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ARAŞTIRMA MAKALESİ / RESEARCH ARTICLE

EVALUATION OF THE POLYPHENOL CONTENTS AND ANTIOXIDANT ACTIVITY OF PROPOLIS EXTRACTED WITH DIFFERENT TECHNIQUES

Farklı Tekniklerle Ekstrakte Edilen Propolisin Polifenol İçerikleri Ve Antioksidan Aktivitelerinin Değerlendirilmesi

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ABSTRACT

Propolis is classified as an opotherapeutic medicine due to the botanical origin of the resins. The chemical composition of propolis is greatly influenced by the honeybee species, botanical source and extraction techniques. Within this frame, we compared the same propolis' polyphenol contents and antioxidant activities prepared with different techniques. Four types of extracts were prepared. The first type was prepared classically by ethyl alcohol (*POH)*. The second and third types were extracted by sterile distilled water kept as both sterilised (*PS)* and non-sterilized (*PN)*. The fourth one was prepared with full vacuumed and dried propolis with honey (*PH*). The antioxidant activity of extracts was evaluated with DPPH radical scavenging, ABTS radical cation scavenging, Cupric ion reducing antioxidant capacity. Also total phenolic and flavonoid content of extracts were investigated. POH extract showed significantly high content of total phenol and flavonoids which followed by PN, PS and PH. POH showed approximately two times higher activity on DPPH radical (IC50=4,39µg/mL) compared with quercetin as references. The highest activity on DPPH is shown by POH with 4,39 µg/mL of IC50 value which was followed by aqueous extracts 18,08. The lowest activity was shown by PS with 4,39 µg/mL of IC50 value. The highest scavenging activity against ABTS radical cation was shown by POH (73,37 mg TE/g extract) and the lowest activity was shown by PS (34,21 mg TE/g extract). According to the results, the new aqueous extraction technique is promising with relatively high polyphenol contents and antioxidant activities. Also honey with propolis can be an alternative product, although it has relatively lower values of antioxidant activity.

Keywords: Propolis, Antioxidant, Honey, A new aqueous *extraction*

ÖZ

Propolis, arılardan gelen organik salgıların karmaşık kimyasal bileşimi nedeniyle opoterapotik bir ilaç olarak sınıflandırılır. Propolisin kimyasal bileşimi, bal arısı türü, botanik kaynak ve ekstraksiyon tekniklerinden büyük ölçüde etkilenmektedir. Bu çerçevede toplanan ham propolisten farklı tekniklerle hazırlanmış ekstaraktlar, polifenol içerikleri ve antioksidan aktiviteleri bazında karşılaştırılmıştır. Dört tip ekstrakt hazırlanmıştır. Birinci tip klasik olarak etil alkol (POH) ile hazırlanmıştır. İkinci ve üçüncü tipler, hem sterilize edilmiş (PS) hem de sterilize edilmemiş (PN) olarak tutulan steril damıtılmış su ile özütlenmiştir. Dördüncüsü ise tamamen vakumlanmış ve kurutulmuş ballı propolis (PH) ile hazırlanmıştır. Ekstraktların antioksidan aktivitesi, DPPH radikal süpürücü, ABTS radikal katyon

süpürücü, Kuprik iyonu azaltan antioksidan kapasite, toplam fenolik içerik ve toplam flavonoid içerik deneyleri ile değerlendirilmiştir. POH özütü, önemli ölçüde yüksek toplam fenol ve flavonoid içeriği göstermiş ve bunu PN, PS ve PH izlemiştir. POH, referans olarak kuersetin ile karşılaştırıldığında DPPH radikali (IC50=12,24 µg/mL) üzerinde yaklaşık iki kat daha yüksek aktivite göstermiştir. DPPH üzerindeki en düşük aktivite, 56,72 µg/mL IC50 değeri ile PS tarafından gösterilmiştir. En yüksek aktivite POH (271,75 mg GAE/g ekstraktı) tarafından gösterilirken, bunu su ekstraktları takip etmiş ve en düşük değer HP'ye ait olarak tespit edilmiştir. ABTS radikal katyonuna karşı en yüksek süpürme aktivitesi POH (73,37 mg TE/g özü) ve en düşük aktivite PS (34,21 mg TE/g özü) ile gösterilmiştir. Sonuçlara göre, nispeten yüksek polifenol içerikleri ve antioksidan aktiviteleri ile yeni su ekstraksiyon tekniğinin umut vericidir. Ayrıca propolisli balın, nispeten daha düşük değerlere sahip olmasına rağmen alternatif bir ürün olarak tüketilebileceği düşünülmektedir.

Anahtar kelimeler: Propolis, Antioksidan, Bal, Su bazlı yeni bir ekstraksiyon

GENİŞLETİLMİŞ ÖZET

Amaç: Bu çalışmanın amacı, besin takviyesi olarak kullanılmakta olan propolisin su bazlı ekstraktından hazırlanan üç farklı ürünün (balla karıştırılmış propolis, su ile ekstrakte edilmiş ve sonrasında sterilize edilmiş ve edilmemiş propolis), geleneksel yöntemlerden biri olan alkol içerisinde çözme ile üretilmiş propolis ile toplam fenol, flavonoid içeriğini ve antioksidan aktivitesini karşılaştırmaktır.

Gereç-Yöntem: Ham propolis, 2018 ve 2019 yıllarında Türkiye'de Tunceli-Ovacık bölgesinden 9 alt bölgede 12 farklı arılıktan toplanmıştır. Ham proprolisin alkol bazlı ekstrasyonunda %99 saf etil alkol kullanılmıştır (POH). Propolis, tam karanlık koşullarda %10 ham propolis ile %90 çözücü kombinasyonunda 4 hafta boyunca alkolde bekletilmiştir. Su bazlı hazırlanan propolis için T.C. Tarım ve Orman Bakanlığı tarafından 2020 yılında tescil edilen (kayıt no: 007395.20.03.2020) yöntemle pH'ı 4.6 olan steril distile su ile ekstraksiyon gerçekleştirilmiştir. Buradan elden edilen ürün sonrasında steril edilmiş (PS) ve edilmemiş (PN) olarak şişelenmiş, ayrıca aynı yöreden toplanan bal ile karıştırılarak (dördüncü bir ürün olarak-PH) saklanmıştır. Ekstraktların antioksidan aktivitesi, DPPH radikal süpürücü, ABTS radikal katyon süpürücü, Kuprik iyonu azaltan antioksidan kapasite, toplam fenolik içerik ve toplam flavonoid içerik deneyleri ile değerlendirilmiştir.

Bulgular ve Sonuç: Toplam fenol ve flavonoid içerikleri karşılaştırıldığında; alkol ile hazırlanan örnek, su ile hazırlanan örneklerde, steril edilmeyen örnek, steril edilen örnek ve bal ile hazırlanan karışım olarak bir sıralama bulunmuştur. POH, referans olarak kuersetin ile karşılaştırıldığında DPPH radikali (IC50=12,24 µg/mL) üzerinde yaklaşık iki kat daha yüksek aktivite göstermiştir. DPPH üzerindeki en düşük aktivite, 56,72 µg/mL IC50 değeri ile su ile hazırlanan steril örnekte tarafından görülmüştür. En yüksek aktivite POH (271,75 mg GAE/g ekstraktı) tarafından gösterilirken, bunu su ekstraktları takip etmiş ve en düşük değer HP'ye ait olarak tespit edilmiştir. ABTS radikal katyonuna karşı en yüksek süpürme aktivitesi POH (73,37 mg TE/g özü) ve en düşük aktivite PS (34,21 mg TE/g özü) ile gösterilmiştir. Sonuçlara göre, nispeten yüksek polifenol içerikleri ve antioksidan aktiviteleri ile yeni su ekstraksiyon tekniğinin umut vericidir. Ayrıca propolisli balın, nispeten daha düşük değerlere sahip olmasına rağmen alternatif bir ürün olarak tüketilebileceği düşünülmektedir.

INTRODUCTION

Propolis is the general name of the resinous substance collected by honey bees from different plant sources or wounds on plants (Çelemli Gençay 2013). These resins then mixed with their waxes and β-glucosidase in the hive. The new material is using by the bee community to coat and strength the inside walls of their hive (Zhang et al. 2011, Simone-Finstrom et al. 2017). Propolis is also used to seal holes, cracks, narrow the burrow entrance hole to prevent the entry of invasive insects, and reduce microbial growth inside the hive.
It prevents the humidity and temperature in the interior of the hive to be kept constant as a barrier to factors such as wind and precipitation (Bhargava et al. 2021).

The chemical composition of propolis can be qualified as complex. Approximately three hundreds of compounds have been identified lately in propolis samples of different origins (Pereira et al. 2015, Salgueiro and Castro 2016, Lorenzon et al. 2018). Among these compounds, flavonoids (flavonols, flavanones, flavanonols, chalcones, dihydrochalcones, isoflavones, isodihydroflavones, flavans and neoflavonoids) are the leading active propolis components, which are responsible for a large part of their biological activity (Huang et al. 2014, Hernandez Zarate et al. 2018, Santos-Buelga et al. 2017). Total flavonoid content can be used as an index to evaluate the quality of propolis. If the flavonoid content is less than 11%, it is classified as low quality, if it is 11-17% and higher, it is classified as good quality and high quality, respectively. (Gardana et al. 2007). Propolis consists of resinous substances such as flavonoidaglycones, phenolic acids and their esters, waxes, which are a mixture of long-chain non-polar compounds, essential oils, pollen, vitamins, minerals, amino acids and fatty acids (Alvarez-Suárez et al. 2010, Escuredo et al. 2013). Propolis contains a large number of enzymes such as adenosine triphosphatase, acid phosphatase, glucose-6-phosphatase and succinic dehydrogenase (Lotfy 2006, Pasupuleti et al. 2017). Propolis also contains β-glucosidase which hydrolyzes flavonoid glycosides into aglycones (Li et al. 2018, Araghi et al. 2021). Propolis is described as an opotherapeutic drug due to the complex chemical composition of the organic secretions of honey bees. (Zenebom and Pascuet 2005, Machado et al. 2017). Raw propolis is very difficult to use due to its hard, brittle, poor solubility and low oral bioavailability (Elbaz et al. 2016, Dallabona et al. 2020). In the last three decades, the number of studies on the pharmacological and chemical properties of propolis has increased. From the end of the 20th century, what is known about the chemical properties of propolis began to change. By the 1960s, it was known that propolis was chemically complex, and that it was a very stable compound. It has been understood that the chemical composition of propolis can vary according to bee species, botanical origin and extraction methods. The quality of propolis depends on many biotic and abiotic variables such as beekeeping practices, product processing and storage conditions. (EFSA 2010). The age, gender, physiology and sometimes lifestyle of the user can also affect the effect of propolis on human health (Dezmirean et al. 2021).

The chemical composition of propolis may also vary with the eco-flora in the region where it is produced (Salatino et al. 2005). Honey bees use secretions from various parts of plants to produce propolis. Due to these differences in plant origin, the complexity and chemical diversity of propolis is directly related to the eco-flora of the area where propolis is produced (Bankova et al. 2014).

This leads to the classification of propolis as different types (e.g. 14 types in Brazil). Propolis-specific components produced in temperate regions of the world are flavonoids without B-ring substituents such as chrysin, galangin, pinocembrin, pinobanksin (Christov et al. 2006, Salatino et al. 2011, Santos-Buelga et al. 2017).

Caffeic acid phenethyl ester, one of the main components of temperate zone propolis, has broad biological activity, including inhibition of nuclear factor κ-B. In general, it inhibits cell proliferation by stopping the cell cycle and inducing apoptosis (Huang et al. 2014, Ristivojević et al. 2015).

In tropical region propolis, especially Brazilian green propolis (CAS: 9009-62-5), the dominating chemical components are prenylated phenylpropanoids (e.g., artepillin C) and diterpenes (Midorikawa et al. 2001, Paviani et al. 2010). The common characteristic of propolis produced in the Pacific and African regions is geranyl flavanones (Bankova 2005, Salatino et al. 2011). Anatolian propolis also differs in terms of its chemical content in parallel with the sources used by honey bees. The propolis derived from *Ferula* spp, *Pinaceae* spp and *Cupressaceae* spp are rich in monoterpenes, sesquiterpenes and diterpenes. (Uzel et al. 2005).

There are several different methods (not the solvents) of extraction models that occur for propolis (Bankova et al. 2021). Some of these methods are commercial while some are just for research. Ethanol is the most commonly used solvent as it has greater extraction capacity. While the propolis removes approximately 50-60% of its components, the classical aqueous extraction method only removes 10% (Park 1998).

The aim of this study is to evaluate the total phenol and flavonoid content antioxidant activity of ethanol extract of propolis, propolis mixed with honey, aqueous sterilised and non-sterilized extract of propolis and assess the chemical composition of it.

MATERIALS AND METHODS

Preparation of propolis

The raw propolis was collected from Tunceli-Ovacık region in Turkey from 12 different apiaries in 9 sub-localities during 2018 and 2019. In the first step, all of the raw propolis was broken or grated and divided into smaller pieces. Then propolis was washed down with water and the mixture was cooled slowly. During this cooling wax and resin were separated from the mixture with sieves, propolis was moved to a separate area. The cleaned propolis was used for extraction via different solvents.

Extraction techniques

Four types of extracts were prepared. All the extractions were made in registered GMP production laboratories where all the equipment and methods were fully calibrated and validated in 2020 and 2021.

Ethanol extraction: For ethyl alcohol extraction, 99% pure double filtered absolute ethyl alcohol produced by (Botafarm Ltd.) were used. The propolis was extracted in the alcohol for 4 weeks in combination of 10% raw propolis to 90% solvent in full dark conditions.

Aqueous extraction: The propolis was extracted by sterile distilled water with pH of 4.6 with a special method developed by Dr. Aytekin which is registered by the Republic of Turkey Ministry of Agriculture and Forestry in 2020 (Reg. No: 007395.20.03.2020). This method includes raw steps of the following; It was heated at 45-50°C (12 hours), then left to infuse (2 hours), stirred from time to time. Distilled water propolis ratio was used as 10%. The mixture was cooled slowly, the acidity was lowered and kept for 12 hours in dark conditions. It was brought to normal pH and filtered four times. The filtrate was collected after each filtration. It was heated in a separate bowl and filtered again. The aqueous mixture obtained here was combined with the other mixture. This mixture was stirred from time to time and kept in the oven at 45°C for a while. The mixture was drained. Raw filtered. Then the mixture was divided into sterilised (S1) and non-sterilized (NS1) groups. NS1 group was bottled and covered with a lid immediately and S1 is bottled after sterilisation. One bottle is open and sterilised and revored in Class 10000 Clean room the other is covered with airtighed cups. We used the less effective type of sterilisation in closed glass vials and used hot vapour under high pressure which is 121°C.

Honey mixed with propolis: The cleaned propolis was dried and full water was evaporated by an industrial type of Vacuum Freeze Dryer model GZL2 (2012) in 12h F-12hD-6hFD conditions. The full vacuumed and dried propolis were mixed with honey (10% propolis and 90% honey from the same apiaries) and kept at room temperature until analysis. For antioxidant assays 5 gr of honey mixed propolis were macerated with 99% ethanol (100 mL) at room temperature for a day and then were filtered. Solvent from the samples was removed using a rotary evaporator.

Antioxidant activity

Determination of total phenolic contents (TPC): TPCs of different propolis and mixture extracts were evaluated by the Folin-Ciocalteu's colorimetric methodhodology (Slinkard and Singleton, 1977) using regression equation of calibration curve (Y= 0.0114x + 0.1427, R2: 0.9986) and expressed in gallic acid equivalents: (GAE) / 1g of extract. Folin-Ciocalteau's reagent was diluted with distilled water (1:10) and then 100 µL of solution was mixed with 20 µL of propolis extract. Then different concentrations of reference dissolved in ethanol. Finally, 80 µl %7.5 of Na_2CO_3 solution was added. Final mix was left at room temperature for two hours in the dark. Then at 765 nm the absorbance was measured.

Determination of total flavonoid contents: Aluminium chloride colorimetric methodhodology (Chang et al., 2002) was used. Flavonoid content of propolis extracts were calculated according to the equation (y=0.0055x+0.1098, R2=0.9983) obtained from the calibration curve as quercetin equivalent (mg/g extract). 25 µl of extract and different concentrations of reference dissolved in ethanol were mixed with 75 µl of 95% ethanol, 5 µl of 10% $AlCl_3$, 5 µl of 1 M KCH_3COO and 140 µl of distilled water. After incubation at room temperature for an half hour, the absorbance of the reaction mixture was measured at 415 nm. Quercetin was used as reference.

DPPH radical scavenging capacity assay: According to Brand-Williams et al. (1995). DPPH radical scavenging capacities of propolis extracts were tested at 12.5, 25, 50 and 100 µg/mL concentrations. The inhibition percentage of extracts on DPPH were calculated. 1mM DPPH reagent (1,1-diphenyl-2-picrylhydrazyl) was solved in ethanol and then 50 µL of this solution was mixed with 150 µL of different concentrations of the propolis extract and Quercetin as reference. The reaction mixture was incubated at room temperature for an half hour in the dark and at 517 nm the absorbance was measured. Radical scavenging activity was expressed as the inhibition percentage and was calculated using the following formula: Inhibition %=[(A_{blank}−A_{sample})/A_{blank}]×100, where A_{blank} is the absorbance of the blank (containing ethanol instead of sample) and A_{sample} is the absorbance of the extracts or reference. IC_{50} value for each extract was calculated from the plotted graph of scavenging activity against the concentrations of the sample.

ABTS radical cation scavenging activity assay: According to Re et al. (1999), ABTS was dissolved in water to a 7 mM concentration. $ABTS^{\cdot+}$ was generated by reacting ABTS stock solution with 2.45 mM $K_2S_2O_8$ and allowing the mixture to stand in the dark at room temperature for 12-16 hours. $ABTS^{\cdot+}$ solution was diluted with ethanol to an absorbance of 0.700 ± 0.02 nm at 734 nm before use. 200 µL of this solution was mixed with 20 µL of the extract and different concentrations of reference dissolved in ethanol. The reaction mixture was incubated for 6 minutes at room temperature in the dark, then absorbance was measured at 734 nm. ABTS radical cation scavenging activities of the propolis extracts were determined in accordance with the equation (y=0,9051x+2,9872, R^2=0.995) of Trolox calibration curve.

Cupric ion reducing antioxidant capacity (CUPRAC) assay: According to Apak et al. (2004), 50 µL of CuCl2 solution (1.0x10- 2 M), 50 µL of neocuproine solution (7.5x10-3 M), 50 µL of NH4Ac buffer solution at pH 7.0 (1.0 M) were mixed and then 25 µL of extracts or different concentrations of reference (800 µg/mL to 25 µg/ mL) and 25 µL of distilled water were added to the initial mixture, separately. The absorbance of the final solution was measured at 450 nm after 30 minutes keeping at room temperature in the dark. Cupric ion reducing antioxidant capacities of the propolis extracts were determined according to the equation (y=0.014x+0.0569, R2=0.9998) as gallic acid equivalent (mg/g extract).

All total phenol, flavonoid content and antioxidant assay was carried out in three repeats.

Statistical analysis

Principal component analysis (PCA) was performed with PAST (Hammer et al. 2001). The four groups were evaluated with their five-character sets.

RESULTS

According to our study, the amount of total phenolics and flavonoid contents in propolis extracts varied from 58,09 ± 2,58 (PH); 286,95 ± 39,1 (POH); 125,61 ± 1,42 (PS); 142,24 ± 16,79 (PN) mg GAE/g extracts and 95,73 ± 9,55; 444,33 ± 20,82; 103,21 ± 21,24; 106, 76± 19,29 mg QE/g respectively (Table 1). These results clearly demonstrated that POH extract showed significantly high content of total phenol and flavonoids which flowed by PN and PS. Lowest amount of total phenol and flavonoid belong to honey-propolis composition extract. As presented in Table 1, the amount of total phenolics and flavonoid contents in propolis extracts varied from 58,09 to 286,95 mg GAE/g extracts and from 95,73 to 444,33 mg QE/g respectively. These results clearly demonstrated that POH extract showed significantly high content of total phenol and flavonoids which flowed by PN and PS. Lowest amount of total phenol and flavonoid belong to honey-propolis composition extract.

Table 1. Total phenolic and flavonoid contents of propolis extracts (POH: ethyl alcohol, PS: extracted by sterile distilled water kept as sterilized, PN*:* extracted by sterile distilled water kept as non-sterilized, PH: prepared with full vacuumed and dried propolis with honey *GAE: Gallic acid equivalent,**QE: Quercetin equivalent)

Tablo 1. Propolis ekstraktlarının toplam fenolik ve flavonoid içerikleri (POH: etil alkol, PS: steril olarak saklanan steril damıtılmış su ile ekstrakte edilen, PN: sterilize edilmemiş olarak saklanan steril damıtılmış su ile ekstrakte edilen, PH: tamamen vakumlanmış ve bal ile kurutulmuş propolis ile hazırlanan *GAE: Gallik asit eşdeğeri,**QE: kuersetin eşdeğeri)

Extracts	Total phenolic content (mg GAE*/g extract)	Total flavonoid content (mg QE**/g extract)
PH	58,09 ± 2,58	95,73± 9,55
POH	286,95 ± 39,1	444,33± 20,82
PS	125,61 ± 1,42	103,21± 21,24
PN	142,24 ± 16,79	106,76± 19,29

In ABTS and DPPH assay honey mixed propolis, after ethanol extract of propolis has highest activity compared with aqueous extract of propolis. This activity can be caused by honey composition. Honey alone displays significant antioxidant activity, similar to many plants (Gheldof et al. 2002). POH extract showed significantly high content of total phenol and flavonoids which followed by PN, PS and PH. POH showed approximately two times much higher activity on DPPH radical (IC50=12.24 μg/mL) compared with quercetin as references. The lowest activity on DPPH is shown by PS with 56,72 μg/mL of IC50 value. The highest activity was shown by POH (271,75 mg GAE/g extract) which was followed by aqueous extracts and lowest value belonged to HP. The highest scavenging activity against ABTS radical cation was shown by POH (73,37 mg TE/g extract) and the lowest activity was shown by PS (34,21 mg TE/g extract). All propolis extracts showed concentration-dependent inhibitory activity against DPPH radical. IC50 values for DPPH radical scavenging capacity are presented in Table 3. A lower IC50 value belongs to POH (IC50=4.39 μg/mL) which corresponds to a higher antioxidant activity of the extract. POH showed approximately 2 times higher activity on DPPH radical (IC50=12.24 μg/mL) compared with quercetin, as references. The lowest activity on DPPH is shown by PS with 56,72 μg/mL of IC50 value.

ABTS radical cation scavenging activities of the propolis extracts were expressed in terms of Trolox equivalent antioxidant capacity (TEAC) in Table 3. A higher TEAC value corresponds to a greater antioxidant activity of the propolis extracts. The highest scavenging activity against ABTS radical cation was shown by PoH (73,37 mg TE/g extract) and the lowest activity was shown by PS (34,21 mg TE/g extract).

Table 2. The inhibitory effects of propolis extracts on DPPH radical (POH: ethyl alcohol, PS: extracted by sterile distilled water kept as sterilised PN*:* extracted by sterile distilled water kept as non-sterilized, PH: prepared with full vacuumed and dried propolis with honey)

Tablo 2. Propolis ekstraktlarının DPPH radikali üzerindeki inhibitör etkileri (POH: etil alkol, PS: steril olarak saklanan steril damıtılmış su ile ekstrakte edilen, PN: sterilize edilmemiş olarak saklanan steril damıtılmış su ile ekstrakte edilen, PH: tamamen vakumlanmış ve bal ile kurutulmuş propolis ile hazırlanan).

Propolis extracts	IC_{50} value (μg/ml)
PH	18,08
POH	4,39
PS	56,72
PN	47,65
Quercetin	10,83

Table 3. The inhibitory effects of propolis extracts on ABTS radical cation and cupric ion reducing antioxidant capacity (CUPRAC) (POH: ethyl alcohol, PS: extracted by sterile distilled water kept as sterilised PN*:* extracted by sterile distilled water kept as non-sterilized, PH: prepared with full vacuumed and dried propolis with honey *TEAC: Trolox equivalent antioxidant capacity, **SD: Standard deviation, ***GAE: Gallic acid equivalent)

Tablo 3. Propolis ekstraktlarının ABTS radikal katyonu ve kuprik iyonu antioksidan kapasitesini (CUPRAC) azaltıcı etkisi. (POH: etil alkol, PS: steril olarak saklanan steril damıtılmış su ile ekstrakte edilen, PN: sterilize edilmemiş olarak saklanan steril damıtılmış su ile ekstrakte edilen, PH: tamamen vakumlanmış ve bal ile kurutulmuş propolis ile hazırlanan *TEAC: Trolox eşdeğeri antioksidan kapasitesi, **SD: Standart sapma, ***GAE: Gallik asit eşdeğeri)

Extract	TEAC* (mg TE/g extract) (ABTS)	Percentage of inhibition ± SD** against ABTS radical cation	Antioxidant capacity (mg GAE***a/g extract)
PH	65,13 ± 2,33	61,94 ± 2,11	56,78 ± 2,08
POH	73,37 ± 0,31	69,40 ± 3,4	271,75 ± 5,71
PS	34,35 ± 2,07	33,95 ± 1,87	205,23 ± 5,11
PN	34,62 ± 1,64	34,32 ± 1,49	126,13 ± 7,44

Cupric ion reducing antioxidant capacities of the propolis extracts were given in Table 3. The highest activity was shown by POH (271,75 mg GAE/g extract) which was followed by aqueous extract and lowest value belonged to HP.

PCA reduced the dimensionality of our multivariate data to two principal components and it was visualised with minimal loss of information, by using scatter diagram (Fig. 1).

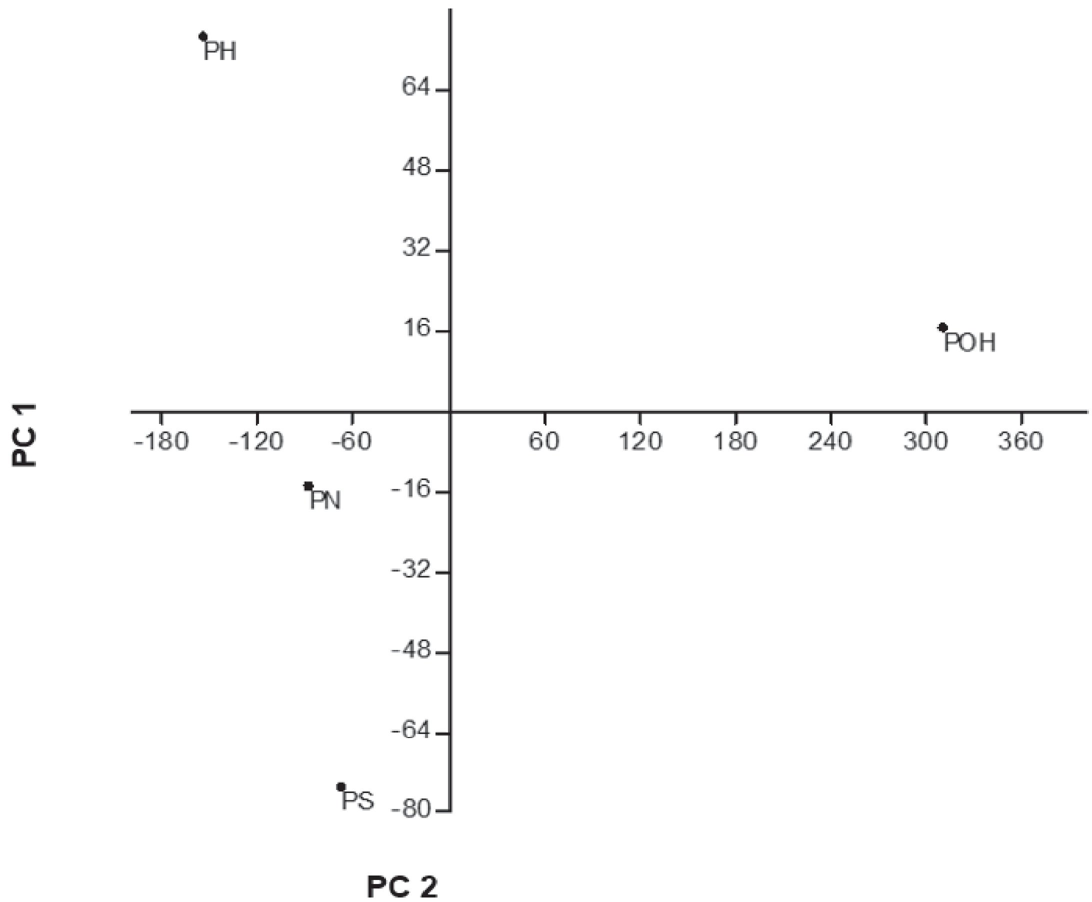

Figure 1. PCA scatter diagram (POH: ethyl alcohol, PS: extracted by sterile distilled water kept as sterilised PN*:* extracted by sterile distilled water kept as non-sterilized, PH: prepared with full vacuumed and dried propolis with honey).

Şekil 1. Temel bileşenler analizi saçılım grafiği (POH: etil alkol, PS: steril olarak saklanan steril damıtılmış su ile ekstrakte edilen, PN: sterilize edilmemiş olarak saklanan steril damıtılmış su ile ekstrakte edilen, PH: tamamen vakumlanmış ve bal ile kurutulmuş propolis ile hazırlanan).

DISCUSSION

Propolis can be classified biologically according to its producer (depending on the bee species) or botanical origin (depending on the plants used by bees). This resinous substance also can be classified chemically. Differentiation among the extraction methods and solvents can give us varied combinations- even if we use the same propolis with exactly the same biological origin.

According to Nalbantsoy et al. (2022), several external factors are present in the production process of propolis. Within this frame, we aim to evaluate different extraction techniques by comparing antioxidant properties of the same propolis with the use of DPPH, ABTS+ and CUPRAC methods. As an opotherapeutic medicine or a human diet supplement, the most important groups we gain from propolis are polyphenolic compounds, especially flavonoids. The antioxidant activity of propolis appears to be largely influenced by both total polyphenol and total flavonoid contents (Sun et al. 2015, Socha et al. 2014, Narimane et al. 2017). Değirmencioğlu et al. reported in their study that with 19 samples from Turkey, total phenolic content found 11.24 -172.98 mg GAE/g and total flavonoid content was 3.88 -58.31 mg QE/g (Değirmencioğlu et al. 2019). Another research carried out with 23 propolis samples from Turkey the total flavonoid content were determined between 21,28- 152,56 mg QE/g and total phenolic content was found between 34,53-259,4 mg GAE/g (Özkök et al. 2021). Güzelmeriç et al. worked with 47 samples produced in Black Sea Region of Turkey and reported that total phenolic content values between 37.25 ± 0.72- 592.57 ± 22.39 mg GAE/g; total flavonoid content values between 14.60 ± 0.57- 125.58 ± 0.58 mg QE/g (Güzelmeriç et al. 2021). The total phenolics and flavonoid contents in our propolis samples are found relatively higher compared with the other studies held in Turkey (Özkök et al. 2021). All propolis types have very low solubility in water and are soluble in organic solvents, because resins are relatively apolar (Bankova et al. 2021). Beside this fact, maybe because of the botanical origin of our samples, water extractions and dried propolis with honey have higher values than some of the samples in other studies extracted with ethanol. Gençay-Çelemli et al. found that the total phenolic compound of the five samples varies between 27.56±0.05 and 171.93±0.28 mg GAE/g. Also, it was added that total phenolic and flavone-flavonol contents were found highest in the sample that sourced from the taxa belonging to the Brassicaceae family, which is contrary to common belief since the phenolic content of chestnut propolis is higher (Gençay-Çelemli et al. 2019).

According phytochemical research on propolis extract, there is generally a positive correlation between the total phenol and flavonoid content in propolis extraction and their antioxidant activity (Güzelmeriç et al. 2021, Degirmencioglu et al. 2019 Gençay et al. 2019,). Phenolic compounds are likely to contribute to the radical scavenging activity of these extracts. According to the results, the new aqueous extraction technique is promising with relatively high polyphenol contents and antioxidant activities. Besides honey with propolis could be an alternative product, although it has relatively lower values. Probably due to the fact that the amount of propolis extract added to honey was not large enough to significantly increase these parameters as it was done before by Osés et al. 2015.

Total phenol and flavonoid contents in non-sterilized aqueous extract of propolis is slightly higher than sterilised aqueous extract. This may result from damage, reduction or alteration of some compounds during the sterilisation process. They show almost the same activity in the ABTS assay whereas in CUPRAC and DPPH assay the antioxidant capacity of sterilised aqueous extract is higher than non-sterilized extract. As we know, the antioxidant capacity of propolis is dependent on its content, but the studies generally aim to compare antioxidant potential of different propolis extract. Although it is a fact that the antioxidant capacity of ethyl alcohol extractions is higher than the others, many commercial ethanol extracted propolis preparations can cause oral mucosal ulceration or gastrointestinal health problems. Moreover, despite the method differences, the results indicated that Tunceli propolis has a relatively high total phenol and flavonoid content compared to other region in Turkey and subsequently possess a high antioxidant potential (Apak et al. 2004; Özkök et al. 2021, Güzelmeriç et al. 2021).

Conclusion

Propolis, as a nutritious product, provides a rich source of nutrients, such as mineral elements, proteins, and antioxidant compounds. The antioxidant capacity of propolis is related to the flavonoid, mineral, and protein contents which derived from botanical origins of the product and extraction solvents. Among the studied samples, ethyl alcohol extraction of propolis possess highest content of phenol and flavonoid, as well as the highest antioxidant activity. The aqueous extractions also have significant antioxidant capacities. In future studies, there is a need to investigate the eco-floral effect of the antioxidant content of propolis Tunceli-Ovacık region in detail.

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