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ORIGINAL PAPER

Effects of various water-based biocompatible solvents on the extraction of propolis from Artvin, Türkiye: Physicochemical properties, biological activity, and phenolic profile

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Abstract

Propolis is a multicomponent material collected by honeybees (*Apis mellifera* L.) from different plants to insulate and repair their hives, protect against microorganisms and foreign bodies, and maintain a sterile environment. This natural product, which attracts attention due to its wide range of biological effects, is often examined in scientific studies using ethanolic extracts, as its bioactive components are mostly soluble in ethanol. The limited bioactive component yield of water extracts, which are more biocompatible and safer compared to ethanol extracts, can be increased by using co-solvents including propylene glycol and glycerol. In this research, we focused on the antioxidant potentials and antimicrobial properties exhibited by different solvent profiles of aqueous (WPe), propylene glycol: aqueous (WPGPe), and glycerol: aqueous (WGPe) extracts of propolis. For this purpose, the physicochemical profile of the extracts, including soluble solids content (SSC), pH, titration acidity (TA), color, total phenolic and total flavonoid contents (TPC and TFC), antimicrobial activity, antioxidant activity, and phenolic component profiles, were subsequently determined using RP-HPLC-UV. L*, a*, and b* values of the extracts ranged between 41.17 and 50.66, 0.00 and 2.17, and 17.90 and 22.09, respectively. The SSC, pH, and TA values were highest for WPGPe and lowest for WPe. Caffeic acid, *p*-coumaric acid, and ferulic acid were the prominent phenolic acids in all extracts. In terms of total phenolic and total flavonoid content, as well as DPPH, ABTS, and FRAP antioxidant activity analyses, the WPGPe was found to be the most superior extract at a statistically significant level ($p<0.01$). Principal component analysis and hierarchical cluster analysis revealed that WPGPe was richer in terms of individual phenolic compounds and other measured biological activity analyses, and therefore formed a statistically distinct group from the other extracts. The antimicrobial capacity of the extracts was evaluated against two Gram (-) bacteria, two Gram (+) bacteria, one yeast and one mould. This study offers practical guidance for the food and nutraceutical sectors on identifying suitable solvents and processing conditions for developing efficacious propolis-based formulations.

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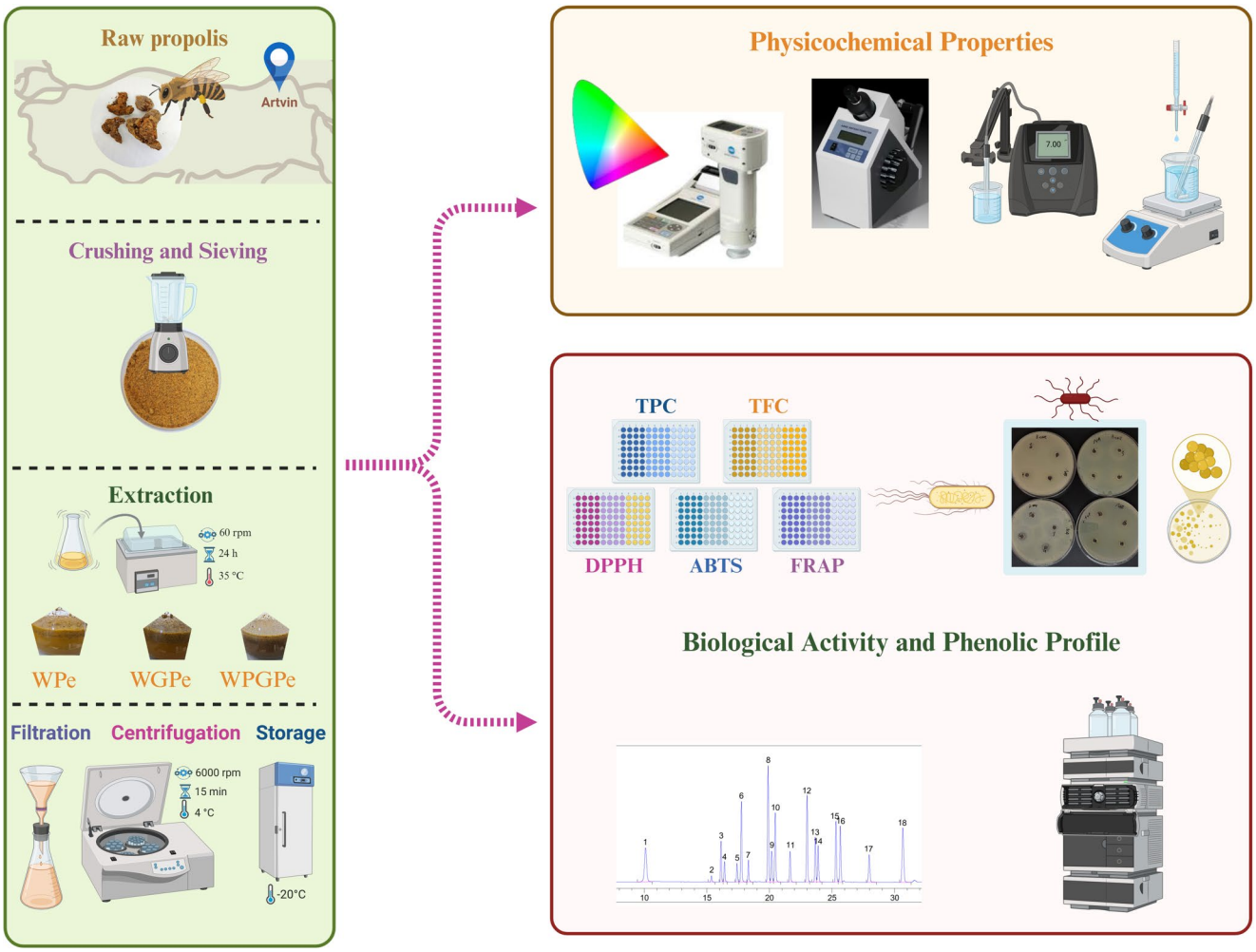
Graphical Abstract



Keywords Propolis · Extraction · Solvent · Bioactive compounds · Antioxidant activity · Antimicrobial activity

Introduction

Propolis, a functional food produced by honeybees from diverse plant sources, is a natural product with a sticky structure [1]. Honeybees (*Apis mellifera* L.) collect small pieces of gummy, resinous, and balsamic substances from flowers, branches, leaf buds, and tree bark with their mandibles, mixing them with their saliva and forming a lump. Subsequently, they transport it to the hive utilizing their legs. Propolis is finally formed after mixing resinous substances with the enzymes of the saliva glands and waxes [1–4]. This natural resource is found in the hive in abundance during late summer and autumn, as the bees start to seal their nest in preparation for winter. Honeybees are remarkable architects, using propolis for several vital purposes within their hives. Propolis is utilized by honeybees to seal holes and cracks, and close gaps, thereby securing their nest due to its strong adhesive properties [4, 5]. Propolis also plays an essential role in maintaining the hive's stability and protecting it from invaders, creating a defensive barrier within the hive. Furthermore, propolis is used as an antiseptic that defends larvae, combs, and honey from pathogens and parasites [1].

Propolis is a resin-like substance that is synthesized by honeybees, forming a remarkable natural product. Its appearance, color, and consistency can vary greatly, making it a unique material. The quality of propolis is significantly affected by a range of factors, including its geographical origin, the timing of collection, the season, the types of vegetation involved, climatic conditions, and the health of the bees [6]. Artvin, known for its rich floral diversity and significance in beekeeping, was chosen as the source of propolis in this study. The region's diverse plant life contributes significantly to the phenolic profile and biological activity of

propolis, and previous studies have highlighted that Artvin propolis has high antioxidant capacity, flavonoid content, and chemical compound concentration [7, 8]. Propolis can display a wide range of colors, from yellow and orange to green and dark brown. This variation is influenced by variety factors, including its source. Propolis is solid and brittle when temperatures drop. However, as the temperature rises above 20 °C, it transforms into a softer and stickier substance, thanks to its resin-like properties [4, 9].

Over 300 distinct substances have been detected in different propolis samples [10–12]. The chemical profile of, propolis includes approximately half resins (which are rich in phenolic acids andflavonoids). The remaining part consist of waxes (30%), essential oils (10%), pollen (5%), and other organic substances (5%) [3].

Propolis is primarily composed of phenolic compounds that play a crucial role in its various biological activities [1]. Most of the bioactive compounds found in propolis are concentrated in its balsamic portion, which is rich in polyphenols. These bioactive components include fatty and phenolic acids and their esters, aldehydes, aromatic alcohols, flavonoids, terpenes, β-steroids, stilbenes, lignin, tannins, and amino acids [13, 14]. Propolis is rich in a variety of compounds, including coumarins, ketones, and several acids such as benzoic and cinnamic acid. It also contains benzyl alcohol, vanillin, and a range of flavonoids like pinocembrin, pinobanksin, quercetin, galangin, and apigenin, along with caffeic acid and its esters, campheride, isovaniline, chrysin, pinocembrin, galangin, and quercetin, while common phenolic acids are ferulic acid, caffeic acid, and *p*-coumaric acid [12, 15, 16]. Propolis induces the body's production of antioxidant enzymes, such as superoxide dismutase, glutathione reductase, and catalase [17]. These compounds contribute to a broad range of therapeutic properties, including antioxidant, antimicrobial, antifungal, anti-inflammatory, antiviral, hepatoprotective, immunomodulatory, antiseptic, antidepressant, anticarcinogenic activity [1, 6, 7, 18].

Moreover, propolis offers significant protection against chronic diseases—including cancer, cardiovascular conditions, and diabetes mellitus, primarily by mitigating oxidative damage [19]. Recent studies have highlighted that natural compounds found in propolis, especially phenolics and terpenes, exhibit remarkable antioxidant capacities and play a key role in shielding cells from oxidative stress [20, 21].

Several studies have demonstrated that propolis exhibits antimicrobial properties against various microorganisms, parasites, bacteria, viruses, and yeasts. Propolis tends to exhibit more substantial antimicrobial properties against Gram-positive bacteria compared to Gram-negative bacteria. This difference in activity has been attributed to the outer membrane structure of Gram-positive bacteria [22, 23]. The bioactivity of propolis largely depends on its chemical composition, which can vary significantly due to factors like geographic location, the types of plants involved, climate conditions, and the specific bee species that produce it [24].

Unlike bee products such as honey, bee bread, and pollen, propolis cannot be directly consumed as a food product without being processed [2]. Propolis, known for its bioactive compounds, often presents challenges in consumption due to its strong, bitter taste and resinous texture. To enhance palatability, various processing techniques have been employed. Microencapsulation, particularly through methods like spray drying with wall materials such as ovalbumin, gelatin, sodium alginate, or maltodextrin, effectively masks the unpleasant taste by forming protective coatings around the propolis, allowing for controlled release and improved solubility [25, 26]. Moreover, the incorporation of volatile herbal components during propolis extraction and the application of spray-drying techniques are effective strategies to overcome the unpleasant taste and odor that limit propolis use in food products. The addition of herbal volatiles helps mask the characteristic smell of propolis, while spray-drying with carriers such as maltodextrin converts the extract into a stable powder form, reducing bitterness and aroma intensity. These approaches not only improve the sensory acceptability of propolis but also preserve its bioactive compounds and enhance its applicability in functional food formulations [27].

The extraction process is essential for fully harnessing the bioactive components found in propolis. Selecting the suitable extraction method and solvent is crucial for producing high-quality and cost-effective products that contain propolis [28]. The chemical composition of propolis extracts will be influenced by the type of extraction solvent used, the composition and ratios of the solvents, temperature, the pH level of the solvents, and the specific extraction methods applied [16, 29]. Ethanol is the leading solvent for extracting bioactive compounds from propolis. It's favored for its accessibility, affordability, eco-friendliness, industrial approval, and high compatibility with propolis's predominantly lipophilic compounds [14, 30]. However, traditional ethanol extracts have drawbacks. Their alcohol content limits use for sensitive populations, like young children and pregnant women. Additionally, while water is safe, it's inefficient; it dissolves only about 10% of propolis components, significantly reducing its effectiveness [30, 31]. To overcome these limitations, researchers are exploring alternative, more biocompatible solvents. This has led to investigations into substances like glycerol and propylene glycol [32]. These alternatives are gaining traction, particularly for applications that require alcohol-free extracts, opening up promising opportunities to broaden propolis's therapeutic and commercial applications.

The purpose of this research was to evaluate and compare the antioxidant and antimicrobial characteristics, as well as the flavonoid contents andphenolic profiles, of propolis extracts obtained from crude propolis using three different solvents: water, a glycerol-water mixture, and a propylene glycol-water mixture.

Materials and methods

Material

Raw propolis was obtained from the Artvin Beekeepers Association in August 2023. The origin of propolis is Aydın village of the Ardanuç district of Artvin (41°02′37″ N, 42°05′44″ E). Upon collection, the propolis was stored at −20 °C until use to preserve its properties.

Phenolic compound standards (gallic acid, chlorogenic acid, caffeic acid, *p*-coumaric acid, ferulic acid, naringin, myricetin, resveratrol, quercetin, naringenin, catechin, chrysin, luteolin, rutin, sinensetin, apigenin, diosmetin, trans-sinapic acid and fumaric acid) used in the study were obtained from Sigma–Aldrich Chemie GmbH (Steinheim, Germany). All other reagents were of analytical grade and were purchased from Sigma-Aldrich (St.Louis, MO, USA).

Microorganism strains used in the antimicrobial activity analysis, including *Escherichia coli* BC 1402, *Salmonella typhimurium* (ATCC 14028), *Staphylococcus aureus* ATCC 29,213, *Bacillus cereus* (ATCC 10987), *Candida albicans* ATCC 1223 and *Aspergillus niger* ATCC 16,888, were obtained from Ataturk University Food Microbiology Laboratory Culture Collection. The mentioned strains were confirmed with the help of the Sherlock Microbial Identification System (version 4.0, MIDI Inc., Newark, DE, USA), BIOLOG MicroStation™ Identification System (Biolog Inc., Hayward, CA, USA), and classical identification methods specified in Bergey's Determinative Bacteriology guide (Holt, 1994). DMSO (dimethyl sulfoxide) and media used in antimicrobial activity analysis were supplied by Merck KGaA (Darmstadt, Germany). Amphotericin B and ofloxacin were purchased from Sigma-Aldrich (St. Louis, USA).

Methods

Extraction procedure of propolis

The extracts were preparation followed the procedure of Topdas et al. [33] with slight adjustments. Initially, raw propolis was finely milled using a Waring grinder (HGB2WTS3, USA) and then passed through a stainless steel sieve (0.45 mm) to obtain the powdered fraction. A portion of sieved propolis powder (25 g) was weighed into amber-colored 500 mL volume glass bottles, and 250 mL of pure water, a propylene glycol: water mixture (1:1, v/v), and a glycerol: water mixture (1:1, v/v) were added, respectively. The obtained propolis and solvent mixtures were shaken in a shaking water bath (JSR, JSSB-30T, Korea) at 35 °C and 60 rpm for 24 h and then filtered using Whatman No. 1 filter paper. The filtrates were centrifuged for 15 min at 6000 rpm and 4 °C using a refrigerated centrifuge (Hettich, Mikro 22R, Germany). The resulting supernatants were transferred to amber falcon tubes. The extraction procedure was repeated 3 times. The samples were stored at −20 °C until furter analysis.

Physicochemical properties

The soluble solids content (SSC) of the extracts was determined using a digital refractometer (WYA 2S, Shanghai Physico-Optical Instrument Factory, China). pH analysis was performed by immersing the pH meter (OHAUS Starter 3100, USA) probe into 20 mL of the sample. Titratable acidity (as citric acid) was performed using the electrometric titration method reported by Cemeroğlu [34]. Before the analysis, the digital refractometer was calibrated based on the refractive index of pure water at 20 °C (1.3330), and 4.01, 7.01, and 10.0 buffer solutions were used to calibrate the pH meter. A three-dimensional colorimeter (Konica Minolta CR-400, Korea) was utulized for assesing color parameters. Following calibration using the insturment's standard white referance plate, the L* (0: black, 100: white), a* (< 0 green, > 0 red), and b* (< 0 blue, > 0 yellow) values of the extracts were measured on a white background at 20 ± 2 °C [35].

Total phenolic content (TPC)

TPC analysis was performed with slight modifications to the method reported by Singleton et al. [36]. First, 0.1 mL of water (WPe), glycerol: water (WGPe), and propylene glycol: water (WPGPe) extracts were taken, and 2.5 mL of 0.2 N FCR (Folin-Ciocalteu reagent) was pipetted and vortexed. Following a3 min period, 2 mL of a 7.5% Na_2CO_3 solution was added and the mixture was vortexed and incubated for 2 h at ambient room temperature in the dark. After incubation, absorbances were recorded at 760 nm by using a visible spectrophotometer (PG Instruments T60V, UK). The TPC of the extracts was calculated gallic acid equivalent mg GAE (Gallic Acid Equivalent)/g sample using the equation obtained from a graph prepared with gallic acid standards at concentrations of 10 to 500 µg/mL. The analysis was performed in triplicate for each sample.

Total flavanoid content (TFC)

TFC of the propolis extracts was analyzed following the protocol reported by Koçak et al. [37]. A 0.25 mL aliquot of the extract samples was taken, and 1.25 mL of pure water

and 0.075 mL of 0.05 g/mL $NaNO_2$ were added, followed by vortexing. After waiting for 6 min, 0.15 mL of 0.1 g/mL $AlCl_3 \cdot 6H_2O$ was added, vortexed, and left to stand for an additional 5 min. 0.5 mL of 1 M NaOH was added and allowed to stand for 15 min. At the end of the waiting period, the sample absorbances were recorded with a visible spectrophotometer at 510 nm. The TFC was calculated in terms of quercetin equivalent mg QE (Quercetin Equivalent)/g sample using an equation derived from the calibration curve prepared with the quercetin standards prepared in the range of 10–1000 mg/L. The analysis was conducted in triplicate.

Antioxidant activity analyses

DPPH (1,1-diphenyl-2-picrylhydrazyl) free radical scavenging activity analysis was performed using the method reported by Balaydin et al. [38]. For this purpose, aliquots of each extract (10–30 µg/mL) were placed into test tube, and the final volume was adjusted to 2 mL using ethanolfollowed by the addition of 0.5 mL of the prepared DPPH stock solution (0.1 mM) was added. The vortexed tubes were left at room temperature and in the dark for 30 min. Then, absorbances were measured at a wavelength of 517 nm. The analysis was performed in triplicate for each sample. % Inhibition values were calculated using Eq. (1) [33, 39]. A calibration curve was created by determining the inhibition% values for different concentrations of Trolox (5–25 µM), and the antioxidant capacity of each samples was reported as mg Trolox Equivalent (TE)/g sample, as derived from this calibration curve [34, 40].

The procedure reported by Köksal et al. [41] was used to determine the ABTS (2,2′-azino-bis(3-ethylbenzthiazolin-6-sulfonic acid) free radical scavenging activity.The ABTS + radical solution was prepared by adding a 2.45 nM potassium persulfate solution to 2 mM ABTS solution and maintaining it under continuous stirring for 16 h at ambient temperature in the dark conditions. Each extract was added to the test tubes at a concentration of 10–30 µg/mL. The total volume was brought to 2.5 mL using the prepared ABTS•+ radical solution. Then, the tubes were vortexed and kept in the dark for 6 min. Absorbances were determined at a wavelength of 734 nm with a visible spectrophotometer. The analysis was conducted in triplicate. The inhibition% values were calculated using Eq. (1) [33, 39]. A calibration curve was created by determining the inhibition% values for different concentrations of Trolox (5–25 µM), and the antioxidant capacity was expressed as mg TE/g sample according to this curve [34, 40].

$$Inhibition\,\% = [(A_{DPPH\ or\ ABTS} - A_{Sample})/A_{DPPH\ or\ ABTS}] \times 100 \quad (1)$$

Where $A_{DPPH\ or\ ABTS}$ is the absorbance of the DPPH or ABTS blank sample (depending on the analysis performed), and A_{sample} represents the absorbance value of the extract.

For FRAP (Ferric Ion Reducing Power) analysis, the procedure reported by Koçak et al. [37] was applied. The analysis was carried out with a daily prepared acetate buffer solution (1 L was prepared using 3.1 g sodium acetate + 16 mL acetic acid adjusted to pH 3.6), TPTZ (2,4,6-tripyridyl-s-triazine) solution (prepared with 0.156 g TPTZ in 50 mL ethanol) and 100 mL solution prepared with 0.5404 g $FeCl_3 \cdot 6H_2O$ and 2 mL HCl (37% m/m). The aforementioned solutions were combined in a 10:1:1 (v: v:v) ratio to form the FRAP reagent. A 25 µL was taken from the sample, and 2.475 mL of FRAP reagent was added. The vortexed mixture was held in the dark for 4 min before measuring its absorbance at 593 nm using visible spectrophotometer. The results were expressed as mg TE/g sample based on equation derived from the calibration curve prepared with Trolox standard solutions in the range 5 to 25 µM. The analysis was performed in triplicate for each sample.

Phenolic component profile by HPLC

The phenolic component profile of the extracts was analyzed with modifications to the method reported by Kara et al. [42] by using an Agilent 1260 Infinity Series HPLC (High-Performance Liquid Chromatography) system, equipped with a 1260 quantitative pump, 1260 Infinity standard autosampler, and 1260 VWD UV detector (set at 280 nm). A reverse-phase C18 column (100 Å, 5 µm, 250 × 4.6 mm inner diameter) was used for chromatographic separation. The column temperature was 30 °C. The mobile phases were ultra-pure water containing 0.03% phosphoric acid (pH 2.5) (A) and acetonitrile (B). The flow rate of the mobile phase was maintained at 0.6 mL/min, with an injection volume of 5 µL. The total analysis and post-run times were 43 min and 2 min. The gradient programme was initiated by maintaining mobile phase A at 95% for the first 8 min, followed by a gradual decrease to 85%, 79%, 65%, 60%, 48% and 33% at 8, 10, 15, 17, 20, and 36 min, respectively. From this point onwards, mobile phase A was increased back to 95% at 36.1 min and maintained at this level until 43 min. Between 43 and 45 min, the system was transferred to the post-run phase. Standard phenolic compounds were dissolved in a mixture of 80% methanol and 20% pure water (v/v) and prepared for analysis. The identification of phenolic compounds was confirmed by comparing the retention times and UV spectra with those of reference standards, and data analysis was performed using ChemStation software. All samples were filtered through 0.45 µm membrane filters before being introduced into the device.

Calibration curves were created for each phenolic compound using solutions prepared by serially diluting standard solutions in the range of 1–50 ppm. The calibration curves were plotted based on the relationship between

concentration (x-axis) and peak area (y-axis) obtained. To determine the sensitivity of the method, the limit of detection (LOD, 3.3 × SD/m) and limit of quantification (LOQ, 10 × SD/m) values were calculated. These limit values were determined based on the standard deviation (SD) of the measurement data obtained at the lowest standard concentration and the slope (m) of the calibration curve.

Antimicrobial activity analysis

In the experimental plan designed to evaluate the antimicrobial activities of the extracts across different cell types, two of the six strains were Gram (-) bacteria, two were Gram (+) bacteria, one was yeast, and one was mold. First, the isolates stored at −80 °C were cultured on PCA (Plate count agar) for bacteria and PDA (Potato Dextrose Agar) for yeast and mold and checked for contamination. Thus, the prepared fresh cultures were diluted in 0.85% sterile physiological saline and adjusted to a standard density of 0.5 McFarland using a densitometer (McFarland, DEN-1 Biosan, Latvia). The determined dose for the extracts was 100 µg/10 µL, while for ofloxacin and amphotericin B, used as positive controls, it was 5 µg/10 µL. 50% DMSO (Dimethyl sulfoxide) was included as a negative control and to prepare doses. Antimicrobial activity analysis was conducted using the well diffusion (agar-well) method as described by Topdas et al. [43]. For this purpose, 100 µL of bacteria, yeast, and mold suspensions were transferred to nutrient agar (for bacteria) and PDA (for yeast and mold) media. Extracts and 100 µL of positive and negative controls were loaded into the opened 5 mm diameter wells, and the petri dishes were incubated at 30 °C for 24–48 h for bacteria, 30 °C for 24–48 h for yeast, and 30 °C for 72–96 h for molds. After incubation, the inhibition zones surrounding the wells were measured in mm and recorded. The analysis was performed in triplicate for each sample.

Statistical analysis

The dataset was statistically analyzed through one-way ANOVA using SPSS version 25.0 (IBM, Armonk, New York, USA). Duncan test, one of the multiple comparison tests, was applied to determine significant differences between solvent types. All findings are presented as mean ± standard deviation, with differences regarded as significant at the $p < 0.01$. To examine the structural pattern of the data related to phenolic components in more detail, Principal Component Analysis (PCA) was performed using SIMCA 14.1 software (MKS UMETRICS, Umea, Sweden). Bi-plot, Loading Scatter Plot, Score Scatter Plot, and Hierarchical Cluster Analysis were created to evaluate the distributional behavior and trend characteristics of propolis extracts and variables in the data matrix, and were analyzed.

Results and discussion

Physicochemical properties

According to Table 1, noteworthy variations are observed in the color parameters evaluated by means of the CIE Lab* system for extracts produced with different solvent combinations ($p < 0.01$). The L* values of the extracts ranged between 41.17 and 50.66. The L* value decreases with solvents other than water, which means that the mentioned extracts are darker in color. These differences observed in the L* values reflect the effect of the solvent system on the pigment solubility. The highest a* (red-green axis) value was measured in WPGPe (2.17). These values indicate that the propylene glycol/water solvent is significantly more effective in extracting reddish pigments. The highest b* value (22.09) was observed in WPGPe, while WPe and WGPe had close results with b* values of 17.90 and 18.43, respectively, and were statistically in the same group. The b* values obtained show that the glycerol/water mixture supports the dissolution of yellowish pigments (e.g., flavonoid or carotenoid derivatives). When all color parameters are evaluated together, it is thought that the color properties of the extract are directly related to the solvent type and that the measured differences can provide important clues about the pigment solubility and phenolic compound content.

The water-soluble dry matter values measured in WPe, WGPe, and WPGPe varied from 2.00 to 9.70, and the values determined showed statistically significant differences depending on the solvent combinations used ($p < 0.01$). Notably, WPGPe exhibited the highest SSC value (9.70), which may be attributed to propylene glycol, a dihydric alcohol with a hydrophilic and slightly viscous nature,

Table 1 Some physicochemical properties of propolis extracts

Samples	L*	a*	b*	SSC (°Brix)	pH	Titratable Acidity (%)
WPe	50.66 ± 0.09^{a}	0.22 ± 0.05^{b}	17.90 ± 0.49^{b}	2.00 ± 0.10^{c}	4.19 ± 0.01^{c}	0.12 ± 0.00^{c}
WGPe	48.88 ± 1.44^{b}	0.00 ± 0.01^{c}	18.43 ± 0.77^{b}	4.60 ± 0.20^{b}	4.28 ± 0.01^{b}	0.14 ± 0.01^{b}
WPGPe	41.17 ± 0.19^{c}	2.17 ± 0.09^{a}	22.09 ± 0.20^{a}	9.70 ± 0.20^{a}	4.48 ± 0.02^{a}	0.28 ± 0.01^{a}
Significance	**	**	**	**	**	**

• WPe: water-based propolis extract; WGPe: glycerol: water-based propolis extract; WPGPe: propylene glycol: water-based propolis extract

• Values with different superscript letters$^{(a, b, c)}$ in within the same column indicate are significantly different (**p < 0.01)

enhancing the extraction of soluble components, particularly polyphenols. Indeed, Conceição et al. [44] reported that increasing the propylene glycol concentration in the solvent enhanced process efficiency, which they attributed to the chemical affinity of propylene glycol to the compounds in the sample. The lower water-soluble dry matter content of WPe compared to WGPe and WPGPe (2.00) may be associated with the limited dissolving capacity of water. These findings demonstrate that the choice of solvent influences not only the color of the extracts but also the total content of extractable solids.

Upon evaluating the pH and titratable acidity data, it was found that the solvent factor exhibited a statistically significant effect ($p < 0.01$). The pH value, reflecting the hydrogen ion concentration of the extracts, varied between 4.19 and 4.48, depending on the solvent's chemical structure and the nature of the dissolved substances. While WPGPe exhibited the highest pH value, WPe revealed the lowest. This increase may be due to propylene glycol facilitating the dissolution of less acidic components. The titratable acidity data of the extracts showed a similar trend to the pH data. While WPe showed the lowest titratable acidity value (0.12%), WPGPe reached the highest value (0.28%). This difference between the extracts reflects the change in the type and quantity of dissolved organic acids with the effect of the solvent composition. It can be stated that the use of propylene glycol is more effective in extracting components, such as weak organic acids or free phenolic acids, compared to water and glycerol [45].

TPC, TFC, and antioxidant capacity

The composition of propolis is significantly influenced by botanical sources, geographical location, seasonal variations, and collection timing. Apart from this, the most significant factor influencing the chemical composition is the type of plant materials found in their ecosystems. For these reasons, the composition of propolis can also serve as a geographical fingerprint of the ecosystem in which it is located. Polyphenols, especially flavonoids and phenolic acids, dominate the chemical composition of propolis, and the biological effects of propolis are related to these compounds [46].

Different solvents are used in the preparation of propolis extracts. At this point, 1,2-propylene glycol, polyethylene glycol (200, 400, or 600), or glycerol solvents, which are particularly useful among polar solvents among propolis solvents, stand out with their relative non-toxicity, safety, and widespread use as pharmaceutical excipients or diluents and moisturizers in many drug products [16]. For these reasons, a mixture of 1,2-propylene glycol and glycerol with water was used in this study.

In the present study, TPC, TFC, and antioxidant activities of propolis extracts were determined, and the results of these analyses are given in Table 2. TPCs of propolis extracts were determined between 14.12 and 53.70 mg GAE/g sample, and TFCs were determined between 14.63 and 54.68 mg QE/g sample. Upon evaluating the TPC and TFC data, it was found that the solvent factor had a statistically significant effect ($p < 0.01$). TPC and TFCs of propolis extracts were ranked as WPGPe > WGPe > WPe (Fig. 1). It was observed that the use of propylene glycol-water solvent in the extraction significantly increased the TPC and TFC values compared to other solvents. In previous studies, ethanol, water, or ethanol: water, methanol, and petroleum ether extracts were generally used for propolis extraction [47–51]. It was observed that differences in TPC and TFC amounts existed in previous studies due to variations in solvent, extraction method, and standards used in calculations [51, 52]. For these reasons, a complete comparison could not be made. However [51], reported that they determined TFC values ranging from 11.46 to 61.64 mg QE/g in propolis extracts prepared using 70% ethanol solvent. Despite the solvent difference, the TFC values of this study are also in this range. Similar to this study, Ramanauskien et al. [53] reported that 1,2-propylene glycol is a highly effective solvent for propolis extraction, yielding high levels of extracted polyphenols. At the same time, Conceição et al. [44] reported that propylene glycol in the solvent has a chemical affinity to the compounds found in the sample. Additionally, it has been reported that using propylene glycol as a solvent is generally more effective in extracting

Table 2 TPC, TFC, and antioxidant capacity (DPPH, ABTS, and FRAP) of propolis extracts

Samples	TPC (mg GAE/g sample)	TFC (mg QE/g sample)	DPPH (mg TE/g sample)	ABTS (mg TE/g sample)	FRAP (mg TE/g sample)
WPe	14.12 ± 0.56^{c}	14.63 ± 0.41^{c}	9.54 ± 0.60^{a}	26.02 ± 0.71^{a}	13.77 ± 0.18^{a}
WGPe	21.33 ± 0.90^{b}	20.83 ± 0.08^{b}	13.85 ± 2.04^{b}	38.72 ± 3.53^{b}	18.15 ± 0.58^{b}
WPGPe	53.70 ± 0.95^{a}	54.68 ± 0.70^{a}	39.68 ± 4.58^{c}	96.10 ± 4.33^{c}	44.00 ± 3.01^{c}
Significance	**	**	**	**	**

• WPe: water-based propolis extract; WGPe: glycerol: water-based propolis extract; WPGPe: propylene glycol: water-based propolis extract

• Means with different letters$^{(a, b, c)}$ in the same column are significantly different (**p < 0.01)

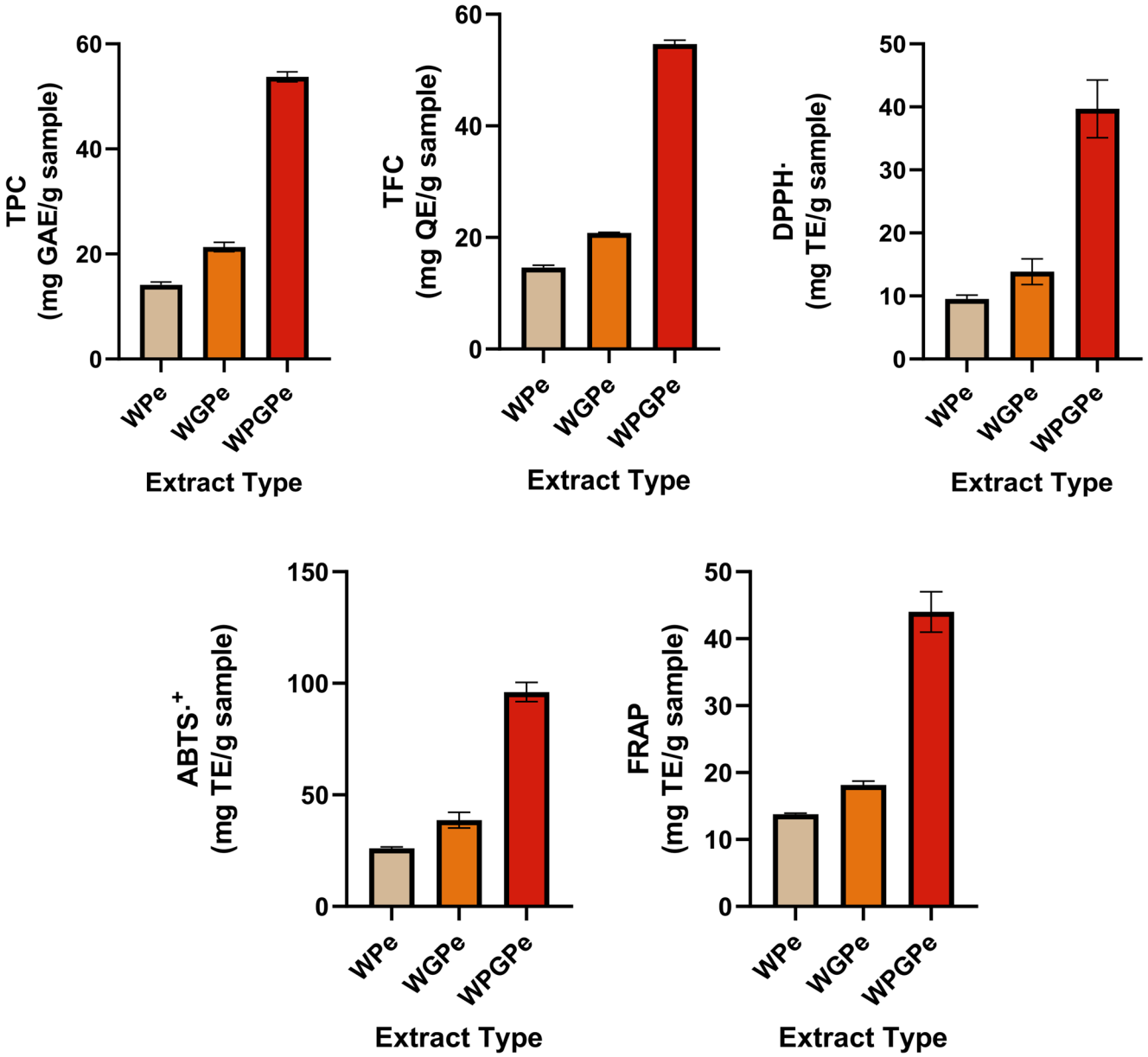


Fig. 1 TPC, TFC, DPPH, ABTS, and FRAP compositions of propolis extracts according to extract type. *In figure: WPe: water-based propolis extract; WGPe: glycerol: water-based propolis extract; WPGPe: propylene glycol: water-based propolis extract

components, such as weak organic acids or free phenolic acids, than water and glycerol [45].

Propolis has been associated with many biological activities, including antioxidant, antiproliferative, antitumoral, antimicrobial, immunomodulatory and anti-inflammatory, effects. Additionally, propolis shows significant potential for used as a therapeutic agent. However, its direct use may be limited due to its low water solubility and poor bioavailability; considering this, the chemical components of propolis need to be extracted by extraction with various types of solvents (such as methanol, ethanol, chloroform, dichloromethane, or hydroalcoholic). The polarity of the solvent must be carefully selected since the chemical components extracted will depend on it. Chemical compounds, such as fatty acids and sterols, can be obtained using less polar solvents, including ether and dichloromethane. Conversely, using more polar solvents enables the extraction of more polyphenols, which are defined as biologically active molecules in propolis [54].

The antioxidant activity of propolis extracts was calculated between 9.54 and 39.68 mg TE/g with DPPH, 26.02–96.10 mg TE/g sample with ABTS method, and 13.77–44.00.77.00 mg TE/g sample with FRAP (Table 2). As shown in Table 2, the solvent factor had a statistically

significant effect on the antioxidant activity data ($p < 0.01$). When the DPPH, ABTS, and FRAP results were examined for antioxidant activity, it was seen that the order WPGPe > WGPe > WPe occurred in all antioxidant analyses (Fig. 1). Similar to the TPC and TFC results, using propylene glycol: water solvent led to an increase in antioxidant activity. Different results have been recorded with different calculation methods for propolis extracts from different regions prepared with different solvents in the literature [48, 51, 55]. The antioxidant activity results obtained are generally consistent with the values in the literature regarding the antioxidant capacities of propolis extracts. In a study conducted by Altuntas et al. [56] using propolis collected from various provinces of Türkiye, higher DPPH results of 46.70–228.20.70.20 mg TE/g were determined, which are greater than our findings. Özenirler and Zare [57], in their study, found ABTS values in propolis ethanol extracts ranging from 34.35 to 73.37 mg TE/g, which is parallel to our results. In fact, when looking at the highest ABTS value detected (96.10 mg TE/g), our results were even higher. Xavier et al. [58], in their study with propolis samples from northeastern Bahia state, Brazil, determined FRAP values in propolis extracts with different solvents ranging from 20.10 to 219.00 mg TE/g. When comparing the ranges of this study with the current study, it's observed that the lowest value (13.77 mg TE/g) in our study is lower, while the highest value (44.00 mg TE/g) falls within their range.

Antimicrobial activity

Results are reported in Table 3. The effectiveness of propolis extracts varied depending on both the type of microorganism and the solvent employed. Propolis extracts usually strong antibacterial activity against Gram-positive bacteria; while their effectiveness against Gram-negative bacteria is limited and concentration [59, 60]. In this study, all types of solutions (WPe, WGPe, and WPGPe) were found to be inactive against *S. typhimurium*, *C. albicans*, and *(A) niger.* The results of the study also demonstrated that water-extracted propolis (WPe) was not effective against the studied strains of microorganisms. Kubiliene et al. [61] compared the antimicrobial activity of ethanol, water, and olive oil extracts of propolis in their study. As a result of the study, it was determined that the water extract did not have antimicrobial activity. According to Acar and Gülendağ [62], ethanol-based propolis showed significantly stronger antibacterial activity than the water-based extract against 26 Gram-positive and 10 Gram-negative foodborne bacteria. While the ethanol extract inhibited several bacterial genera, including *Staphylococcus* and *Listeria*, the water-based propolis showed little to no inhibitory effect. These results highlight the greater effectiveness of ethanol-based propolis, especially against Gram-positive bacteria. In this study, the glycerol solution of propolis (WGPe) exhibited antibacterial activity against *E. coli*, *B. cereus S. aureus*, and. The propylene glycol solution of propolis (WPGPe) was active against Gram-positive bacteria, which are *S. aureus* and *B. cereus.* Furthermore, another study found that propolis extracted with propylene glycol had strong antibacterial and moderate antifungal activity, similar to ethanol-based extracts, due to its high content of bioactive constituents. In contrast, water-based extracts showed minimal antimicrobial effects, making propylene glycol a more effective and eco-friendlier alternative [45]. In this study, propolis extracts exhibited no inhibitory effect on *A. niger*, suggesting a lack of antifungal activity against this species. The study by Haile and Dekebo [63] showed that Haramaya propolis has strong antifungal properties, particularly against *A. niger*. The ethanolic extract contains compounds such as benzenamine and paromomycin, which are likely responsible for this activity. At a concentration of 10 mg/mL, the propolis effectively inhibited *A. niger*. According to a study conducted by Veronica et al. [64], among the three extracts of propolis, the glycerin extract demonstrated superior antimicrobial activity at all tested concentrations, particularly exhibiting strong antibacterial effects against *E. faecalis* and *S. aureus*. In contrast, the water-based extracts were notably effective against *C. albicans*. These findings suggest that both glycerin and water are promising, biocompatible solvents for propolis extraction in endodontic applications, and that the choice of extraction vehicle significantly influences the antimicrobial effectiveness of the propolis. A study by Ramanauskien et al. [53] found that propylene glycol-based propolis solutions exhibited notable antimicrobial activity, especially against *S. aureus* and *B. subtilis*. These solutions were also effective against other tested microorganisms, though less potent than ethanol-based extracts. In contrast, water-extracted propolis and propolis in a water-ethanol-propylene glycol mixture showed no significant antimicrobial effects. The findings emphasize that the choice of solvent greatly influences the

Table 3 Antimicrobial activities of propolis extracts

Samples	*Escherichia coli*	*Staphylococcus aureus*	*Bacillus cereus*	*Salmonella typhimurium*	*Candida albicans*	*Aspergillus niger*
WPe	-	-	-	-	-	-
WGPe	41.60±0.10	39.17±0.06	39.83±0.15	-	-	-
WPGPe	-	17.90±0.10	13.20±0.10	-	-	-
Positive Control	31.90±0.10	32.90±0.10	27.00±0.20	33.87±0.23	10.93±0.06	12.8±0.20

WPe: water-based propolis extract; WGPe: glycerol: water-based propolis extract; WPGPe: propylene glycol: water-based propolis extract

antimicrobial properties of propolis, with propylene glycol-based formulations showing promise and warranting further investigation into their composition and preparation methods.

Phenolic profile

The discovery of the importance of phenolic compounds for human health has led to the extraction, characterisation, and evaluation of the biological activities of these compounds and their potential therapeutic applications [65–69]. Although the qualitative and quantitative composition of propolis varies depending on its geographical and botanical origin, phenolic compounds are recognised as its major bioactive components [70–72]. The characterisation of phenolic compounds and their biological effects in ethanol and methanol extracts prepared from propolis samples from many different geographical regions such as Greece, China, Iran, Romania, Thailand, New Zealand and Türkiye have been extensively investigated and validated in the last two decades [73–78]. Turkish propolis is very rich in bioactive compounds due to its rich floral flora [79], resulting from the country's unique geographical location (intersection of Euro-Siberian, Iran-Turanian, and Mediterranean regions). However, literature reviews have shown that there are limited quantitative studies on the characterization of phenolic compounds in propolis samples originating different regions of Türkiye [77, 80–82]. Among the existing studies, the lack of research conducted with water, propylene glycol: water, and glycerol: water extracts of propolis obtained from Artvin Province reveals a scientific gap in the characterization of propolis specific to this region.

Phenolic compound composition of propolis extracts prepared using glycerol: water and propylene glycol: water solvents, which are preferred in addition to water because they are green solvents recommended to eliminate the use of classical organic solvents and to produce extracts suitable for the food industry, was analysed by RP-HPLC-UV method. The phenolic compound composition of the samples was determined using 18 phenolic compound standards (gallic acid, epigallocatechin, chlorogenic acid, catechin, epicatechin, caffeic acid, rutin, *p*-coumaric acid, trans-sinapic acid, ferulic acid, myricetin, resveratrol, luteolin, quercetin, apigenin, diosmetin, sinensetin and chrysin) (Table 4; Fig. 2). As presented in Table 4, epigallocatechin, chlorogenic acid, catechin, epicatechin, trans-sinapic acid, myricetin, luteolin, quercetin, diosmetin, and sinensetin were not detected in any extract. The predominant phenolic compound of WPe and WGPe was ferulic acid (968.82 and 1190.44 µg/g propolis, respectively), while chrysin (2334.41 µg/g propolis) was found in WPGPe. Ferulic acid, the the methoxy form of caffeic acid, is recognized for its ability protect DNA and lipids against oxidation triggered by reactive oxygen species [74]. The data reported by Duymaz et al. [83] for ferulic acid in 70% ethanol, propylene glycol, and L-arginine extracts of propolis originating from Türkiye (Yalova), at 1.29 ± 0.06, 1.22 ± 0.05, and 1.52 ± 0.11 mg/g raw propolis, respectively, are compatible with our study.

Table 4 Phenolic profile and RP-HPLC-UV analysis parameters of propolis extracts

Compound	RT	Equation	R^2	LR	LOD	LOQ	Concentration (µg/g propolis)		
							WPe	WGPe	WPGPe
Gallic acid	10.103	y = 23.355x –1.102	1.00000	1–50	0.02	0.07	98.98	18.12	63.12
Epigallocatechin	15.364	y = 1.895x + 0.331	0.99993	1–50	0.21	0.63	nd	nd	nd
Chlorogenic acid	16.128	y = 12.257x + 0.426	0.99999	1–50	0.07	0.21	nd	nd	nd
Catechin	16.409	y = 6.376x + 3.208	0.99981	1–50	0.10	0.30	nd	nd	nd
Epicatechin	17.406	y = 5.746x + 0.217	0.99999	1–50	0.04	0.13	nd	nd	nd
Caffeic acid	17.754	y = 26.078x + 4.284	0.99998	1–50	0.02	0.06	918.82	828.39	610.96
Rutin	18.317	y = 6.766x + 0.839	0.99999	1–50	0.07	0.20	31.69	47.04	61.16
p-Coumaric acid	19.888	y = 39.386x + 3.417	0.99999	1–50	0.02	0.07	895.42	1073.73	1512.36
Trans-sinapic acid	20.173	y = 10.658x + 1.108	0.99998	1–50	0.04	0.12	nd	nd	nd
Ferulic acid	20.457	y = 23.966x + 0.344	0.99998	1–50	0.02	0.06	968.82	1190.44	1976.83
Myricetin	21.646	y = 10.658x –1.147	0.99998	1–50	0.36	1.09	nd	nd	nd
Resveratrol	23.003	y = 30.513x + 1.585	0.99999	1–50	0.01	0.04	198.69	227.42	375.05
Luteolin	23.657	y = 14.926x + 0.163	0.99999	1–50	0.04	0.13	nd	nd	nd
Quercetin	23.885	y = 12.546x –0.213	1.00000	1–50	0.31	0.93	nd	nd	nd
Apigenin	25.309	y = 19.761x + 2.660	0.99999	1–50	0.17	0.52	nd	65.28	340.54
Diosmetin	25.658	y = 19.245x + 2.326	0.99999	1–50	0.15	0.46	nd	nd	nd
Sinensetin	27.963	y = 12.067x –0.026	0.99999	1–50	0.07	0.20	nd	nd	nd
Chrysin	30.653	y = 27.932x + 0.941	0.99999	1–50	0.01	0.04	17.15	240.10	2334.41

*RT: Retention time (min); R^2: Correlation coefficient; LR: Linear range µg/mL; LOD: Limit of detection (µg/mL); LOQ: Limit of quantification (µg/mL); WPe: water-based propolis extract; WGPe: glycerol: water-based propolis extract; WPGPe: propylene glycol: water-based propolis extract

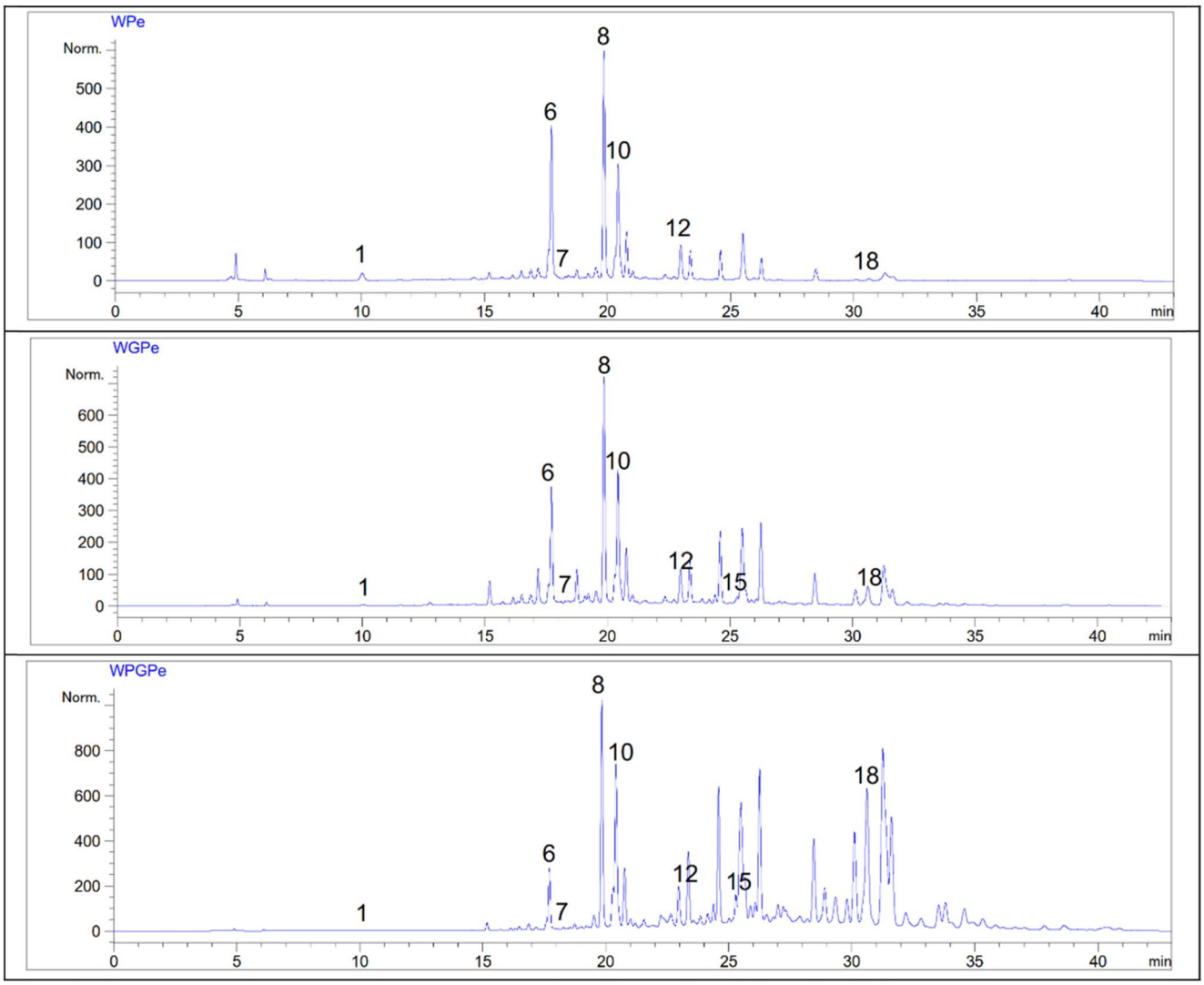


Fig. 2 Optimum RP-HPLC chromatographic profiles of propolis extracts recorded at 280 nm. Gallic acid (1), Epigallocatechin (2), Chlorogenic acid (3), Catechin (4), Epicatechin (5), Caffeic acid (6), Rutin (7), *p*-Coumaric acid (8), Trans-sinapic acid (9), Ferulic acid (10), Myricetin (11), Resveratrol (12), Luteolin (13), Quercetin (14), Apigenin (15), Diosmetin (16), Sinensetin (17) ve Chrysin (18)

Kubiliene et al. [84] determined ferulic acid levels of 78.4, 161.9, and 798.9 µg/mL in aqueous, polyethylene glycol-aqueous, and ethanolic extracts of propolis collected from Lithuania, respectively. Aliyazicioglu et al. [85] determined ferulic acid levels in the methanolic extracts of 20 propolis samples from Türkiye with 12 botanical origins, ranging from 22.33 to 712.69 mg/100 g, in accordance with our present study data. Similarly, close ferulic acid values were reported in propolis from North Macedonia, Greece, and Brazil [72, 74]. Ozdal et al. [77] reported transferulic acid at 373 ± 9.62 ppm only in propolis from the Marmara Region, among 70% ethanolic extracts prepared from propolis samples sourced from different geographical regions of Türkiye.

The HPLC data of WPe, WGPe, and WPGPe extracts reveal a significant difference in chrysin levels among the extracts. Therefore, the choice of solvent in propolis extraction is critically important for the concentration of chrysin. Chrysin, also known as 5,7-dihydroxy-2-phenyl-4 H-chromen-4-one, is a natural flavonoid found in various plants. It has been reported to be found in honey, propolis, and many plant extracts [86]. Its anti-inflammatory, hepatoprotective, neuroprotective, antioxidant, and anticancer properties have been reported [86, 87]. However, despite its beneficial effects on health, as a lipophilic flavonoid, it has limited solubility in solvents with high polarity, such as water, and therefore exhibits low absorption [88]. Co-solvents, such as polyethylene glycol, propylene glycol, and alcohol, are used to increase solubility [89]. In the present study, the lowest chrysin level was 17.15 µg/g propolis in WPe, while the WGPe sample had a concentration about 10 times lower than WPGPe (240.10 µg/g propolis). In many studies in the literature, chrysin was not reported in

aqueous, polyethylene glycol-aqueous, methanol, and ethanolic extracts of propolis from different countries [61, 74, 77, 78, 85]. Duymaz et al. [83] determined the level of crysin in 70% ethanol and monopropylene glycol extracts of propolis originating from Türkiye as 4.30 ± 0.10 and 4.33 ± 0.12 mg/g raw propolis, respectively. The fact that the chemical characteristics of propolis are closely linked to the plant species available in its local environment and samples taken from different geographical and climatic regions naturally causes the phenolic compounds it contains to vary.

Similar to chrysine, apigenin (4′,5,7-trihydroxy-flavone) is a flavonoid with very low water solubility (1.35 µg/mL) [90]. Studies have mostly focused on the effects of this flavonoid on various types of cancers [91–93]. WGPe and WPGPe contained apigenin at levels of 65.28 µg/g propolis and 340.54 µg/g propolis, but it was not detected in WPe. The apigenin concentration reported by Duymaz et al. [83] as 1.87 ± 0.10 mg/g raw propolis for the mono-propylene glycol extract of propolis was significantly higher than the values obtained in our study. This is thought to be because the solvent used in the extraction was pure (not mixed with water). Consistent with our results, Ozdal et al. [77] reported the apigenin level in a 70% aqueous-ethanol extract of propolis obtained from the Marmara, Central Anatolia, Eastern Anatolia, and West Mediterranean regions of Türkiye, classified into 11 groups according to their origin, between 33 ± 0.85 and 528 ± 13.61 ppm by LC-MS/MS. In addition, the researchers detected gallic acid in propolis in only four groups, ranging from 90 ± 2.32 to 374 ± 11.4 ppm. In our samples, the gallic acid levels of the extracts were ranked as WPe (98.98 µg/g propolis) > WPGPe (63.12 µg/g propolis) > WGPe (18.12 µg/g propolis), and the result obtained only in WPe was compatible with the mentioned study [77]. In the study carried out by Aliyazicioglu et al. [85] on propolis from different cities of Türkiye (Kastamonu, Eskişehir, Ereğli, Zonguldak, İzmir, Erzurum, Sinop, and Artvin), the amounts of gallic acid were reported at levels ranging from 0.87 to 7.01 mg/100 g propolis, which is close to our present study.

In pressurised liquid extracts of propolis collected from Bingöl, Rize, Tekirdağ and Van by Erdogan et al. [80] with ethanol: water: HCl (34.44 ± 2.08, 28.54 ± 1.44, 13.18 ± 0.53, and 35.86 ± 1.90 mg/kg, respectively) were higher than the water extract in our study but lower than the glycerol: water and propylene glycol: water extracts. In the current study, caffeic acid was found to be the most abundant in water extracts (918.82 µg/g propolis), similar to gallic acid. Our findings are above the values of caffeic acid reported by Erdogan et al. [80] in the range of 284.43 ± 13.11 to 546.66 ± 21.08 mg/kg and are below the values reported by Lagouri et al. [74], which ranged from 1.30 ± 0.11 to 4.17 ± 0.27 mg/g (except Rhodes, 80% methanol extract). Omer et al. [82] reported caffeic acid, trans-ferulic acid, o-coumaric acid, morin, kaempferol, and chrysin as the major phenolic constituents of powdered water extracts of Turkish propolis originating from the West Black Sea Region of Türkiye (Samsun) obtained at low temperatures (+ 4, −80, and − 196 °C). They reported caffeic acid levels as 812.60 and 1734.35 mg/mL.

P-coumaric acid (4-hydroxy-cinnamic acid) is the most abundant form of coumaric acid, which is the hydroxy derivative of cinnamic acid [94]. The *p*-coumaric acid content of the extracts ranged from 895.42 µg/g propolis (WPe) to 1512.36 µg/g propolis (WPGPe). Our results were significantly higher than the *p*-coumaric acid values reported by Erdogan et al. [80] in pressurized liquid extracts of propolis from Bingöl, Rize, Tekirdağ, and Van (Türkiye) (17.14 ± 1.0, 23.28 ± 2.97, 22.08 ± 3.42, and 88.75 ± 4.17 mg/kg, respectively). The *p*-coumaric acid levels reported by Pellati et al. [95], ranging from 20 to 1350 ppm in propolis extracts, were compatible with our study. Rutin (quercetin-3-O-rutinoside), also known as vitamin P, is a flavonoid with lipophilic character and is soluble in organic solvents such as pyridine, methanol, and ethanol [96]. Its solubility in water is very low (0.125 g/L) [97]. WPGPe had the highest rutin content (61.16 µg/g propolis), followed by WGPe and WPe, respectively. Our results were lower than the rutin levels determined by Coneac et al. [98] for water extract of Romanian propolis (0.3 mg/g propolis) and by Zehra et al. [99] for ethanol extracts of Azerbaijani propolis (between 7.80 and 683,000 mg/100 g); while they were compatible with the values reported by Siripatrawan et al. [78] in the range of 13.47 to 72.42 ppm in 30%, 40%, 50% and 70% ethanol aqueous Thai propolis extracts. It was observed that no rutin was reported in propolis from Portugal [45, 100], Bulgaria [101], and Iran [102].

Resveratrol (3,4’,5-trihydroxy-trans-stilbene) is a polyphenolic compound belinging to the stilbenoid class found in more than 70 plants, but most commonly found and extracted from grapes. It is known to have immune-enhancing, anti-aging, anti-obesity, neuroprotective, cardioprotective, and cancer-protective effects [103]. The extracts contained resveratrol in the range of 198.69 µg/g propolis (WPe) to 375.05 µg/g propolis (WPGPe). Omer et al. [82] did not determine resveratrol in water extracts of Turkish propolis (Black Sea Region of Türkiye, Samsun) extracted at + 4 °C; however, they reported 140.48 and 107.20 mg/mL in samples extracted at − 80 °C and − 196 °C, respectively. In many studies in the literature, resveratrol was determined in Turkish propolis [77, 80, 81, 104, 105], Iranian propolis [73], Chinese propolis [106], Polish propolis [107], Greek propolis [74], and Lithuanian propolis [84].

Popova et al. [105], vanillin, *p*-coumaric acid, ferulic acid and caffeic acid and ester derivatives of phenolic acids such

as benzyl *p*-coumarate, benzyl ferulate and various coumaroyl glycerol derivatives by GC-MS in 70% ethanol extract of propolis originating from the region (Artvin, Türkiye) where propolis was obtained in our present study. In the literature, phenolic compounds detected in propolis studies originating from various locations in Türkiye include naringenin, chrysin, acacetin, ferulic acid, dimethyl caffeic acid, isoferulic acid, 4-vinylphenol, and 2-methoxy-4-vinylphenol in Erzurum propolis; tectocrisin, pinocembrin, chrysin, 4 H-1-benzopyran-4-one, 3,5,7-trihydroxy-2-phenyl, methylhomovanillate, and isoferulic acid in Bursa and Hatay propolis [108, 109]. Ozdal et al. [77] reported that luteolin, 3,4-dimethoxycinnamic acid, *p*-coumaric acid, caffeic acid phenethyl ester, pinobanksin, and naringenin were the dominant phenolic compounds in the ethanol extracts of Turkish propolis. Erdogan et al. [80] reported that chlorogenic acid, caffeic acid, gallocatechin, epicatechin gallate, myricetin, and catechin are the major phenolic compounds found in of propolis originating from Bingöl, Rize, Tekirdağ, and Van provinces in Türkiye. Sha et al. [106] determined by HPLC-UV that the dominant components of Chinese propolis were caffeic acid, isoferulic acid, 3,4-dimethoxycinnamic acid, pinobanksin 5-methyl ether, pinocembrin, benzyl caffeate, chrysin, and galangin. Milek et al. [107] reported that extracts of south-eastern Poland propolis containing ethanol, acetone, propylene glycol, glycerol, water, PEG (Polyethylene glycol) 400, and lecithin aqueous contained phenolic acids consisting mainly of caffeic, *p*-coumaric, ferulic, benzoic acid, and derivatives, and flavonoids including pinobanksin, sacuranetin, pinocembrin, and galangin by HPLC. The researchers reported significant differences between the extracts, especially in terms of *p*-coumaric acid, the dominant component. They also noted that the water extracts were almost entirely composed of phenolic acids and that this extract was richer in phenolic components than the extracts prepared with 50% glycerol [107]. In addition, cinnamic, gallic, hydroxybenzoic, gentisic, vanillic, and chlorogenic acids, as well as their various derivatives, have been reported in Polish propolis by various researchers [110–112]. The differences between WPe, WGPe, and WPGPe, which we analyzed in all the mentioned studies and the present study, can be attributed to the plant diversity in the geographical region where propolis is obtained, the extraction solvent, the various temperature and time treatments, and the extraction methodologies.

Principal component analysis (PCA), hierarchical cluster analysis (HCA), and correlation

PCA, which is a statistical technique that allows visualising the similarities and differences between samples by reducing the size of multivariate data, was used in this the present study to distinguish the chemical and biological differences between water, glycerol: water and propylene glycol: water extracts of propolis in terms of TPC, TFC, antioxidant activity (DPPH, ABTS, and FRAP) and phenolic component profile at statistical level and to reveal which parameters play a determining role in this differentiation. The score scatter plot, loading plot, and biplot images of PCA analysis are presented in Fig. 3A-C, respectively. The PCA results demonstrated that the proportions of variance explained by the first and second components were R2X [1] (91.20%) and R2X [2] (8.75%), respectively, and these two components accounted for a total of 99.95% of the variance in the dataset. This very high rate shows that the study data are highly compatible with PCA. When the score scatter plot (Fig. 3A) is analyzed, the clear separation of WPGPe from WGPe and WPe in terms of PC1 indicates a distinct separation between the sample groups based on the measured parameters.

Gallic acid and caffeic acid are separated from other compounds in the upper left side (negatively) of the Loading scatter plot (Fig. 3B). This shows that these two compounds are significantly separated from other compounds and are among the variables that make the most substantial contribution to the PCA model. Similarly, rutin is also positioned alone in the lower right side of the loading scatter plot. Therefore, it has a distinct profile from other phenolic compounds in terms of PC1 and PC2 components, and it stands out as a distinguishing variable. The fact that *p*-coumaric acid, ferulic acid, resveratrol, apigenin, and chrysin are positioned very close to each other on the right side of the graph (Fig. 3B) shows that they are concentrated together in similar extract groups.

PCA and correlation results (Figs. 3 and 4) were consistent with each other. In the loading scatter plot (Fig. 3B), significant and high positive correlations were determined among components. Remarkably, high positive correlations were found between the following pairs: TPC and TFC, TPC and resveratrol, TFC and ABTS, FRAP and resveratrol, DPPH and resveratrol, ABTS and resveratrol, ferulic acid and apigenin, resveratrol and apigenin ($r=1.000$, $p<0.05$); TPC and DPPH, TPC and ferulic acid, TPC and FRAP, TPC and apigenin, FRAP and ABTS, FRAP and apigenin, FRAP and chrysin, DPPH and ABTS, DPPH and apigenin, DPPH and chrysin, ABTS and ferulic acid ($r=0.999$, $p<0.05$); TFC and ferulic acid, TFC and chrysin, resveratrol and chrysin, resveratrol and ferulic acid ($r=0.998$, $p<0.05$); DPPH and ferulic acid, FRAP and ferulic acid, *p*-coumaric acid and ferulic acid ($r=0.997$, $p<0.05$) (Fig. 4, heatmap visual). The strong correlations of some phenolic compounds (e.g., resveratrol and ferulic acid) with both TPC and TFC, alongside antioxidant activity assesments, highlight these compounds as key indicators of the biological activity of the extracts.

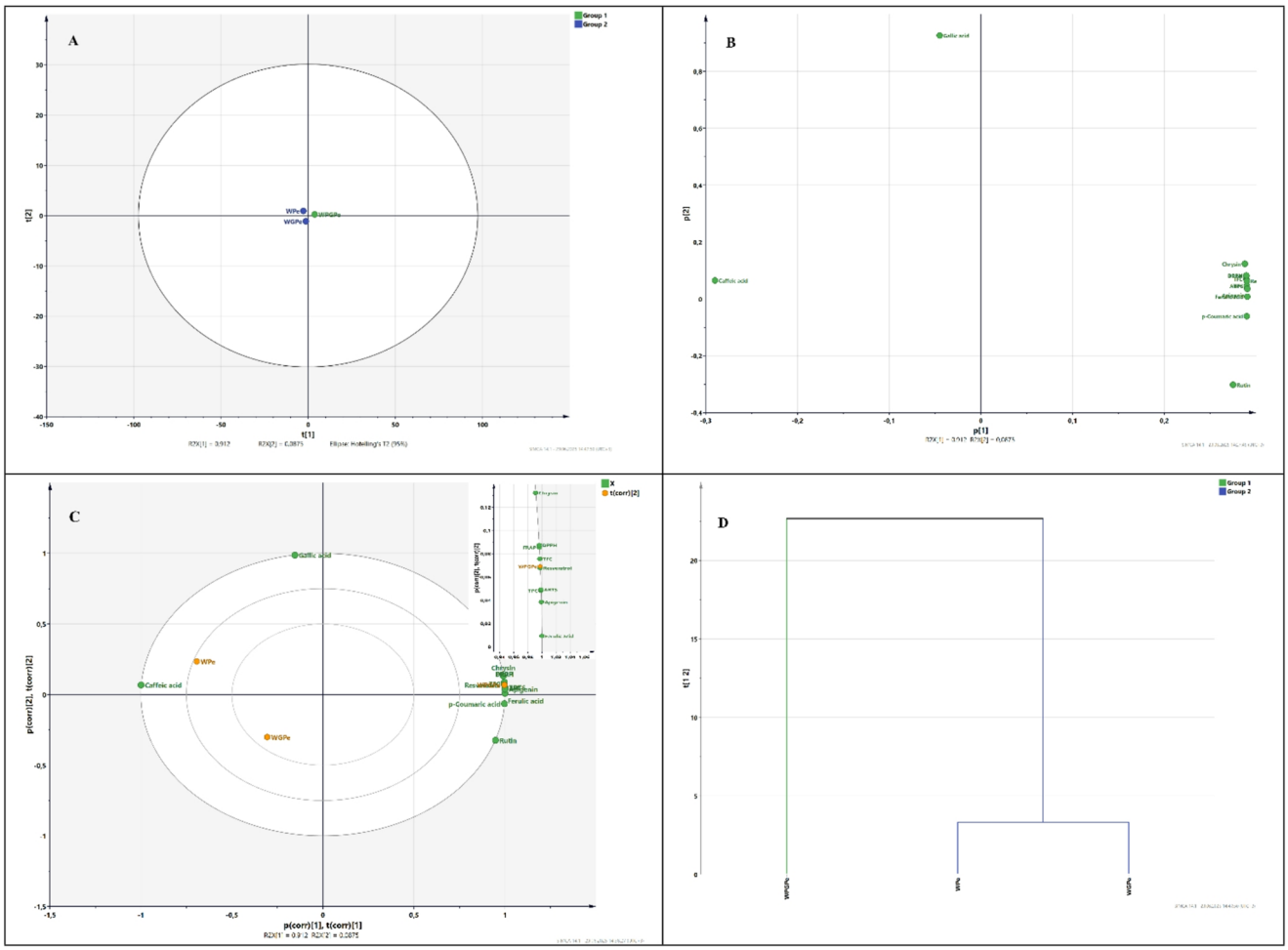


Fig. 3 Score scatter plot (**A**), Loading scatter plot (**B**), biplot (**C**) of the PCA, and Dendrogram of the HCA

The biplot graph presented in Fig. 3(C) shows that WPe is located close to caffeic acid and partially to gallic acid. Thus, the caffeic acid content of this extract is higher than that of others (918.82 and 98.98 µg/mL, respectively, Table 4). WGPe is located in the lower-left quadrant of the PCA plane and is far from all bioactive parameters (TPC, TFC, DPPH, ABTS, and FRAP) and most of the phenolic compounds. This situation shows that WGPe is the sample with the lowest contribution to the PCA. WPGPe is located on the positive PC1 axis of the biplot and very close to the central orbit. This extract is very close to the bioactive parameters TPC, TFC, DPPH, FRAP, and ABTS, as well as phenolic compounds such as resveratrol, apigenin, ferulic acid, and chrysin. Therefore, the propylene glycol: water extracts of propolis presented a rich and balanced profile in terms of the aforementioned parameters. As a result, WPGPe can be evaluated as one of the strongest samples in terms of functional properties, being at the center of antioxidant parameters and some basic phenolic compounds on the PCA.

Additionally, HCA (dendrogram or PLS-Trees) was applied to create hierarchical clusters based on the similarity between the extracts and to visually verify the grouping of similar samples in the matrices resulting from PCA (Fig. 3D). By default, the tree on the horizontal axis of the dendrogram is sorted by cluster size. The separation of clusters is made based on the point where the distance increases suddenly on the vertical axis, and groups with similar characteristics are created accordingly. As seen in Figs. 1 and 3D clusters formed in the dendrogram. While WPGPe was located in the first cluster, WPe and WGPe were grouped in the second cluster. The coexistence of WPe and WGPe indicates a higher level of similarity between these extracts, while it suggests that WPGPe has distinct properties from the others. When the cluster structure and distribution of compounds were examined, particularly the TPC, TFC, FRAP, DPPH, and ABTS data of WPGPe, as well as the contents of *p*-coumaric acid, ferulic acid, resveratrol, apigenin, and chrysin, were significantly different from those of the other extracts (Tables 2 and 4). The observed separation

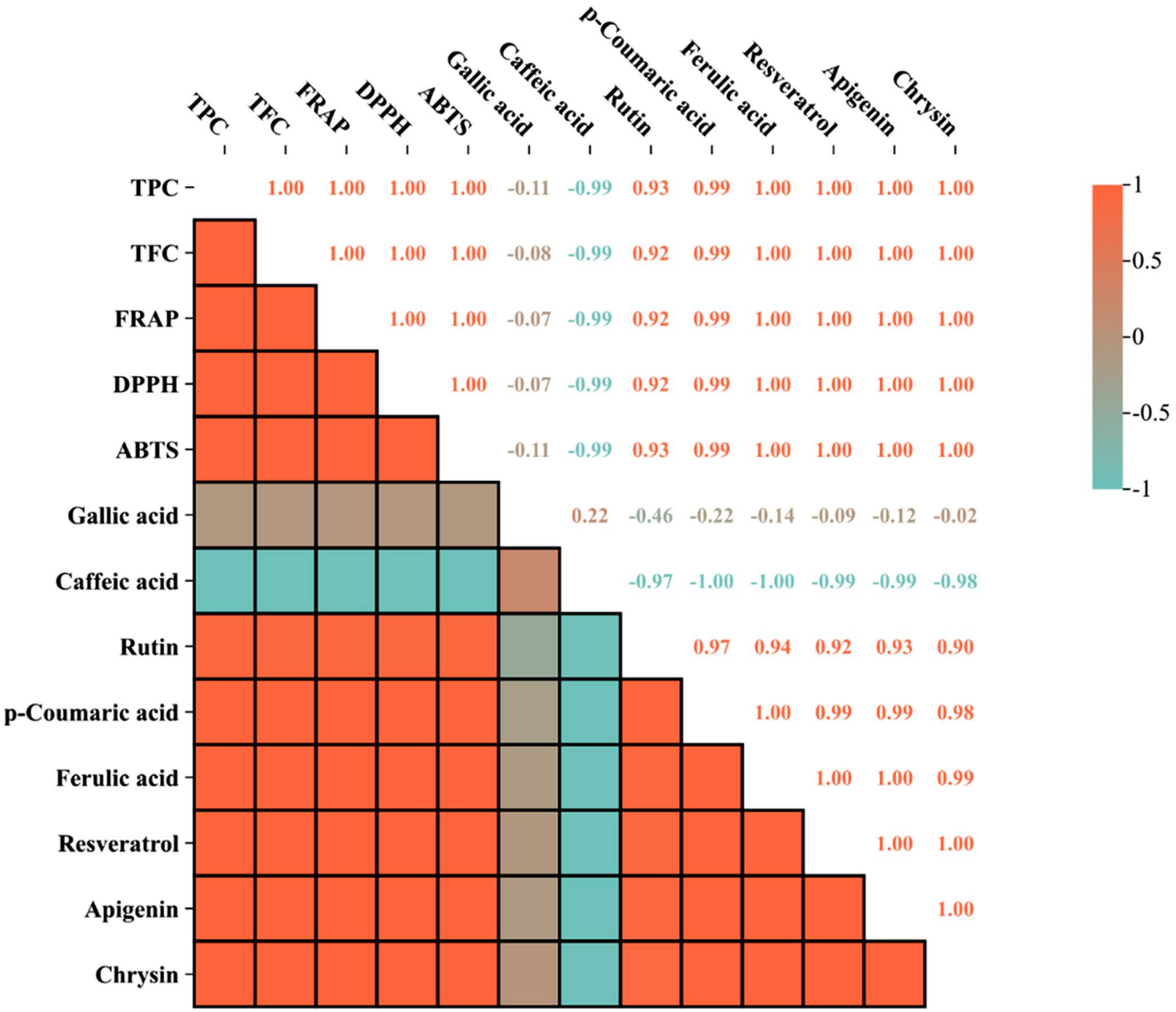


Fig. 4 Heat map of correlations of TPC, TFC, antioxidant activity, and phenolic compound profile data of extracts. The color scale reflects the direction of correlation, changing from positive to negative, from orange to blue

between the clusters was consistent with the PCA results. The agreement between the results of both multivariate methods emphasizes the differences in antioxidant, total phenolic, and flavonoid amounts, in addition to the phenolic compounds profiles of propolis extracts prepared with different solvents. This suggests that the type of solvent plays a crucial role in determining the biological and chemical properties of the extract.

Conclusion

This study is the first to determine the most suitable solvents for extracting propolis from Artvin Province in northeastern Türkiye, specifically water, propylene glycol-water, and glycerol-water, which exhibit higher biocompatibility compared to ethanol. In this direction, the physicochemical properties of the extracts (color, soluble solid content, pH, and titratable acidity) were determined, along with their TPC and TFC values, antioxidant analyses (ABTS, DPPH, and FRAP assays), antimicrobial activity, and a phenolic compound profile. Additionally, PCA and HCA were performed to investigate the effect of the extraction procedure on the antioxidant properties and phenolic compounds present in propolis extracts. WPGPe was significantly different ($p<0.01$) from WGPe and WPe in terms of physicochemical analyses, total phenolic and flavonoid contents, antioxidant activity, and phenolic compound profile. Antimicrobial activity results showed that all extracts were ineffective against *S. typhimurium*, *C. albicans*, and *A. niger*. However, WGPe

was effective against *E. coli*, *S. aureus*, and *Bacillus cereus*, and WPGPe was effective against *S. aureus* and *B. cereus*. Our results demonstrated that the choice of solvent plays an important role in shaping the biological and chemical properties of the extract, and that the use of safer, biocompatible solvents, such as those that are not ethanol, is both practical and sustainable. Propylene glycol and glycerol are renewable, low-toxicity compounds widely accepted in the food and pharmaceutical industries. The implementation of such eco-friendly extraction systems could reduce the environmental footprint of propolis production and support the development of sustainable methods for bioactive ingredient. The extracts obtained in our study have the potential to be evaluated in the food, health, and cosmetic fields and can also guide future studies. Despite the promising results, this study has certain limitations. The solvent systems were tested under laboratory conditions; however, long-term stability or sensory acceptability in formulated products was not evaluated. Future studies should focus on optimizing solvent ratios for maximum bioactive recovery while assessing the bioavailability and consumer acceptability of the resulting extracts.

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Declarations

Competing interest The authors state that they have no competing financial interests or personal relationships that could have influence the work presented in this manuscript.

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References

1. Y. Tumbarski, I. Ivanov, M. Todorova et al., Phenolic content, antioxidant activity and in vitro anti-inflammatory and antitumor potential of selected Bulgarian propolis samples. Biomedicines (2025). https://doi.org/10.3390/biomedicines13020334
2. Z. Bakkaloğlu, M. Arıcı, Effects of propolis extraction with different solvents on total phenolic Content, antioxidant capacity and antimicrobial activity. Acad. Food. **17** (2019). https://doi.org/10.24323/akademik-gida.667272
3. M. El-Sakhawy, A. Salama, S.A. Mohamed, Propolis applications in food industries and packaging. Biomass Convers. Biorefin. **14**, 13731–13746 (2024). https://doi.org/10.1007/s13399-023-04044-9
4. M. Kieliszek, K. Piwowarek, A.M. Kot et al., Recent advances and opportunities related to the use of bee products in food processing. Food Sci. Nutr. **11**, 4372–4397 (2023). https://doi.org/10.1002/fsn3.3411
5. W. Siheri, S. Alenezi, J. Tusiimire, D.G. Watson, *The Chemical and Biological Properties of propolis, in Bee products–chemical and Biological Properties* (Springer, 2025), pp. 209–269
6. K. Pant, A. Sharma, H.K. Chopra, V. Nanda, Impact of biodiversification on propolis composition, functionality, and application in foods as natural preservative: a review. Food Control **155**, 110097 (2024). https://doi.org/10.1016/j.foodcont.2023.110097
7. S. Ufuk, M. Şengül, The effects of different extraction methods and solvents on antioxidant properties of propolis. Black Sea J. Agric. **6**, 386–393 (2023). https://doi.org/10.47115/bsagriculture.1273535
8. E. İzol, M.A. Yılmaz, İ. Gülçin, Chemical characterization by chromatography techniques and comprehensive biological activities of Artvin bee products. ChemistrySelect. **10**, e202501545 (2025). https://doi.org/10.1002/slct.202501545
9. A.M. Silva, B. Rocha, M.M. Moreira et al., Biological activity and chemical composition of propolis extracts with potential use in vulvovaginal candidiasis management. Int. J. Mol. Sci. (2024). https://doi.org/10.3390/ijms25052478
10. M.H. Al-Mamoori, S.G. Okbagabir, E.N. Pakina, M. Zargar, The biological effectiveness of crude propolis extract in inhibiting the growth of phytopathogens. RUDN J. Agron. Anim. Ind. **19**, 592–601 (2024). https://doi.org/10.22363/2312-797X-2024-19-4-592-601
11. B. Karagul, S. Ugras, P. Karagul et al., Analysis and evaluation of quality parameters of commercial propolis products using a new high performance liquid chromatography (HPLC) method and comparison of antimicrobial properties. Microchem. J. (2024). https://doi.org/10.1016/j.microc.2024.111023
12. A. Vaseghi, R.A. Parchin, K.R. Chamanie et al., Encapsulation of propolis extracted with methylal in the chitosan nanoparticles and its antibacterial and cell cytotoxicity studies. BMC Complement. Med. Ther. (2024). https://doi.org/10.1186/s12906-024-04472-8
13. Z. Can, Y. Kara, S. Kolayli, I. Çakmak, Antioxidant activity and phenolic composition of propolis from Marmara region, Turkey. J. Apicult. Res. **63**, 542–548 (2024). https://doi.org/10.1080/00218839.2022.2157582
14. L.S. Contieri, L.M.D. Mesquita, V.L. Sanches et al., Recent progress on the recovery of bioactive compounds obtained from propolis as a natural resource: Processes, and applications. Sep. Purif. Technol. **298**, 121640 (2022). https://doi.org/10.1016/j.seppur.2022.121640
15. S. El-Guendouz, B. Lyoussi, M.G. Miguel, Insight on propolis from mediterranean countries: chemical composition, biological activities and application fields. Chem. Biodivers. **16**, e1900094 (2019). https://doi.org/10.1002/cbdv.201900094
16. J. Suran, I. Cepanec, T. Masek et al., Propolis extract and its bioactive Compounds-From traditional to modern extraction technologies. Molecules. **26** (2021). https://doi.org/10.3390/molecules26102930
17. A.A. Attia, R.H. ElMazoudy, N.S. El-Shenawy, Antioxidant role of propolis extract against oxidative damage of testicular tissue induced by insecticide chlorpyrifos in rats. Pestic. Biochem. Physiol. **103**, 87–93 (2012). https://doi.org/10.1016/j.pestbp.2012.04.002

18. N. Zullkiflee, H. Taha, A. Usman, Propolis: its role and efficacy in human health and diseases. Molecules (2022). https://doi.org/10.3390/molecules27186120
19. V.P. Chavda, A.Z. Chaudhari, D. Teli et al., Propolis and their active constituents for chronic diseases. Biomedicines (2023). https://doi.org/10.3390/biomedicines11020259
20. A. Necip, I. Demirtas, S.E. Tayhan et al., Isolation of phenolic compounds from eco-friendly white bee propolis: Antioxidant, wound-healing, and anti-Alzheimer effects. Food Sci. Nutr. **12**, 1928–1939 (2024). https://doi.org/10.1002/fsn3.3888
21. N. Orsolic, M.J. Jembrek, Molecular and cellular mechanisms of propolis and its polyphenolic compounds against cancer. Int. J. Mol. Sci. **23** (2022). https://doi.org/10.3390/ijms231810479
22. S. Araba, H. Özparlak, Propolis. Selçuk Üniversitesi Fen Fakültesi Fen Dergisi **48**, 1–9 (2022). https://doi.org/10.35238/sufefd.1068425
23. S. Islam, E.A. Hussain, S. Shujaat et al., Antibacterial potential of propolis: molecular docking, simulation and toxicity analysis. Amb Express. **14** (2024). https://doi.org/10.1186/s13568-024-01741-0
24. M.Y. Abduh, T.R. Shafitri, E. Elfahmi, Chemical profiling, bioactive compounds, antioxidant, and anti-inflammatory activities of Indonesian propolis extract produced by *Tetragonula laeviceps*. Heliyon **10**, e38736 (2024). https://doi.org/10.1016/j.heliyon.2024.e38736
25. C. Jansen-Alves, F.N. Victoria, C.D. Borges, R.C. Zambiazi, Encapsulation of propolis extract in ovalbumin protein particles: characterization and in vitro digestion. Nat. Prod. Res. **38**, 1766–1770 (2024). https://doi.org/10.1080/14786419.2023.2214945
26. C.A. Ligarda-Samanez, D. Choque-Quispe, E. Moscoso-Moscoso et al., Microencapsulation of propolis and honey using mixtures of maltodextrin/tara gum and modified native potato starch/tara gum. Foods **12**, 1873 (2023). https://doi.org/10.3390/foods12091873
27. M. Miłek, E. Sidor, R. Bonikowski et al., An innovative method of obtaining herbal propolis extracts and application of its spray-dried form as natural food preservatives. International Journal of Food Science **2025**, 5546802 (2025). https://doi.org/10.1155/ijfo/5546802
28. V. Bankova, B. Trusheva, M. Popova, Propolis extraction methods: a review. J. Apic. Res. **60**, 734–743 (2021). https://doi.org/10.1080/00218839.2021.1901426
29. T. Ghumro, S.Q. Memon, A. Mallah et al., Design of experiment for extraction optimization and antioxidant assay studies of biologically active compounds of propolis. J. Apicult Res. 1–9 (2024). https://doi.org/10.1080/00218839.2024.2394744
30. B. Trusheva, H. Petkov, M. Popova et al., Green approach to propolis extraction: natural deep eutectic solvents. Comptes Rendus l'Academie Bulg. Sci. **72**, 897–905 (2019). https://doi.org/10.7546/CRABS.2019.07.06
31. N.E. Bayram, Y.C. Gercek, S. Bayram, B. Togar, Effects of processing methods and extraction solvents on the chemical content and bioactive properties of propolis. J. Food Meas. Charact. **14**, 905–916 (2020). https://doi.org/10.1007/s11694-019-00340-z
32. C.S. Funari, A.T. Sutton, R.L. Carneiro et al., Natural deep eutectic solvents and aqueous solutions as an alternative extraction media for propolis. Food Res. Int. **125**, 108559 (2019). https://doi.org/10.1016/j.foodres.2019.108559
33. E.F. Topdas, M. Sengul, A. Taghizadehghalehjoughi, A. Hacimuftuoglu, Neuroprotective potential and antioxidant activity of various solvent extracts and essential oil of *Ferula orientalis* L. J. Essent. Oil-Bear. Plants **23**, 121–138 (2020). https://doi.org/10.1080/0972060x.2020.1729247
34. B. Cemeroğlu, Gıda analizleri. Gıda Teknolojisi Derneği Yayınları 2007. 173–177
35. I.A. Karakütük, M. Sengül, M. Zor, S. Aksoy, The effects of using different plant species and sweeteners (stevia and sucrose) in sherbet production on chemical and sensory quality of sherbet. J. Food Meas. Charact. **17**, 5308–5321 (2023). https://doi.org/10.1007/s11694-023-02041-0
36. V.L. Singleton, R. Orthofer, R.M. Lamuela-Raventós, Analysis of total phenols and other oxidation substrates and antioxidants by means of Folin-Ciocalteu reagent. Methods Enzymol. **299**, 152–178 (1999). https://doi.org/10.1016/S0076-6879(99)99017-1
37. E. Koçak, E. Demircan, B. Özçelik, Antioxidant capacities and phenolic profiles of Ottoman strawberry fruit and Ottoman strawberry jam. Ecol. Life Sci. **13**, 119–130 (2018). https://doi.org/10.12739/NWSA.2018.13.3.5A0101
38. H.T. Balaydin, I. Gülçin, A. Menzek et al., Synthesis and antioxidant properties of diphenylmethane derivative bromophenols including a natural product. J. Enzym Inhib. Med. Ch. **25**, 685–695 (2010). https://doi.org/10.3109/14756360903514164
39. M. Sengul, H. Unver, E.F. Topdas et al., Evaluation of antioxidant properties and phenolic and aromatic profiles of Cornelian Cherry pestil samples prepared with sucrose and stevia addition. J. Food Process. Pres. **46** (2022). https://doi.org/10.1111/jfpp.16681
40. M. Zor, I.A. Karakutuk, M. Sengul et al., Role of different oils and cooking materials on chemical compounds and antioxidant properties of garlic. J. Food Meas. Charact. **18**, 8314–8329 (2024). https://doi.org/10.1007/s11694-024-02803-4
41. E. Köksal, I. Gülçin, S. Beyza et al., In vitro antioxidant activity of silymarin. J. Enzyme Inhib. Med. Chem. **24**, 395–405 (2009). https://doi.org/10.1080/14756360802188081
42. Y. Kara, Z. Can, S. Kolayl, Applicability of phenolic profile analysis method developed with RP-HPLC-PDA to some bee product. Braz Arch. Biol. Techn. **65** (2022). https://doi.org/10.1590/1678-4324-2022210384
43. E.F. Topdas, M. Sengul, B. Cetin, Influence of extraction methods and solvents on the antimicrobial activity of pickled. J. Food Saf. Food Qual. **72**, 100–106 (2021). https://doi.org/10.2376/0003-925X-71-XX
44. E.F. Conceição, A.A. Novo, C.N. Kunigami et al., Microwave-Assisted Solid–Liquid extraction using propylene glycol as a solvent in the recovery of bioactive compounds from Umbu seeds. Biology Life Sci. Forum. **26**, 77 (2023). https://doi.org/10.3390/Foods2023-15152
45. A.S. Freitas, A. Cunha, P. Parpot et al., Propolis efficacy: the quest for eco-friendly solvents. Molecules **27**, 7531 (2022). https://doi.org/10.3390/molecules27217531
46. A.K. Balasubramaniam, A. Elangovan, M.A. Rahman et al., Propolis: a comprehensive review on the nature's polyphenolic wonder. Fitoterapia (2025). https://doi.org/10.1016/j.fitote.2025.106526
47. I.B.S. Cunha, A.C.H.F. Sawaya, F.M. Caetano et al., Factors that influence the yield and composition of Brazilian propolis extracts. J Brazil Chem Soc **15**, 964–970 (2004). https://doi.org/10.1590/S0103-50532004000600026
48. M. El Alı, A. Jaber, Z. Dorra et al., Chemical analysis and antioxidant activity of four propolis samples collected from different regions of Lebanon. J. Apitherapy Nat. **4**, 1–21 (2021). https://doi.org/10.35206/jan.828140
49. L. Moreira, L.G. Dias, J.A. Pereira, L. Estevinho, Antioxidant properties, total phenols and pollen analysis of propolis samples from Portugal. Food Chem. Toxicol. **46**, 3482–3485 (2008). https://doi.org/10.1016/j.fct.2008.08.025
50. B.A. Rocha, P.C.P. Bueno, M.M.D.L.L. Vaz et al., Evaluation of a Propolis Water Extract Using a Reliable RP-HPLC Methodology and *In Vitro* and *In Vivo* Efficacy and Safety Characterisation. Evid-Based Compl Alt, 2013, 70451 (2013). https://doi.org/10.1155/2013/670451
51. S. Kolayli, C. Birinci, Y. Kara et al., A melissopalynological and chemical characterization of Anatolian propolis and an assessment of its antioxidant potential. Eur. Food Res. Technol. **249**, 1213–1233 (2023). https://doi.org/10.1007/s00217-023-04208-x

52. I. Escriche, M. Juan-Borrás, Standardizing the analysis of phenolic profile in propolis. Food Res. Int. **106**, 834–841 (2018). https://doi.org/10.1016/j.foodres.2018.01.055
53. K. Ramanauskien, A.M. Inkèniene, V. Petrikait, V. Briedis, Total Phenolic Content and Antimicrobial Activity of Different Lithuanian Propolis Solutions. Evid-Based Compl Alt, 2013, (2013). https://doi.org/10.1155/2013/842985
54. P. Mendez-Pfeiffer, J. Juarez, J. Hernandez et al., Nanocarriers as drug delivery systems for propolis: a therapeutic approach. J. Drug Deliv. Sci. Technol. **65**, 102762 (2021). https://doi.org/10.1016/j.jddst.2021.102762
55. F. Ay, H.İ Güler, S. Çanakçı, A. Beldüz, Investigation of the inhibition of SARS-CoV-2 spike RBD and ACE-2 interaction by phenolics of propolis extracts. Journal of Apitherapy and Nature **7**, 85–106 (2024). https://doi.org/10.35206/jan.1471090
56. Ü. Altuntas, I. Güzel, B. Özçelik, Phenolic constituents, antioxidant and antimicrobial activity and clustering analysis of propolis samples based on PCA from different regions of Anatolia. Molecules **28**, 1121 (2023). https://doi.org/10.3390/molecules28031121
57. Ç. Özenirler, G. Zare, Evaluation of the polyphenol contents and antioxidant activity of propolis extracted with different techniques. Uludag Bee J. **22**, 176–187 (2022). https://doi.org/10.31467/uluaricilik.1106415
58. J.D. Xavier, I.B. Valentim, F.O.S. Camatari et al., Polyphenol profile by UHPLC-MS/MS, anti-glycation, antioxidant and cytotoxic activities of several samples of propolis from the northeastern semi-arid region of Brazil. Pharm. Biol. **55**, 1884–1893 (2017). https://doi.org/10.1080/13880209.2017.1340962
59. J.M. Sforcin, Biological properties and therapeutic applications of propolis. Phytother. Res. **30**, 894–905 (2016). https://doi.org/10.1002/ptr.5605
60. M. Turhan, Effects of Extraction Methods Used on Propolis, in *Methods of Biochemical Analysis of Bee Products*. (2024), pp.163–182. https://doi.org/10.69860/nobel.9786053359326.8
61. L. Kubiliene, V. Laugaliene, A. Pavilonis et al., Alternative preparation of propolis extracts: comparison of their composition and biological activities. BMC Complement. Altern. Med. (2015). https://doi.org/10.1186/s12906-015-0677-5
62. B.O. Acar, E. Gülendağ, Comparison of antimicrobial activities of ethanol-and water-based propolis extracts on various foodborne pathogens by agar-well diffusion method. Res. Agric. Sci. **54**, 130–136 (2023). https://doi.org/10.5152/AUAF.2023.23148
63. K. Haile, A. Dekebo, Chemical composition and antimicrobial activity of Haramaya propolis (bee glue), Ethiopia. Int. J. Pharm. Sci. Res. **4**, 734 (2013)
64. G. Veronica, B.T. Reddy, U.K.C. Birapu et al., Comparative evaluation of antimicrobial efficacy of triple antibiotic paste and propolis extract using three different vehicles as intracanal medicament: an in vitro study. J. Dr YSR Univ. Health Sci. **13**, 323–329 (2024). https://doi.org/10.4103/jdrysruhs.jdrysruhs_161_23
65. O.R. Alara, N.H. Abdurahman, C.I. Ukaegbu, Extraction of phenolic compounds: a review. Curr. Res. Food Sci. **4**, 200–214 (2021). https://doi.org/10.1016/j.crfs.2021.03.011
66. Z.W. Liu, X.A. Zeng, M. Ngadi, Enhanced extraction of phenolic compounds from onion by pulsed electric field (PEF). J. Food Process. Preserv. (2018). https://doi.org/10.1111/jfpp.13755
67. M. Nardini, Phenolic compounds in food: characterization and health benefits. Molecules (2022). https://doi.org/10.3390/molecules27030783
68. L.H. Shi, W.R. Zhao, Z.H. Yang et al., Extraction and characterization of phenolic compounds and their potential antioxidant activities. Environ. Sci. Pollut. Res. **29**, 81112–81129 (2022). https://doi.org/10.1007/s11356-022-23337-6
69. M. Zemestani, M. Rafraf, M. Asghari-Jafarabadi, Chamomile tea improves glycemic indices and antioxidants status in patients with type 2 diabetes mellitus. Nutrition **32**, 66–72 (2016). https://doi.org/10.1016/j.nut.2015.07.011
70. V. Bankova, M. Popova, B. Trusheva, Plant sources of propolis: an update from a chemist's point of view. Nat. Prod. Commun. **1**, 1023–1028 (2006)
71. V.S. Bankova, S.L. de Castro, M.C. Marcucci, Propolis: recent advances in chemistry and plant origin. Apidologie **31**, 3–15 (2000). https://doi.org/10.1051/apido:2000102
72. Y.K. Park, S.M. Alencar, C.L. Aguiar, Botanical origin and chemical composition of Brazilian propolis. J. Agric. Food Chem. **50**, 2502–2506 (2002). https://doi.org/10.1021/jf011432b
73. H. Afrouzan, A. Tahghighi, S. Zakeri, A. Es-Haghi, Chemical composition and antimicrobial activities of Iranian propolis. Iran. Biomed. J. **22**, 50–65 (2018). https://doi.org/10.22034/ibj.22.1.50
74. V. Lagouri, D. Prasianaki, F. Krysta, Antioxidant properties and phenolic composition of Greek propolis extracts. Int. J. Food Prop. **17**, 511–522 (2014). https://doi.org/10.1080/10942912.2012.654561
75. K.R. Markham, K.A. Mitchell, A.L. Wilkins et al., HPLC and GC-MS identification of the major organic constituents in New Zeland propolis. Phytochemistry **42**, 205–211 (1996). https://doi.org/10.1016/0031-9422(96)83286-9
76. M. Oroian, F. Ursachi, F. Dranca, Influence of ultrasonic amplitude, temperature, time and solvent concentration on bioactive compounds extraction from propolis. Ultrason. Sonochem. (2020). https://doi.org/10.1016/j.ultsonch.2020.105021
77. T. Ozdal, F.D. Ceylan, N. Eroglu et al., Investigation of antioxidant capacity, bioaccessibility and LC-MS/MS phenolic profile of Turkish propolis. Food Res. Int. **122**, 528–536 (2019). https://doi.org/10.1016/j.foodres.2019.05.028
78. U. Siripatrawan, W. Vitchayakitti, R. Sanguandeekul, Antioxidant and antimicrobial properties of Thai propolis extracted using ethanol aqueous solution. Int. J. Food Sci. Tech. **48**, 22–27 (2013). https://doi.org/10.1111/j.1365-2621.2012.03152.x
79. E. Guzelmeric, P.I. Yuksel, B.K. Yaman et al., Comparison of antioxidant and anti-inflammatory activity profiles of various chemically characterized Turkish propolis sub-types: which propolis type is a promising source for pharmaceutical product development? J. Pharm. Biomed. Anal. **203**, 114196 (2021). https://doi.org/10.1016/j.jpba.2021.114196
80. S. Erdogan, B. Ates, G. Durmaz et al., Pressurized liquid extraction of phenolic compounds from Anatolia propolis and their radical scavenging capacities. Food Chem. Toxicol. **49**, 1592–1597 (2011). https://doi.org/10.1016/j.fct.2011.04.006
81. M. Kartal, S. Kaya, S. Kurucu, GC-MS analysis of propolis samples from two different regions of Turkiye. Z. Naturforsch. C **57**, 905–909 (2002). https://doi.org/10.1515/znc-2002-9-1025
82. H.H.S. Omer, I. Demirtas, T. Ozen, Investigation of phenolic contents and bioactivities of water-based extracts prepared from cryogenically pulverized Turkish propolis. J. Food Meas. Charact. **17**, 1586–1601 (2023). https://doi.org/10.1007/s11694-022-01716-4
83. G.M. Duymaz, G. Duz, K. Ozkan et al., The evaluation of L-arginine solution as a solvent for propolis extraction: the phenolic profile, antioxidant, antibacterial activity, and in vitro bioaccessibility. Food Sci. Nutr. **12**, 2724–2735 (2024). https://doi.org/10.1002/fsn3.3953
84. L. Kubiliene, A. Jekabsone, M. Zilius et al., Comparison of aqueous, polyethylene glycol-aqueous and ethanolic propolis extracts: antioxidant and mitochondria modulating properties. BMC Complement. Altern. Med. **18**, 165 (2018). https://doi.org/10.1186/s12906-018-2234-5
85. R. Aliyazicioglu, H. Sahin, O. Erturk et al., Properties of phenolic composition and biological activity of propolis from Turkey. Int.

J. Food Prop. **16**, 277–287 (2013). https://doi.org/10.1080/10942912.2010.551312
86. R. Ali, M. Abul Kalam, W. Qamar et al., Chrysin-loaded Soluplus-TPGS mixed micelles: optimization, characterization and anticancer activity against hepatocellular carcinoma cell line. J. Drug Deliv. Sci. Technol. (2024). https://doi.org/10.1016/j.jddst.2024.106371
87. Y. Yao, L. Chen, J.T. Xiao et al., Chrysin protects against focal cerebral ischemia/reperfusion injury in mice through attenuation of oxidative stress and inflammation. Int. J. Mol. Sci. **15**, 20913–20926 (2014). https://doi.org/10.3390/ijms151120913
88. H. Kim, H.W. Kim, S. Jung, Aqueous solubility enhancement of some flavones by complexation with cyclodextrins. Bull. Korean Chem. Soc. **29**, 590–594 (2008). https://doi.org/10.5012/bkcs.2008.29.3.590
89. S. Nagaraja, G.M. Basavarajappa, M. Attimarad, S. Pund, Topical nanoemulgel for the treatment of skin cancer: proof-of-technology. Pharmaceutics (2021). https://doi.org/10.3390/pharmaceutics13060902
90. J.J. Zhang, D.P. Liu, Y.T. Huang et al., Biopharmaceutics classification and intestinal absorption study of apigenin. Int. J. Pharm. **436**, 311–317 (2012). https://doi.org/10.1016/j.ijpharm.2012.07.002
91. S.M. Nabavi, S. Habtemariam, M. Daglia, S.F. Nabavi, Apigenin and breast cancers: from chemistry to medicine. Anti-Cancer Agent Me. **15**, 728–735 (2015). https://doi.org/10.2174/1871520615666150304120643
92. F. Ali, F. Rahul, Naz et al., Health functionality of apigenin: A review. Int. J. Food Prop. **20**, 1197–1238 (2016). https://doi.org/10.1080/10942912.2016.1207188
93. D. Tang, K.L. Chen, L.Q. Huang, J. Li, Pharmacokinetic properties and drug interactions of apigenin, a natural flavone. Expert Opin. Drug Metab. **13**, 323–330 (2017). https://doi.org/10.1080/17425255.2017.1251903
94. N. Schultheiss, M. Roe, S.X.M. Boerrigter, Cocrystals of nutraceutical-coumaric acid with caffeine and theophylline: polymorphism and solid-state stability explored in detail using their crystal graphs. Crystengcomm. **13**, 611–619 (2011). https://doi.org/10.1039/c0ce00214c
95. F. Pellati, G. Orlandini, D. Pinetti, S. Benvenuti, HPLC-DAD and HPLC-ESI-MS/MS methods for metabolite profiling of propolis extracts. J. Pharm. Biomed. Anal. **55**, 934–948 (2011). https://doi.org/10.1016/j.jpba.2011.03.024
96. S.S. Narasagoudr, V.G. Hegde, R.B. Chougale et al., Physicochemical and functional properties of rutin induced chitosan/poly (vinyl alcohol) bioactive films for food packaging applications. Food Hydrocolloids **109**, 106096 (2020). https://doi.org/10.1016/j.foodhyd.2020.106096
97. C.A. Pedriali, A.U. Fernandes, L.D. Bernusso, B. Polakiewicz, The synthesis of a water-soluble derivative of Rutin as an antiradical agent. Quim. Nova **31**, 2147–2151 (2008). https://doi.org/10.1590/S0100-40422008000800039
98. G. Coneac, E. Gafiţanu, D. Hădărugă et al., Flavonoid contents of propolis from the West side of Romania and correlation with the antioxidant activity. Chem. Bull. Politehnica Univ. (Timisoara). **53**, 56–60 (2008)
99. C. Zehra, O. Yildiz, H. Şahin et al., Phenolic profile and antioxidant potential of propolis from Azerbaijan. Mellifera. **15**, 16–28 (2015)
100. S.I. Falcão, M. Vilas-Boas, L.M. Estevinho et al., Phenolic characterization of Northeast Portuguese propolis: usual and unusual compounds. Anal. Bioanal. Chem. **396**, 887–897 (2010). https://doi.org/10.1007/s00216-009-3232-8
101. S. Kumazawa, T. Hamasaka, T. Nakayama, Antioxidant activity of propolis of various geographic origins. Food Chem. **84**, 329–339 (2004). https://doi.org/10.1016/S0308-8146(03)00216-4
102. S. Mohammadzadeh, M. Sharriatpanahi, M. Hamedi et al., Antioxidant power of Iranian propolis extract. Food Chem. **103**, 729–733 (2007). https://doi.org/10.1016/j.foodchem.2006.09.014
103. B.X. Ren, M.X.Y. Kwah, C.L. Liu et al., Resveratrol for cancer therapy: challenges and future perspectives. Cancer Lett. **515**, 63–72 (2021). https://doi.org/10.1016/j.canlet.2021.05.001
104. N. Sahinler, O. Kaftanoglu, Natural product propolis: chemical composition. Nat. Prod. Res. **19**, 183–188 (2005). https://doi.org/10.1080/14786410410001704877
105. M. Popova, S. Silici, O. Kaftanoglu, V. Bankova, Antibacterial activity of Turkish propolis and its qualitative and quantitative chemical composition. Phytomedicine **12**, 221–228 (2005). https://doi.org/10.1016/j.phymed.2003.09.007
106. N. Sha, H.L. Huang, J.Q. Zhang et al., Simultaneous quantification of eight major bioactive phenolic compounds in Chinese propolis by High-Performance liquid chromatography. Nat. Prod. Commun. **4**, 813–818 (2009). https://doi.org/10.1177/1934578X0900400615
107. M. Milek, R. Bonikowski, M. Dzugan, The effect of extraction conditions on the chemical profile of obtained raw poplar propolis extract. Chem. Pap. **78**, 6709–6720 (2024). https://doi.org/10.1007/s11696-024-03567-3
108. N. Duran, M. Muz, G. Culha et al., GC-MS analysis and antileishmanial activities of two Turkish propolis types. Parasitol. Res. **108**, 95–105 (2011). https://doi.org/10.1007/s00436-010-2039-z
109. S. Silici, S. Kutluca, Chemical composition and antibacterial activity of propolis collected by three different races of honeybees in the same region. J. Ethnopharmacol. **99**, 69–73 (2005). https://doi.org/10.1016/j.jep.2005.01.046
110. K. Pobiega, A.M. Kot, J.L. Przybyl et al., Comparison of the chemical composition and antioxidant properties of propolis from urban apiaries. Molecules. **28**, 6744 (2023). https://doi.org/10.3390/molecules28186744
111. R. Socha, D. Galkowska, M. Bugaj, L. Juszczak, Phenolic composition and antioxidant activity of propolis from various regions of Poland. Nat. Prod. Res. **29**, 416–422 (2015). https://doi.org/10.1080/14786419.2014.949705
112. M. Wozniak, L. Mrówczynska, P. Kwasniewska-Sip et al., Effect of the solvent on propolis phenolic profile and its Antifungal, Antioxidant, and in vitro cytoprotective activity in human erythrocytes under oxidative stress. Molecules. **25** (2020). https://doi.org/10.3390/molecules25184266

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