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Determination of Phenolic Contents and Antioxidant Activities in Different Solvents of Hatila Valley and Macahel Bee Products in Artvin, Turkey

Tuğba Nigar BOZKUŞ[1]✍
[1]Laboratory Technology Program, Artvin Vocational School, Artvin Coruh University, Artvin, Türkiye
[1]https://orcid.org/0000-0001-9613-6911
✉: tubisco@hotmail.com

ABSTRACT

This study determines the extracts of bee products prepared with different solvents in terms of phenolic content and antioxidant activity of the Hatila Valley and Macahel region of Artvin, Turkey. For this purpose, water and ethanol extracts of royal jelly and honey; water, ethanol, 70% ethanol, propylene glycol, and 70% propylene glycol extracts of pollen and propolis have been analyzed by total polyphenol and flavonoid content and ferric-reducing power. The results of the analysis indicated that Macahel honey and propolis had higher phenolic content and antioxidant activity than Hatila honey and propolis. The propolis of both regions, whose mixtures were prepared, was determined as Macahel propolis > Hatila + Macahel propolis > Hatila propolis in terms of polyphenol and flavonoid content and ferric reducing power. Although propolis samples with 70% ethanol and 70% propylene glycol had higher solubility, the lowest solubility was in water. These differences vary depending on the geographical location, botanical variations of the region where the bee products are collected, and the solvent used for the extraction.

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Türkiye Artvin İli Hatila Vadisi ve Macahel Arı Ürünlerinin Farklı Çözücülerdeki Fenolik İçeriklerinin ve Antioksidan Aktivitelerinin Belirlenmesi

ÖZET

Bu çalışma, Türkiye'nin Artvin ili Hatila Vadisi ve Macahel yöresinin farklı çözücülerle hazırlanan arı ürünlerinin ekstraktlarını fenolik içerik ve antioksidan aktivite yönünden belirlemektedir. Bu amaçla arı sütü ve balın su ve etanol ekstraktları; polen ve propolisin su, etanol, %70 etanol, propilen glikol ve %70 propilen glikol ekstraktları, toplam polifenol ve flavonoid içerik ve demir indirgeyici güç açısından analiz edilmiştir. Analiz sonuçları Macahel balı ve propolisinin Hatila balı ve propolisine göre daha yüksek fenolik içeriğe ve antioksidan aktiviteye sahip olduğunu göstermiştir. Karışımları da hazırlanan her iki yörenin propolisi polifenol ve flavonoid içerik ve demir indirgeyici güç bakımından Macahel propolisi > Hatila + Macahel propolisi > Hatila propolisi olarak belirlenmiştir. %70 etanol ve %70 propilen glikollü propolis örnekleri daha yüksek çözünürlüğe sahip olmakla birlikte en düşük çözünürlük suda olmuştur. Bu farklılıklar, arı ürünlerinin toplandığı bölgenin coğrafi konumu, botanik çeşitliliği ve ekstraksiyon için kullanılan çözücüye bağlı olarak değişmektedir.

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INTRODUCTION

Considering that natural products can help protect human health, they are in high demand by consumers, the food industry, and researchers today (Agüero et al., 2011). Bee products are particularly notable because of their increasing importance among these natural products and their widespread use in various sectors. Bee products are royal jelly and bee venom, which the bee secretes directly from its body, and honey, pollen,

and propolis, which are products that the bee collects from plants and partially adds to their body secretions (Rita Elkins, 2011). The biological properties of bee products vary depending on the vegetation and geographical characteristics of the region where they are collected (Kaskoniene, 2010). Bee products can be used to prevent pathological conditions such as inflammation, diabetes, cancer, and heart diseases and to protect human health due to their antioxidant, antimicrobial, antibacterial, antiviral, anti-inflammatory, and anticancer properties (Kumazawa et al., 2004; Korkmaz, 2008; Premratanachai & Chanchao, 2014). Natural bee products with high antioxidant capacity contain pharmacologically effective flavonoids, and various phenolic and aromatic compounds (Buratti et al., 2007; Olszewski et al., 2010; Nori et al., 2011).

Honey is a natural sweet substance that honeybees produce from the nectar of flowers, the secretions of living parts of plants, or the excrement of plant-sucking insects on living parts of plants, which honeybees collect, transform, and combine with certain substances (Mendes et al., 1998; Kahraman et al., 2010). While honey contains approximately 70-80% carbohydrates, 18-20% water, and 1-2% protein, organic acids, phenolic compounds, and mineral substances, it contains more than 200 chemical components (Saxena et al., 2010; Otmani et al., 2021). Bee pollen is formed because of mixing flower pollen collected by bees with nectar and secretions from the hypopharyngeal glands (Oliveira et al., 2013). Bee pollen has a very rich content in terms of proteins, various amino acids, carbohydrates, saturated/unsaturated fatty acids, lipids, sterols, vitamins, terpenes, phenolic substances, enzymes, and minerals (Villanueva et al., 2002; Campos et al., 2010; Fuenmayor et al., 2014; Conte et al., 2017). Propolis is a sticky, resinous natural substance collected by honeybees from various plant sources. Bees use propolis to seal holes in honeycombs, smooth the inner walls of the hive, and protect the entrance from outsiders (Burdock, 1998). Propolis contains more than 300 chemical components such as polyphenols (flavonoid aglycones, phenolic acids and esters, phenolic aldehydes, alcohols, and ketones), sesquiterpene quinones, coumarins, steroids, and inorganic compounds (Khalil, 2006). Royal jelly is a thick and milky secretion secreted from the hypopharyngeal and mandibular glands of young worker bees (*Apis mellifera* L.) and is used to feed larvae (Isidorova et al., 2009). Royal jelly generally consists of 60-70% water, 9-18% protein, 7-18% carbohydrates, 3-8% lipids and minerals, vitamins, and essential amino acids (Sabatini et al., 2009).

Artvin is one of the most valuable gene centers that preserve and house the pure Caucasian bee breed in Turkey's beekeeping sector. Thanks to its rich plant diversity and ecosystem, the Macahel (Camili) region, which was declared the first "Biosphere Reserve" of Turkey by UNESCO, and the Hatila Valley, which hosts around 1300 rich and diverse plant species with endemic characteristics, are among the most important regions of Artvin in terms of beekeeping (Eminağaoğlu, 2015; Anonymus, 2021).

It has been determined that there are a limited number of studies conducted by different researchers on bee products from the Artvin region in the literature (Popova et al., 2005; Silici et al., 2005; Girgin et al., 2009; Silici et al., 2010; Aliyazıcıoglu et al., 2013; Keskin et al., 2019). In addition, while there are no studies on the bee products of Hatila Valley, one of the two important regions of Artvin, there are very few studies on the Macahel (Camili) region (Özen et al., 2010; Temiz et al., 2013; Sarıkahya et al., 2021). However, none of these studies included a study in which bee product samples from Hatila Valley and Macahel were investigated in terms of phenolic content and antioxidant activity.

The extraction solvent is as important as the extraction method in determining the phenolic content and antioxidant activities of bee products (Cunha et al., 2004; Sforcin, 2007; Bozkuş & Değer, 2022). Extraction methods can affect the activity of bee products, as different solvents can dissolve and extract different components in other bee products (royal jelly, honey, pollen) just like in propolis (Sforcin, 2007). Therefore, in this study, water, ethanol, and propylene glycol were preferred as solvents for the extraction of bee products, which have not been proven to be harmful to health. In the literature, there is no study in which bee product extracts dissolve best among these solvents and the difference between these solvents is revealed. This study aimed to determine the amounts of total polyphenol and total flavonoid content and antioxidant activities of water, ethanol, 70% ethanol, propylene glycol, and 70% propylene glycol extracts from bee product samples (honey, pollen, royal jelly, propolis) obtained from Hatila Valley and Macahel and to determine the best solvent for the samples of propolis. At the same time, the comparison of the phenolic content and antioxidant activities of all Hatila Valley bee products, the evaluation of Hatila Valley and Macahel propolis, and their mixtures in terms of phenolic content and antioxidant activity are among the objectives of this study. In this sense, this study is the first research in this field.

MATERIAL and METHOD

Preparation of Bee Product Extracts

Extracts of natural bee product samples obtained from the Macahel and Hatila Valley of Artvin province in Turkey were prepared as follows;

Hatila royal jelly: 15 g of Hatila royal jelly sample was weighed and 25 mL of pure water and ethanol were

added to it. These samples were coded as HRJ_W and HRJ_E.

Hatila honey: 15 g of Hatila honey sample was weighed and 25 mL of pure water and ethanol was added. These samples were coded as HH_W and HH_E.

Macahel honey: 15 g of Macahel honey sample was weighed and 25 mL of pure water and ethanol were added to it. These samples were coded as MH_W and MH_E.

Hatila pollen: 2.5 g of Hatila pollen sample was weighed and 25 mL of pure water, ethanol, and propylene glycol were added each. These samples were coded as $HPol_W$, $HPol_E$, and $HPol_{PG}$.

Hatila propolis: 2.5 g of Hatila propolis sample was weighed and 25 mL of pure water, ethanol, propylene glycol, 70% ethanol, and 70% propylene glycol were added to it. These samples were coded as $HPro_W$, $HPro_E$, $HPro_{PG}$, $HPro_E$(70%), $HPro_{PG}$(70%).

Macahel propolis: 2.5 g of Macahel propolis sample was weighed and 25 mL of pure water, ethanol, propylene glycol, 70% ethanol, and 70% propylene glycol were added. These samples were coded as MProW, MProE, MProPG, $MPro_E$(70%), $MPro_{PG}$(70%).

Hatila + Macahel propolis: 1.25 g of Hatila propolis sample and 1.25 g of Macahel propolis sample were weighed and 25 mL of pure water, ethanol, propylene glycol, 70% ethanol, and 70% propylene glycol were added. These samples were coded as $H+MPro_W$, $H+MPro_E$, $H+MPro_{PG}$, $H+MPro_E$(70%), $H+MPro_{PG}$(70%).

Royal jelly and honey samples were vortexed, kept in an ultrasonic bath for 10 minutes, and left to incubate in a shaker incubator at 25°C at 150 rpm for 24 hours with continuous shaking to dissolve. Pollen and propolis samples were vortexed and incubated in a shaker incubator at 60°C at 150 rpm for 24 hours with continuous shaking to dissolve. After 24 hours of incubation, royal jelly, honey, pollen, and propolis extracts were centrifuged at 2057 g for 10 minutes and filtered with filter paper. Thus, 600 mg/mL water and ethanol stock royal jelly and honey extracts, 100 mg/mL water, ethanol and propylene glycol stock pollen extracts, and 100 mg/mL water, ethanol, 70% ethanol, propylene glycol, and 70% propylene glycol stock propolis extracts were prepared. The extracts were stored in the refrigerator at +4 °C in the dark to be used in the necessary experiments.

Determination of the Total Polyphenol Content

The total polyphenol content of the extracts was determined spectrophotometrically according to the modified Folin-Ciocalteu method (Horzic et al., 2009). According to this method, 12.5 µL of bee product extract, 62.5 µL of 1:10 diluted Folin-Ciocalteu reagent, and 125 µL of 20% sodium carbonate solution (Na_2CO_3) were added to a 96-well microplate. After 30 minutes of incubation at room temperature and in the dark, absorbance was measured at 700 nm on a microplate reader. The total polyphenol content was calculated using a calibration curve constructed with gallic acid as standard. The results are given in mg gallic acid (GA) / g sample.

Determination of the Total Flavonoid Content

The total flavonoid content of the extracts was determined using the aluminum chloride colorimetric method (Chang et al., 2002). According to this method, 20 µL of bee product extract, 172 µL of 80% ethanol, 4 µL of 10% aluminum chloride ($AlCl_3$), and 4 µL of 1 M potassium acetate (KCH_3COO) solution were added to a 96-well microplate. After 40 minutes of incubation at room temperature and in the dark, the absorbance was measured at 415 nm in a microplate reader. The total flavonoid content was calculated using a calibration curve constructed with quercetin as standard. Results are given as mg quercetin (Q) / g sample.

Determination of Ferric (Fe^{3+}) Reducing Antioxidant Power

Antioxidant activity with the ferric (Fe^{3+}) reducing power method was determined by modifying the method proposed by (Moreira et al., 2008). 40 µL of bee product extract, 100 µL of 0.2 M phosphate buffer (pH: 6.6), and 100 µL of potassium hexacyanoferrate [$K_3Fe(CN)_6$] were added to each of 1.5 mL microtubes and incubated at 50 °C for 20 minutes in the dark. After incubation, the microtubes were cooled. 100 µL of 10% trichloroacetic acid (TCA) was added to the mixtures in the microtube and this mixture was centrifuged at 3000 g for 10 minutes. 100 µL of the upper phases of the centrifuged samples were taken from the 96-well microplate and 100 µL of distilled water and 20 µL of iron (III) chloride ($FeCl_3$) were added to them. The final mixture was incubated for 5 minutes at room temperature and in the dark. After incubation, the absorbance was measured at 700 nm in a microplate reader. The ferric (Fe^{3+}) reducing antioxidant power was calculated using a calibration curve generated with trolox as standard. Results are given as mg trolox (T) / g sample.

Statistical Analysis

The results were expressed in the form of arithmetic mean ± standard deviation (S.D); n = 4. Data were statistically evaluated using the R program (Version 4.3). To reveal the relationship between the groups, normality analysis was performed, and it was seen that the data were normally distributed. Data were evaluated with one-way analysis of variance (ANOVA) and t-test. Based on the results of this analysis, the Games-Howell post-hoc test analysis was used among the significant groups, and those with $p<0.05$ were considered significant.

RESULTS and DISCUSSION

In this study, bee product samples obtained from Hatila Valley and Macahel were prepared in different solvents and analyzed to evaluate their polyphenol and flavonoid content and antioxidant activities. The results of the determination of the total polyphenol content of the extracts are given as mg GA/g sample in Table 1, the results of the determination of the total flavonoid content are given as mg Q/g sample in Table 2 and the results of the determination of the ferric (Fe^{3+}) reducing antioxidant power are given as mg T/g sample in Table 3.

Table 1. Total polyphenol content of bee product extracts (mg GA/g sample)
Çizelge 1. Arı ürünü ekstraktlarının toplam polifenol içeriği (mg GA/g örnek)

Sample Code	Water Extract	Ethanol Extract	Propylene Glycol Extract	70% Ethanol Extract	70% Propylene Glycol Extract
HRJ	0.50 ± 0.02	0.43 ± 0.01			
HH	0.12 ± 0.02	0.15 ± 0.04			
MH	0.20 ± 0.01	0.19 ± 0.02			
HPol	4.76 ± 0.09	17.24 ± 0.28	18.93 ± 0.63		
HPro	6.59 ± 0.13	21.11 ± 0.07	29.41 ± 0.46	24.24 ± 0.64^{a}	27.01 ± 0.76^{a}
MPro	10.07 ± 0.24^{b}	76.04 ± 0.88^{b}	74.06 ± 1.58^{b}	94.04 ± 1.84^{a,b}	99.12 ± 2.75^{a,b}
H+MPro	8.59 ± 0.15^{b}	67.06 ± 1.54^{b}	66.65 ± 0.92^{b}	68.76 ± 2.11^{a,b}	72.31 ± 3.78^{a,b}

HRJ: Hatila royal jelly, HH: Hatila honey, MH: Macahel honey, HPol: Hatila pollen, HPro: Hatila propolis, MPro: Macahel propolis, H+MPro: Hatila+Macahel propolis, GA: Gallic acid. Each value is expressed as mean ± S.D., n =4. a: It differs significantly in terms of solvent ($p<0.05$). b: It differs significantly in terms of location ($p<0.05$).

Table 2. Total flavonoid content of bee product extracts (mg Q/g sample)
Çizelge 2. Arı ürünü ekstraktlarının toplam flavonoid içeriği (mg Q/g örnek)

Sample Code	Water Extract	Ethanol Extract	Propylene Glycol Extract	70% Ethanol Extract	70% Propylene Glycol Extract
HRJ	0.16 ± 0.03	0.05 ± 0.02			
HH	0.02 ± 0.01	Not detected			
MH	0.03 ± 0.00	Not detected			
HPol	0.79 ± 0.13	2.31 ± 0.03	2.56 ± 0.11		
HPro	0.67 ± 0.13	6.52 ± 0.28	7.98 ± 0.13	5.38 ± 0.26^{a}	4.52 ± 0.30^{a}
MPro	0.43 ± 0.06^{b}	15.51 ± 0.28^{b}	17.59 ± 0.38^{b}	25.93 ± 0.79^{a,b}	16.81 ± 0.59^{a,b}
H+MPro	0.54 ± 0.10^{b}	10.63 ± 0.77^{b}	11.32 ± 0.55^{b}	16.59 ± 0.62^{a,b}	11.49 ± 0.31^{a,b}

HRJ: Hatila royal jelly, HH: Hatila honey, MH: Macahel honey, HPol: Hatila pollen, HPro: Hatila propolis, MPro: Macahel propolis, H+MPro: Hatila+Macahel propolis, Q: Quercetin. Each value is expressed as mean ± S.D., n =4. a: It differs significantly in terms of solvent ($p < 0.05$). b: It differs significantly in terms of location ($p < 0.05$).

Table 3. Ferric (Fe^{3+}) reducing antioxidant power of bee product extracts (mg T/g sample)
Çizelge 3. Arı ürünü ekstraktlarının demir (Fe^{3+}) indirgeyici antioksidan gücü (mg T/g örnek)

Sample Code	Water Extract	Ethanol Extract	Propylene Glycol Extract	70% Ethanol Extract	70% Propylene Glycol Extract
HRJ	0.38 ± 0.04	0.26 ± 0.01			
HH	0.60 ± 0.03	0.13 ± 0.04			
MH	0.69 ± 0.04	0.23 ± 0.01			
HPol	7.96 ± 0.26	36.24 ± 1.12	47.01 ± 0.81		
HPro	11.10 ± 0.15	20.12 ± 0.21	33.43 ± 0.21	25.97 ± 0.42^{a}	33.36 ± 0.62^{a}
MPro	17.82 ± 0.25^{b}	141.74 ± 0.93^{b}	174.76 ± 0.42^{b}	125.21 ± 1.89^{a,b}	170.48 ± 1.80^{a,b}
H+MPro	14.40 ± 0.21^{b}	88.74 ±0.87^{b}	132.82 ± 0.50^{b}	92.27 ± 0.97^{a,b}	107.88 ± 0.69^{a,b}

HRJ: Hatila royal jelly, HH: Hatila honey, MH: Macahel honey, HPol: Hatila pollen, HPro: Hatila propolis, MPro: Macahel propolis, H+MPro: Hatila+Macahel propolis, T: Trolox. Each value is expressed as mean ± S.D., n =4. a: It differs significantly in terms of solvent ($p < 0.05$). b: It differs significantly in terms of location ($p < 0.05$).

Although it varies depending on the solvent used in the study, it was detected in the range of the total polyphenol content of honey samples is 0.12 ± 0.02 – 0.20 ± 0.01 mg GA/g honey, the total flavonoid content is 0.02 ± 0.01 – 0.03 ± 0.00 mg Q/g honey, and the amount of ferric reducing antioxidant power is 0.13 ± 0.04 – 0.69 ± 0.04 mg T/g honey. It was determined that between linden honey obtained from Hatila Valley and chestnut honey obtained from Macahel, chestnut honey has higher phenolic content and antioxidant activity compared to linden honey. Likewise, although it varies depending on the extraction solvent used, it was determined the total polyphenol content of Hatila, Macahel and Hatila + Macahel propolis samples is 6.59

± 0.13 – 99.12 ± 2.75 mg GA/g propolis, the total flavonoid content is 0.43 ± 0.06 – 25.93 ± 0.79 mg Q/g propolis, and the amount of ferric reducing antioxidant power between 11.10 ± 0.15 – 174.76 ± 0.42 mg T/g propolis. Depending on the solvent used, it was determined in the range the total polyphenol content of pollen samples is 4.76 ± 0.09 – 18.93 ± 0.63 mg GA/g pollen, the total flavonoid content is 0.79 ± 0.13 – 2.56 ± 0.11 mg Q/g pollen, and the amount of ferric reducing antioxidant power is 7.96 ± 0.26 – 47.01 ± 0.81 mg T/g pollen, while the highest amounts were detected in the propylene glycol extract. It was determined that the total polyphenol, total flavonoid, and ferric-reducing antioxidant power of royal jelly samples were higher in the water extract than in the ethanolic extract and varied according to the extraction solvent, respectively: 0.43 ± 0.01 – 0.50 ± 0.02 mg GA/g royal jelly, 0.05 ± 0.02 – 0.16 ± 0.03 mg Q/g royal jelly was between 0.26 ± 0.01 – 0.38 ± 0.04 mg T/g royal jelly.

In terms of supporting this study, in various studies with different types of honey, it has been reported that the amount of polyphenolic substances in chestnut honey is higher than in other honey (Al-Mamary et al., 2002; Aljadi & Kamaruddin, 2004; Küçük et al., 2007). Tezcan et al. (2011) determined the total phenolic content of 10 different honey samples obtained from the Black Sea region of Turkey in the range of 0.36 ± 0.02 – 1.14 ± 0.02 mg GA/g honey. From this study, it was concluded that dark-colored honey such as chestnut honey show higher antioxidant values depending on their total phenolic compound content. In another study conducted by Al et al. (2009) using water extracts of 24 different kinds of honey in Romania, they determined that the total polyphenol content of the honey was in the range of 2.00 – 125.00 mg GA/100 g honey and the total flavonoid content was in the range of 0.91 – 28.25 mg Q/100 g honey. Furthermore, they reported that the highest flavonoid content was found in honey with multiple floral sources. In another study conducted by Bertoncelj et al. (2007) with the 7 most common types of honey in Slovenia, it was found that all honey types contain phenolic compounds and have antioxidant activity. The total phenolic content and antioxidant activity showed great differences between different types of honey. It was determined that the total amount of phenolic content and antioxidant activity was the lowest in light-colored acacia and linden honey, and the highest in darker honeys such as fir, spruce, and forest honey. Phenolic compounds have been observed to be responsible for the antioxidant activity of honey and it has been determined that there is a significant relationship between antioxidant activity and phenolic content (Bertoncelj et al., 2007).

Raw propolis is not an easily consumed mixture due to the resin- and wax-like substances in its structure. For this reason, it is necessary to reveal the biologically active components in its structure with the applied extraction method (Pietta et al., 2002; Özkök et al., 2021). Solvents such as ethanol, glycerol, polyethylene/polypropylene glycol, glycerol, and water are used in the extraction process (Özkök et al., 2021). Since different solvents can dissolve and extract different components in propolis, propolis extraction methods can affect propolis activity (Sforcin, 2007). In the study conducted by Bozkuş and Değer (2022), Turkish propolis was collected from different provinces of Turkey and mixed. The water, ethanol, glycerol, dimethyl sulfoxide (DMS, O), and acetone extracts of Turkish propolis were found to have total polyphenol content in the range of 19.7 ± 0.29 – 141.2 ± 9.99 mg GA/g propolis, total flavonoid content in the range of 1.3 ± 0.12 – 55.3 ± 6.63 mg Q/g, and ferric reducing power in the range of 26.2 ± 8.57 – 273.8 ± 11.62 mg T/g propolis. Depending on the amount of phenolic content and antioxidant activity, it was determined that propolis dissolved best in DMSO, followed by ethanol, acetone, glycerol, and water. As in the previous study, in this study, the ethanol extract of propolis was found to be higher in terms of phenolic content and antioxidant activity than the water extract. In a study by Silva et al. (2012) conducted in three different regions of Portugal, hydroalcoholic extracts of propolis were found to have significantly higher polyphenol and flavonoid content compared to methanol and water extracts. Findings like the study conducted by Silva et al. (2012) were also determined in this study, and it was found that 70% ethanol and 70% propylene glycol extracts of propolis generally had higher phenolic content and antioxidant activity than ethanol, propylene glycol, and water. The total polyphenol content ranged from 87.15 ± 4.80 – 277.17 ± 7.50 mg GA/g propolis in hydroalcoholic extracts, 58.61 ± 3.10 – 181.31 ± 4.71 mg GA/g propolis in methanol extracts, and 18.52 ± 1.35 – 72.15 ± 1.20 mg GA/g propolis in water extracts. The total flavonoid content ranged from 25.15 ± 2.53 – 142.32 ± 4.52 mg Q/g propolis in hydroalcoholic extracts, 13.62 ± 2.49 – 135.51 ± 4.18 mg Q/g propolis in methanol extracts, and 6.34 ± 0.55 – 42.30 ± 2.10 mg Q/g propolis in water extracts.

The results obtained from this study were found to be compatible with each other when compared with the data in the literature in terms of the amount of polyphenol and flavonoid content. In addition, since different modified methods have been applied in the literature in terms of ferric-reducing power, it was seen that the results were in line with the literature data when compared only with propolis.

In the study, when Hatila Valley propolis, Macahel propolis, and Hatila + Macahel propolis mixture were compared in terms of polyphenol and flavonoid content and ferric reducing power, it was determined that the order was Macahel propolis > Hatila + Macahel

propolis > Hatila propolis. In addition, it was determined that there was a statistically significant difference between 70% ethanol and 70% propylene glycol and water, and the lowest solubility was in water. Only water, ethanol, and propylene glycol solvents were used in this study. However, the use of different solvents or solvent combinations can increase or alter the extraction of different components, which can affect the antioxidant activity and phenolic content. Therefore, this study is important for the optimization of extraction procedures and the most effective use of bee products, depending on the effects of different solvents on the phenolic content and antioxidant activity of bee products.

Kroyer and Hegedus (2001) found the total polyphenol content of the pollen samples collected from various places and prepared as water, ethanol, and methanol-water (1:1) extracts between 7.4 ± 0.2 – 9.7 ± 0.3 mg GA/g pollen. The total polyphenol content of the pollen mixture was determined as 8.2 mg GA/g pollen. When this study is compared with the study conducted by Kroyer and Hegedus (2001), the total polyphenol content of the aqueous and ethanol extracts of pollen was determined in the range of 4.76 ± 0.09 – 17.24 ± 0.28 mg GA/g pollen, so the results were different.

Although there are many studies on honey, pollen, and propolis in the literature, there are not many studies on the amount of phenolic content and antioxidant activity of royal jelly. Nagai and Inoue (2004) stated that royal jelly is a rich mixture of protein and fatty acids, and the amount of phenolic substances is not very high. In another study, six samples of royal jelly were collected from the Mediterranean region (Morocco, Portugal, and Spain), and the total polyphenol content in their water extracts ranged from 3.0 ± 0.1 –9.0 ± 0.8 mg GA/g royal jelly. The total flavonoid content ranged from 0.1 ± 0.0 – 0.5 ± 0.0 mg Q/g royal jelly (El-Guendouz et al., 2020). When the values in this study were compared with the study conducted by El-Goundez et al. (2020), the polyphenol content of the water extract of royal jelly was determined as 0.50 ± 0.02 mg GA/g royal jelly, while the flavonoid content amount was determined as 0.43 ± 0.01 mg Q/g royal jelly, and the results were found to be compatible with each other.

In this study, when Hatila Valley bee product samples were compared in terms of polyphenol and flavonoid content and antioxidant activity, it was determined that the order was propolis > pollen > royal jelly > honey. There are many studies in the literature showing that propolis has a high amount of polyphenols (Kumazawa et al., 2004; Choi et al., 2006; Ahn et al., 2007; Moreira et al., 2008; Kalogeropoulos et al., 2009). Therefore, it is expected that the phenolic content of propolis is high, and the values obtained were found to be compatible with the literature. Furthermore, this study focused only on the Hatila Valley and Macahel bee products. Therefore, the antioxidant activities and phenolic content of the bee products obtained from other regions and different climatic conditions will be different.

The main components responsible for the antioxidant activity of bee products are flavonoids and phenolic compounds, and these antioxidant effects are closely related to their free radical scavenging activities (Eraslan et al., 2008; Kanbur et al., 2009; Hegazi, 2012). Their composition, which varies according to botanical origin, is also responsible for high levels of antioxidant activity (Kanbur et al., 2009). Therefore, both in the studies conducted in the literature and in this study, it was determined that as the amount of phenolic substance increased, the total antioxidant activity increased in parallel.

This study shows that bee products have phenolic content and antioxidant activity, revealing the potential health benefits of these natural products. This can make a significant contribution to existing knowledge in the field of health and nutrition. In addition, this study highlights the potential of bee products for wider use in diet and health applications.

CONCLUSION and RECOMMENDATIONS

Today, bee products are widely consumed as nutritional supplements and are used in the food, pharmaceutical, and cosmetic industries. It is known that honeybee products such as honey, pollen, propolis, and royal jelly are rich in antioxidants and have various antioxidant effects and beneficial functions in the human body.

In this study, when the Hatila Valley bee product samples were compared in terms of polyphenol and flavonoid content and antioxidant activity, it was determined that the order was propolis > pollen > royal jelly > honey and the values obtained were consistent with the literature. Therefore, for bee products, it is possible to say that as the amount of phenolic substance increases, the total antioxidant activity increases in parallel. Additionally, when Hatila Valley propolis, Macahel propolis, and Hatila + Macahel propolis were compared in terms of phenolic content and antioxidant activity, it was determined that the order was Macahel propolis > Hatila + Macahel propolis > Hatila propolis. In addition, it was determined that there was a significant difference between 70% ethanol and 70% propylene glycol and water, and while the solubility of these solvents was higher, the solvent with the lowest solubility was water. It can be concluded from this study that extracts with less potency are mixed with more active extracts, resulting in lower activity than extracts with high activity, but higher activity than extracts with low activity. This strengthens the idea that such propolis mixtures can be suitable for different applications in terms of various sectors such as the food, medical and

cosmetic industries. Therefore, because of the mixtures obtained, it will be possible to provide maximum benefit from this natural product, as well as increase its value both biologically and economically.

Since Hatila Valley and Macahel bee products have a rich phenolic content and therefore high antioxidant activity, they can potentially be used in applications such as food additives, nutritional supplements, or functional foods in terms of food science and technology. This study also shows that the phenolic content and antioxidant activity of bee products can vary depending on the type of solvent. This can be an important factor in the selection of solvents used in the extraction and processing of bee products, not only in the food industry but also in various sectors such as pharmaceuticals and cosmetics.

In conclusion, this study is important for the conservation and sustainability of local ecosystems, as it provides important information on the local biodiversity of certain regions such as the Hatila Valley and Macahel.

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Author's Contributions

The article has a single author.

Conflict of Interest Statement

The author declares that there is no conflict of interest in this study.

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