

## Investigation of Phenolic Compounds and Antioxidant Capacity of Bee Pollen Collected from Different Geographical Regions in Turkey

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### ABSTRACT

The aim of this study was to evaluate the antioxidant activity, total phenolic acid, and total flavonoid content in bee pollen collected from different regions of Turkey. A total of 81 bee pollen samples from 81 beekeepers were collected from 6 different regions of Turkey. Total phenolic content of pollen samples was determined in mg gallic acid equivalent gr pollen sample (mgGAE/gr) and total flavonoid analysis was made with a spectrophotometric method in mg based on quercetin (QE/g) concentration. Total antioxidant level was conducted according to free radical scavenger effects of pollen on DPPH (1,1-diphenyl-2-picrylhydrazyl) as an indirect method. According to the results of the study, total phenolic acid levels of pollen differed between 7.81 and 57.69 (mg GAE/g), total flavonoid content differed between 3.72 and 4.97 (mg QE/g) and DPPH differed between 55.18 and 94.08 (mg/ml). It is considered that the study data will contribute to the presentation of the chemical components of Turkish bee pollen.

**Keywords:** Antioxidant, apitherapy, bee pollen, flavonoid, phenolic.

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### Türkiye'nin Farklı Coğrafi Bölgelerinden Toplanan Arı Poleninin Fenolik Bileşikleri ve Antioksidan Kapasitelerinin Araştırılması

### ÖZ

Bu çalışmada, Türkiye'nin değişik bölgelerinden toplanan bal arısı polenlerin toplam fenolik asit, flavonoid düzeyleri ve total antioksidan kapasiteleri araştırılmıştır. Polen numuneleri Türkiye'nin farklı bölgelerden 81 arıcıdan toplam 81 örnekten temin edilmiştir. Toplam fenolik madde içeriği kuru ağırlığın gramı başına mg gallic asit eşdeğeri (mgGAE/gr) ve toplam flavonoid analizi ise gramı başına quercetin (mgQE/gr) konsantrasyon temel alınarak spektrofotometrik bir yöntem ile yapılmıştır. Total antioksidan düzeyi indirekt metot olarak polenin DPPH (1,1-diphenyl-2-picrylhydrazyl) üzerindeki serbest radikal süpürücü etkilerine göre yapılmıştır.

Çalışmamızda elde edilen bulgulara göre polenin total fenolik asit düzeyleri 7.81 ile 57.69 (mgGAE/g), toplam flavonoid düzeyleri 3.72 ile 4.97 (mgQE/g) ve DPPH ise 55.18 ile 94.08 (mg/ml) aralığında değişkenlik göstermiştir. Çalışma verilerinin Türk arı poleninin kimyasal yapısının ortaya konulmasında katkı sağlayacağı düşünülmektedir.

**Anahtar Kelimeler:** Antioksidan, apiterapi, arı poleni, flavanoid, fenolik

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## INTRODUCTION

For centuries, human beings have used bee products for therapeutic purposes in addition to their use as food products. Bee pollen forms in the antenna of flowering plants with the combination of pollen which is the male reproductive organ with a role in pollination and bee secretion and it is one of the primary nutritional sources used for colony development (Almeid-Munadion et al. 2005). Depending on different plant sources, the pollen obtained has been reported to consist of approximately 200 compounds. Pollen, which includes an average of 1,6% phenolic compounds, also has a high variation of flavonoids, leukotrienes, catechins, phenolic acid, flavonol and flavonol glycosides (Asfova et al. 2001; Saraiva et al. 2018). Studies have shown that phenolic compounds are responsible for bee pollen's anti-microbial (Morais et al. 2011), anti-inflammatory (Maruyama et al. 2010; Lopes et al. 2019), anti-mutagenic (Tohamy et al. 2014; AL-Yuosef et al. 2020), anti-fungal (Marinova and Tcharbanov, 2011), anti-nociceptive and anti-oxidant (Freire et al. 2012; Ghouizi et al. 2020) activities. These activities depend completely on the chemical structure and biochemical characteristics of bee pollen (Mosaic et al.2019). In other words, the concentration and components of phenolic compounds also change the quality of pollen's different biological activity (Freire et al. 2012). Therapeutic activity bee pollen shows are not valid for all pollens in the same level because the chemical content of bee pollen changes depending on the source of plants. In addition to collecting pollens from different types of flowers, bees also transfer different chemical substances within this plant to pollen content (Nagai et al.2002; Nisbet et al. 2009). Thus, the chemical composition of pollen differs depending on the type of plant. Chemical composition and biochemical features of pollen differ based on the types of plants from which pollen is collected, geographical source and season (Morgano et al. 2012). This change also changes the composition of the active substances of especially pollen. This study examines the total phenolic compounds and antioxidant capacity of bee pollens collected from different regions of Turkey.

## MATERIAL and METHOD

Pollen samples to be used in the study, a total of 81 samples from 81 beekeepers were collected from different regions of Turkey as Central and Eastern Black Sea (13), Marmara (13), Central Anatolia (16), Mediterranean (13), Aegean (13), and Eastern-

Southern Anatolia (13) between May and July. In the selection of pollen samples, pollen diversity, different regions and being free from environmental pollution were taken into consideration. The beekeepers from whom the samples were collected were registered to bee re-registration system. The pollens which were brought to the laboratory under healthy conditions were dried in a stove at 40 oC for 48 hours. They were ground and powdered and to prepare the methanol extracts of pollens 1 gram was taken from each sample and solubilized in 25 ml distilled methanol (4% methanol stock pollen solutions).

### Total Phenolic Matter Amount Analysis

Total phenolic concentration level was applied by modifying Folin-Ciocalteu method (Bertoncelj et al., 2007). Total phenolic content of pollen samples was determined in mg gallic acid equivalent gr pollen sample (mgGAE/gr) by using Folin-Ciocalteu reactive.

### Total Flavonoid Matter Amount Analysis

Total flavonoid analysis was determined with the modification of the method developed by Chang et al.,(2002). Absorbances prepared by being based on quercetin (mgQE/g) concentration in mg in 1 gram pollen were read in a spectrophotometer at 415 nm.

### DPPH Radical Scavenger Activity Measurement

Antioxidant activity of pollen samples was measured by a small modification of the method suggested by Meda et al.,(2005) by depending on the ability of catching DPPH radical. Antioxidant activity was calculated as the % inhibition of DPPH radical by making use of the following equation:

$$\% \text{ inhibition} = (\text{Control absorbance} - \text{Sample absorbance}) \times 100.$$

### Statistical Analyses

The data obtained from the study were analyzed with variance analysis (ANOVA) technique and differences between averages were determined with Duncan multiple comparison test. Statistical assessments were made by using SPSS program.

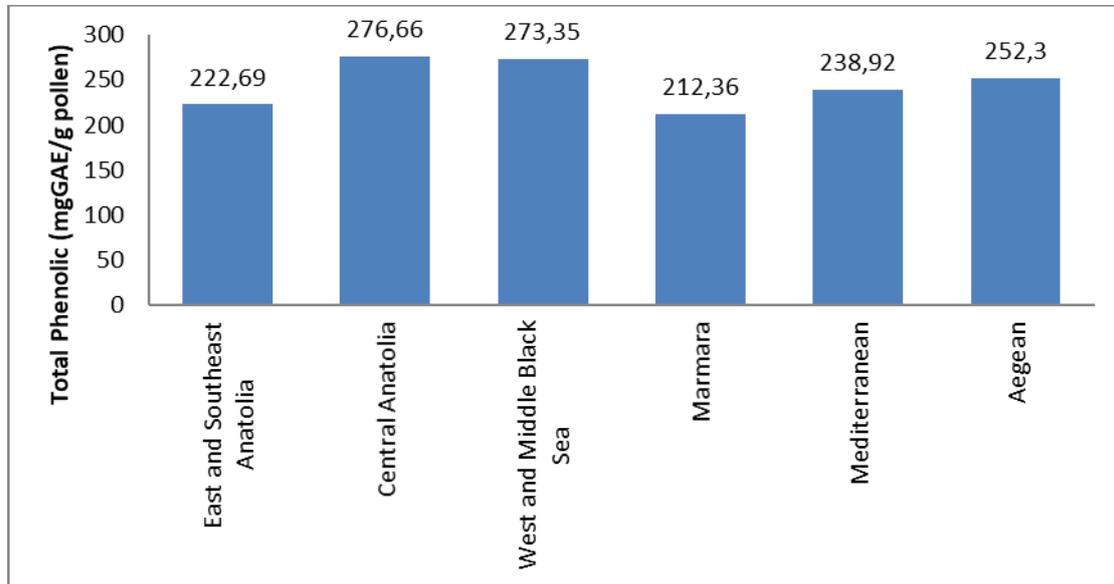
## RESULTS

### Total Phenolic Matter Amount:

Total phenolic content in pollen samples was found to differ between  $21.23 \pm 1.38$  and  $27.66 \pm 2.91$  (mg GAE/g pollen). However, no statistically significant difference was found between regions ( $P < 0.05$ ) (Table 1 and Figure 1).

**Table 1** Bölgeler arası polen ekstraktların toplam fenolik, flavonoid ve antioksidan düzeyleri  
**Table 1** Total phenolic, flavonoid and total antioxidant levels of pollen extracts of regions.

Regions	Total phenolic (mg GAE/g pollen)	Min-Max	Total flavonoid (mg QE/g pollen)	Min-Max	Antioxidant, DPPH-sc <sub>50</sub> (mg/ml)	Min-Max
E.Southeast Anatolia	22.26 ± 2.36	8.88 – 34.13	4.19 ± 0.53	2.20 - 7.79	76.14 ± 2.97	55.18 - 91.54
Central Anatolia	27.66 ± 2.91	15.19 – 57.69	4.14 ± 0.45	0.05 - 8.66	74.97 ± 3.22	46.93 - 94.08
Black Sea	27.33 ± 2.32	16.88 – 45.82	3.72 ± 0.25	2.41 - 5.40	77.93 ± 3.63	41.65 - 94.08
Marmara	21.24 ± 1.38	16.01 – 32.52	4.89 ± 0.32	3.26 - 6.57	77.04 ± 3.62	42.28 - 89.43
Mediterranean	23.89 ± 2.69	12.96 – 45.80	4.97 ± 0.64	0.85 - 8.91	77.48 ± 2.14	68.92 - 88.16
Aegean	25.23 ± 3.45	7.81 – 52.98	4.96 ± 0.52	2.78 - 8.16	69.49 ± 5.61	36.15 – 92.18
<b>Total</b>	<b>24.60± 2.52</b>	<b>-</b>	<b>4.48 ± 0.45</b>	<b>-</b>	<b>75.51± 3.53</b>	<b>-</b>

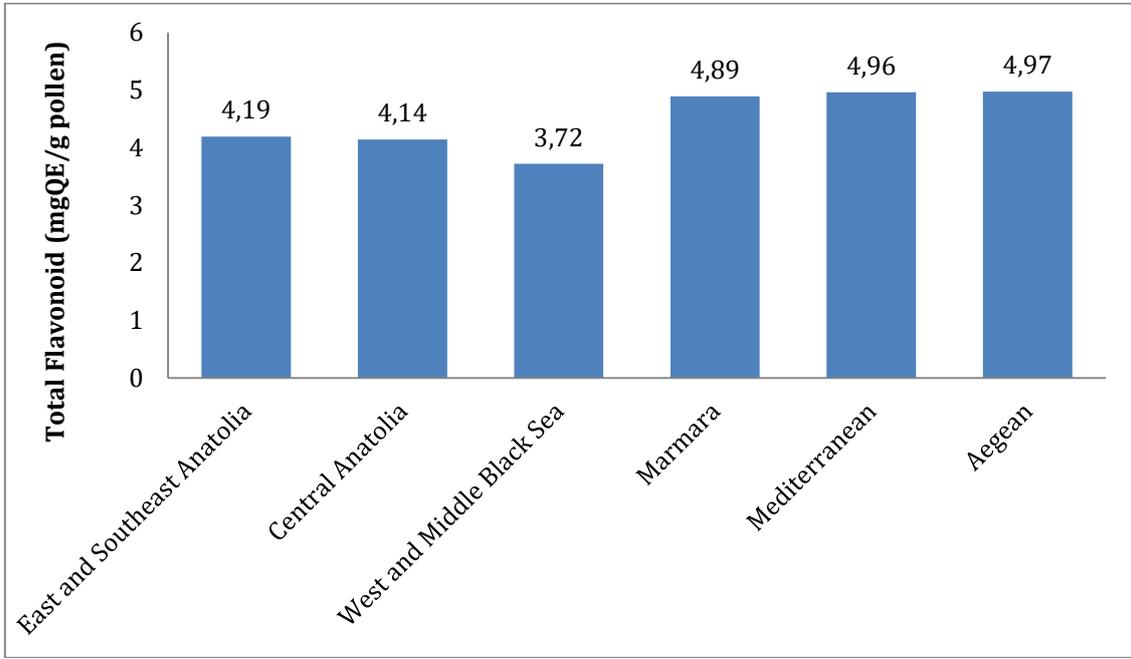


**Şekil 1.** Bölgeler arası polen ekstraktların fenolik madde miktarı  
**Figure 1.** Total phenolic amount in pollen extracts of different regions

**Total Flavonoid Matter Amount:**

Total flavonoid levels found in pollen samples of groups are presented in Table 1 and Figure 2. According to the data obtained, the lowest value between groups was found in the Eastern and

Central Black Sea region ( $3.72 \pm 0.25$  QE/g), while the highest value was found in Aegean and Mediterranean pollen ( $4.97 \pm 0.64$  ve  $4.96 \pm 0.52$ ) ( $P < 0.05$ ).

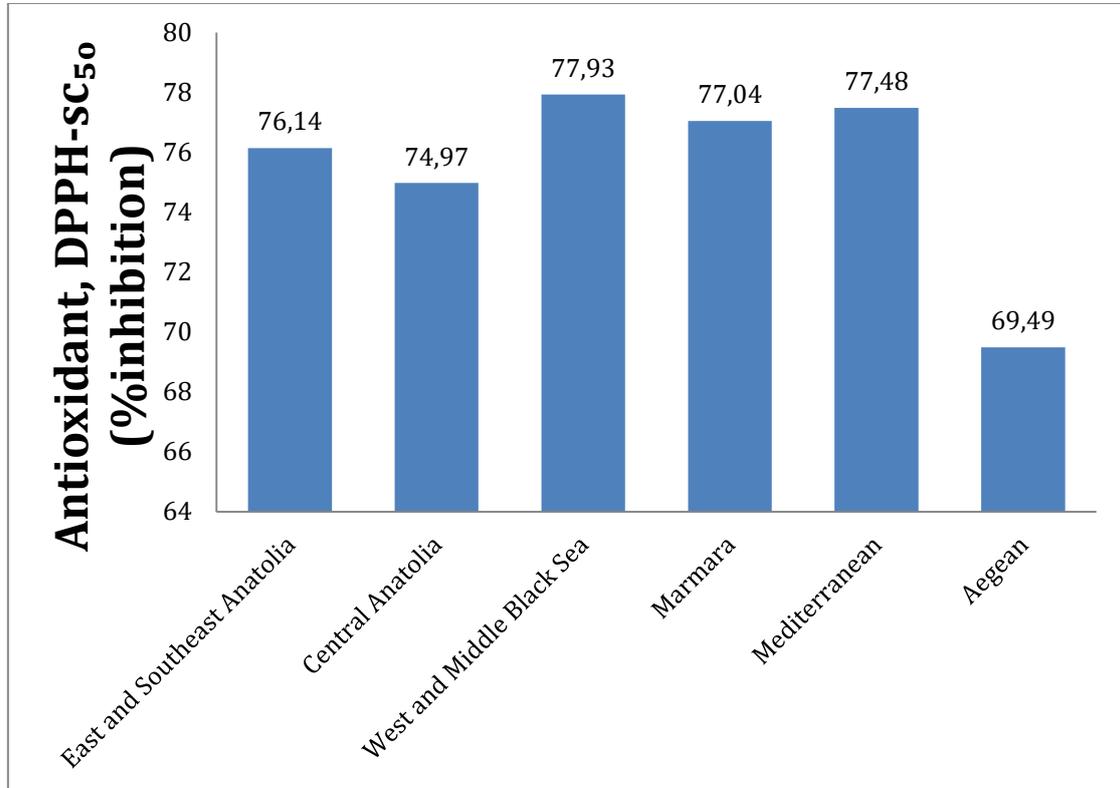


**Şekil 2.** Bölgeler arası polen ekstraktların flavonoid analiz sonuçları  
**Figure 2.** Flavonoid analysis results in pollen extracts of regions

### Total Antioxidant Activity

Average values of the total antioxidant activity are shown in Table 1 and Figure 3. The comparison of

radical scavenging activity in pollen groups showed that pollens of the Aegean region had the highest antioxidant capacity ( $69.49 \pm 5.61$ ) ( $P < 0.05$ ).



**Şekil 3.** Bölgeler arası polen ekstraktların antioksidan analiz sonuçları  
**Figure 3.** Antioxidant analysis results of pollen extracts of regions

## DISCUSSION

In our study, the average pollen the total phenolic content of groups in bee pollen samples collected from different regions of Turkey was found as  $24.60 \pm 2.52$  mg/g. These values were found to differ between minimum 7,81 and maximum 57,69 mg/g. No statistically significant difference was found between the comparison of groups. When the phenolic matter intensity obtained in the study was compared with other countries, total phenolic content of pollens collected from the southeast of Brazil was reported to differ between 41,5 and 213,2 mg/g (Freire et al. 2012). In a study conducted in the North of the United States of America by LeBlanc et al. (2009), pollen phenolic acid levels were reported as 15,91-34,85 mg/g. In a study conducted in Poland Kraków, total phenolic matter from different plants was found to differ between 1293-8243 mg/100g (Leja et al. 2007), while it was reported as  $10,49 \pm 0,3$  mg/gr on average in Greek pollen (Graikou et al. 2011). In a study conducted in Venezuela, polyphenol concentration was reported to be between 396,7 and 1286,7 GAE/100 g (Pérez-Pérez et al. 2012). In pollens obtained from plants in 5 natural parks in Portugal, phenolic compound content was found to differ between 10,5 and 16,8 mg GAE/g (Morais et al. 2011). In another study conducted in Spain, phenolic content of pollens obtained from different regions was reported to differ between 18,55mg/g and 32,15mg/g (Pascoal et al. 2014). The average total phenolic acid in Algeria was calculated as  $30,46 \pm 8,22$  mg GAE/g (Rebiai et al. 2012). In a study conducted in Romania (Marghitas et al. 2009), found total phenolic level was between  $4,4 \pm 0,1$  and  $16,4 \pm 0,3$  mg GAE/g. In another study conducted in Lithuania, this rate was reported as between 24,4-38,9 mg GAE/g (Kaskoniene et al. 2015). Pollen phenolic matter concentration in the present study is found to have a high value when compared with other literature. In the study, total flavonoid content of the regions was found as  $4.48 \pm 0.45$ . According to these data, the lowest value was found in eastern and central Black Sea region ( $3.72 \pm 0.25$  QE/g), while the lowest value was found in Aegean and Mediterranean pollen as ( $4.97 \pm 0.64$ ) and ( $4.96 \pm 0.52$ ). When these data were compared with other countries, Spanish pollen flavonoid concentration was found to differ between 3.92 and 10.14 mg/g (Pascoal et al. 2014), while this value was calculated as  $8.92 \pm 5.5$  mg/g in Algeria (Rebiai et al. 2012). In their study they conducted in Romania (Marghitas et al. 2009), found total flavonoid concentration as  $0.6 \pm 0.03$  and  $13.6 \pm 0.2$  (mg/g). In a study conducted by LeBlanc. et al.(2009) in the North of America, pollen total flavonoid was reported to differ between (2.54- 5.48 mgQE/gr). In a study conducted in Poland, total flavonoid was reported to be between

(171- 1068 mg/ 100gr) (Leja et al. 2007). In their study conducted in Lithuania, (Kaskoniene et al. 2015) reported that this value differed between 7.3 - 10.0 mg/g range. In the study, the comparison of radical scavenger activity between groups showed that Aegean region's pollen had higher antioxidant capacity when compared with other regions ( $69.49 \pm 5.61$ ). When antioxidant capacity of Turkish pollen was compared with other literature, % inhibition in Romanian pollen was reported as between  $0.135 \pm 0.01$  and  $2.81 \pm 0.03$  (Marghitas ve ark. 2009), while it was recorded as between 30.7 and 34.9 in Lithuania honey (Kaskoniene et al. 2015). In another study, it was suggested that seasons were effective on pollen chemical compound and antioxidant level could change between spring (12.8) and fall (90.4) (Freirer et al. 2012). In a study conducted in Poland, total antioxidant activity was found to differ a lot (6.8% – 86.4) (Leja et al. 2007). In the United States of America, DPPH % inhibition values were found to differ between (19.76 -90.45) in methanol extraction (LeBlanc. et al. 2009). In Mexico pollen, these values were found as  $0.39 \pm 0.05$  IC50 ( $\mu\text{g/ml}$ ) (Almaraz-Abarca et al. 2007). In a study conducted in Brazil, this value was found as  $104.5 \pm 0.5$  in yellow pollen and as  $106.1 \pm 1.3$  EC50 ( $\mu\text{g/ml} \pm \text{SD}$ ) in brown pollen (SarmantoSilva et al. 2006). In studies conducted in Turkey, (Avşar et al. 2016) reported total phenolic content as between  $.02 \pm 0.26$  mg GAE g<sup>-1</sup> and  $103.8 \pm 6.72$  mg GAE g<sup>-1</sup> and radical scavenger activity as IC50= 0.093-19.5 mg/mL. In another study, phenolic level was reported as between  $24.151 \pm 1.062$  and  $105.206 \pm 2.550$  (mg GAE/100g), while total antioxidant activity was reported as between  $1.144 \pm 0.010$  and  $3.320 \pm 0.028$   $\mu\text{g/mL}$  (Aygul ve ark. 2016). Ulusoy (2010) reported that total antioxidant activity of Anzer pollen samples was between 0.65 and 5.98 mg/ml and total phenolic matter amount was between 44,07 and 124,10 mg/g.

The factors affecting different values between studies are plants source, season, storing and extraction method (Kroyer and Hegedus., 2001; SarmantoSilva et al., 2006; LeBlanc. et al. 2009; Freire et al. 2012).

## CONCLUSION

In this study, total phenolic matter compounds and antioxidant levels of bee pollens from different regions of Turkey were found. The results obtained were compared with the biochemical data of the bee pollens produced in other countries. From the results obtained, it is possible to state that the most important reasons for the difference in bee pollen biochemical values between regions and countries were plant source, soil, season and storing and extraction method.

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