PHENOLIC COMPOSITION AND ANTIOXIDANT PROPERTIES OF ANZER HONEY FROM BLACK SEA REGION OF TURKEY

Türkiye'nin Karadeniz Bölgesinden Anzer Balının Fenolik Bileşimi ve Antioksidan Özellikleri

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ABSTRACT

Anzer honey is produced on the Anzer plateau, known for its rich flora, in the Eastern Black Sea region of Turkey. It is well known across the world, and is believed to be of high medical value.

This study is the first detailed research in which the polyphenolic profile responsible for the bioactive properties of Anzer honey was determined, melissopalynological analysis was performed, and in which total phenolic contents (TPC), total flavonoid contents (TFC), and total antioxidant activities were identified. The ferric (III) reducing antioxidant power (FRAP) assay and 1,1-diphenyl-2-picrylhydrazil (DPPH) free radical scavenging test were used to determine antioxidant activity. The total mean phenolic content of Anzer honey was 26.92 GAEE/100g, and the total flavonoid content was 2.79 mgQE/100g. Anzer honeys' mean FRAP and DPPH values were 110.11 μmoLTrolox/100g and 49.12 mg/mL, respectively. The phenolic acids and flavonoids of Anzer honey were determined using high-performance liquid chromatography (HPLC). Of the 19 standard compounds used in the analysis, pinocembrin, hesperidin, chrysin, protocatechuic acid, p-coumaric acid, catechin, caffeic acid phenyl ester (CAPE), p-OH benzoic acid, and caffeic acid as major compounds, while myricetin, luteolin, rutin, resveratrol, epicatechin, f-cinnamic acid, ferulic acid, and gallic acid were identified as minor compounds. Daidzein and syringic acid were not detected. Based on these findings, Anzer is a flower honey noteworthy for the rich variety of its polyphenols.

Key words: Anzer honey, antioxidant, phenolics, flavonoids

ÖZ

Anzer balı, Türkiye'nin Doğu Karadeniz bölgesinde zengin bitki örtüsü ile ünlü Anzer platosunda üretilmektedir. Anzer balı dünyaca tanınan bir bal olup, tıbbi değerinin yüksek olduğuına inanılmaktadır. Bu çalışma Anzer balının biyoaktif özelliklerinden sorumlu polifenolik profilinin belirlendiği melissopalinojik analiz, toplam fenolik madde, toplam flavonoid madde ve toplam antioksidan aktivitelerinin tespit edildiği ilk detaylı araştırmadır. Antioksidan aktive demir (III) indirgeyici antioksidan güç testi (FRAP) ve 1,1-difenil-2-pikrilhidrazil (DPPH) serbest radikali temizleme testi ile ölçüldü. Anzer balının toplam fenolik içeriğinin ortalama 26.92 GAE/100g ve toplam flavonoid madde miktarının 2.79 mg QE/100g olduğu tespit edildi. Anzer ballarının ortalama FRAP ve DPPH değerleri

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sirasıyla 110,11 μmolTrolox/100g ve 49,12 mg/mL olarak bulundu. Anzer balının fenolik asitleri ve flavonoidleri yüksek performanslı sıvı kromatografisi (HPLC) ile belirlendi. Analizde kullanılan 19 adet standart bileşikten daidzein ve şiringik asit hariç, pinosembrin, hesperidin, krisin, protokatekuık asit, p-kumarik asit, kateşin, kafeik asit fenil ester (CAPE), p-OH benzoik asit, kafeik asit major bileşen, mirisetin, luteolin, rutin, resveratrol, epikaçeşin, t-sinantam asit, ferulik asit ve gallik asit ise minor bileşen olarak tespit edildi. Bu sonuçlara göre, Anzer balı içeriğinde polifenolik maddelerin çeşitliliği bakımından dikkat çeken özellikli sahip bir çiçek balıdır.

Anahtar kelime: Anzer balı, Antioksidan, Fenolikler, Flavonoidler

GENİŞLETİLMİŞ ÖZET

Amaç: Anzer Doğu-Karadeniz Bölgesinde yer alan Rize-Ikizdere ilçesine bağlı 2300 rakıma sahip bir yayla olup, çok zengin çiçek florası ile bilinmektedir. Bölgenin her 15 gün de bir değişen bitki florasından dolayı çok sayıda endemik ve endemik olmayan bitki türlerine ev sahipliği yapmaktadır. Bu coğrafya da üretilen Anzer balı Türkiye'de ve dünyada tanınan bir bal olup, tıbbi değerinin yüksek olduğuna inanılmaktadır. Anzer balı halk arasında farenj, bademcik iltihabı, mide ülseri ve yara tedavisi gibi rahatsızlıklarda geleneksel olarak kullanılmaktadır. Sınırlı miktarda üretilen bu balın biyolojik değerinin yüksek olduğu inanılmaktadır. Balın biyolojik değeri yapısında yer alan sekonder metabolitlerden ileri gelmektedir. Polifenoller balda bulunmaktadır en önemi sekonder metabolitler olup bu çalışmada Anzer balının polifenollerini araştırıldı. Elde edilen veriler literatürdeki çiçek balları ile karşılaştırılarak, Anzer balının farklı özeliliklerinin ortaya çıkarması amaçlandı.

Gereç ve Yöntem: Ekim 2018'de, Rize Tarım Kredi Kooperatifi (Türkiye) yardımıyla S.S. Çiçekli Köyü (Anzer) Anzer yaylasındaki arıcılardan 11 farklı Anzer balı örneği temin edildi. Bu etiketli balların melissopalinolojik analizi, toplam fenolik madde, toplam flavonoid madde miktarları, polifenolik kompozisyonları, antioksidan özellikleri belirlendi. Antioksidan aktivite, demir(III) indirgeyici antioksidan güç testi (FRAP) ve 1,1-difenil-2-pikrilhidrazil (DPPH) serbest radikali temizleme testi ile ölçüldü. Anzer ballarının yüksek performanslı sıvı kromatografisi (HPLC-UV) ile yapılan fenolik kompozisyon analizinde 19 adet fenolik standart bileşik (daidzein ve şiringik asit, pinosembrin, hesperidin, krisin, protokatekuık asit, p-kumarik asit, kateşin, kafeik asit fenil ester (CAPE), p-OH benzoik asit, kafeik asit major bileşen, mirisetin, luteolin, rutin, resveratrol, epikaçeşin, t-sinantam asit, ferulik asit ve gallik asit) kullanıldı.

Bulgular: Mikroskopik olarak yapılan melissopalinolojik analiz sonucu Anzer balında hiç bir polenin %45'in üzerinde olmadığını ve Lamiacea, Fabacea, Apiacea, Roccacea, Asteracea, Ericacea, Lillacea polenlerinin major seviyede Thymus, Rumex, Onobrychis, Cistus, Plantago, Ranunculus, Rhododendron, Myosotis, Geranium polenlerinin ise minor seviyede olduğu tespit edildi. Anzer balının toplam fenolik madde miktarlarının 19,50 ile 38,30 mgGAE/100g ve toplam flavonoid madde miktarları 2,03 ile 3,66 mgQE/100g arasında değişmektedir. Umunelere anzer etrafındaki bitkilerin biosin absurdan 92.53 ile 124,33 mgTrolox/100g arasında ve serbest radikal temizleme aktivitesi (DPPH) 38,04 ile 64,12 mg/mL arasında olduğu tespit edildi. Balların fenolik bileşimlerinin analizi sonucunda anzer balının dairde ve şiringik asit hariç sırasıyla pinosembrin, hesperidin, krisin, protokatekuık asit, p-kumarik asit, kateşin, kafeik asit fenil ester (CAPE), p-OH benzoik asit, kafeik asit, mirisetin, luteolin, rutin, resveratrol, epikaçeşin, t-sinantam, ferulik asit ve gallik asit kullanıldı.

Sonuç: Anzer balı içeriğinde polifenolik maddelerin çeşitliliği bakımından dikkat çekici özellikli sahip bir çiçek balıdır. Balların fenolik bileşimlerinin analizi sonucunda anzer balının dairde ve şiringik asit hariç sırasıyla pinosembrin, hesperidin, krisin, protokatekuık asit, p-kumarik asit, kateşin, kafeik asit fenil ester (CAPE), p-OH benzoik asit, kafeik asit major düzeyde, mirisetin, luteolin, rutin, resveratrol, epikaçeşin, t-sinantam, ferulik asit ve gallik asit minor düzeyde tespit edildi. Balın fenolik bileşimleri bunun esas olmayan bitki florasından ileri geldiği düşünülmektedir. Baladı major seviyede bulunan pinosembrin, hesperidin ve krisin gibi flavonoid yapıtı doğal bileşiklerin geniş spektrumu biyolojik aktiviteleri bu balın tıbbi değerini artırmaktadır.
INTRODUCTION

Anzer honey is particularly well known for its curative properties. It is also unique since it is produced from the nectar of approximately 500 flowers from the Anzer Plateau near İkizdere, Rize in the Eastern Black Sea region of Turkey (Tezcan et al. 2011). Depending on climatic conditions, *Apis mellifera* bees collect the honey from July to August in general. Although Anzer honey predominantly contains *Thymus* spp, various secondary and trace pollen grains from other plants have also been identified. The mixed pollen grains largely consist of *Thymus* spp, *Campanula* spp, *Trifolium* spp, *Geranium* spp, *Lotus* spp, *Salvia officinalis*, *Heracleum* spp, *Myosotis* spp, and *Lamium* spp (Sorkun et al. 1989). This heterofloral honey is the best known and most expensive honey in Turkey and is produced in very limited quantities. Another reason for the high value of Anzer honey is that the bees in the region are Caucasian bees (*Apis mellifera caucasica*). Local inhabitants believe that Anzer honey can be used in the treatment of pharyngitis, tonsillitis, ulcers, wounds and abrasions (Şekeroğlu et al. 2007). These are more docile, cold-resistant and industrious than other bees and can collect nectar from deep tube flowers using their long proboscis (Kara et al. 2012). The medicinal property of honey results from the presence of various secondary molecules, mostly phenolic compounds, such as phenolic acids and flavonoids. Polyphenols are responsible for biological properties such as antioxidant, antimicrobial, antiviral, antifungal and anticarcinogenic and anti-diabetic activity (Cianciosi et al. 2018). In addition to sugars, all honeys contain numerous organic acids, proteins, SH-containing amino acids, α-tocopherol, ascorbic acid, phenolic acids, flavonoids, anthocyanin, and honey enzymes (Deng et al. 2018; Vasić et al. 2019). Composition of honey may vary depending on the flora, the geographical region, and the time of harvest. Relatively few studies have investigated the antioxidant properties of Anzer honey. Anzer honey has been reported to protect the rat stomach against ethanol-induced increased vascular permeability, which may be associated with its ascorbic acid content (Doğan and Kolanakaya 2005). Another study reported that Anzer honey possesses a high antioxidant capacity with an efficient sulfhydryl source, and exhibits a marked protective effect against hepatic injury in rats (Korkmaz and Kolanakaya 2009). In another study of Anzer honey, sugar and organic acid composition were reported, but phenolic compositions were not mentioned (Tezcan et al. 2011). Therefore, since there is not enough information about the content of Anzer honey in the literature, especially secondary metabolites, polyphenolic composition and antioxidant properties of the honey was evaluated.

MATERIALS AND METHODS

Chemicals
The chemical standards employed were all HPLC-grade pure. The common phenolic compounds were supplied by Sigma–Aldrich (Munich, Germany), 2,4,6-Tripyridyl-s-triazine (TPTZ), Folin–Ciocalteu’s phenol reagent, 1,1-diphenyl-2-picrylhydrazyl (DPPH), and Trolox (6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid) were supplied by Sigma Chemical Co. (St Louis, MO, USA). Acetonitrile, methanol and ethanol were purchased from Sigma–Aldrich Co. (St. Louis, MO, USA). UV–VIS Spectrophotometer (Thermo Scientific Evolution TM 201; USA) was used in all absorbance measurements.

Honey Samples
Eleven Anzer honey samples were obtained from the beekeepers on the Anzer Plateau in October, 2018. Melissopalynological characterization was performed using the standardized technique developed by Louveaux et al. (1978). In this method, pollen was classified according to percentages; dominant pollen constituting 45% or more of the total pollen grains, secondary pollen (16–44%), important minor pollen (3–15%), or minor pollen (less than 3%). Briefly, approximately 5 g of honey sample was dissolved (50 mL) by the addition of 99% methanol. The mixture was continuously stirred with a shaker (Heidolph Promax 2020, Schwabach, Germany) at room temperature for 24 hours, and then sonicated for 4 hours with an ultrasonicator. The mixture passed through filter paper and concentrated in a rotary evaporator (IKA-Werke, Staufen, Germany) at 40°C. The residue was redissolved in methanol and kept at 4°C until used for phenolic compound analysis (Çakir et al. 2018).

Total Phenolic Content Measurement
TPC were measured based on Folin–Ciocalteu’s method (Singleton and Rossi. 1965) using gallic acid (GA) as standard. Firstly, 100 µL of various concentrations of gallic acid and sample solutions were diluted with 500 µL 0.2 N Folin–Ciocalteu

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The antioxidant properties of the different honeys were assessed using the method described by Benzie and Strain (1996). The reducing power of ferric tripyridyltriazine (Fe-III-TPTZ) complex (FRAP) method relies on the calculation of samples’ iron-reducing capacities. The FRAP reagent consisted of a mixture of 25 mL of 300 mM acetate buffer, with 2.5 mL of 10 mM TPTZ solution in 40 mM HCl and 2.5 mL of 20 mM FeCl₃·6H₂O solution. Next, 300 µL freshly prepared FRAP reagent was mixed with 100 µL of honey sample prior to incubation for 4 min at 37°C. The last absorbance was read at 595 nm against reagent blank with distilled water. Trolox was employed as standard, and the radical scavenging activity of DPPH was expressed as SC₅₀, defined as the concentration (mg/mL) needed to inhibit 50% of the DPPH radical scavenging activity. SC₅₀ values are calculated using linear regression analysis, lower values indicating greater antioxidant activity.

**Total Flavonoid Measurement**

TFC were calculated by means of a spectrophotometric method using quercetin as standard (Fukumoto and Mazza 2000). Briefly, 500 µL mL of quercetin (1, 0.5, 0.25, 0.125, 0.0625, and 0.03125 mg/mL) and 0.5 mL samples, 100µL of 10% Al (NO₃)₃ and 100µL of 1 M NH₄CH₃COO were added to a test tube. This mixture was incubated at room temperature for 40 min and the absorbance was measured against a blank at 415 nm. The TFC was calculated as mg of quercetin equivalents per 100 g honey sample.

**Ferric Reducing/Antioxidant Power Assay**

The antioxidant properties of the different honeys were assessed using the method described by Benzie and Strain (1996). The reducing power of ferric tripyridyltriazine (Fe-III-TPTZ) complex (FRAP) method relies on the calculation of samples’ iron-reducing capacities. The FRAP reagent consisted of a mixture of 25 mL of 300 mM acetate buffer, with 2.5 mL of 10 mM TPTZ solution in 40 mM HCl and 2.5 mL of 20 mM FeCl₃·6H₂O solution. Next, 300 µL freshly prepared FRAP reagent was mixed with 100 µL of honey sample prior to incubation for 4 min at 37°C. The last absorbance was read at 595 nm against reagent blank with distilled water. Trolox was employed as standard, and the radical scavenging activity of DPPH was expressed as SC₅₀, defined as the concentration (mg/mL) needed to inhibit 50% of the free radical scavenging activity. SC₅₀ values are calculated using linear regression analysis, lower values indicating greater antioxidant activity.

**DPPH Radical-Scavenging Activity**

The radical scavenging activity of the methanolic honey samples was determined using the 1,1-diphenyl-2-picrylhydrazil (DPPH) radical assay. This technique relies on measuring the change from purple to yellow that takes place in the DPPH solution as the radical is neutralized by the antioxidants (Molyneux 2004). Briefly, varying concentrations of 0.75 mL of honey extracts were combined with 0.75 mL of 0.1 mM of DPPH in methanol. The resulting mixture was stored in a lightless environment for 30 min, after which the absorbance at 517 nm was measured using a spectrophotometer. Trolox was used as standard, and the radical scavenging activity of DPPH was expressed as SC₅₀, defined as the concentration (mg/mL) needed to inhibit 50% of the free radical scavenging activity. SC₅₀ values are calculated using linear regression analysis, lower values indicating greater antioxidant activity.

**RP-HPLC Analysis and Sample Preparation**

Firstly, the methanolic residue was dissolved in 15 mL acidified distilled water (pH 2). Liquid-liquid extraction was carried out with 5×3 mL diethyl ether and 5×3 mL ethyl acetate, consecutively. Both diethyl ether and ethyl acetate phases were incorporated and dried by rotary evaporation (IKA-Werke, Staufen, Germany) at 40°C. The pellet was resuspended in 2 mL methanol, filtered with syringe filters (Rc membrane, 0.45 µm), and injected to HPLC (Elite LaChrom Hitachi, Japan). Each specimen was injected into the HPLC system with a reverse phase C18 column (150 mm 4,6 mm, 5 mm; Fortis) at 280 and 340 nm. The mobile phase consisted of (A) 2% acetic acid in water and (B) acetonitrile: water (70:30). The sample injection volume was 20 µL. The column temperature was set at 30°C, and the flow rate at 1,5 mL/min. The programmed solvent began with a linear gradient held at 95% A for 3 min, decreasing to 80% A at 10 min, 60% A at 20 min, 20% A at 30 min and finally 95% A at 50 min. A standard chromatogram for the 19 phenolic standards (gallic acid, protocatechuic acid, p-OH benzoic acid, catechin, caffeic acid, syringic acid, epicatechin, p-coumaric acid, ferulic acid, rutin, myricetin, resveratrol, daidzein, lutein, t- cinnamic acid, hesperidin, chrysin, pinocembrin, and CAPE) applied at HPLC-UV is shown in Figure 1 (Çakir et al. 2018).
Figure 1. HPLC-UV chromatograms of phenolic standards

**Results**

Melissopalynological findings of the study is showed that the honeys contained pollens from different family types at levels less than 45%, including Lamiaceae, Fabaceae, Apiaceae, Roraceae, Asteracea, Ericaceae, And Liliaceae. The major pollens detected in the honey samples were *Thymus*, *Rumex*, *Onobrychis*, *Cistus*, *Plantago*, *Ranunculus*, *Rhododendron*, *Myosotis*, and *Geranium*.

Total phenolic contents, total flavonoids and antioxidant results are given in Table I. TPC ranged from 19.50 to 38.30 mg GAE/100g honey, and the mean value was determined as 26.92 mg GAE/100g. Flavonoids, an important subclass of polyphenols, were also measured as totally in the honey samples, the results were ranged from 2.03 to 3.66 mg QE/100g and a mean value of 2.79 mg QE/100g. FRAP ranged from 92.53 to 124.33 μmol Trolox/100 g and the mean value was determined as 110.11 μmol Trolox/100g. DPPH ranged from 35.30 to 64.12 mg/mL, and a mean value of 49.12 mg/mL (Table 1).

Nineteen phenolic standards were used to measure the phenolic profiles of the honey samples with UV-HPLC, and all standards except for syringic acid and daidzein were detected at different concentrations. High levels of pinocembrin, hesperidin and chrysin were detected, together with moderate levels of protocatechuic acid, p-coumaric acid, catechin, caffeic acid phenyl ester (CAPE), p-OH benzoic acid and caffeic acid, and lower levels of epicatechin, rutin, t-cinnamic acid, gallic acid, myricetin, luteolin, ferulic acid, and resveratrol. The results are given in Table 2.
**DISCUSSION**

Honey is an important food source and an important source of bioactive components. Honey compounds may vary depending on the geographical features of the region of production, the plant flora, the harvest time, and the mode of production (Džugan et al., 2018). The bioactive properties of honey largely derives from the secondary metabolites it contains, and these are known to be maximally affected by the plant flora (Juszczak et al., 2016, Kaygusuz et al., 2016). Since Anzer honey is produced in a region with a rich plant flora, it is regarded as healthy, with a pleasant taste and aroma. The bioactive

| Standards (µg/100g) | A1 | A2 | A3 | A4 | A5 | A6 | A7 | A8 | A9 | A10 | A11 | X±SD |
|---------------------|----|----|----|----|----|----|----|----|----|-----|-----|------|
| Gallic Acid         | 89 | 26 | -  | 27 | -  | 28 | 42 | -  | 24 | 42  | 15  | 20±16|
| Protocatequic Acid  | 560| 33 | 804| 626| 1601|763|670|2155|478|506|193|853±583|
| p-OH Benzoic Acid  | 96 | 125|131|130|90 | 110|123|151 |96 | 109|125|119±17|
| Catechin            | 52 | 396|203|407|475 |338|189|1200|300|208|825|450±320|
| Caffeic Acid        | 25 | 185|128|100|087 |153|136|92 |85 | 088|66 |110±37|
| Syringic Acid       | -  | -  | -  | -  | -  | -  | -  | -  | -  | -   | -   | -    |
| Epicatechin         | 52 | 276|018|079|008 | -  |17  |28  |20 |1  |20 |47±83|
| p-Coumaric Acid     | 63 | 1405|193|1230|177 |1024|563 | -  |693 |174|214|562±501|
| Ferulic Acid        | 10 | 31 |29 |38 |028 | -  | -  |31  |22 |26 |22 |26±4|
| Rutin               | -  | 120|24 |154|064 |116 |41  | -  |129 |5  | -  |65±56|
| Myricetin           | 61 | 34 |60 |88 |104 |112 |39  |130 |55 |204|88 |96±22|
| Resveratrol         | 51 | 32 |19 |44 |67  |80  |34  |90  |28 |69 |32 |49±23|
| Daidzein            | -  | -  | -  | -  | -  | -  | -  | -  | -  | -   | -   | -    |
| Luteolin            | 15 | 94 |84 |100|79  |87  |77  |87  |66 |61 |63 |80±13|
| 3-Cinnamic Acid     | 4  | 65 |16 |54 |20  |42  |40  |33  |23 |13 |32 |34±16|
| Hesperidin          | 9236|2810|4420|3910|1210|1804|6408|4310|4500|3810|1810|4020±2293|
| Chrysins            | 5726|6810|2810|1820|4730|6410|2301|1720|5320|4230|1820|3972±1949|
| Pinocembrin         | 5205|4720|6200|3601|3814|5520|6420|3000|4601|6000|4133|4837±1132|
| CAPE                | 89 | 230|466|320|55  |430 |230 |90  |70 |120|204|209±144|

(-): not detected
components of this honey produced in very limited quantities and sold at very high prices are unknown. The purpose of this study was to perform a biological characterization of Anzer honey’s secondary metabolites in particular polyphenolic compositions and associated antioxidant characteristics, and thus both to fill a gap in the scientific literature and also to contribute to bee-keeping in Turkey. Palynological studies revealed that Anzer honey is a blossom honey of the class of hetero floral honeys. The pollen analyses were consistent with the data in the literature (Sorkun et al. 1989).

The purity and naturalness of honey is determined by such parameters as moisture, conductivity, optic rotation, acidity, pH, color value, sugar ratios, HMF, and proline, according to the honey codices (Juszczak et al. 2016, El Sohaimy. 2015). However, the real value of honey is associated with the variety and quantities of the secondary metabolites it contains, and other parameters not used in this study. The variety and quantity of polyphenolic compounds passing through flowers is an important parameter in defining the geographical sign of honey. The polyphenols contained in honey depend on the flora in the region, and these are the essential compounds responsible for the honey’s color and taste, sensory characteristics, and biological activity values. Bioactive compounds with phenolic structures, like phenolic acids, flavonoids, and anthocyanin are natural compounds that reduce the risk of oxidative damage in living cells, are capable of scavenging free radicals, reducing inflammation, and activating the immune system (Cianciosi et al. 2018, Manach et al. 2004). The mean total polyphenol content of Anzer honey in the present study was 26.92 mg GAE/100g, a value consistent with flowers honeys in the literature. The mean total phenolic content of Jerusalem thorn honeys (Karaçalı) collected from the Marmara region of Turkey in our previous study was 53 mg GAE/100g (Malkoç et al. 2019). Another study of monofloral honeys in Turkey reported total polyphenolic contents of 25 mg GAE/100g in clover honey, 16 mg GA/100g in acacia honey, 41 mg GAE/100 g in linden honey, 23 mg GAE/100 g in rhododendron honey, and 98 mg GAE/100g in chestnut honey (Can et al. 2015). The mean total flavonoid content of Anzer honey in this study was 2.79 mgQE/100g, a value higher than those reported for blackthorn, acacia, rhododendron, clover and milk vetch honeys (Can et al. 2015).

The antioxidant capacities of the honeys in this study were measured using the FRAP method. The honeys reduced iron (III) at varying concentrations, and these values ranged between 92 and 124 (µmolTrolox/100g) according to the Trolox standard. Honeys’ antioxidant values were tested using DPPH, a commercial radical test. The mean DPPH scavenging activity was determined as mean 49.12 mg/mL. These results show that Anzer honey has greater free radical-scavenging and antioxidant activities than those of numerous honeys cited in the literature (Anand et al. 2018). The antioxidant value of the honey derives from the small quantity of ascorbic acid in its structure and more from the presence of polyphenolic substances. For example, chestnut and oak honeys, regarded as possessing large amounts of polyphenolic substances, have been reported to exhibit DPPH radical neutralizing capacities of 20 mg/mL and 12 mg/mL, respectively (Can et al. 2015). To summarize, these honeys’ reduction capacities vary within a narrow range, the values determined are compatible with the data in the literature, and the honey has a higher antioxidant capacity than many floral honeys.

Polyphenolic composition with HPLC-UV revealed that of the Anzer honeys studied contained varying quantities of all phenolic compounds, except for the standard syringic acid and daidzein. Based on mean values, the phenolic substances found at the highest levels were pinocembrin (4837±1132 µg/100g), hesperidin (4020±2293 µg/100g), and chrysin (3972±1949 µg/100g). In this study, phenolic compounds of protocatechuic acid, p-coumaric acid, catechin, CAPE, p-OH benzoic acid and caffeic acid were detected at moderate levels, myricetin, luteolin, rutin, resveratrol, epicatechin, t-cinnamic acid, ferulic acid and gallic acid were detected in minor levels.

These phenolic acids and flavonoids detected in Anzer honeys are important antioxidant and anti-inflammatory molecules, and are responsible for the honey’s apitherapeutic properties. Hesperidin, pinocembrin, chrysin, coumaric acid, caffeic acid and CAPE are the effective agents of propolis, and their significantly elevated levels in Anzer honey were striking (Huang et al. 2014). Studies have determined that these phenolic compounds possess medical properties. For example, pinocembrin, detected at the highest concentration in Anzer honey, is an important flavonoid with proven pharmacological activity in neurodegenerative and cardiovascular diseases (Nyokat et al. 2017). Hesperedin is a flavonoid largely found in citrus fruits.
possessing good anti-inflammatory and antioxidant properties (Parhiz et al. 2015, Iranshahi et al. 2015), while chrysin has been reported to be an important anti-tumoral agent (Kasala et al. 2015). The phenolic acids such as protocatechuic acid and caffeic acid are powerful antidiabetic agents (Spiliotiet et al. 2014), p-coumaric acid and caffeic acid reduce oxidative damage caused by hypoxia with their neuroprotective effects (Cruz et al. 2016), and catechin and p-OH benzoic acid exhibit antioxidant properties by scavenging superoxide radicals (Afroz et al. 2016, Velika and Kron 2012). In addition, the preponderance of the compounds in the honey may use as a promising marker in determining the botanical origins of the honey. For example, quercetin is the main flavonoid compound in sunflower honey, 8-(methoxy) kaempferol in rosemary honey, hesperidin in citrus honey, and naringenin and luteolin in lavender honey (Kaškonienė and Venskutonis 2010).

CONCLUSION
The results are showed that Anzer honey is a heterofloral blossom honey and contains a wide range variety polyphenolic and flavonoid compounds. The presence of polyphenolic substances increases the medicinal value of honey and provides high antioxidant, antimicrobial and anti-inflammatory properties.

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