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ARAŞTIRMA MAKALESİ / RESEARCH ARTICLE

A STUDY ON FREE-RADICAL SCAVENGING ACTIVITY, INDIVIDUAL PHENOLIC COMPOUNDS AND ELEMENT CONCENTRATION OF PROPOLIS

Propolisin Serbest Radikal Temizleme Aktivitesi, Bireysel Fenolik Bileşik İçeriği ve Element Konsantrasyonu Üzerine Bir Çalışma

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ABSTRACT

The aim of this study was to assess the quality of five propolis samples obtained from Turkey (TP), China (CP), Brazil (BP1, BP2), and Ethiopia (EP). The phenolic compounds of the propolis were identified and quantified using the liquid chromatography-tandem mass spectrometry technique (LC-MS/MS). In addition, quality parameters such as total flavonoid content, total phenolic content, free-radical scavenging activity and element contents were investigated. As a result of LC-MS/MS analysis, the extracts were sorted as TP> BP1> BP2> CP> EP in terms of the total concentration of individual phenolic compounds. Chlorogenic acid was determined as the dominant compound in BP1 and EP, *p*-coumaric acid as the dominant compound in the BP2 and trans-ferulic acid as the dominant compound in the samples from CP and TP. The concentration of DPPH was higher in TP whereas the ABTS concentration was almost similar to other propolis extracts. The contents of potassium (K), calcium (Ca), iron (Fe), magnesium (Mg) and sodium (Na) in the propolis samples were in the range of 2416.75-14416.02 mg/kg, 8.52-613.25 mg/kg, 102.66-1425.82 mg/kg, 523.84-7336.74 mg/kg and 57.65-191.15 mg/kg, respectively. Consequently, it is again supported that chemical characteristics and activity of propolis varies according to its geographical origin with this study.

Keywords: Chinese propolis, Ethiopia propolis, Brazilian propolis, Turkish propolis, Element composition, Mineral content, Phenolic compounds.

ÖZ

Bu çalışmada, Türkiye (TP), Çin (CP), Brezilya (BP1, BP2) ve Etiyopya'dan (EP) elde edilen beş propolis örneğinin kalitesi değerlendirildi. Propolisin fenolik bileşikleri sıvı kromatografi kütle spektrometresi (LC-MS/MS) ile kantitatif olarak tespit edildi. Buna ilave olarak, total flavonoid içerik, total fenolik içerik, antioksidan aktivite ve element içeriği (ICP-MS ile) gibi kalite parametleri de araştırıldı. LC-MS/MS analizinin sonuçlarına göre propolis ekstraktları 24 adet bireysel fenolik bileşiğin toplam konsantrasyonu açısından TP> BP1> BP2> CP> EP olarak sıralandı. BP1 ve EP için klorogenik asit, BP2 için p-kumarik asit, CP ve TP için ise trans-ferulik asit baskın bileşikler olarak tespit edildi. TP'de DPPH konsantrasyonu en yüksek iken, ABTS konsantrasyonu ise diğer propolis örnekleri ile benzerlik gösterdi. Propolis numunelerindeki potasyum (K), kalsiyum (Ca), demir (Fe), magnezyum (Mg) ve sodyum (Na) içeriklerinin sırasıyla 2416.75-14416.02 mg/kg, 8.52-613.25 mg/kg, 102.66–1425.82 mg/kg, 523.84–7336.74 mg/kg and 57.65-191.15 mg/kg arasında değişiklik gösterdiği belirlendi. Sonuç olarak bu çalışma ile propolisin kimyasal bileşimi ve aktivitesinin coğrafi kökenine göre değiştiği tekrar desteklendi.

Anahtar kelimeler: Çin propolisi, Etiyopya propolisi, Brazilya propolisi, Türkiye propolisi, Element içerik, Mineral içerik, Fenolik bileşikler.

GENiŞLETiLMiŞ ÖZET

Amaç: Propolis bal arıları tarafından bitkilerin farklı kısımlardan toplanan reçine benzeri yapışkan koyu renki bir maddedir. Bu ürün arılar tarafından kovan savunması, koloni sağlığının korunması, yapı malzemesi vb. bir çok amaç doğrultusunda kullanılmaktadır. Bununla birlikte insanlar tarafından bu ürün yüzyıllardır kovandan toplanarak sağlık koruyucu/destekleyici ve tedavi edici gibi özellikleri nedeniyle kullanılmaktadır. Bu çalışmada farklı ülkelerden (Türkiye, Çin, Brezilya, Etiyopya) elde edilen propolis örneklerinin, total flavonoid içerik, total fenolik içerik ve serbest radikal temizleme aktiviteye ek olarak elemental içerik ve bireysel fenolik bileşik konsantrasyonu bakımından değerlendirilmesi amaçlandı.

Materyal ve Metot Propolis örneklerinin serbest radikal temizleme aktivitelerinin tespit edilmesi için DDPH ve ABTS testleri kullanıldı. Propolis örneklerinin total flavonoid içeriği alüminyum klorür ve toplam fenolik madde içeriği ise Folin-Ciocalteu metodu ile tespit edildi. Propolis örneklerinde Li, B, Be, Mg, Na, Al, Cu, Ca, Zn, Cr, K, V, Mn, Co, Fe, Ga, Ni, Sr, In, Rb, Ru, Ag, Cs, Pd, Cd, Pt, Ba, Hg, Tl ve Au olmak üzere toplamda 29 element indüktif olarak eşleşmiş plazma kütle spektrometresi (ICP-MS) ile tespit edildi. Bununla birlikte 24 adet fenolik bileşiğin (2,5-dihidroksibenzoik asit, 2-hidroksi transsinnamik asit, kafeik asit, kateşin, epikateşin, klorogenik asit, etil gallat, gallik asit, isorhamnetin, kamferol, luteolin, mirisetin, naringin, P-kumarik asit, phlorizin, propil gallat, protokateşik asit, kuersetin, resveratrol, rutin, salisilik asit ve sinapik asit, siringik asit, trans ferulik asit) kalitatif ve kantitatif olarak tespiti, sıvı kromatografi-kütle spektrometresi/kütle spektrometresi (LC-MS/MS) kullanılarak gerçekleştirildi.

Sonuç ve Tartışma: LC-MS/MS analizi sonucunda ekstraktlar, incelenen 24 adet bireysel fenolik bileşiğin toplam konsantrasyonu bakımından Türkiye > Breziya 1> Brezilya 2> Çin > Etiyopya propolisi olarak sıralandı. Brezilya 1 ve Eyiyopya propolisinde major bileşik olarak klorojenik asit, Brezilya 2 propolisinde major bileşik olarak p-koumarik asit tespit edilirken, Çin ve Türkiye orijinli propolis örneğinde major bileşik olarak trans-ferulik asit tespit edildi. Bununla birlikte Türkiye propolisinde Çin propolisinden farklı olarak kafeik asit miktarı oldukça yüksek olarak belirlendi. Propolis numunelerinde makroelementlerden potasyum (K), kalsiyum (Ca), demir (Fe), magnezyum (Mg) ve sodyum (Na) içerikleri sırasıyla 2416,75-14416,02 mg/kg, 8,52-613,25 mg/kg, 102,66–1425,82 mg/kg, 523,84–7336,74 mg/kg ve 57,65-191,15 mg/kg arasında tespit edildi. Genel olarak, bu çalışma propolisin kimyasal içeriğinin üretildiği coğrafi bölgeye bağlı olarak benzerlik ve farklılıklara sahip olduğunu gösterdi. Bu durum, farklı coğrafi bölgelerdeki arıların propolis yapmak için kullandığı bitkisel kaynaklardan ileri gelebilir. Ayrıca elde edilen sonuçlar propolis örneklerinin antioksidan aktiviteye sahip olduğu ve propolisin diyetlerde bir antioksidan kaynağı olarak takviye gıda şeklinde kullanılabileceği destekledi. Bununla birlikte, propolisin kimyasal içeriğindeki bileşiklerin katkılarını anlamak için daha fazla araştırma yapılmalıdır.

INTRODUCTION

Propolis is a resinous substance collected by bees and used in their hives as a protective agent and a building material. This resin is gathered from different types of plants by honey bees (*Apis mellifera* L.) that form it into pellets with their mandibles, probably mixing it with secretions of their salivary glands and beeswax (Alamyel et al. 2018). Until 2018, more than 850 compounds have been identified in the chemical content of propolis (Šturm and Ulrih 2019). Propolis is usually composed of 50% resin, 30% wax, 10% essential oils, 5% pollen and 5% other substances, however, the chemical content of propolis varies depending on factors such as botanical and geographical origin (Wang et al. 2016)

Several different types of propolis have been defined with respect to their chemical profile, plant and geographical origin. In the tropics, where poplars are not abundant, bees seek different floral sources for the production of resin (Coelho et al. 2017). There are various different types of propolis that are available, such as poplar propolis which is most often produced from *Popoulus nigra* L. in Europe, North America, non-tropic regions of Asia, New Zealand, Green; Brazilian propolis predominantly produced from *Baccharis dracunculifolia* DC. in Brazil; Birch propolis produced from *Betula verrucosa* Ehrh. in Russia; Red propolis produced from *Dalbergia* spp. in Cuba, Brazil and Mexico; Mediterranean propolis produced from Cupressaceae in Sicily, Greece, Crete and Malta; Clusia propolis produced from *Clusia* spp. in Venezuela and Cuba; and Pacific propolis produced

from *Macaranga tanarius* in the Pacific region. The major components of these different types of propolis are flavanones, flavones, cinnamic acids and esters for poplar propolis; diterpenic acids and prenylated *p*-coumaric acids for Brazilian propolis; flavones and flavonols for Birch propolis; isoflavonoids for Red propolis; diterpenes for Mediterranean propolis; polyprenylated benzophenones for Clusia propolis; c-prenyl-flavanones for Pacific propolis (Sforcin and Bankova 2011).

Propolis is well known for its diverse and beneficial biological effects such as antibacterial (Bayram et al., 2017, Temiz et al. 2011)), antifungal (Silici et al. 2005), anti-Inflammatory (Kolaylı et al. 2016), and antioxidant (Temizer et al., 2017). It is also known to stimulate wound healing, reduce tumefaction and suppresses pain (Zilius et al. 2016). Phenolic compounds are essential bioactive components of propolis (Vargas-Sanchez et al. 2015). Phenolics, aromatic alcohols, terpenes, and aldehydes are principal components of propolis and their existence in propolis defines the quality of the propolis as well as its pharmacological property and possible application areas (Zilius et al. 2016). However, there is limited information about the presence/level of elements that can significantly affect the quality of propolis (González-Martín et al. 2015). Therefore, it is extremely important to provide detailed information on the element content of propolis in order to ensure its safe usage in different areas.

As natural products that contain propolis are rich in bioactive components such as minerals, vitamins, polyphenols, amino acids, many efforts have been made recently to use these products in commercial products. Products rich in minerals and polyphenols are of great interest in many fields such as food, cosmetics and medicine, as some of the mineral elements listed as bioactive compounds act as cofactors in most enzymatic events in plants, animals and humans (Kuppusamy et al. 2016). The aim of the present study was to assess the total polyphenol and flavonoid contents, antioxidant activities and elemental composition of the ethanol extracts of propolis obtained from four different countries.

MATERIAL AND METHODS

Propolis samples

Five propolis samples used in the study were obtained commercially from Turkey (TP), Brazil (BP1, BP2), China (CP) and Ethiopia (EP) in 2017.

Preparation of propolis extracts

Extracts were prepared according to Zhou et al. (2015) with some modification. 1.5 g raw propolis sample was pulverized and then added in 10 mL ethanol (95%). Then, ultrasonic assisted extraction was performed in an ultrasonic cleaning bath for 60 min at 40 °C. The mixture was centrifuged for 30 min. The supernatant was transferred in a pear-shaped flask and this procedure was repeated twice. Supernatants were combined and the total volume adjusted to 25 mL with ethanol (95%). The final mixture was filtrated through a 0.45 µm membrane.

Total phenolic assay

The content of total phenolic compounds was performed according to the Folin-Ciocalteu method proposed by Magalhaes et al. (2010) with some modification. 50 µL extract, 50 µL Folin-Ciocalteu reagent (1:5, v/v) and 100 µL sodium hydroxide solution (0.35 M) were added in each well, respectively. After 3 minutes, absorbance was recorded at 760 nm. The results were expressed as gallic acid equivalent (mg GAE/g).

Total flavonoid assay

Total flavonoid analysis of the extracts was performed as Zhishen et al. (1999) with some modification. Accordingly, 1 mL extract was mixed with 0.3 mL $AlCl_3.6H_2O$ (10%) after the addition of 0.3 mL $NaNO_2$ (5%). 2 mL NaOH (1 M) and 2.4 mL distilled water were added then mixture was stirred with vortex. The absorbance was measured at 510 nm. Total flavonoid content was expressed as mg quercetin equivalent (mg QE/g).

Determination of free-radical scavenging activity

1,1-diphenyl-2-picrylhydrazyl (DPPH) radical scavenging *assay*

The DPPH assay was based on the 96-well plate assay described by Herald et al. (2012) with some modifications. 15 µL extract and 185 µL of DPPH solution (150 µmol L^{-1}) were mixed, and vortexed for 10 s. Absorbance was measured at 517 nm after being stored in the dark for 45 minutes at room temperature. The results were expressed as mg

Trolox equivalent antioxidant capacity per g of samples (mg TE/g).

2,2'-azino-bis-3-ethylbenzthiazoline-6-sulphonic acid (ABTS) radical scavenging assay

The ABTS radical cation was reacted with 2.45 mM potassium persulfate and left in the dark at room temperature for 12-16 h before use. ABTS solution was diluted with ethanol to an absorbance of 0.70 at 734 nm and equilibrated at 30 °C. The extracts were first diluted with 1 mL sample and 1 mL of ABTS solution, then with methanol to a total volume of 4 mL. The tubes were stored at room temperature for 6 min. Then, absorbance was measured at 734 nm The results were expressed as mg Trolox equivalent antioxidant capacity per g of samples (mg TE/g) (Re et al. 1999).

Determination of individual phenolic compounds

Liquid chromatography-tandem mass spectrometry (LC-MS/MS) was used for detection individual phenolic compounds (2,5-dihydroxybenzoic acid, 2-hydroxytranscinnamic acid, caffeic acid, catechin, epicatechin, chlorogenic acid, ethyl gallate, gallic acid, isorhamnetin, kaempferol, luteolin, myricetin, naringin, *p*-coumaric acid, phlorizin, propyl gallate, protocatechuic acid, quercetin, resveratrol, rutin, salicylic acid, sinapic acid, syringic acid, trans ferulic acid). LC was performed using an Agilent 6460 (Agilent Technologies, Waldbronn, Germany) LC system. Chromatographic separation was carried out with an Agilent Zorbax SB-C8 column (150 × 3.0 mm, 3.5 µm particle size). MS/MS analyses were accomplished on an Agilent LC-MS (Agilent Technologies, Waldbronn, Germany) 6460 triple quadruple mass spectrometer equipped with an electrospray ionization (ESI) interface.

Determination of element profiles of propolis samples by inductively coupled plasma mass spectrometry (ICP-MS)

ICP-MS analysis was performed at the Central Research Laboratory of Bayburt University. In this study all reagents used for the elemental analysis of samples were of analytical grade. The element standard solutions were prepared by diluting a stock solution of 1000 mg/L of lithium (Li), boron (B), beryllium (Be), magnesium (Mg), sodium (Na), copper (Cu), calcium (Ca), zinc (Zn), chromium (Cr), potassium (K), vanadium (V), manganese (Mn), cobalt (Co), iron (Fe), gallium (Ga), nickel (Ni), strontium (Sr), indium (In), rubidium (Rb), ruthenium (Ru), silver (Ag), cesium (Cs), palladium (Pd), cadmium (Cd), platinum (Pt), barium (Ba), mercury (Hg), thallium (Tl) and gold (Au). 0.5 g of propolis sample, 9 mL of nitric acid (Sigma Aldrich, Germany) and 1 mL of hydrogen peroxide (Sigma Aldrich, Germany) were mixed. Then, the digestion procedures were carried out in a microwave digestion system. The final volume was completed to 50 mL with ultrapure water. Analysis of 30 elements was carried out by inductively coupled plasma mass spectrometry ICP-MS (7800 Series from Aigelent) (Oroian et al. 2015).

Statistical analysis

Analysis of variance (ANOVA) and post hoc Tukey's test tests were utilized for analysis of total phenolic, total flavonoid and antioxidant activity data.

RESULTS

In this study, 24 compounds were identified from different propolis extracts and the quantitative value of each compound was determined. BP1 had a higher concentration of chlorogenic acid (791.69 mg/100g) and *p*-coumaric acid (495.67 mg/100 g). In the same way, the sample obtained from Turkey (TP) had a higher concentration of kaempferol (156.28 mg/100 g) and quercetin (428.9 mg/100 g) compared to the other propolis samples.

The highest total concentration (1808.65 mg/100 g) of screened 24 individual phenolics was found to be in the TP sample (Table 1). While caffeic acid was a major component in the TP sample, at a concentration of 238.52 mg/100 g, it was a minor component in the propolis sample obtained from China (CP), at a concentration of 0.76 mg/100 g. In addition, the concentration of isorhamnetin in the CP sample was higher (110.33 mg/100 g) compared to that in the TP sample, which was found at concentration of 90.58 mg/100g. The component resveratrol, which was lacking in TP, BP1, BP2 and EP was present in CP (23.27 mg/100 g). Trans ferulic acid and quercetin as major component were both presents in the CP and TP samples in important concentrations when compared to the BP1, BP2 and EP samples.

Table 1. Phenolic composition (mg/100g) of propolis extracts

COMPOUNDS	BP1	BP2	EP	CP	TP
2,5-dihydroxybenzoic acid	7.76	2.70	5.18	12.57	1.76
2-hydroxytranscinnamic acid	nd	nd	nd	nd	nd
Caffeic acid	243.13	34.99	36.59	0.76	238.52
Catechin	nd	nd	nd	2.28	5.80
Epicatechin	nd	nd	nd	2.52	6.41
Chlorogenic acid	791.69	226.2	619.15	3.94	2.18
Ethyl gallate	0.004	1.44	0.08	0.14	0.07
Gallic acid	14.48	180.63	37.69	7.35	10.88
Isorhamnetin	2.91	23.25	1.42	110.33	90.58
Kaempferol	74.54	80.79	12.79	66.60	156.28
Luteolin	2.45	93.17	11.95	42.80	90.94
Myricetin	0.66	32.54	3.70	7.49	2.47
Naringin	nd	nd	nd	nd	nd
***p*-coumaric acid**	495.67	279.20	6.13	8.07	84.01
Phlorizin	nd	0.38	nd	0.62	0.41
Propyl gallate	nd	nd	nd	nd	nd
Protocatechuic acid	172.39	88.53	254.10	37.95	60.67
Quercetin	62.21	165.80	27.42	201.38	428.90
Resveratrol	nd	nd	nd	23.27	nd
Rutin	1.67	72.54	1.36	1.05	7.32
Salicylic acid	12.98	4.55	3.10	27.23	0.61
Sinapic acid	nd	nd	nd	11.59	nd
Syringic acid	nd	nd	nd	nd	nd
Trans ferulic acid	23.70	10.29	0.20	701.60	620.84
TOTAL	**1655.35**	**1296.62**	**1020.86**	**1241.26**	**1808.65**

nd: not detected

The TP sample differed with the EP sample in many ways. Firstly, the main components of the EP sample were determined as chlorogenic acid (619.2 mg/100 g) and protocatechuic acid (254.1 mg/100 g), while these components were available in much smaller concentration in the TP sample. Conversely, the TP sample contained greater concentrations of trans ferulic acid (620.84 mg/100 g), quercetin (428.9 mg/100 g) and caffeic acid (238.52 mg/100 g) compared to the EP sample, which contained these compounds in minor concentrations. In addition, the TP sample included catechin, epicatechin and phlorizin, which were absent in the EP sample. It was determined that the EP and CP samples were completely different in many aspects. The CP sample had trans ferulic acid (701.6 mg/100 g),

quercetin (201.38 mg/100 g) and isorhamnetin (110.33 mg/100 g) constituting the largest percentage of its component. These components were also present in the EP sample, however, they were considered as a minor component owing to their low concentrations (0.2, 27.42 and 1.42 mg/100 g, respectively).

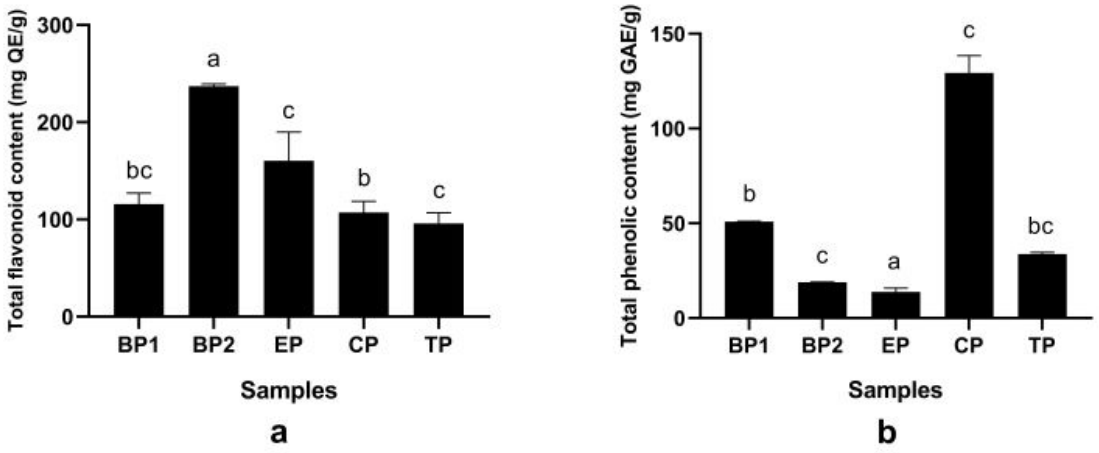

Figure 1. (a) Total flavonoid content of propolis extracts (mg QE/g). (b) Total phenolic content of propolis extracts (mg GAE/g)

The highest total phenolic levels were detected in the CP, BP1 and TP samples, while the lowest levels were found in the BP2 and EP samples, respectively (Figure 1b). The flavonoid content of the propolis extracts varied from 95.966 to 237.201 mg QE/g (Figure 1a). Among all the samples, the BP2 sample had the highest flavonoid content at 237.17 mg QE/g, which was followed by the EP sample at 160.471 mg/QE g, the BP1 sample at 115.834 mg/QE g, the CP sample at 107.244 mg/QE g and the TP sample at 95.966 mg/QE g. The descriptive statistics and comparison results for the DPPH assay are given in Figure 2a. The TP sample showed higher DPPH value compared to the other samples. The DPPH value for the BP2 sample was 75.907 mg TE/g, which made it the lowest among all of the samples. The ABTS value of the EP sample was determined as the lowest (Figure 2b).

As seen in Table 2, the main elements in propolis samples were found to be potassium (K), calcium (Ca), iron (Fe), magnesium (Mg), and sodium (Na). The two most significant elements in the propolis samples were the K macro element with a concentration of 2416.75-14416.02 mg/kg followed by the Mg macro element with a concentration of 523.84-7336.74 mg/kg. The highest levels of K were found in the BP1 and EP samples. The highest concentrations of Na, Zn, and Fe were found to be 191.15, 74.95 and 1425.82 mg/kg, respectively in the TP sample.

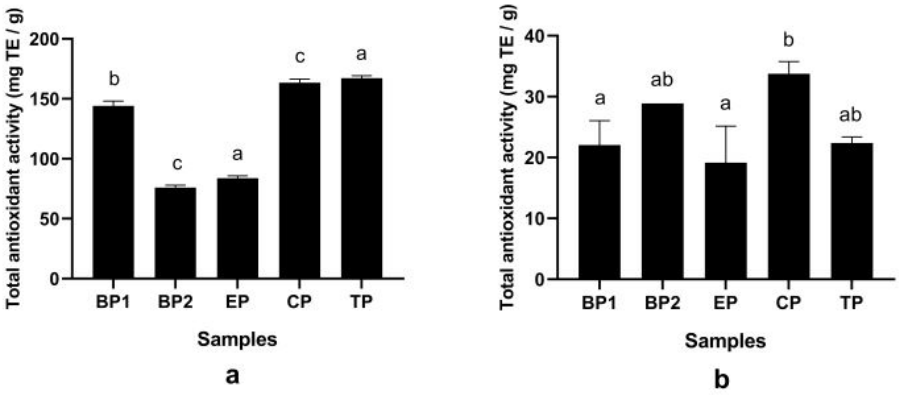

Figure 2. (a) Results of DPPH assay of propolis extracts (mg TE/g). (b) Results of ABTS assay of propolis extracts (mg TE/g)

Table 2. Elemental compositions of propolis samples (mg/kg)

Elements	BP1	BP2	EP	CP	TP
Li	0.0147	0.06	0.18	0.16	0.64
Be	nd	0.004	0.01	0.008	0.01
B	20.16	14.16	27.62	5.07	13.81
Na	57.65	115.14	159.68	182.91	191.15
Mg	1024.64	820.33	7336.74	523.84	1008.47
K	14416.02	6011.08	6087.20	2416.75	2607.41
Ca	293.61	219.66	613.25	8.52	415.24
V	0.05	0.55	2.14	0.39	4.35
Cr	0.16	0.80	2.26	0.56	3.08
Mn	28.18	60.69	52.52	2.50	35.66
Fe	102.66	347.75	861.97	287.01	1425.82
Co	0.03	0.07	0.68	0.14	1.10
Ni	3.51	3.11	1.36	2.18	2.59
Cu	9.01	5.11	3.68	1.47	4.29
Zn	33.21	25.01	14.10	8.00	74.95
Ga	0.02	0.15	0.36	0.05	0.46
Rb	73.89	23.81	5.79	1.72	3.09
Sr	9.25	6.32	27.79	0.09	9.004
Ru	nd	nd	nd	nd	nd
Pd	nd	nd	0.004	nd	nd
Ag	nd	nd	0.006	nd	0.004
Cd	0.66	0.65	1.97	1.07	1.53
In	0.04	0.05	0.01	0.08	0.03
Cs	0.07	0.27	0.04	nd	0.10
Ba	10.68	8.79	18.69	0.006	22.63
Pt	nd	nd	nd	nd	nd
Au	nd	nd	nd	nd	nd
Hg	nd	nd	nd	nd	nd
Tl	nd	nd	nd	nd	nd

*nd: not detected (<0.000)

DISCUSSION

The potent chemical components associated with propolis are the phenolic compounds, which differ in concentration and structure depending on factors including the geographical location of production, the season of production, the sources of flora used. Propolis, which shows powerful antioxidant activity, contains antioxidative compounds such as caffeic acid, chlorogenic acid, gallic acid, *p*-coumaric acid, kaempferol, quercetin, protocatechuic acid, and trans-ferulic acid. Flavonoids and phenolic acid esters, especially caffeic acid, are known for their antioxidant, antiviral and antibacterial activity (Pietta 2000; Rao et al. 1992; Tapia et al. 2004, Ahn et al. 2007). The findings of this study showed that regional origin greatly affects the phenolis of the propolis (Table 1). The propolis samples obtained from Brazil (BP1 and BP2) were found to be different in terms of their constituents. The BP1 and BP2 samples had two major components, however the concentration levels of these components differed. Correspondingly, the outcomes of this study were in line with the results of Salatino et al. (2005) who found that the components of propolis also differ among the propolis obtained from the same location. The BP and TP samples differed in regard to the concentration of trans ferulic acid, quercetin, kaempferol, luteolin and isorhamnetin, which was particularly found at a significant concentration in the TP sample compared to the BP1 and BP2 samples, in which it was found only in minute concentrations. Overall, these findings are in agreement with the findings of Teixeira et al. (2010) who determined that Brazilian propolis is rich in phenolics. Components of ethanolic extract of Brazilian green propolis are artepillin C, *p*-coumaric acid, ferulic acid, 4-Hydroxy 3-prenylcinnamic acid, kaempferide, caffeic acid, kaempferol, hesperitin sakuranetin, isorhamnetin, and pinocembrin (Szliszka et al. 2013). Quercetin, benzoic acid, ferulic acid, caffeic acid and coumaric acid were determined in high concentrations in the Turkish propolis samples, while chlorogenic acid, vanillic acid, syringic acid, epicatechin, rutin, and o-coumaric acid were found to be in small amounts and catechin was not found at all (Aliyazıcıoglu et al. 2013).

Propolis is a natural source of phenolic compounds, which are associated with important health benefits. The total content of phenols provides an index of various measurable properties of propolis such as antioxidant capacity, antibacterial activity and ability to scavenge free radicals (Gardini et al. 2018). The variation of total phenolic and flavonoid content for propolis samples from different locations was quite large. The total phenolic and total flavonoid content in the extracts showed statistically significant differences in accordance with the regions and ranged from 13.764 to 129.368 mg GAE/g. Similar to the present study, Wang et al. (2016) found the total phenolic content of propolis extract obtained from China to be 132.1 ± 3.28 mg/GAE g. They also revealed that the total phenolic content of a propolis extract obtained from Brazil was higher (126.8 ± 4.12 mg GAE/g) than the value obtained in the present study. Furthermore, they determined that the total flavonoid content of Chinese (32.5 ± 0.53 mg/QE g) and Brazilian (53.0 ± 0.22 mg QE/g) propolis samples were lower than the values determined in the present study. The total phenolic data, presented in Figure 1b, were in agreement with the data obtained for the propolis samples obtained in China (42.9 ± 0.8- 302 ± 8.3 mg GAE/g) (Ahn et al. 2007) and Turkey (0.1038-86.807 mg GAE/g), but lower than those obtained for the propolis samples obtained in Brazil (307.63±0.92-398.31±11.15 mg GAE/g) (De Oliveira Reis 2019). On the other hand, the total phenolic content of the EP sample used in the present study was higher than the content determined by Liben et al. (2018). Similarly, the results of the present study suggested that the total phenolic and flavonoid contents of the propolis samples varied by region. This indicates that the phenolic compounds present in the chemical structure of the different plant sources, most likely in flora, are included in the chemical structure of propolis and consequently are an important factor in determining the quality of propolis.

The DPPH values for the samples evaluated in this study were between 75.907 and 167.225 mg TE/g. Similarly, Banskota et al. (2000) reported that the DPPH activity of propolis obtained from China was higher than that of the propolis obtained in Brazil. The ABTS values of the samples ranged from 19.163 to 33.747 mg TE/g. The results of the present study are compatible with the results put forward by Yang et al. (2011), who studied the high antioxidant activity in propolis obtained from China. It is possible that in regions where climatic conditions vary, there are sources of phenolic compounds with bioactive properties that vary accordingly. Therefore, propolis samples produced in different regions of the World cannot be expected to be the same in terms of chemical content and thus biological capacity. These differences may be reflected in the antioxidant

activity of the propolis and may cause the biological spectra to vary, as supported by the results of this study.

Mineral diversity is reflected in the composition of propolis through the transfer of the mineral composition of the soil to the plants from which the propolis is obtained. For this reason, plant sources affect the elemental composition of propolis to a great extent (Lovaković et al. 2018). As a result, the elemental content of propolis is used to develop distinctive features and reliable traceability methods of the geographical areas where it was produced as an indicator of environmental pollution (Golubkina et al. 2016). In the present study, when the elemental composition of the propolis samples were examined, it was observed that although there were quantitative differences, they had qualitatively similar content. All of the samples lacked ruthenium (Ru), platinum (Pt), gold (Au), mercury (Hg) and thallium (Tl). Moreover, minor concentrations of lithium (Li), beryllium (Be), vanadium (V), chromium (Cr), cobalt (Co), nickel (Ni), copper (Cu), gallium (Ga), strontium (Sr), cadmium (Cd), indium (In), cesium (Cs), palladium (Pd), silver (Ag) were determined in the propolis samples. Overall, the concentrations of many elements in the TP sample were generally higher than those in the other samples. There are limited reports on the mineral content of propolis. Gong et al. (2012) determined that Ca, Mg, K, Fe, Na and Zn levels in the propolis samples obtained from Brazil were higher than 160 mg/kg, which is accordance with the results of the present study. In contrast, the present study also found lower concentrations of Zn and Na in the BP2 sample and a lower concentration of Fe in the BP1 sample. Dogan et al. (2006) investigated the content of Na, K, Ca, Mg, N, Cu and Zn in propolis samples from Turkey and found that the highest element rate in all of the samples to be Na. However, the present study found that the highest element rate in the TP sample was K. Dogan et al. (2006) also determined the Ca content of the propolis samples at a lower concentration than those obtained in the present study. Cantarelli et al. (2011) reported that trace element level provides sufficient information for the identification of propolis. Similarly, in this study, although differences in the trace element contents of the propolis samples were observed, more samples were required to make a clear geographic distinction. The elemental composition of the propolis samples may vary depending on many factors such as vegetation, environmental factors (pollution, industrialization, etc.), beekeeping equipment, beehive production material (plastic, wood, etc.) and differences in sample collection methods (scraping or trapping method, etc.).

CONCLUSION

In this study, the major components of the propolis samples were detected as trans ferulic acid and quercetin for the TP and CP samples, chlorogenic acid for the EP and BP1 samples and *p*-coumaric acid for the BP2 sample. Caffeic acid, which is an important component for the quality determination of propolis, was found to be the highest in the BP1 and TP samples, respectively. In addition, the results confirmed that the individual phenolics of propolis contribute to antioxidant activity in particular, and that propolis can also be used as a supplement in diets as an antioxidant source. It is thought that the elemental compositions of propolis samples can be important in distinguishing their regional origin and also providing an idea of the quality of the product and where it should be used. Overall, this study confirmed that the chemical content of propolis has significant differences depending on the geographical location it was produced. The reason for this could be that bees in different geographical locations use different flowers and trees to make the propolis. However, further research must be conducted to understand the contributions of the valuable compounds of propolis.

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