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Determination of Total Phenolic Compounds and Antioxidant Activity of Turkish Propolis Extracted by Different Methods

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Abstract: Propolis, also known as bee glue, has a wide range of biological activities and physiological properties. Propolis is a product collected by bees during honey making, and due to its resinous structure it cannot be consumed in its raw form and can be made suitable for consumption by separating unwanted substances. Many methods can be used to extract bioactive compounds from raw propolis, but since there may be differences between these methods in terms of extraction efficiency, an effective method should be used. In this study, the total phenolic content (TPC) and antioxidant activity of propolis samples extracted by microwave assisted ethanolic extraction (MAE), ultrasonic assisted ethanolic extraction (UAE), and supercritical fluid (SCF) extraction methods were investigated. In the experiments, TPC was analyzed by Folin-Ciocalteu method and antioxidant activity tests were performed using DPPH radical scavenging and CUPRAC methods. As a result of the analyses, the TPC of the samples was found in the range of 42.83-83.88 mg GAE/g sample, DPPH radical scavenging activity was in the range of 22.97-37.30 mg TE/g sample and antioxidant capacity obtained by CUPRAC method was in the range of 143.83-259.69 mg TE/g sample. In line with the values obtained, even though MAE and UAE were determined to have the highest phenolic content and antioxidant capacity among the propolis extracts, no significant difference was found between them, but as a result of the literature information, it was understood that the content of propolis varies according to climatic conditions and geographical origin and that MAE and UAE can be preferred in order to increase the extraction efficiency, while the procedures of the methods should be given more importance.

Keywords: Propolis, phenolics, extraction, antioxidant activity, ultrasound.

Farklı Yöntemler ile Ekstrakte Edilmiş Propolis Örneklerinin Toplam Fenolik Madde ve Antioksidan Aktivitelerinin Belirlenmesi

Özet: Arı tutkalı olarak da bilinen propolis, çok çeşitli biyolojik aktivitelere ve fizyolojik özelliklere sahiptir. Propolis, reçinemsi yapısı nedeniyle arılar tarafından bal yapımı sırasında toplanan, ham haliyle tüketilemeyen ve istenmeyen maddelerin ayrıştırılmasıyla tüketime uygun hale getirilebilen bir üründür. Ham propolisten biyoaktif bileşiklerin ekstraksiyonu için birçok yöntem kullanılabilir ancak bu yöntemler arasında ekstraksiyon verimi açısından farklılıklar olabileceğinden etkili bir yöntem kullanılmalıdır. Bu çalışmada, mikrodalga destekli etanolik ekstraksiyon (MAE), ultrasonik destekli etanolik ekstraksiyon (UAE) ve süperkritik sıvı (SCF) ekstraksiyon yöntemleri ile ekstrakte edilen propolis örneklerinin toplam fenolik içeriği (TPC) ve antioksidan aktivitesi araştırılmıştır. Deneylerde, Toplam Fenolik madde içeriği Folin-Ciocalteu yöntemi ile analiz edilmiş ve antioksidan aktivite testleri ise DPPH radikal süpürme ve CUPRAC yöntemleri kullanılarak gerçekleştirilmiştir. Analizler sonucunda örneklerin toplam fenolik madde içeriği 42.83-83.88 mg GAE/g örnek aralığında, DPPH radikal süpürme aktivitesi 22.97-37.30 mg TE/g örnek aralığında ve CUPRAC yöntemi ile elde edilen antioksidan kapasitesi 143.83-259.69 mg TE/g örnek aralığında bulunmuştur. Elde edilen değerler doğrultusunda propolis ekstraktları arasında MAE ve UAE'nin en yüksek fenolik madde içeriğine ve antioksidan kapasiteye sahip olduğu belirlense de aralarında anlamlı bir fark bulunamamış ancak literatür bilgileri neticesinde propolis içeriğinin iklim koşullarına ve coğrafi kökene göre değişkenlik gösterdiği ve ekstraksiyon veriminin arttırılması için MAE ve UAE'nin tercih edilebileceği, yöntemlerin prosedürlerine ise daha fazla önem verilmesi gerektiği anlaşılmıştır.

Anahtar Kelimeler: Propolis, fenolik madde, ekstraksiyon, antioksidan aktivite, ultrason.

Article

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1.Introduction

Propolis, also called bee glue, is a resinous and strongly adhesive natural substance collected by honey bees (*Apis mellifera* L.) from leaves, plant nectar and buds of trees and plants, mixed with enzymes secreted by bees and pollen, and used by bees to smooth the inner walls of the hive and protect the hive as well as to keep the temperature inside the hive constant (Kalogeropoulos et al., 2009, Pasupuleti et al., 2017). At the same time, its antiseptic properties prevent microbial infection of larvae, honey combs and combs and its antibiotic properties protect the health of the hive from disease in a bee, despite the large number of bees in a cramped environment (Kuropatnicki et al., 2013.). Propolis, which have been used as a herbal treatment method since ancient times and can be obtained from many different plant sources, are found in various chemical compositions with the effect of geographical features, vegetation and seasonal changes, and more than 300 compounds have been identified in their content (Ozdal et al., 2019; Potkonjak et al., 2012). In general, its structure consists of 50-60% resin and wax, 30-40% beeswax, 5-10% essential oils, 5% pollen grains, microelements and vitamins (Rufatto et al., 2017). Several studies have shown that propolis extracts have antioxidant, antimicrobial, anti-inflammatory, immunomodulatory, anti-tumor, anti-viral and other biological activity properties (Göç et al., 2018; Altuntas et al., 2023). Especially these properties are attributed to the phenolic acids and alcohols, flavanoids, terpenes and sesquiterpenes found in propolis (Machado et al., 2015; Oldoni et al., 2015). Propolis has limited consumption in its raw form due to its resinous structure, needs to be extracted to make it consumable (Keskin et al., 2018). For this reason, the substances in raw propolis should be removed by extraction and the polyphenolic fractions that contribute the most to its therapeutic properties should be preserved (Erdogan et al., 2011). Especially ethanol extracted propolis is produced and used as raw material in antioxidant capsules, throat sprays, cosmetics and toothpastes and as antibacterial, antiviral, antioxidant, anticancer and anti-inflammatory agents (Aliyazıcıoğlu et al., 2013). Propolis can be extracted by various methods to obtain its substances effectively. In this direction, microwave-assisted extraction (MAE), ultrasonic-assisted extraction (UAE) and supercritical fluid extraction (SCFE) methods can be applied to compare with simple ethanolic extraction (Jang et al., 2009; Haminiuk et al.,2012; Machado et al., 2015). Traditional methods include extraction by simple steam distillation, vacuum distillation, but these methods require high temperature and energy consumption and may result in loss of desired compounds (Tylkowski et al., 2010). Although ethanol extraction is the most widely used method, more and more people are allergic to ethanol, which may limit its use (Biscaia and Ferreira, 2009). However, as Trusheva et al. (2007) indicated that, it is generally accepted to use 70% ethanol as solvent for the extraction of phenolics, as in most commercial products, and microwave-assisted extraction and ultrasonic extraction methods have been developed for faster and more efficient extraction of organic substances. Even though ethanol is the first choice solvent due to its chemical properties in relation to the matrix, ethyl ether, water, methanol and chloroform can also be used for the extraction of certain compounds, while alternative methods such as supercritical fluid extraction can be used in addition to traditional methods because they show the desired properties and can adjust the solvent strength and process selection (Machado et al., 2015).

For solvent selection, reconstituted ethanol was indicated as a better choice for the extraction of phenolic compounds, especially flavanoids, from crude propolis, and it was also proved that ethanol concentration also affected the extraction efficiency, with extracts using 70% ethanol having higher flavanoid and phenolic acid content than those using 96% ethanol (Woźniak, et al., 2019). In addition, in order to increase the extraction efficiency, ethanol-treated samples were extracted and filtered for 15 and 30 minutes under a microwave producing 2450 MHz with a maximum wattage of 800 watts, and for 3 hours with ultrasound as another method (Jang et al., 2009). Furthermore, in microwave-assisted extraction, the propolis solution was microwaved 2 or 3 times for 10 seconds each to reduce the loss due to high temperature, while for ultrasonic-assisted extraction, an ultrasonic bath was used for 10 and 30 minutes to maintain a constant temperature (Trusheva, 2007). In a study, supercritical extraction method was carried out by passing CO_2 at a flow rate of 1 g/minute and 0,5, 10 and 15% ethanol as co-solvent at 150, 200 and 250 bar pressures 3 times for 30 minutes at temperatures of 20, 35 and 50°C, respectively (Paviani et al., 2012). Due to its resinous structure, raw propolis dissolves best in ethanol and is offered to consumers with many products, but the use of propolis extracts obtained with ethanol is limited due to the harmful effects of alcohol consumption and the pungent taste and odor it gives to the final product (Keskin et al., 2019). In addition, pressurized liquid extraction is considered as a rapid analysis method because of its positive impact on the environment due to high input, automation and low solvent consumption, and because pressurized liquids remain liquid at boiling points and allow extraction at high temperatures (Erdogan et al., 2011). The supercritical fluid method, which can be used instead of traditional methods in propolis extraction, is a clean technology and has promising properties such as its capacity to preserve antioxidant properties due to its low temperature (Paviani et al., 2012).

The aim of the present study was to determine the amount of phenolic substances and antioxidant activity of propolis extracts obtained by different methods and to determine the effectiveness of various extraction methods. In this direction, ethanol extraction, microwave assisted extraction, ultrasonic assisted extraction and supercritical CO_2 extraction methods. The antioxidant activities of the obtained propolis extracts were determined by using 1,1-diphenyl-2-picrylhydrazyl (DPPH) radical scavenging method and copper (II) ion reduction based antioxidant capacity method (CUPRAC). In addition, the total phenolic content was determined and all the results were statistically compared and evaluated.

2. Materials and Methods

2.1 Materials

In this experiment, crude propolis sample obtained from local producers from Istanbul, Çatalca region was used in ground form stored in a closed form at -20°C. Ethanol, Folin Ciocalteau reactant, Sodium carbonate (Na_2CO_3), Gallic acid; DPPH (1,1-diphenyl-2-picrylhydrazyl), methanol, Trolox ((±)-6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid), Copper (II) chloride dihydrate ($CuCl_2.2H_2O$), Neocuproin (2,9-

dimethyl 1,10-phenanthroline) and Ammonium acetate (NH_4Ac) were purchased from Sigma Aldrich company (Germany).

2.2 Methods

2.2.1. Preparation of propolis extracts

Firstly, the propolis sample stored at -20°C and stuck together due to moisture was pulverized again with a grinder. For ethanolic, microwave-assisted and ultrasonic-assisted extractions, 3 grams of each were weighed and extracted with 25 mL ethanol/water (70/30) solution for 24 hours at room temperature under sealed conditions. Then, 3 of the extracts were filtered with prepared filter paper and transferred to a falcon tube for the extraction with stirring; 3 were treated in a microwave oven for 10 seconds 3 times at 30 second intervals and then filtered with filter paper and the remaining 3 extracts were subjected to ultrasonic stirring for 10 min each and then filtered with filter paper. The collected extracts were placed in falcon tubes and kept overnight at -20°C and centrifuged at 10000 rpm for 10 minutes to remove the remaining sediment. The extracts were stored sealed at -20°C until analysis was performed. For supercritical extraction, 10 grams of the ground sample was exposed to a 6 g/min flow of CO_2 at 50°C and 5% ethanol/water (70/30) solution in this flow and extracted at initial pressures of 150, 250 and 350 bar for a total of 2 hours 30 minutes.

2.2.2. Determination of total phenolic compound

Spectrophotometric assay was performed using the Folin-Ciocalteu method according to the Altuntas et al. (2023). Samples obtained by 4 different extraction techniques, each with 3 parallels, were diluted 1:50 with ethanol/water (70/30) solution. Then, 200 µL of diluted sample, 1.5 mL of Folin-Ciocalteu reagent diluted 1:10 with distilled water and 1.2 mL of Na_2CO_3 solution (7.5 g Na_2CO_3/100 mL distilled water) were added to each test tube with an automatic pipette. The mixture in the tubes was quickly homogenized by vortex and incubated in the dark for 45 minutes. At the end of 45 minutes, 300 µL of sample was placed in the wells and the absorbance measured at 765 nm. For the preparation of the calibration graph, gallic acid solutions in the concentration range of 0.08-0.2 mg/mL were substituted for the sample in the same way and the calibration curve was obtained and given in terms of standard gallic acid equivalent (GAE).

2.2.3. Determination of DPPH radical scavenging activity

The antioxidant content in propolis extracts was determined by using DPPH method according to the Apak et al. (2004). Samples obtained by 4 different extraction techniques, each with 3 parallels, were diluted 1:50 with ethanol/water (70/30) solution. Then, 100 µL of diluted sample and 2 mL of DPPH solution (3.943 mg/100 mL methanol) were added to each test tube. The mixture in the tubes was quickly homogenized with a vortex and incubated in the dark for 30 min. At the end of 30 minutes, 300 µL of sample was added to the wells and the absorbance was measured at 517 nm. For the preparation of the calibration graph, Trolox solutions in the concentration range of 0.08-0.2 mg/mL were substituted for the sample in the same way and the calibration curve was obtained and expressed in terms of standard Trolox Equivalent (TE).

2.2.4. Copper (II) ion reduction based antioxidant capacity method (CUPRAC)

The determination of antioxidant substances in propolis extracts was carried out using the CUPRAC method according to the Altuntas et al (2023). Samples obtained by 4 different extraction techniques, each with 3 parallels, were diluted 1:50 with ethanol/water (70/30) solution. Then, 100 µL of diluted sample, 1 mL of $CuCl_2.2H_2O$ solution (0.1748 g/100 mL distilled water), 1 mL Neocuproin (0.156 g/100 mL ethanol) and 1 mL NH_4Ac (7.708 g/100 mL distilled water) were added to each test tube. The mixture in the tubes was quickly homogenized by vortexing and incubated in the dark for 30 minutes. At the end of 30 minutes, 300 µL of sample was placed in the wells and absorbance was measured at 450 nm. For the preparation of the calibration graph, Trolox solutions in the concentration range of 0.08-0.2 mg/mL were substituted for the sample in the same way and the calibration curve was obtained and expressed in terms of standard Trolox Equivalent (TE).

2.2.5. Statistical analysis

Antioxidant activation and phenolic substance results obtained from the analyses were compared using one-way analysis of variance (ANOVA) with Minitab® 18 statistical software program and the accuracy and significance of the results were examined. Tukey's comparison test was used to examine the differences between the results at 95% confidence interval. Regression analysis was also performed using Microsoft Excel 2016 program.

3. Results and Discussion

3.1. Total Phenolic Compound

The amounts of total phenolic compound (TPC) of propolis extracts obtained by various methods were expressed in terms of Gallic acid equivalent (GAE) per gram sample. The TPC of the samples were calculated as the average and standard deviation value and is shown in Table 3.1.

Table 3.1. Results of total phenolic content.
Tablo 3.1. Toplam fenolik fadde içeriği sonuçları.

Extraction method	TPC (mg GAE/g sample)
Ethanolic Extraction	71.64±1.2
MA Extraction	83.88±2.3
UA Extraction	83.62±3.9
SCF Extraction	42.83±10.3

According to the results, TPC of the samples was found to be 71.64, 83.88, 83.62 and 42.83 mg GAE/g sample for ethanolic extraction, microwave assisted extraction, ultrasonic assisted extraction and supercritical CO_2 extraction, respectively. The amount of phenolic substances was found to be close between the methods and relatively low in the sample with supercritical CO_2 extraction. According to ANOVA one-way analysis of variance, there was no significant difference between the propolis extracts in TPC. When the results were analyzed with Tukey test at 95% confidence interval, the differences between the samples were not statistically significant ($p<0.05$). The results of the ANOVA test analysis of variance are given in Table 3.4. The lack of significant differences between the methods can be explained by the fact that no precautions were taken to keep the temperature constant during the experiments and the loss of antioxidant and phenolic substances due to the sensitivity of the

compounds in propolis to heat and therefore the yield of microwave and ultrasonic assisted extractions did not lead to a more significant difference. As observed by Jang et al. (2009), some degradation of some substances may have occurred in this study as a result of exposing the sample to excessive heat, such as the lower concentration of phenolics after 14 days of extraction than after 12 hours, which may have been due to dissolution or degradation of phenolic substances.

Differences between supercritical extraction conditions and other extraction conditions should also be taken into account when comparing the results. According to Silva et al. (2012), the phenolic content of hydro-alcoholic (80% ethanolic) extracts varied from 87.15±4.80 to 277.17±7.5 mg/g from region to region and these values were lower in methanolic and aqueous extraction methods. The values found in this study were between 42.83 and 83.88 mg/g and considering the geographical factors, the phenolic content values of the samples prepared in 70% ethanol were relatively lower. On the other hand, in another study, the total phenolic content of propolis samples extracted with pure ethanol was found to be 10.673 mg/g (Temizer et al., 2017). In line with these data, it can be stated that the results obtained as higher are not completely shaped by error sources but stand out due to the effect of regional differences.

In another study conducted to measure the effectiveness of ultrasonic (UAE) and microwave-assisted extractions (MAE), the total phenolic content varied between 35.9-52.0 mg/g and 24.4-40.4 mg/g, respectively, while the extraction with 70% ethanol was 43.0-44.0 mg/g, moreover, it was observed that the amount of phenolics in the samples exposed to MAE for a longer time was lower (Trusheva et al., 2007). Therefore, it can be said that the data obtained with ultrasonic and microwave-assisted extractions are at an acceptable level. In a study conducted with supercritical extraction, the total phenolic amount varied between 62.21-80.3 mg/g depending on the amount of ethanol passed as a co-solvent and it was determined that the yield of 1% ethanol was higher than 2% ethanol (Machado, 2015). Even though the values found in this study are relatively low, they are acceptable because the amount of phenolics varies depending on the solvent ratio, pressure and temperature.

3.2. DPPH Radical Scavenging Activity

In the determination of antioxidant activity, DPPH radical scavenging method was used and the required calibration graph was created using Trolox standard. Antioxidant activity values of propolis extracts were given in terms of Trolox equivalent (TE). The results of the samples were given as average values and strandard deviation, and antioxidant activities were indicated according to the extraction method as seen in Table 3.2.

Table 3.2. Results of the DPPH radical scavenging activity.
Tablo 3.2. DPPH radikal süpürme aktivitesi sonuçları.

Extraction method	DPPH (mg TE/g sample)
Ethanolic Extraction	37.30±4.2
MA Extraction	35.65±2.3
UA Extraction	35.31±0.4
SCF Extraction	22.97±4.8

The antioxidant activity determination with the concentrations calculated from the absorbances measured by removing DPPH radical was found as 38.30, 35.65, 35.31 and 22.97 mg TE/g sample for ethanolic extraction, microwave assisted extraction, ultrasonic assisted extraction and supercritical extraction, respectively. Among the methods, DPPH radical scavenging antioxidant activity was found at close values and relatively low in the sample subjected to supercritical CO_2 extraction. There was no significant difference in antioxidant capacity between propolis extracts according to ANOVA one-way analysis of variance with $p>0.05$. When the results were analyzed by Tukey test at 95% confidence interval, the differences between the samples were not statistically significant as in the determination of total phenolic matter. Anova test analysis of variance results are given in Table 3.4.

The reasons for the lack of a significant difference between the findings obtained in the determination of antioxidant activity, as in the analysis of total phenolic matter, can be attributed to uncontrollable reasons such as losses due to high temperature, inability to use the extraction methods in the most effective way and other environmental conditions. In a study in which the extraction was carried out with 60% ethanol and homogenized every day in a dark environment for 6 days, DPPH radical scavenging antioxidant activities were found as 135, 151 and 454 mg TE/g in different propolis samples from Turkey (Yesiltas, 2014). The fact that these values were much higher than the values obtained in the study gave an idea about the efficiency of the extraction and it was observed that a longer extraction in a controlled environment could increase the efficiency. As a result of the analysis of ethanolic extracts of propolis, DPPH radical scavenging capacity was found to be 0.33-1.11 mmol TE/g in samples obtained from various regions (Kalogeropoulos et al., 2009). When the values obtained from this study were converted to mmol TE/g, an antioxidant capacity between 0.14-0.17 was determined and it was understood that lower results were obtained.

3.3. Copper (II) Ion Reduction Based Antioxidant Capacity Method (CUPRAC)

The average values of the samples for CUPRAC method based on copper (II) ion reducing activity were taken and the antioxidant activities were shown in Table 3.3 according to the extraction method.

Table 3.3. Results of the CUPRAC tests.
Tablo 3.3. CUPRAC testi sonuçları.

Extraction method	CUPRAC (mg TE/g sample)
Ethanolic Extraction	239.37±16.7
MA Extraction	250.41±7.3
UA Extraction	259.69±3.5
SCF Extraction	143.83±4.9

Antioxidant activity determination with concentrations measured using the CUPRAC method was found to be 239.37, 250.41, 259.69 and 143.83 mg TE/g sample for EE, MAE, UAE and SCFE, respectively. Among the methods, the antioxidant activity determined by CUPRAC method was found to be relatively low in the samples subjected to supercritical fluid extraction. There was no significant difference in antioxidant activity between propolis extracts according to ANOVA one-way analysis of variance with $p>0.05$. When the results were analyzed by Tukey test at 95% confidence interval, the differences between the samples were not statistically significant as in the determination of TPC and DPPH method. The results of the analysis of variance

Anova test are given in Table 3.4.

The CUPRAC method, which generally gives higher results among antioxidant analysis methods, gave a higher result than the other method in this study. In a study conducted with extracts from two different regions, antioxidant activities measured by CUPRAC method were found to be 12-35 mM TE/100 mL (Daraban et al., 2019). In order to compare the studies, the values obtained in this study were converted to mM TE/100 mL and found to be between 38-41 and it was observed that the values were relatively higher. In a study in which TPC was determined between 143-380 mg GAE/g, the antioxidant capacity determined by CUPRAC method was found to be 575-1433 mg TE/g (Yeşiltaş, 2014). In this study, the amount of phenolic substances obtained was found to be 42.83-83.88 mg GAE/g and antioxidant capacity values were found as 143.83-259.37 mg TE/g, although the amount of antioxidant capacity was found to be low, there was no discrepancy between the data.

In our study, although there were quantitative differences in the extraction of phenolic compounds with different polarity ethanol and carbon dioxide, there was no statistically significant difference. The results of Haminiuk et al. (2014), showed that higher contents of phenolic compounds were not obtained either with the most or the least polar solvents where ethanol, methanol and water used (Haminiuk et al., 2014). In line with the previous studies examined, phenolic substances and antioxidant activities were found to be at acceptable values in propolis, although it is understood that they may be at higher amounts, and a linearly increasing relationship was observed between the amount of phenolic substances and antioxidant activity. The antioxidant activity in propolis, which removes unstable free radicals that cause a number of diseases such as aging and immune system disorders in the human body, was determined by removing free radicals added to a certain extent, and the beneficial effect of propolis on health was once again understood.

3.4. Relationship Between Total Phenolic Compounds and Antioxidant Activity Results

In order to examine the relationship between total phenolic compounds and antioxidant activities of the extracts, regression analysis was performed separately for DPPH and CUPRAC methods and the data obtained are summarized in Table 5.4. In addition, the results of the analysis of variance (ANOVA) test are also given in Table 3.4.

The regression analysis results between TPC and DPPH method; TPC and CUPRAC method, respectively, are shown in Table 5.4. The high r^2 values obtained as a result of the comparison of the antioxidant activity methods with the TPC prove the accuracy of the relationship between the experiments.

4. Conclusion

In this study, the total amount of phenolic substances and antioxidant activities of propolis extracts obtained by ethanolic extraction, UAE, MAE and SCFE methods were investigated.

Table 3.4. Relationship between TPC and antioxidant activity results.
Tablo 3.4. Toplam fenolik madde içeriği ile antioksidan aktivite sonuçları arasındaki ilişki.

Extraction Method	TPC (mg GAE/g sample)	DPPH (mg TE/g sample)	CUPRAC (mg TE/g sample)
Ethanolic Extraction	71.64±7.2	37.30±4.2	239.37±16.7
MA Extraction	83.88±2.3	35.65±2.3	250.41±7.3
UA Extraction	83.62±3.9	35.31±0.4	259.69±3.5
SCF Extraction	42.83±0.3	22.97±4.8	143.83±14.9
P value obtained from ANOVA	*0.707*	*0.081*	*0.561*
Regression coefficient (r^2)	-	0.83	0.97

The effectiveness of the methods was compared by determining the content of phenolic substances. In line with the experiments, it was observed that there was no significant difference between the phenolic substance and antioxidant values obtained by extraction in four different methods and their activities were statistically similar. It was revealed that ultrasonic and microwave assisted extractions gave higher values in terms of phenolic matter and antioxidant capacity among the methods. It is understood that various extraction techniques, especially ultrasonic and microwave assisted extraction techniques can be used for the most effective use of these properties.

The efficiency of the four different extraction methods used in this study generally covered the determination of phenolic substances. However, the fact that the yield did not change significantly as a result of ultrasonic and microwave assisted extractions, although it increased, showed that further research should be carried out for these two methods and the conditions affecting the propolis content should be carefully examined and extraction processes should be carried out in accordance with these conditions. In addition, for a more effective and accurate comparison, the amount of solvent, temperature and similar conditions used for samples obtained by supercritical extraction should be applied in the same way for simple and conventional extractions and the differences between them should be indicated.

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6. Conflicts of Interest

The authors declare no conflict of interest.

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