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RESEARCH ARTICLE

Evaluation of Antioxidant Properties and Total Phenolic and Flavonoid Contents of Honey Bee Hive Products Collected from the Ankara Region

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ABSTRACT

In this study, the total phenolic (TP) and total flavonoid (TF) profiles of multifloral honey, bee bread, bee pollen, and drone larvae (apilarnil), which are among the products of bee hives, were determined. In addition, the antioxidant activities of the aforementioned products were determined by 2,2'-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid) (ABTS) assay, ferric reducing antioxidant power (FRAP) assay, and 1,1-diphenyl-2-picrylhydrazyl (DPPH) assay methods. Honey, bee bread, bee pollen, and apilarnil samples collected from 10 honey bee hives in the Ankara region were the material of the study. As a result of the analysis, TP content levels were found to be in the order of bee pollen > bee bread > honey > apilarnil, while TF contents were in the order of bee pollen > bee bread > apilarnil > honey. Considering the results of the DPPH, FRAP, and ABTS assays, the levels of activity were determined in the order of honey > bee pollen > bee bread > apilarnil. The highest antioxidant activity level determined in honey was concluded to be the result of synergistic antioxidant effects of other bioactive complex substances contained in honey. Therefore, we believe that bioactive complex substances that increase the antioxidant activity level of honey should be evaluated in future studies.
Keywords: ABTS assay, Ankara region, DPPH assay, FRAP assay, Total phenol contents, Total flavonoid contents

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Ankara Bölgesinden Toplanan Bal Arısı Kovanı Ürünlerinin Antioksidan Özelliklerinin ve Toplam Fenolik ve Flavonoid İçeriklerinin Değerlendirilmesi

ÖZ

Bu çalışmada, arı ürünleri olan multifloral bal, arı ekmeği, arı poleni ve erkek arı larvası (apilarnil)'in toplam fenolik (TP) ve toplam flavonoid (TF) profilleri ortaya konuldu. Bunun yanı sıra söz konusu örneklerin antioksidan aktiviteleri, 2,2'-azinobis (3-ethylbenzothiazolin)-6-sulfphonate (ABTS) testi ve ferric reducing antioxidant power (FRAP) testi ve 1,1-diphenyl-2-picrylhydrazy (DPPH) testi metotları kullanılarak belirlendi. Çalışmada materyalini Ankara yöresindeki 10 bal arısı kovanından toplanan bal, arı ekmeği, arı poleni ve apilarnil örnekleri oluşturdu. Yapılan analizlerin sonucunda bal arısı ürünlerinin toplam fenolik içerik düzeyine göre sıralaması arı poleni > arı ekmeği > bal > apilarnil olarak bulunurken, bal arısı ürünlerinin toplam flavonoid içeriği düzeyine göre sıralaması ise şu şekildedir: arı poleni > arı ekmeği > apilarnil > bal. Bal arısı kovan ürünlerinin DPPH, FRAP ve ABTS analizine göre sıralaması bal > arı poleni > arı ekmeği > apilarnil olarak belirlendi. Sonuç olarak balın en yüksek antioksidan aktivite düzeyi balın içerdiği diğer biyoaktif kompleks maddelerin sinerjistik antioksidan etkileri olarak değerlendirilmiştir ve bu nedenle balın antioksidan aktivite düzeyini artıran biyoaktif kompleks maddelerin ileriki çalışmalarda değerlendirilmesi gerektiği kanaatindeyiz.
Anahtar Kelimeler: ABTS assay, Ankara yöresi, DPPH assay, FRAP assay, Toplam Fenolik ve Flavonoid içerik.

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INTRODUCTION

The breeding of honey bees has been practiced since ancient times, and it is stated in the scientific literature that honey bee products such as honey, bee pollen, bee bread, and drone larvae (apilarnil) have strong curative properties and are used for various supportive treatments due to their high contents of bioactive molecules (Crane et al. 1990). Scientific studies indicate that honey bee hive products have antioxidant, antibacterial, anti-inflammatory, antitumor, and antiviral effects (Bartkiene et al. 2020, Viuda-Martos et al. 2008). These properties of honey bee hive products are a result of the various phenolic substances and flavonoids, vitamins, and enzymes that these products contain (Dzugan et al. 2018). While complex sugars make up about 80% of honey, which is one of the food items most widely consumed by people around the world, various amino acids, minerals, lipids, sterols, phenolic and flavonoid substances, vitamins, and enzymes are also present in honey and these compounds attract the attention of researchers (Ajibola et al. 2012). Bee pollen, which is a honey bee hive product, contains approximately 4-15% water, 7.5-40% protein, 15-82% sugar, 1.3-7% lipids, and 1-3.5% various vitamins and minerals (Kostic et al. 2015). In addition, similar to honey, bee pollen also contains significant levels of organic acids, phenolic and flavonoid substances, and enzymes (Komosinska-Vassev et al. 2015). Bee bread is reported to contain approximately 21.70-23.33% protein and 57.06-58.89% carbohydrates (Mohammad et al. 2019), as well as various bioactive peptides, minerals and vitamins, organic acids, and phenolic and flavonoid substances, which have been the subject of important studies (Margaoan et al. 2019). Apilarnil, also produced in honey bee hives, contains 9-12% protein, 6-10% carbohydrates, 5-8% lipids (Barnutiu et al. 2013), various vitamins and minerals, phenolic and flavonoid substances, and sex hormones. Generally speaking, the most important compounds responsible for the biological activities of honey bee hive products are thought to be flavonoids and phenolic compounds (Velasquez et al. 2022, Giampieri et al. 2022). The strong antioxidant effects of flavonoids are a result of their free radical scavenging activities (Martinello & Mutinelli 2021, Persuric et al. 2021). For this reason, in the present study, total phenolic (TP), total flavonoid (TF), and total antioxidant levels of samples of these products are examined in the evaluation of the bioactivity levels of honey bee hive products. We believe that it is important to reveal the levels of bioactive substances contained in honey bee hive products and determine the physicochemical structural differences among these products, which vary according to the type of product, region, and year. In this study, the TP and TF profiles of bee hive products including honey, bee bread, bee pollen, and drone larvae (apilarnil) were determined. In addition, the antioxidant activities of these samples were evaluated by 2,2'-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid) (ABTS), ferric reducing antioxidant power (FRAP), and 1,1-diphenyl-2-picrylhydrazyl (DPPH) assay methods.

MATERIALS AND METHODS

Honey, bee pollen, bee bread, and apilarnil samples collected separately from each of 10 honey bee hives in one apiary in the Ayaş district of the Ankara region in June 2022 constituted the material of the study. The bees breed in the hives from which the samples were obtained were Apis mellifera anatoliaca. Samples (0.5 g each for apilarnil, pollen, honey, and bee bread) were brought to the laboratory in propylene transport bags with ice packs and placed quickly in a refrigerator at 4 ° C until analysis was performed.

Preparation of Extracts

Apilarnil samples of 0.5 g in weight were extracted with 5 mL of distilled water or 70% ethyl alcohol. Samples were homogenized with a tissue homogenizer at medium speed (15000 rpm) for 2 minutes. The extracts were then centrifuged for 20 minutes in a Sigma 3-30KS centrifuge (Sigma, Darmstadt, Germany) at 10000 rpm and 4 °C (refrigeration temperature). The collected supernatants were held at -20 °C until analysis. Samples of pollen, honey, and bee bread of 0.5 g in weight were combined with 5 mL of 80:20 analytical grade methanol/double-distilled water , respectively, and the mixtures were vortexed (Biosan Biovorteks VI, Biosan, Riga, Latvia) for 5 minutes. They were then centrifuged in a cooled centrifuge at 4 °C for 10 minutes. Afterwards, the prepared samples were stored at -20 °C until analysis was performed (Wilcynska et al. 2010).

Determination of Total Phenol Concentrations

TP concentration levels were determined according to the Folin-Ciocalteu method as modified by Beretta et al (2005). For this, after adding 900 µL of distilled water and 5 mL of Folin-Ciocalteu reagent to 100 µL of extract, 4 mL of Na2CO3 (75 g/L) was added to the mixture 4 minutes later. These mixtures were incubated for 2 hours and activity levels were determined at 750 nm with a Shimadzu UV-1201 Uv-Vis spectrophotometer (Shimadzu, Kyoto, Japan). The total amount of phenolics was calculated as mg gallic acid equivalent (GAE) in 100 g of extract (Bertoncelj et al. 2007, Diminis et al. 2010).

Determination of Total Flavonoid Concentrations

Using the method developed by Dewanto et al. (2002), mixtures were evaluated colorimetrically using aluminum chloride. For this, 1.25 mL of distilled water, 75 µL of 5% NaNO3, and 150 µL of 10% AlCl3 were added to 250 µL and this mixture was incubated for 2 hours and spectrophotometrically evaluated at 765 nm.

FRAP assay

For FRAP assays, the method modified by Benzie and Strain (1996) was used. The working principle of this method is based on the reduction of a ferrous 2,4,6-tripyridyl-s-triazine complex to its colored form in the presence of antioxidants. To prepare the FRAP reagent, 40 mM HCL, 2.5 mL of 20 mM FeCl3, and 2.5 mL of 10 mM TPTZ solution in a total volume of 25 mL were mixed. The pH of the mixture was adjusted to 3.6 with 0.3 M sodium acetate. Subsequently, 200 µL of extract was mixed with 1.5 µL of FRAP reagent and incubated for 10 minutes, and the absorbance of the reagent was measured spectrophotometrically at 593 nm.

ABTS assay

For ABTS assays, the method modified by Re et al. (1999) was used. ABTS cation radicals were synthesized by the reaction of 7 mM ABTS solution with 2.45 mM potassium persulfate solution. The mixture was kept in the dark at room temperature for 16 hours. The ABTS+ solution was then diluted with distilled water until an absorbance of 0.7 at 734 nm was reached. The extract was added to this solution immediately after preparing ABTS+ solution aliquots of 2.0 µL to obtain final concentrations between 0 and 100 µg/mL. After 10 minutes, percent inhibition was calculated for each concentration at 734 nm.

DPPH assay

DPPH assays were conducted based on the free radical scavenging effect of DPPH for extracts prepared using an indirect method. DPPH (0.1 mM) was prepared in methanol in a volume of 1 mL and 3 mL of methanolic extract was added. With reference to ascorbic acid, the measurements were modified at 520 nm by spectrophotometer and they were carried out according to the methods of Meda et al. (2005) and Diminis et al. (2010) (Wilczynska et al. 2010). As positive controls, 1.8 mL of DPPH solution and 0.2 mL of ascorbic acid/Trolox solution were used. Antioxidant activity (%) was calculated using the absorption values of the samples against the negative control at 517 nm.

RESULTS AND DISCUSSION

In this study, the values of TP and TF contents, DPPH assay measurements, ABTS assay measurements, and FRAP assay measurement were obtained for the analysis of honey, bee bread, bee pollen, and apilarnil samples. TP levels were measured as 31.1±1.75 mg quercetin equivalent (Qeurcetin (QE))/100 g, 11.37±0.87 mg QE/100 g, 29.36±5.1 mg QE/g, and 47.5±3.62 mg QE/100 g for honey, bee bread, bee pollen, and apilarnil, respectively. TF levels were measured as 2.51±0.42 mg GAE/100 g, 3.52±0.53 mg GAE/g, 5.11±0.72 mg GAE/g, and 14.35±3.2 mg GAE/100 g, respectively. DPPH assay measurements yielded values of 58.61±3.24 SC 50 mg/mL, 0.48±0.02 SC 50 mg/mL, 1.33±0.26 SC 50 mg/mL, and 4.93±0.95 SC 50 mg/mL, respectively. The ABTS assay results were 38.3±2.27 SC 50 mg/mL, 0.31±0.01 SC 50 mg/mL, 24.8±7.47 SC 50 mg/mL, and 35.89±4.06%, respectively. The values of the FRAP assay measurements were determined as 101.97±9.12 µmol Trolox/g, 39.71±1.06 µmol Trolox/100 g, 84.36±28.87 µmol Trolox/100 g, and 0.59±12.73 mmol/100 g, respectively. It is important to consider the TP and TF contents in determining the antioxidant capacity of honey bee hive products. Didaras et al. (2021) reported the TP contents of bee bread samples collected from 18 different regions of Greece as ranging between 2.34±0.22 and 5.27±0.00 mg GAE/g bee bread. Bakour et al. (2017) determined the average TP contents of bee bread samples from Morocco to be 14.88±0.98 mg GAE/g. Malkoç et al. (2019) determined the average total TF contents to be 2.79 mg QE/100 g in measurements that they performed for 11 different Anzer honey samples collected from the Black Sea region of Turkey. Rocchetti et al. (2018) reported TP values of 4.20±0.40-29.60 mg GAE/g for 32 pollen samples from the Marche region of Italy. Socha et al. (2016) found the mean TP level to be 0.47±0.04 mg GAE/g for five samples of multifloral honey collected from the southern regions of Poland. Sawicki et al. (2022) reported average TP levels of 11.77±0.15 mg QE/g for bee pollen samples and 0.07±0.00 mg QE/g for honey samples collected from the Kujawy region of Poland. The same researchers revealed that the TP levels of bee bread were 60% lower than those of other bee hive products. Rzepecka-Stojko et al. (2015) found the average TP level of three pollen samples collected from southern Poland to be 20.22 mg QE/g. Mayda et al. (2020) reported the mean TP level of five honey bee hive product samples collected from five different regions of Turkey as 2.62-4.44 mg QE/g. Saral et al. (2019) analyzed samples collected from the Tekirdağ, Ankara, Hatay, and Artvin regions of Turkey and reported mean TP levels ranging from 28±5 to 58±27 mg GAE/100 g for honey samples and 41±14 to 1258±505 mg GAE/100 g for bee pollen samples. Silici (2019) reported the average TP content of six apilarnil samples collected from the Kayseri region of Turkey as 834.05 mg GAE/100 g. Sidor et al. (2021) obtained average TP levels ranging between 144.8±16.6 and 399.3±14.6 mg GAE/100 g for three apilarnil samples obtained from southeastern Poland.

The levels of flavonoid substances in honey bee hive products have also been revealed in various studies. Didaras et al. (2021) determined that the levels of flavonoid contents of bee bread samples collected from 18 different regions of Greece ranged between 6.49±0.04 and 14.64±0.26 mg QE/g. Bakour et al. (2017) reported the TF contents in bee bread samples collected in Morocco as 1.67±0.12 mg QE /g. Malkoç et al. (2019) found that the average value of TF for 11 different Anzer honey samples collected from the

Black Sea region of Turkey was 2.79 mg QE/100 g. Saral et al. (2019) analyzed honey bee hive products collected from the Tekirdağ, Ankara, Hatay, and Artvin regions and determined average TF levels of 1±1 to 5±2 mg QE/100 g honey for samples and 253±64 to 499±99 mg QE/100 g for bee pollen samples. Sidor et al. (2021) reported TF levels of three apilarnil samples obtained from southeastern Poland ranging between 15.0±4.8 and 57.2±4.1 mg/100 g.

There are various studies in which the antioxidant properties of honey bee hive products were revealed using DPPH assay, ABTS assay, and FRAP assay methods. Didaras et al. (2021) found the IC50 value of the DPPH assay to range between 0.18±0.02 and 1.25±0.04 for bee bread samples collected from 18 different regions of Greece. Bakour et al. (2017) determined the mean IC50 value of the DPPH assay to be 0.05±0.01 mg/mL for bee bread collected in Morocco. Malkoç et al. (2019) reported an average DPPH level of 49.12 mg/mL for measurements performed for 11 different Anzer honey samples in the Black Sea region of Turkey. Rocchetti et al. (2018) determined the DPPH levels of 32 pollen samples collected from the Marche region of Italy to range between 11.9±6.4 and 108.7±6.1. Saral et al. (2019) analyzed honey bee hive products collected from the Tekirdağ, Ankara, Hatay, and Artvin regions and they reported DPPH levels of 30.90±1.3 to 155.70±76.68 SC 50 mg/mL for honey samples and 0.47±0.5 to 0.47±0.51 SC 50 mg/mL for bee pollen samples. Sidor et al. (2021) found the DPPH activity of three apilarnil samples obtained from southeastern Poland to range from 6.3±1.3% to 20.5±0.1%. Previous studies have confirmed that the ABTS assay reflects the scavenging capacity of antioxidants by means of a free radical (i.e., ABTS). Didaras et al. (2021) determined the average IC50 values of the ABTS assay to be 0.38-1.80 for bee bread samples collected from 18 different regions of Greece. Bakour et al. (2017) reported the average ABTS IC50 level to be 0.08±0.05 mg/mL for bee bread samples collected in Morocco. Rocchetti et al. (2018) reported ABTS levels of bee pollen samples collected from the Marche region of Italy within the range of 48.8±14.1 to 224.6±18.6 μmol TE/g DW. Sidor et al. (2021) found that ABTS activities of apilarnil samples collected from southeastern Poland ranged between 32.1±0.5% and 71.3±0.4%. The FRAP assay analysis method is based on the evaluation of a change in the color of a solution as a result of the conversion of ferric (Fe3+) ions in that solution into ferrous (Fe2+) ions by the antioxidants in the environment, and this method has been used in many previous studies. Zuluaga et al. (2015) reported an average value of 46.1±13.0 FRAP μmol Trolox/g for bee bread. Sahin and Kemal (2019) reported average FRAP assay results of 72.38±0.21 μmol FeSO4.7H2O/g for samples of bee pollen. Malkoç et al. (2019) determined an average FRAP level of 110.11 μmol Trolox/100 g for 11 different Anzer honey samples collected from the Black Sea region. Saral et al. (2019) analyzed honey bee hive products collected from the Tekirdağ, Ankara, Hatay, and Artvin regions and found the FRAP assay levels of honey samples to range from 1.37±0.17 to 1.37±0.17 μmol FeSO4.7H2O/g while the values obtained for bee pollen ranged from 8.69±1.64 to 84.89±10.09 μmol FeSO4.7H2O/g. Sidor et al. (2021) found the average FRAP levels of three apilarnil samples obtained from southeastern Poland to be between 0.4±0.1 and 1±0 mmol/100 g. The data obtained from the present study are generally similar to the results of previous studies on this subject. However, in addition to differences in analysis methods used, such as the DPPH assay, ABTS assay, and FRAP assay, variables such as extraction method, botanical species, and geographical conditions may have roles in the levels of phenolic and flavonoid compounds determined in honey bee hive products and may explain the differences detected in the antioxidant properties of these products.

In this study, it was determined that the samples could be ranked in the order of bee pollen > bee bread > honey > apilarnil according to the levels of TP contents in these samples. In the evaluation of the TF levels of the collected samples, an order of bee pollen > bee bread > apilarnil > honey was obtained. In evaluations of the data obtained from DPPH, FRAP, and ABTS assays, the collected samples were ranked as honey > bee pollen > bee bread > apilarnil, respectively. It was determined that the antioxidant capacity of honey was higher than that of the other considered products, which could be attributed to the synergistic effects of bioactive substances contained in honey.

CONCLUSION

This study was undertaken to reveal the TP and TF profiles of honey, bee bread, bee pollen, and apilarnil collected from honey bee hives in the Ankara region of Turkey and to determine the antioxidant activities of these products using ABST, FRAP, and DPPH assays. It is thought that the present findings will serve as a guide for future studies. In addition, this study has provided an up-to-date assessment of the bioactivity levels of honey bee hive products in the Ankara region. We believe that our finding of higher antioxidant capacity for honey samples compared to bee bread, bee pollen, and apilarnil samples can be attributed to the synergistic effects of other bioactive complex substances contained in honey, and this result will be evaluated in further studies using honey.

Çıkar çatışması: Yazarlar bu yazı için gerçek, potansiyel veya algılanan çıkar çatışması olmadığını beyan etmişlerdir.

Yazarların Katkı Oranı: 1EK%50, 1SS%50

Yazarlar makaleye eşit oranda katkı sağlamış olduklarını beyan etmişlerdir.

Çalışmamız etik kurul onayı gerektirmemektedir.

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