by stingless bees on canine osteosarcoma cells in vitro. Evidence-based complementary and alternative medicine: eCAM, 73-7386.

[14] SFORCIN, J M (2007) Propolis and the immune system: a review. Journal of ethnopharmacology, 113, 1-14.

[15] BUYUKBERBER, M; SAVAS, M. C; BAGCI, C; KORUK, M; GULSEN, M. T; TUTAR, E; BILGIC, T; DEVECI, R; KUCUK, C (2009) The beneficial effect of propolis on cerulein-induced experimental acute pancreatitis in rats. The Turkish journal of gastroenterology, 20, 122-128.

[16] KITAMURA, H; NAOE, Y; KIMURA, S; MIYAMOTO, T; OKAMOTO, S; TODA, C; SHIMAMOTO, Y; IWANAGA, T; MIYOSHI, I (2013) Beneficial effects of Brazilian propolis on type 2 diabetes in ob/ob mice: Possible involvement of immune cells in mesenteric adipose tissue. Adipocyte, 2, 227-236.

[17] BARROS, M P; SOUSA, J P; BASTOS, J K; ANDRADE, S F (2007) Effect of Brazilian green propolis on experimental gastric ulcers in rats. Journal of ethnopharmacology, 110, 567-571.

[18] BARROS, M P; LEMOS, M; MAISTRO, E L; LEITE, M F; SOUSA, J P; BASTOS, J K; ANDRADE, S F (2008) Evaluation of antiulcer activity of the main phenolic acids found in Brazilian Green Propolis. Journal of ethnopharmacology,120, 372.

[19] YILDIZ, O; KARAHALIL, F; CAN, Z; SAHIN, H; KOLAYLI, S (2013) Total monoamine oxidase (MAO) inhibition by chestnut honey, pollen and propolis. Journal of Enzyme Inhibition and Medicinal Chemistry, 29(5):690-4.

[20] CAI, Y Z; MEI, S; JIE, X; LUO, Q; CORKE, H (2006) Structure-radical scavenging activity relationships of phenolic compounds from traditional Chinese medicinal plants. Life sciences, 78, 2872-2888.

[21] MOHAMMADZADEH, S; SHARIATPANAHI, M; HAMEDI, M; AHMADKHANIHA, R; SAMADI, N; OSTAD, S. N (2007) Chemical composition, oral toxicity and antimicrobial activity of Iranian propolis. Food Chemistry, 103, 1097-1103.

[22] GREGORIS, E; STEVANATO, R (2010) Correlations between polyphenolic composition and antioxidant activity of Venetian propolis. Food and chemical toxicology, 48, 76-82.

[23] HAVSTEEN, B H (2002) The biochemistry and medical significance of the flavonoids. Pharmacology & therapeutics, 96, 67-202.

[24] KÜÇÜK, M; KOLAYLI, S; KARAOĞLU, Ş; ULUSOY, E; BALTACI, C; CANDAN, F (2007) Biological activities and chemical composition of three honeys of different types from Anatolia. Food Chemistry, 100, 526-534.

[25] HUANG, W Y; CAI, Y Z; ZHANG, Y (2010) Natural phenolic compounds from medicinal herbs and dietary plants: potential use for cancer prevention. Nutrition and cancer, 62, 1-20.

[26] SFORCIN, J M; FERNANDES, A; JR. LOPES, C A; BANKOVA, V; FUNARI, S R (2000) Seasonal effect on Brazilian propolis antibacterial activity. Journal of ethnopharmacology, 73, 243-9.

[27] DAUGSCH, A; MORAES, C S; FORT, P; PARK, Y K (2008) Brazilian red propolis—chemical composition and botanical origin. Evidence-based complementary and alternative medicine: eCAM 5, 435-441.

[28] SHIMOMURA, K; SUGIYAMA, Y; NAKAMURA, J; AHN, M R; KUMAZAWA, S (2013) Component analysis of propolis collected on Jeju Island, Korea. Phytochemistry, 93, 222-229.

[29] DE VILLIERS, A; LYNEN, F; CROUCH, A; SANDRA, P (2004) Development of a Solid-Phase Extraction Procedure for the Simultaneous Determination of Polyphenols, Organic Acids and Sugars in Wine. Chromatographia, 59, 403-409.

[30] SINGLETON, V L; Orthofer, R; Lamuela-Raventós, R M (1999) Analysis of total phenols and other oxidation substrates and antioxidants by means of folin-ciocalteu reagent. In Methods in enzymology, Lester, P., Ed. Academic Press: 1999; Vol. 299, pp 152-178.

[31] BENZIE, I F; STRAIN, J J (1999) Ferric reducing/antioxidant power assay: direct measure of total antioxidant activity of biological fluids and modified version for simultaneous measurement of total antioxidant power and ascorbic acid concentration. Methods in enzymology, 299, 15-27.

[32] MOLYNEUX, P (2004) The use of the stable free radical diphenylpicrylhyrazyl (DPPH) for estimating antioxidant activity. The Songklanakarin Journal of Science and Technology, 26, 211–219.

[33] BARBARIC, M; MISKOVIC, K; BOJIC, M; LONCAR, M B; SMOLCIC-BUBALO A, DEBELJAK Z; MEDIC-SARIC M, J (2011) Chemical composition of the ethanolic propolis extracts and its effect on HeLa cells. Journal of ethnopharmacology, 135, 772-778.

[34] CHAN, G C; CHEUNG, K W; SZE, D M (2013) The immunomodulatory and anticancer properties of propolis. Clinical reviews in allergy & immunology, 44, 262-273.

[35] SALOMAO, K; DANTAS, A P; BORBA, C M; CAMPOS, L C; MACHADO, D G; Aquino Neto, F R; de Castro, S L (2004) Chemical composition and microbicidal activity of extracts from Brazilian and Bulgarian propolis. Letters in applied microbiology, 38, 87-92.

[36] GULCIN, I; BURSAL, E; SEHITOGLU, M H; BILSEL, M; GOREN, A C (2010) Polyphenol contents and antioxidant activity of lyophilized aqueous extract of propolis from Erzurum, Turkey. Food and chemical toxicology, 48, 2227-38.

[37] KUMAZAWA, S; HAMASAKA, T; NAKAYAMA, T (2004) Antioxidant activity of propolis of various geographic origins. Food Chemistry, 84, 329-339.

[38] THIRUGNANASAMPANDAN, R; RAVEENDRAN, S B; JAYAKUMAR, R (2012) Analysis of chemical composition and bioactive property evaluation of Indian propolis. Asian Pacific journal of tropical biomedicine, 2, 651-654.

[39] FALCAO, S I; VALE, N; GOMES, P; DOMINGUES, M R; FREIRE, C; CARDOSO, S M; VILAS-BOAS, M (2013) Phenolic profiling of Portuguese propolis by LC-MS spectrometry: uncommon propolis rich in flavonoid glycosides. Phytochemical analysis: PCA, 24, 309-318.

[40] LIN, J P; YANG, J S; LU, C C; CHIANG, J H; WU, C L; LIN, J J; LIN, H L; YANG, M D; LIU, K C; CHIU, T H; CHUNG, J G (2009) Rutin inhibits the proliferation of murine leukemia WEHI-3 cells in vivo and promotes immune response in vivo. Leukemia research, 33, 823-828.

[41] VISKUPICOVA, J; STROSOVA, M; STURDIK, E; HORAKOVA, L (2009) Modulating effect of flavonoids and their derivatives on sarcoplasmic reticulum Ca2+-ATPase oxidized by hypochloric acid and peroxynitrite. Neuro endocrinology letters, 30 Suppl 1, 148-151.

[42] YANG, N; REN, G J (2008) Application of near-infrared reflectance spectroscopy to the evaluation of rutin and D-chiro-Inositol contents in tartary buckwheat. Journal of agricultural and food chemistry, 56, 761-764.