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Evaluation of antioxidant activity of bee products of different bee races in Turkey

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Abstract: Bee products with high bioactive properties have a very important place in human nutrition. Biochemical properties of these products such as honey, pollen, and propolis vary according to flora, season, bee race, and production methods. In this study, the biological activities of honey, bee pollen, and propolis samples from four bee races in Turkey (*Apis mellifera caucasica*, *Apis mellifera anatoliaca*, *Apis mellifera syriaca*, and *Apis mellifera carnica*) were examined. Besides the determining of total polyphenol and total flavonoid contents, the antioxidant capacity was investigated by three different assays: DPPH (1,1-diphenyl-2-picrylhydrazyl) radical-scavenging activities, CUPRAC (cupric reducing antioxidant capacity) and FRAP (ferric reducing antioxidant power). The rank order of antioxidant potencies was as follows in the four bee races: propolis > pollen > honey. The polyphenol content of the products was evaluated and *Apis mellifera syriaca* for honey, *Apis mellifera anatoliaca* for pollen, and *Apis mellifera carnica* for propolis were found higher than the others. When bee races were compared, it was seen that flora is a more effective factor than bee race for antioxidant capacity.

Key words: *Apis mellifera*, honey, propolis, pollen, bee race, antioxidant capacity

1. Introduction

Turkey has a rich flora with 163 families, 1225 genera, and 9000 species [1]. It is thus a country with high potential for beekeeping, considering the diversity of flowers and climates. It is the country with the second highest honey production after China[1]. Morphological, physiological, and behavioral characteristics in terms of classification of 24 bee races were determined in the world. Turkey has 4 widely spread bee races, including *Apis mellifera carnica*, *Apis mellifera caucasica*, *Apis mellifera syriaca*, and *Apis mellifera anatoliaca*. *Apis mellifera caucasica* is raised in Turkey's northeast area to Samsun, *Apis mellifera syriaca* in a very small area near the Turkey-Syria border, *Apis mellifera carnica* in the Thrace region of Turkey, and *Apis mellifera anatoliaca* in the remaining part of Turkey [2].

Honey is a sweet natural substance produced by honeybees (*Apis mellifera*). The composition of honey is related to geographical conditions including flora, climate, and environmental conditions [3]. Honey has been used in folk medicine in the treatment of burns and wounds, asthma, and ulcers [4].

Propolis is produced by honeybees from resins and gums and is used to protect hives from pathogens [5,6]. Propolis is rich in phenolic compounds that have biological properties such as antibacterial, antiinflammatory, antioxidant, and antitumor effects [7–9]. Pollen is the male organ cells of flowers. Bee pollen is produced by mixing these pollens with nectar and bee secretions. Bee pollen contains high amounts of protein, carbohydrates, lipid, minerals, and vitamins [10]. It has a significant amount of phenolics in addition to other nutrients [11,12], which show special bioactive properties such as antibacterial [13,14], antifungal [13], and antioxidant [15,16]. It has also been reported that pollen increases testosterone levels and sperm count [17].

Reactive oxygen species (ROS) and other free radicals cause diseases including cardiovascular and neurological disorders and cancer [18,19]. Antioxidants protect the human body from ROS and other free radicals before they attack biological cells [20]. Natural antioxidants such as polyphenols in the human diet come from bee products, cereals, plants, and fruits, and they contain different

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compounds that possess high antioxidant activities and multiple biological effects [21,22]. Therefore, in recent years, studies on antioxidant substances in foods and plants have increased.

To date, researchers have focused predominantly on the bioactivities of bee products such as pollen, propolis, and honey; however, no study has been conducted about the effects of bee races (*Apis mellifera caucasica*, *Apis mellifera anatoliaca*, *Apis mellifera syriaca*, and *Apis mellifera carnica*) on the biochemical quality of bee products. Therefore, in our study we aimed to identify the total polyphenol and total flavonoid contents and the antioxidant activity of honey, propolis, and bee pollen samples collected from different honeybee races in Turkey.

2. Materials and methods

2.1. Supply of materials and preparation of extracts

Honey, propolis, and pollen samples were supplied by local beekeepers with the support of the Beekeepers Association of Turkey and the Provincial Directorate of Agriculture and the Ministry of Forestry of Artvin. Samples were obtained from each race's own area on 2–3 August 2017 (Table 1). Five samples were taken from each region for each bee product. The sampling process was done by selecting different hives. All samples were kept at 4 °C until analysis time.

About 10 g of honey and about 5 g of pollen and propolis were weighed. Honey samples were stirred and pollen samples were blended. Frozen propolis was ground until powdered.

All samples were extracted with methanol (Sigma-Aldrich, Germany) at room temperature for 24 h in the dark. The extracts were filtered with Whatman filter paper No. 4 and then the filtrates of the samples were stored at 4 °C until analysis.

2.2. Determination of total polyphenol and total flavonoid content

The total polyphenol content of samples was determined with the Folin–Ciocalteu colorimetric method [23]. First, 20 µL of extract, 400 µL of 0.5 N Folin–Ciocalteu reagent (Fluka Chemie, Switzerland), and 680 µL of distilled water were added and vortexed. Following 3 min of incubation, 400 µL of 10% Na_2CO_3 solution (Merck, Darmstadt, Germany) was added. After incubation at room temperature for 2 h, absorbances of samples were measured at 760 nm. The results were expressed as mg gallic acid equivalents (GAE)/100 g samples.

Total flavonoid content of samples was determined according to the method of Chang et al. [24]. First, 0.5 mL of extract was mixed with 4.3 mL of solvent, 0.1 mL of 10% $AlCl_3$ (Sigma-Aldrich), and 0.1 L of 1 M NH_4CH_3COO solution (Sigma-Aldrich). The mixture was shaken and incubated for 40 min, and then absorbance was measured at 415 nm. The flavonoid contents of samples were represented as mg quercetin equivalents (QUE)/100 g of sample.

2.3. Determination of antioxidant activities

The ferric reducing ability of methanolic extracts was analyzed by the method of Benzie and Strain [25]. Iron(II) sulfate heptahydrate (Sigma-Aldrich) was used as a standard. FRAP reagent was prepared as a 10:1:1 mixture of three solutions: 300 mM acetate buffer (pH 3.6), 10 mM TPTZ (in 40 mM HCl) (Fluka Chemie), and 20 mM $FeCl_3$ (Merck). For this, 3 mL of FRAP reagent, 100 µL of extract, and 100 µL of distilled water were added to a flask and vortexed (IKA Lab Dancer). Absorbance values at 593 nm were recorded after waiting 4 min at room temperature. FRAP value was expressed as µmol $FeSO_4.7H_2O$ per gram of sample.

The CUPRAC value of extracts was analyzed by the method of Apak et al. [26]. First, 1 mL of 10 mM copper(II) chloride solution (Merck) was mixed with 1 mL of 7.5 mM neocuproine (Merck), 1 mL of 1 M ammonium acetate buffer (pH 7.0), 200 µL of distilled water, and 900 µL of sample solution. Absorbance was measured at 450 nm after 30 min. Trolox (Sigma-Aldrich) was used as a standard. The results were represented as mmol Trolox/g of sample.

DPPH scavenging ability of samples was estimated using the procedure of Yu et al. [27]. Trolox was used as a standard and the values were expressed as SC_{50} (mg sample/mL). Samples (0.75 mL, various concentrations) were mixed with 0.7 mL of DPPH (0.1 mM in methanol) (Sigma-Aldrich) and vortexed (IKA Lab Dancer). After incubation in the dark at room temperature for 50 min, absorbance at 517 nm was recorded.

Table 1. Source of bee product samples.

Bee race	Samples	Region
Apis mellifera carnica	Honey, pollen, and propolis	Tekirdağ, Thrace
Apis mellifera anatoliaca	Honey, pollen, and propolis	Ankara, Kızılcahamam, Central Anatolia
Apis mellifera syriaca	Honey, pollen, and propolis	Hatay, Samandağ, Southeastern Anatolia
Apis mellifera caucasica	Honey, pollen, and propolis	Artvin, Camili, Eastern Black Sea

2.4. Statistical analysis

All data are reported as mean ± SD. Significant differences between the mean values were analyzed with ANOVA tests. Duncan tests were used among the groups. Differences showing a level of $P < 0.05$ were considered to be statistically significant.

3. Results and discussion

In our study, bee products (honey, pollen, and propolis) from four different bee races (*Apis mellifera caucasica*, *Apis mellifera anatoliaca*, *Apis mellifera syriaca*, and *Apis mellifera carnica*) were used. Table 1 shows the regions from which bee products were supplied and Table 2 shows the coding of the samples.

Turkey is geographically located in three floristic areas including the European-Siberian floristic area, Mediterranean floristic area, and Iran-Turan floristic area [28]. Samples of *Apis mellifera caucasica* were taken from the Camili region of Artvin. The Camili (Macahel) region, which was the first biosphere reserve of Turkey, was included in a biodiversity and sustainable natural resource method (GEF) project in 2000 due to its rich flora and fauna. It was included in the World Biosphere Reserves Network by UNESCO on 29 June 2005 and the Ministry of Agriculture and Forestry declared this area a "Pure Caucasian Bee Genetic Region" [29]. Samples of *Apis mellifera anatoliaca* were taken from Kızılcahamam in Ankara. It has a terrestrial climate at an altitude of 975 m and a transition climate because it is located between the Central Anatolia and Western Black Sea regions. Soğuksu National Park, which is located in the district, has important plant richness and it has been determined that 428 plant species are naturally found in Kizilcahamam.[2] Samples of *Apis mellifera syriaca* were taken from Hatay. It is located within the Mediterranean climate zone. There are 175 endemic and 1246 species in Hatay Province [30]. Samples of *Apis mellifera carnica* were taken from Tekirdağ, a province bordering the Marmara Sea and Black Sea in the northwest of Turkey. A Mediterranean climate is dominant on the Marmara coast in Tekirdağ. Oak and hornbeam trees are seen in the south, while beech trees are common in the north. Rhododendrons are available in the north. Sunflower is also grown in many parts of the province [31].

Propolis, honey, and bee pollen are all known for their potent antioxidant properties [32–36]. We intended to investigate the effect of the factor of honeybee race on the antioxidant properties of bee products. For this purpose, total polyphenol and total flavonoid contents were analyzed in honey, pollen, and propolis samples from each bee race (Table 3). SH and AH had statistically higher total phenolic content in honey samples and the total phenolic contents were 58 mg GAE/100 g and 49 mg GAE/100 g, respectively. In pollens, APo was found to have the highest total phenolic content with a value of 1258 mg GAE/100 g (see Table 2 for codes).

The statistically highest total flavonoid content was found in CarH (4 mg QUE/100 g) and in SH (5 mg QUE/100 g). Both TP and TF values of propolis samples of

Table 2. Coding of samples.

Name of bee race	Race code	Sample	Final code
Apis mellifera syriaca	S	Honey	SH
		Pollen	SPo
		Propolis	SPr
Apis mellifera anatoliaca	A	Honey	AH
		Pollen	APo
		Propolis	APr
Apis mellifera caucasica	Cau	Honey	CauH
		Pollen	CauPo
		Propolis	CauPr
Apis mellifera carnica	Car	Honey	CarH
		Pollen	CarPo
		Propolis	CarPr

Table 3. Total phenol and total flavonoid results of bee products.

Sample code	TP (mg GAE/100 g)	TF (mg QUE/100 g)
SH	58 ± 27^{b}	5 ± 2^{c}
AH	49 ± 10^{b}	3 ± 1^{b}
CauH	28 ± 5^{a}	1 ± 1^{a}
CarH	32 ± 2^{a}	4 ± 1^{c}
SPo	738 ± 131^{b}	253 ± 64^{a}
APo	1258 ± 505^{c}	390 ± 10^{b}
CauPo	47 ± 15^{a}	261 ± 76^{a}
CarPo	41 ± 14^{a}	499 ± 99^{c}
SPr	1879 ± 228^{a}	294 ± 50^{a}
APr	8550 ± 1237^{b}	337 ± 96^{a}
CauPr	9905 ± 1087^{c}	380 ± 78^{a}
CarPr	$11{,}769 \pm 1248^{d}$	1190 ± 216^{b}

[a,b,c,d] Values with different letters within a column are significantly different at $P < 0.05$.
Values are means ± standard deviations for triplicate determination.

[2] Kalkınma Bakanlığı, Ankara, Turkey, 2016. http://www.ankaraka.org.tr/tr/attachment/Fizibilite%20Raporu.pdf?i=0&newsId=3783 (in Turkish).

the *Carnica* bee race were high. The total phenolic content for CarPr was found as 11,769 mg GAE/100 g. The total flavonoid content for CarPr was 1190 mg QUE/100 g. Pollen samples of the *Carnica* bee race were also found to have high flavonoid contents (499 mg QUE/100 g).

FRAP and CUPRAC methods for reducing ability and the DPPH method for radical scavenging activity were selected to determine the antioxidant capacity (Table 4). According to the results of FRAP analysis, the highest activity in honey and propolis samples was shown in the *Caucasica* bee race (respectively 4.57 µmol/g and 1600.25 µmol/g). Pollen of the *Anatoliaca* bee race was found to have high activity with a value of 84.89 µmol/g. There was no statistically significant difference in CUPRAC values of honey samples ($P < 0.05$). APo had the highest CUPRAC value (84.89 mmol/g) in pollen samples. SPr (0.40 mmol/g) and CarPr (38 mmol/g) had statistically higher CUPRAC values in propolis samples. When the DPPH activities were examined, CauH in honey samples, CarPr in propolis samples, and APo in pollen samples showed high activity.

The composition of propolis is variable, depending on the flora around the hive and the climate in which it is collected [37]. It includes caffeic acid, galangin, quercetin, and chrysin, which have antioxidant activity [38]. In the present study, CarPr showed the highest activity in TP, TF, and DPPH analyses. CauPr had the highest activity in FRAP analysis. SPr and CarPr showed statistically similar activities in CUPRAC analysis. Recently, we reported that propolis showed the highest antioxidant activity among different bee products (honey, pollen, propolis, and royal jelly) [16]. In parallel with our study, Nakajima et al. [39] found that among pollen, propolis, and royal jelly, propolis had the strongest antioxidant effects. In a different study, Mohdaly et al. [40] reported that propolis showed high antioxidant activity when compared with bee pollen and propolis.

Bee pollen is collected by bees from plant flowers, mixed with secretions from the salivary glands or nectar. Pollen is a major food source for growing bee larvae [41]. In our study, APo showed the highest activity in TP analysis, and in TF analysis CarPo had the highest activity. For FRAP, CUPRAC, and DPPH values, APo samples had higher activity than other pollen samples. It has been reported that pollen has high bioactivity, but not as high as that of propolis [16,26,39]. In a previous study, the total phenolic content of Anzer bee pollen was reported to vary between 44.07 and 124.10 mg GAE/g [35]. Fatrcová-Šramková et al. [42] found that monofloral bee pollen has polyphenol contents between 319.31 and 1383.67 mg/kg. LeBlanc et al. [43] reported that the total flavonoid values of the bee pollen of the Sonoran Desert ranged from 5.48 to 2.66 mg QUE/g. In other study, total flavonoid contents in pollen ranged from 7.32 to 7.95 mg QUE/g and DPPH scavenging activity was found to be between 13.87 and 15.04 mg Trolox/g [44]. It was seen that, due to the diversity of bee pollen and supply from different regions, the polyphenol content, flavonoid content, and antioxidant activity could change from one region to another.

Mineral, pollen, and phenolic contents of honey are related to its botanical origin and that also has important effects on the antioxidant activity of honey [45].

Table 4. Antioxidant activity analysis of results of bee products.

Sample code	FRAP (µmol $FeSO_4.7H_2O$/g)	CUPRAC (mmol Trolox/g)	DPPH-SC_{50} (mg/mL)
SH	2.25 ± 1.41^{a}	0.03 ± 0.03^{a}	74.89 ± 24.90^{b}
AH	1.37 ± 0.17^{a}	0.02 ± 0.00^{a}	155.70 ± 76.68^{c}
CauH	4.57 ± 1.33^{b}	0.04 ± 0.05^{a}	30.90 ± 1.93^{a}
CarH	1.94 ± 0.62^{a}	0.01 ± 0.01^{a}	63.46 ± 4.29^{ab}
SPo	25.37 ± 1.69^{b}	0.14 ± 0.03^{b}	1.53 ± 0.22^{c}
APo	84.89 ± 10.09^{d}	0.24 ± 0.04^{d}	0.47 ± 0.51^{a}
CauPo	75.65 ± 18.98^{c}	0.18 ± 0.04^{c}	0.52 ± 0.96^{a}
CarPo	8.69 ± 1.64^{a}	0.02 ± 0.02^{a}	0.84 ± 0.17^{b}
SPr	166.91 ± 12.86^{a}	0.40 ± 0.09^{b}	0.12 ± 0.08^{b}
APr	1317.76 ± 216.35^{b}	0.29 ± 0.08^{a}	0.13 ± 0.01^{b}
CauPr	1600.25 ± 143.76^{c}	0.27 ± 0.08^{a}	0.13 ± 0.01^{b}
CarPr	1432.15 ± 101.18^{b}	0.38 ± 0.14^{b}	0.02 ± 0.02^{a}

^{a,b,c,d} Values with different letters within a column are significantly different at $P < 0.05$.
Values are means ± standard deviations for triplicate determination.

SH and AH honey samples had statistically similar higher activities, and CauH and CarH samples showed lower activity in terms of total phenolic content. SH and CarH had the highest total flavonoid contents. For FRAP and DPPH values, CauH was found to have higher activity than other honey samples, while the CUPRAC values were not statistically significant. Nine Turkish honey samples from different floral sources were investigated by Ulusoy et al. [46] and they reported that the total phenolic contents varied from 66 to 223 mg/g, the antioxidant activities found with CUPRAC ranged from 124.8 to 532 μmol/g, and those with FRAP ranged from 33 to 166 μmol/g. In addition, DPPH scavenging activity expressed as IC_{50} ranged from 84 to 296 μg/mL. One study reported that the total phenolic contents of 62 honey samples varied between 16.02 and 120.04 mg GAE/100 g, ferric reducing activities varied between 0.66 and 4.30 μmol/g, and DPPH scavenging activity varied between 12.56 and 152.40 mg/mL [41].

It was found that antioxidant activities of the bee products varied according to their phenolic contents and could be ordered from highest to lowest as propolis, pollen, and honey. These results are compatible with those of other studies [16,47].

Bioactivity analyses of honey, pollen, and propolis of different bee races were conducted and the effect of bee race on the product characteristics was determined at a level of significance of $P < 0.05$. We cannot say that the products of a single bee race are superior to the bee products of different races. *Apis mellifera syriaca* in honey samples, *Apis mellifera anatolica* in pollen samples, and *Apis mellifera carnica* in propolis samples showed the highest bioactivities. Chemical analysis of propolis collected from three different races of *Apis mellifera* in the same region in Turkey was conducted by Silici and Kutluca. They reported that the three honeybee races (*Apis mellifera caucasica*, *Apis mellifera carnica*, and *Apis mellifera anatolica*) used neither the same nor a single propolis source [48].

It was concluded that, beyond the advantages of physical characteristics of the bee race, floral diversity of bee products is most responsible for bioactivity. The results of this research will encourage researchers who want to conduct similar detailed studies with different bee races in the same location and season.

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