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Araştırma Makalesi / Research Article

Botanic Origin and Antioxidant Activity of Some Bitlis Honeys

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Abstract

Honey is one of the most known beeproduct in worldwide. The aim of this study is to interpret botanic origin, and antioxidant activity of five honey samples from Bitlis region. All the samples were classified as multifloral. The total phenol content (TPC), total flavonoid content (TFC), hydrogen peroxide scavenging activity (HPSA), ferric reducing antioxidant power (FRAP), DPPH radical scavenging activity (DPPH), ferrous ions chelating activity (FICA), ABTS radical cation scavenging activity (ABTS) of honey samples were found between, 165.73-349.41 mg GAE/100 g, 1.26-4.08 mg CAE/100, 201.76-216.59 µg/mL, 52.46-71.2%, 256.41-419.29 µg/mL, 69.44-94%, and 20.06-20.59 µg/mL, respectively. These results were compared with Trolox (TRO), Routine (RUT) and Butylated Hydroxy Anisole (BHA) standard antioxidant components. The reported information gives increased nutrition characteristics to honey and support to the growing of beekeeping, supplying a choice to family income raise.

Keywords: antioxidant activity, beekeeping, Bitlis honey, multifloral.

Bitlis'ten alınan bazı balların Botanik Orijini ve Antioksidan Aktivitesi

Öz

Bal, dünya çapında en iyi bilinen arı ürünlerinden biridir. Bu çalışmanın amacı Bitlis yöresinden alınan 5 bal örneğinin botanik orjinini ve antioksidan aktivitesini değerlendirmektir. Bütün bal örnekleri multifloral olarak sınıflandırılmıştır. Bal örneklerinin antioksidan aktivitesi toplam fenol içeriği, toplam flavonoid içeriği, hidrojen peroksit giderme aktivitesi (HPSA), demir indirgeme antioksidan gücü (FRAP), DPPH radikal giderme aktivitesi (DPPH), demir iyon şelatlama aktivitesi (FICA), ABTS, sırasıyla 165.73-349.41 mg GAE/100 g, 1.26-4.08 mg CAE/100, 201.76-216.59 µg/mL, 52.46-71.2%, 256.41-419.29 µg/mL, 69.44-94%, and 20.06-20.59 µg/mL, olarak sırasıyla bulunmuştur. Bu sonuçlar Trolox (TRO), Rutin (RUT) ve Butillenmiş Hidroksi Anisol (BHA) standart antioksidan bileşenler ile karşılaştırılmıştır. Bu çalışmadan elde edilen sonuçlar, bölgede besin değeri daha yüksek ballar üretilmesini sağlayarak arıcılığın büyümesine destek vermekte ve arıcılık yapan ailelerin gelir düzeyinin yükselmesini sağlamaktadır.

Anahtar Kelimeler: antioksidan aktivite, arıcılık, Bitlis balı, multifloral.

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1. Introduction

Beekeeping is one of the most common agriculture businesses worldwide. Beekeeping offers a comfortable way for people to permit finance from natural sources without damaging local flora. Bee products are specific to location, season, and climatic factors and depend heavily on flora. Turkey covers three different floristic regions and possesses rich vegetation with approximately 120000 plants. Therefore, Turkey is one of the major producers in the honey global market. Bitlis is located at the Irano-Turanian floristic region which has high levels of plant biodiversity. Asteraceae, Fabaceae, Poaceae Caryophyllaceae, Ranunculaceae and Brassicaeae are found most commonly families in Bitlis. Astragalus, Trifolium, Vicia, Veronica, Ranunculus, Gypsophylla, Silene, Alyssum and Centaurea are widely present genus (Altan and Behçet, 1995; Altıok, 2004; Behçet, 1991; Çelik, 2006; Keser and Özgökçe, 2019). These taxa constitute a big potential of melliferous sources for Bitlis region.

Honey that essentially consist of fructose, glucose and sucrose is a natural sweet solution, while also contains other natural products such as enzymes, organic acids, vitamins, minerals, numerous volatile and phenolic compounds (Dżugan et al., 2018; Kireçci and Kireçci, 2018). The chemical composition of honey has been reported to be highly variable and dependent on the native flora as well as climatic conditions (Temizer et al., 2016; Temizer et al., 2019).

Honey has a large spectrum of biological activities and has been used for various purposes by the people because of natural antioxidant and antimicrobial properties (Pasias et al., 2018; Temizer et al., 2016). There are many analytical methods for definition of the antioxidant capacity of honey, a lot of analytical methods are been used. The most generally experiments are total phenolic and flavonoid contents, DPPH, HPSA, FRAP, ABTS, FICA (Al-Hindi Shehata, 2014; Kıvrak and Kıvrak, 2017; Temizer et al., 2018; Temizer et al., 2019).

Beekeeping is a very long-standing and habitual farmer activity for rural societies in Bitlis. However, there is not enough literature on honey produced from Bitlis. To our knowledge this is the first time that honey obtained from Bitlis location have been comprehensively researched. The objective of this study is to assess the antioxidant capacity and botanic origin of honey samples derived from Bitlis, a region with richness of vegetation.

2. Materials and Methods

2. 1. Honey sample collection and pollen analysis

The present study was carried out using honey samples from local beekeepers of Bitlis (Figure 1). The samples were stored at a room temperature (22±2°C) as well as experiment time.

Figure 1. Honey samples collected from localities (Google earth)

A 10 g sample of each honey was conducted following traditional method without acetolysis (Louveaux et al., 1978) The botanic classifications of each honey sample were done by microscopic pollen analysis. The following terms were used for frequency classes: predominant pollen (>45%), secondary pollen (16–45%), important minor pollen (3–15%) and minor pollen (<3%) (Pound et al., 2018). The following equation (Eq. 1) was used to obtain the frequency per taxon.

$$Frequency\ (\%) = \frac{Total\ number\ of\ pollen\ of\ a\ particular\ species}{Total\ number\ of\ observed\ pollen} x100 \quad (1)$$

2. 2. Antioxidant analyses

2.2.1. Preparation of honey solution

A 10 g of honey was added to 50 ml of distilled water and kept in a water bath until the honey dissolved. Finally, total volumes are adjusted to 100 mL by using distilled water.

2.2.2. TFC assays

The TFC of the honey solution were determined according to the colorimetric method described by Chung et al. (2002) with minor modifications. Sample solutions (0.5 mL) were added to a tube containing 1.5 mL of absolute ethanol. To the mixture was added subsequently $AlCl_3.6H_2O$ solution (0.1 mL, 10.0 %) and potassium acetate (0.1 mL, 1.0 mol/L). Distilled water was added to bring the total volume to 5.0 mL and the absorbance was read after 30 min at 415 nm (Optizen Pop UV / Vis Single Beam). Total flavonoids contents were expressed as microgram of catechin equivalent that was obtained from standard graph (R^2=0.9979).

2.2.3. TPC assays

The TPC of samples were analyzed by the Folin&Ciocalteu' s phenol reagent (Folin C) colorimetric method described by Slinkard and Singleton(1977). Sample solutions (0.5 mL) were mixed with 7.0 mL of distilled water and subsequently with Folin C reagent (0.5 mL). After 3 min, Na_2CO_3 solution (3.0 mL, 2.0 %) was added into the mixture. The color developed for 1 h and the absorbance was measured at 760 nm in a spectrophotometer (Optizen Pop UV / Vis Single Beam). Gallic acid was used as the standard, and total phenolic content was expressed as microgram of gallic acid equivalent by using an equation that was obtained from standard gallic acid graph (R^2= 0.9995).

2.2.4. DPPH assays

The DPPH were studied by following a previous report with slowly modified (Blois, 1958). Serially diluted samples (3.0 mL) at the different concentrations (10-100 µg/mL) were added to $DPPH^{\bullet}$ solutions (1.0 mL, 0.2 mM) in ethanol. The mixtures were shaken forcefully and allowed to sit at room temperature for 30 min. Then, absorbance was recorded at 517 nm by using a spectrophotometer (Optizen Pop UV / Vis Single Beam) and the results were expressed as SC_{50} (µg/mL) by linear regression analysis and represent mean of the data.

2.2.5. HPSA assays

The HPSA was assayed according to the method described by Ruch et al. (1989). Briefly, the samples (3.4 mL) were mixed with 0.6 mL of 40 mM H_2O_2 solution (prepared in the same buffer). The absorbance of the mixture was measured at 230 nm versus blind sample after 10 min with UV/VIS spectrophotometer (Optizen, Korea). Phosphate buffer without hydrogen peroxide was used as blank. The decrease in absorbance value showed the high level of hydrogen peroxide scavenging activity designation. The results were expressed as SC_{50} values (µg/mL).

2.2.6. FICA assays

The FICA of the standards and antioxidant material samples were investigated according to Dinis Method (1994) (Dinis et al., 1994). For this reason, sample or standard solutions (0.4 mL) mixed with $FeCl_2$ (0.05 mL, 2 mM). The obtained mixture was kept for 10 min. Then, ferrozine (0.2 mL, 5 mM) and absolute ethanol (3.3 mL) were added to all tubes. The final mixture tubes were vortexed and the absorbance values were measured at 562 nm. The FICA was calculated by using this formula (2):

$$\text{Ferrous Ions Chelating Activity (\%)} = [1 - (A_s/A_c)] \times 100 \quad (2)$$

2.2.7. ABTS assays

$ABTS^{\bullet+}$ scavenging activities of the standards and samples were carried out following by Gökce et al. method (2019). For this assay, ABTS (2.0 mM) and potassium persulfate (2.45 mM) was mixed for generation of ABTS radical cation. The obtained mixture was kept during 16 h at room temperature in the dark. The stable $ABTS^{\bullet+}$ solution can be used during 2 days. The final absorbance was adjusted as 0.750 ± 0.020 at the 30°C and 734 nm. For calibration of absorbance value, PBS (0.1 M pH 7.4) was used for dilution. The final $ABTS^{\bullet+}$ solution (1.0 mL) was mixed with the standard and sample solutions (3.0 mL) at the different concentrations (1-10 µg/mL in PBS). The results were calculated as SC_{50} by linear regression analysis.

2.2.8. FRAP assays

The FRAP was determined by using Oyaizu method (1986) (Oyaizu, 1986). For this experimental method, PBS (2.5 mL, pH 6.6, 0.2 M) and potassium ferricyanide (2.5 mL, 1.0%) were mixed with sample and standard (2.5 mL) solutions, respectively. The obtained mixture tubes were kept at 50°C during 20 min and then TCA (2.5 mL, 10%) were added to all tubes. Finally, 2.5 mL of this solution were mixed with distilled water (2.5 mL) and $FeCl_3$ (0.5 mL, 0.1%). The FRAP was expressed as % by using the absorbance values at 700 nm following the formula (3):

$$FRAP\ (\%) = (A_s/A_c) \times 100 \quad (3)$$

3. Findings and Discussion

Melissopalynological results of 5 honey samples collected from different localities of Bitlis (Figure 1) were summarized in Table 1. Honeys were classified as multifloral due to the frequencies of the pollen per taxa never exceed 45% (Table 1). The pollen spectra of the samples were found to overlap with Bitlis vegetation. Anthemis typ, Taraxacum typ and Astragalus were represented in all of the samples. The secondary taxa were changed in samples which Astragalus and Frangula were in sample 1, Anthemis typ and Lamiaceae in sample 2, Cynoglossum in sample 3, Astragalus in sample 4 (Table 1). Sorkun et al. (1989) determined that pollen grains belonging to the families of Asteraceae, Fabaceae, Fagaceae, Myrtaceae, Malvaceae, Brassicaceae, Scrophulariaceae, Lamiaceae and Oleaceae are the important source of Turkish flower honey (Sorkun et al., 1989). Özkan et al. (2016) argued that melliferous plants described Fabaceae 34 taxa (16%) Asteraceae 22 taxa (11%), Rosaceae 21 taxa (10%) and Labiatae 18 taxa (9%) (Özkan et al., 2016). Özler (2018) claimed that Fabaceae, Rosaceae, Eucalyptus, Centaurea were determined melliferous plants (Özler, 2018). The results obtained in the study were match with previous studies.

Table 1. Pollen spectra of honey samples from Bitlis (%)

Taxa	Sample 1	Sample 2	Sample 3	Sample 4	Sample 5
Asteraceae					
Artemisia		2			5
Anthemis typ	5	25	14	8	10
Taraxacum typ	5	4	8	12	8
Betula		2	2		2
Brassicaceae		15	13	15	6
Boraginaceae				3	2
Cynoglossum	15	6	22		2
Caryophllaceea	4				
Chenopodiaceae	2				2
Fabaceea		8		5	9
Astragalus	32	10	15	21	7
Vicia			12	16	5
Frangula	35			3	10
Lamiaceae (6-colpate)		18		6	5
Juglans	2			2	
Poaceae		2			
Rosaceae		8	14	7	
Rumex				2	
Verbascum					23
Veronica					4

The honey samples have between 165.73-349.41 mg GAE/100g. Juszcak et al. (2016) argued that the total phenol content of the investigated honey samples have from 21.73 to 50.12 mg GAE/100g. Stagos et al. (2018) declared that the TPC of the honey samples ranged from 0.55 to 0.92 mg GAE/gr sample. Dżugan et al. (2018) declared that the honey samples 236.94-1876.58 mg GAE/kg. Nayık and Nanda (2016) determined that total phenolic content 37–117 mg GAE/100 g and total flavonoid content 8–17 mg QE/100 g for 37 honey samples from Kashmir, India. The result. Temizer et al. (2019) determined that total phenolic and total flavonoid amounts of Ordu honeys varied from 32.5-171.05 mg GAE/100g, 1.65-38.75 mg CAE/100g, respectively.

Table 2. Biochemical properties of Bitlis honey samples

Samples	TPC[1]	TFC[2]	FICA[3]	FRAP[3]	HPSA[4]	ABTS[4]	DPPH[4]
Sample 1	349.41	4.08	92.26	68.55	201.76	20.59	359.07
Sample 2	327.86	3.66	93.24	62.23	204.14	20.2	419.29
Sample 3	165.73	1.39	93.62	71.2	210.53	20.17	256.41
Sample 4	283.74	1.88	94	68.42	209.4	20.06	328.95
Sample 5	205.75	1.26	93.89	52.46	216.59	20.14	293.69
BHA			84.25	72.05	193.27	8.42	8.47
RUT			86.80	92.66	122.98	15.54	16.90
TRO			69.44	53.04	445.06	4.18	26.74

[1] mg GAE/100 g; [2]mg CAE/100 g; [3] %; [4] SC_{50} (µg/mL)

Juszcak et al. (2016) determined that the total flavonoid content of the investigated honey samples have from 3.61 to 7.13mg QE/100g. Navajas et al. (2011) studied Mexico honey samples and their total flavonoid contents were found as 29.58 ± 0.49 – 187.08 ± 0.59 RE /100 g sample. Nayik and Nanda (2016) declared that total flavonoid content 8–17 mg QE/100 g. These results are higher than our results. Temizer et al. (2018) determined that total flavonoid contents were 5.51±0.19-8.29±0.05 mg CAE/100 g.

The DPPH of the analysed five honey types was fairly low 256.41-419.29 %) as compared to different states (13.46-53.31%) (Alzahrani et al., 2012). Al-Hindi and Shehata (2014) were determined the DPPH radical scavenging activity as 2.15 – 3.68 µg/mL in Saudi Arabia honey samples. These results are higher. IC_{50} values were found as between 29.388 and 458.450 mg/mL at the end of DPPH radical scavenging activity assay (Ertürk et al., 2014). These results are compatible with our results. These results are compatible with our results. When our samples' DPPH radical removal activities are compared with the standards, we can say that the samples do not show an effective activity.

ABTS radical cation scavenging activities of our samples are ranged from 20.06 – 20.59 µg/mL. Al-Hindi and Shehata (2014) carried out the Saudi Arabia honey samples and ABTS radical scavenging activities were found as 0.36 -1.2 µg/mL. We can see that these results are better. Kıvrak and Kıvrak (2017) determined that DPPH free radical scavenging activity and $ABTS^{+}$ cation radical scavenging activity which were measured as SC_{50}: 54.33 – 99.40 µg/mL and SC_{50}: 10.33 – 41.20 µg/mL, respectively. While DPPH activity is higher, ABTS activity similarity is detailed. The ABTS radical removal activities of our samples are as remarkable as standard compounds.

Sherin et al. (2015) determined the metal-chelating activities for honey samples as 54.2%. Our results are higher. Bellik and Selles (2017) studied two different Algerian honey samples and their results are lower than our results (Bellik Selles, 2017). García-Tenesaca et al. (2017) declared that

FRAP (µmol TE/100 g of honey) 92.05-425.35; DPPH (µmol TE/100 g of honey) 18.22-84.05. While our samples showed FRAP activity close to BHA, lower activity than RUT was determined than TRO. We encounter a similar situation in HPSA.

4. Conclusions and Recommendations

In this study, the honey samples were classified as a multifloral honey. Each honey sample was collected from same location that has unique and different pollen spectra and antioxidant properties from each other. Standard compounds usually showed better antioxidant activity as compared to Bitlis honeys. However antioxidant activity of Bitlis honeys were almost exhibited the stronger than that reported elsewhere. Also, chromatographic analyse methods can be used for identification of dominant component(s) in the future. So information about the contribution of these components to antioxidant activity can be obtained. Moreover, the components, whose structure is not illuminated, can be used in medicine, pharmacology, food sectors etc.

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