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# *Determination of Antioxidant Activities of the Chestnut and Flower Honeys Collected from Eastern Black Sea Region in Turkey*

## *Türkiye' de Doğu Karadeniz Bölgesi' nden Toplanan Kestane ve Çiçek Ballarının Antioksidan Aktivitelerinin Belirlenmesi*

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## Abstract

Honey, a natural product produced by honey bees (*Apis mellifera*), is used as a sweetener and food source for human. Composition of honey varies depending on bee collects nectar type of plants and environmental conditions. In this study, flower and chestnut honeys, collected from different cities (Trabzon, Rize, Bayburt ve Artvin) of the Eastern Black Sea Region, were investigated antioxidant activity. For determination of antioxidant capacity, total phenolic content, Copper (II) Ion Reducing Antioxidant Capacity Assay (CUPRAC), ferric-reducing antioxidant capacities (FRAP) and 2,2-diphenyl-1-picrylhydrazyl (DPPH) radical scavenging capacity tests were selected. The total phenolic content varied from 20.05 to 57.18 mg/g extract as gallic acid equivalent. The antioxidant activities found with CUPRAC ranged from 1.37 to 3.40 µM/g, DPPH˙ scavenging activity as SC50 ranged from 22.36 to 115.53 mg/mL, FRAP value as $FeSO_4$ equivalent were varied from 1.25 to 49.32 µM/g extract. Chestnut honey was found to have higher antioxidant activity than flower honey all analyzes.

**Keywords:** Honey, phenolic content, antioxidant activity, chestnut.

## Özet

Bal, bal arıları *Apis mellifera* tarafından üretilen doğal bir ürün olup, insanlar tarafından tatlandırıcı ve besin kaynağı olarak kullanılmaktadır. Balların bileşimi arının nektar topladığı bitkilerin türüne, çevresel koşullara göre değişim göstermektedir. Çalışmamızda Doğu Karadeniz Bölgesi'nin farklı illerinden (Trabzon, Rize, Bayburt ve Artvin) toplanan çiçek ve kestane ballarının antioksidan aktiviteleri incelendi. Antioksidan kapasitenin belirlenmesi için toplam fenolik madde, Bakır (II) İyonu İndirgeme Esaslı Antioksidan Kapasite (CUPRAC), Demir İndirgeme Antioksidan Kapasite (FRAP) ve 2,2-difenil-1-pikrilhidrazil (DPPH) radikal temizleme testleri seçildi. Toplam fenolik içerik gallik asit eşdeğeri olarak 20.05 ile 57.18 mg/g arasında değişmektedir. CUPRAC ile bulunan antioksidan aktiviteleri 1.37 ile 3.40 µM/g, DPPH˙ temizleme aktivitesi SC50 değeri 22.36 il3115.53 mg/mL arasında değişmekte, $FeSO_4$ eşdeğeri olarak FRAP değeri 1.25 ilae 49.32 µM/g arasında değişmektedir. Kestane balının tüm çiçek balelerine göre daha yüksek antioksidan aktiviteye sahip olduğu bulundu.

**Anahtar kelimeler:** Bal, fenolik madde, antioksidan aktivite, kestane

## 1. INTRODUCTION

Honey is a natural product that is stored by honey bees (Apis mellifera) after being collected from nectars of flowers or from tree sap and changed in stomach, and used as a sweetener and food source by people (Crane, 1983; Özdemir, Dağdemir, Özdemir & Sağdıç, 2008). Honey is an energetic nutrient, and has a biologically active molecule. It contains about 0.1-0.5% phenolic compounds are responsible for its antioxidant, antiinflammatory, antiviral, anticarcinogenic, antitumoral and other biological active properties (Mohammed & Bash, 1997; Molan, 2001).

Honey color varies from light yellow to dark amber. Due to, honey color varies depending on the amount of pollen, phenolic compounds, mineral and hydroxymethyl furfural (HMF). In general, it is reported that dark colored honey has more mineral and phenolic compound content than light colored honey, that has more acidic structures and accordingly has higher biological activity (Bertoncelj, Dobersek, Jamnik & Golob, 2007; Gonzalez-Miret, Terrab, Hernanz & Fernandez-Recamales, 2005; White Jr, 1984). Many honey like chestnut, thyme, pine, purple honeys are reported to be dark colored honeys and have high biological activity (Beratta, Garnata, Ferrero & Orioli, 2005; Can et al., 2015; Socha, Juszczak, Pietrzyk & Fortuna, 2009).

The Eastern Black Sea Region offers great opportunities in terms of the mountains that rise from the sea in a short time, deep valleys, climatic diversity, thousands of plant species, and the Beekeeping Sector with its pristine nature. The aim is to determine the total amount of phenolic substances and antioxidant activities contributing to the determination of the biological value of honey from different sources of Eastern Black Sea Region.

## 2. MATERIAL AND METHODS

### 2.1 Preparation of Samples

Flowers and chestnut honeys were collected from Trabzon, Rize, Artvin and Bayburt in Turkey (Table 1). Honey samples were stored at +4 ° C until they were analyzed. 25 g honey was weighed to prepare the honey extract. 50 mL of methanol was added. It was shaken for one day at room temperature. It was then filtered filter paper (No.1) and extract was analyzed.

Table 1. Tpye, received region and code of honeys

| Received region | Type | Code |
|---|---|---|
| Trabzon, Of | Chestnut | OYK1 |
| Trabzon, Of | Chestnut | OYK2 |
| Rize, Fındıklı | Chestnut | RFK |
| Rize, Fındıklı | Flower | RFC |
| Rize,Anzer Plateau | Flower | RAC |
| Rize, Çayeli | Chestnut | RCK |
| Bayburt, Demirözü | Flower | BDC |
| Bayburt,Maden Village | Flower | BMC |
| Artvin,Macahel Village | Chestnut | MAK1 |
| Artvin,Macahel Village | Chestnut | MAK2 |
| Artvin, Murgul | Flower | MUC |

### 2.2 Total Phenolic Content and Antioxidant Activity Assays

Total phenolic content was determined according to the Folin-Ciocalteu colorimetric method. Gallic acid was used as standard (Slinkard & Singleton, 1977). Folin assay was also based on all phenolic contents in the aquatic solution. According to Benzie and Strain (1999), ferric reducing antioxidant capacities (FRAP) of the samples were determined. FRAP reagent was prepared fresh daily. FeSO4.7H2O was used as standard. The Copper (II) Ion Reducing Antioxidant Capacity Assay (CUPRAC) is based on copper (I) -neocuproine reduction as a result of addition of copper (II) -neopurin complex into solution and result is obtained by measuring absorbance at 450 nm against reference without antioxidant. Trolox was used as a standart (Apak, Güçlü, Özyürek & Karademir, 2004).

DPPH$^{\bullet}$ radical (2,2-diphenyl-1-picrylhydrazyl) is a commercially available radical. The DPPH$^{\bullet}$ radical clearing activity was determined by modifying the method developed by Yu et al. (2002). A methanolic solution of DPPH$^{\bullet}$ radical was prepared as 4 mg/100 mL in our study. Samples and standard (Trolox) were prepared at different concentrations. 750 µL of the extracts at varying concentrations were added to 750 µL of the methanolic DPPH$^{\bullet}$ solution and the absorbance at 517 nm was measured after 50 min of incubation. $SC_{50}$ values were determined.

## 3. RESULTS AND DISCUSSION

According to the Turkish Ministry of Agriculture Food Codex Honey Communiqué (Communiqué No: 2005/49), honey is classified as flower and honeydew. However, the biochemical structure and composition of honey varies depending on the sources from where it is obtained. There is no mention of differences between honey production type (flower, honeydew) and geographical structure.

Composition of honey composition varies according to type of bee, climate, plant diversity and environmental conditions (Küçük et al., 2007). The bee collects pollen from flowers to produce honey, and the antioxidant capacity of these flowers effect the antioxidant capacity of honey. Natural herbs containing high antioxidants have gained importance recently. Honey which is a natural mixture is interesting for this reason.

In this study, chestnut and flower honey collected from Artvin, Trabzon, Rize and Bayburt were determined total phenolic content, FRAP, CUPRAC (Table 2) and DPPH values (Fig. 1). In general, it was found that chestnut honeys have higher activity than flower honeys. It was reported that chestnut honey has a higher phenolic content in studies conducted among different honey species (Aljadi & Kamaruddin, 2004; Al-Mamary, Al-Meeri & Al-Habori, 2002; Can et al., 2015; Küçük et al., 2007).

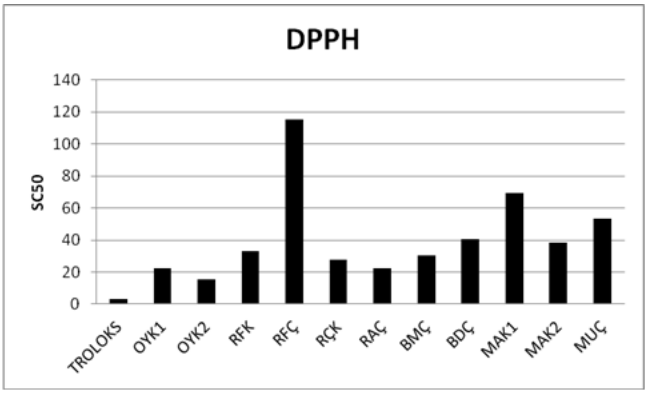


Figure 1. Result of DPPH value of honeys

In a study, it was reported that the total phenolic content of chestnut honey collected from the Eastern Black Sea Region was 430 mg GAE / 100 g. In the same study, it was found that Anzer honey was 240 mg GAE / 100 g and honey collected from Bayburt was 170 mg GAE / 100 g. It was also reported that the DPPH clearing activity of chestnut honey was high, and that of chestnut and Anzer honey were close to each other in terms of FRAP value (Kolaylı, Aliyazıcıoğlu, Ulusoy & Karaoğlu, 2008). These results are compatible with our work. Another study with different flora honeys, total phenolic content (47 ± 1.8 mg GA / 100 g) and the radical scavenging activity were found to be highest in chestnut honey (Sağdıç, Silici & Ekici, 2013). Can et al. (2015) analysis of 14 different flora as soruce of honeys; chestnut, oak and heather honeys had high phenolic and flavonoid contents. These three honeys also showed high activity for FRAP and DPPH analysis.

Ferric reducing power (FRAP) determines the total antioxidant capacity and the reduction capacity is the sum of the reduction powers of the phenolic compounds present in the honey (Küçük et al., 2007). In our study, MAK1 sample was high reduction power and the lowest reduction power was in the RFC sample. Ulusoy, Kolaylı and Sarıkaya (2010) reported that a mixture of chestnut honeys had a higher value of FRAP than flower honeys. Also Anzer honey had the highest activity among the flower honeys.

Copper (II) ion reducind antioxidant capacity (CUPRAC) is an antioxidant measurement method developed by Apak et al. (2004) which started to be used in recent years. As in other analyzes, chestnut honeys were found to have the highest activity in CUPRAC analysis. Sarıkaya, Ulusoy, Öztürk, Tuncel and Kolaylı, (2009) found that CUPRAC values of chestnut honeys were given as 6.5 and 7.7 µM TEAC / mg honey.

The $SC_{50}$ values of our samples range from 15,40-115,53 mg /mL in measurements made on the Troloks® standard precursor. Accordingly, the best DPPH radical scavenging activity was found in OYK2, the lowest scavenging activity was found on the RFÇ (Fig. 1). It is seen that the DPPH activity of chestnut honeys were higher than that of the flowers in parallel with our study (Can et al., 2015; Sarıkaya et al., 2009; Ulusoy et al., 2010).

When we compared colors of the honeys used in our work, chestnut honeys were darker than flower honey. As a matter of fact, it is reported in the literature that dark colored honeys have high activity (Bertoncelj et al., 2007; Can et al., 2015; Gonzalez-Miret et al., 2005). In general, it had found that chestnut honey was higher antioxidant activity than flower honeys.

Table 2. Total phenolic content, CUPRAC and FRAP value of honeys

| Samples | Total phenolic (mg GA /g sample) | FRAP (µmol FeSO4 /g sample) | CUPRAC (mM TEAC/g sample) |
|---|---|---|---|
| **OYK1** | 44.36 ± 2.14 | 23.86 ± 0.51 | 3.40 ± 0.01 |
| **OYK2** | 43.40 ± 11.78 | 5.75 ± 0.17 | 3.31 ± 0.05 |
| **RFK** | 44.25 ± 4.57 | 4.42 ± 0.44 | 2.19 ± 0.09 |
| **RCK** | 45.16 ± 3.57 | 3.08 ± 0.17 | 2.65 ± 0.02 |
| **RFC** | 37.79 ± 6.00 | 1.25 ± 0.00 | 1.50 ± 0.06 |
| **RAC** | 38.09 ± 2.14 | 9.64 ± 0.82 | 2.53 ± 0.09 |
| **BDC** | 36.78 ± 0.00 | 18.69 ± 0.19 | 1.88 ± 0.09 |
| **BMC** | 41.02 ± 6.00 | 19.19 ± 0.67 | 2.00 ± 0.12 |
| **MAK1** | 36.78 ± 1.43 | 49.92 ± 1.33 | 1.37 ± 0.13 |
| **MAK2** | 57.18 ± 5.43 | 33.25 ± 3.47 | 2.21 ± 0.10 |
| **MUC** | 20.05 ± 4.57 | 18.58 ± 0.29 | 1.77 ± 0.01 |

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