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Research Article

Chemical Content by LC–MS/MS, Antiglaucoma, and Antioxidant Activity of Propolis Samples From Different Regions of Türkiye

Ebubekir İzol,[1] **Ercan Bursal**,[2] **İsmail Yapıcı**,[3] **Mustafa Abdullah Yilmaz**,[4] **İsa Yilmaz**,[5] **and İlhami Gülçin**[6]

[1]*Bee and Natural Products R&D and P&D Application and Research Center, Bingol University, Bingöl, Türkiye*
[2]*Faculty of Health, Muş Alparslan University, Muş, Türkiye*
[3]*Central Laboratory Application and Research Center, Gümüşhane University, Gümüşhane, Türkiye*
[4]*Department of Pharmaceutical Chemistry, Faculty of Pharmacy, Dicle University, Diyarbakır, Türkiye*
[5]*Department of Animal Production and Technologies, Faculty of Applied Science, Muş Alparslan University, Muş, Türkiye*
[6]*Department of Chemistry, Faculty of Science, Ataturk University, Erzurum, Türkiye*

Correspondence should be addressed to Ebubekir İzol; eizol@bingol.edu.tr

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Propolis is a sticky substance produced by bees because of the reaction of beeswax, pollen, and bee enzymes. Particularly, their biological activity and chemical content attract attention. Thus, in this study, the total amount of phenolic and flavonoid substances, Fe^{3+}-Fe^{2+}, Cu^{2+} (cupric ions reducing activity [CUPRAC]), and Fe^{3+}-TPTZ (ferric ions reducing antioxidant power [FRAP]) reducing, and $DPPH^{\bullet}$ and $ABTS^{\bullet+}$ scavenging assays in vitro antioxidant properties of propolis samples obtained from four different provinces of Türkiye were determined. In addition, the chemical content of propolis samples was quantitatively determined by LC–MS/MS, and the antiglaucoma property was revealed by hCAII enzyme inhibition. Propolis samples from Ordu presented the highest amounts of total phenolic and flavonoid content (492.3 ± 5.8 and 96.1 ± 2.1, respectively) and also highest antioxidant activity ($DPPH^{\bullet}$ and $ABTS^{\bullet+}$ IC_{50} [μg/mL]: 8.884 ± 0.84 and 4.589 ± 0.80, respectively; Fe^{+3}, CUPRAC, and FRAP: 1.051 ± 0.012, 1.021 ± 0.008, and 0.957 ± 0.007 μg/mL, respectively). hCAII enzyme inhibition was highest in Muş propolis (IC_{50} [μg/mL]: 8.6) as determined. By LC–MS/MS, 53 different components were screened and 35 bioactive components were determined. According to the results, propolis was found to be a raw material because it contains high concentrations of acacetin, chrysin, caffeic acid, and quinic acid (123.824, 24.759, 47.779, and 16.32 mg analyte/g extract, respectively).

Keywords: antiglaucoma; antioxidant; chemical content; LC–MS/MS; propolis

1. Introduction

Propolis is a resinous material released from various plant sources, collected by honeybees, and mixed with different bee secretions to make the final product [1]. Honeybees use propolis materials to seal holes and cracks in beehives, protecting them from microbial infection and extreme weather conditions. The plant sources and geographical conditions could change the biochemical content and activities of propolis samples [2]. The biochemical properties of many propolis samples from different parts of the world have been studied and evaluated. Antioxidant, antimicrobial activities, and phenolic contents of them have been also investigated [3–6].

Propolis is a very important organic product for bees. It has been determined that it has biologically antioxidant, antimicrobial, immunomodulator, anticancer, antibacterial, and antivirus activities [7]. It was determined that antioxidant properties and phenolic content are higher than in most organic products (such as most plants and fruits). These biological effects are seen in humans as well as in bees [8]. These properties make propolis very valuable. The chemical content

of propolis varies according to the components, and the formation of different components is caused by certain effects. These effects are geographic flora, location, climate, and bee species. The general content of propolis includes primary and secondary metabolites such as wax, resin, pollen, essential oils, carbohydrates, terpenoids, alkaloids, amino acids, vitamins, minerals, and phenolics. At the same time, it is stated that most of the bioactive compounds that cause the biological activity of propolis are phenolic and flavonoid compounds [9]. For this reason, it is very important to determine qualitatively and quantitatively the phenolics, which are the most important bioactive components propolis.

Antioxidants are important substances that play a role in the healing of more than 100 diseases [10, 11]. By removing free radicals and reactive oxygen, they prevent their damage to tissues and cells. However, due to the damage caused by synthetic antioxidants, research on natural antioxidants is increasing [12–14]. In scientific studies, it has been stated that phenolic components show antioxidant properties. For this reason, while investigating the antioxidant properties of natural products, phenolic components are also investigated by advanced methods such as LC–MS/MS [15–17].

The carbonic anhydrase (CA) enzymes are a significant enzyme class with many isoenzymes. CA enzymes catalyze the reversible hydration of carbon dioxide (CO_2) to protons and bicarbonate (HCO_3^-). There are many functions performed by CAs in the biological system, such as ureagenesis, lipogenesis, and gluconeogenesis [18]. CA inhibition has therapeutic uses in the treatment of infection, convulsions, glaucoma, and cancer, and it maintains fluid balance in the body, especially in the eyes, kidneys, and stomach. Glaucoma, which is defined as high intraocular pressure, has been determined in studies to be alleviated using carbonic anhydrase II (CAII) inhibitors [19, 20]. Therefore, the CAII enzyme inhibition power of natural products is important for glaucoma disease [21].

This study reports the total phenolic and flavonoid amounts, phytochemical contents, antioxidant activities, and antiglaucoma properties of different propolis samples. The propolis samples were obtained from Muş, Ordu, Manisa, and Iğdır cities, located in different regions of Türkiye. The effective antioxidant properties of ethanol and water extracts of propolis were determined by using five different in vitro bioanalytical methods, including three reducing antioxidant methods (cupric ions reducing activity [CUPRAC], ferric ions reducing antioxidant power [FRAP], and Fe^{3+}-TPTZ reducing assays) and two radical scavenging antioxidant methods ($ABTS^{\bullet+}$ and $DPPH^{\bullet}$) According to the results, the propolis sample gathered from Ordu city had the highest total phenolic and flavonoid contents. Remarkably, the propolis sample gathered from Ordu City had the highest antioxidant activity for all five antioxidant methods. The results supported the association of phenolic compounds with antioxidant activity. In addition, the chemical content and antioxidant and antiglaucoma properties of propolis from four provinces were determined for the first time in this study.

2. Materials and Methods

2.1. Chemicals. Standard chemicals were purchased from Sigma-Aldrich (Steinheim, Germany) for use in LC–MS/MS analysis. Commercial purchases of standards and other chemicals were made from Sigma-Aldrich Chemie GmbH (Steinheim, Germany) for use in antioxidant and enzyme experiments. High-purity hCAII enzyme was isolated using human erythrocytes and the Sepharose-4B tyrosine-sulfanilamide affinity column method.

2.2. Propolis Samples. The propolis samples were obtained from Muş, Ordu, Manisa, and Iğdır cities, located in different regions of Türkiye. The locations have different climates, environments, and plant biodiversity properties. Ordu propolis was gathered from a forest environment that has mainly chestnut trees and rose flowers. Manisa propolis was gathered from a forest environment that has mainly pine trees. Muş and Iğdır propolis samples were gathered from mainly natural and wild flower locations. The propolis samples were collected by İsa Yılmaz from Muş Alparslan University.

2.3. Preparation of the Propolis Extracts. The mixtures of ethanol and water solvents (50:50%) were used to carry out the propolis extracts, according to a previous study. Briefly, the propolis samples (5 g) were mixed with 100 mL of distilled water and an ethanol mixture (1/20: w/v). The mixtures were homogenized by a magnetic stirrer and heated until boiling for 15 min. The homogeneous mixtures were filtered with filter papers. The filtrate samples were dried in a lyophilizer (Labconco, Freezone 1L) at 5 mm Hg at −50°C for preparing the extracts. The lyophilized samples were stored at −30°C until being used.

2.4. Determination of Total Phenolic and Flavonoid Amounts. The total phenolic and flavonoid contents of the samples were determined according to a former study [22]. For the total phenolic content determination, the propolis extracts (0.5 mL) were mixed with Folin–Ciocalteu solution (1.0 mL) and Na_2CO_3 (0.5 mL, 1%). The absorbance of the mixtures was measured at 725 nm after incubation for 2 h at room temperature. Gallic acid was used as a standard, and the total phenolic amounts were given as milligrams of gallic acid equivalents (GAE).

For the total flavonoid content determination, the propolis extracts (0.5 mL) were mixed with ethanol (1.5 mL, 95%), aluminum chloride (1.5 mL, 10%), potassium acetate (0.5 mL, 1.0 M), and distilled water (2.3 mL), respectively. The absorbance of the mixtures was measured at 415 nm after the incubation for 30 min at room temperature. Quercetin used as a standard, and the flavonoid amounts were given as milligrams of quercetin equivalents (QE) per gram of extract [14].

2.5. Antioxidant Activity. The antioxidant activities of the propolis samples were analyzed using five well-known methods to determine the radical scavenging and reducing capacities of the propolis extracts. In vitro CUPRAC method [23], FRAP method [24], and Fe^{3+}-TPTZ reducing assays [25] were performed to evaluate the reducing potentials of the extracts. Furthermore, the radical scavenging activities of the extracts were examined using the 2,2-diphenyl-1-picryl-hydrazyl (DPPH) method [26] and 2,2′-azino-bis3-ethylbenzothiazoline-6-sulfonic acid (ABTS) method [27], with slight modifications as reported in a previous study [28]. The antioxidant potentials were determined by comparison with the standard antioxidant compounds (BHA, BHT, and Trolox). The different concentrations (10–30 μg/mL) of the extracts and reference standards were used to examine the effect of the dose-dependent antioxidant potential of the plant extracts.

2.6. Determination of Chemical Contents by LC–MS/MS. LC–MS/MS analyses were carried out to determine the phenolic contents of propolis samples. For this aim, Shimadzu-Nexera model ultrahigh-performance liquid chromatograph (UHPLC) coupled with a tandem mass spectrometer was used to accomplish quantitative evaluation of 53 phytochemicals. The reversed-phase LC was equipped with an autosampler (SIL-30AC model), a column oven (CTO-10ASvp model), binary pumps (LC-30CE model), and a degasser (DGU- 20A3R model). The details of chromatographic conditions were given in a previous study [29].

2.7. Antiglaucoma Assay. The antiglaucoma property was determined by the method of İnci et al. [19]. Accordingly, acetazolamide, a common hCAII inhibitor, and its stock solution were tested at 25°C, 3 min, and 348 nm. The hCAII inhibition potential of the extracts was calculated using the activity (%)–compound plot. The IC_{50} value was determined based on activity (%) versus compound plot.

3. Results and Discussion

3.1. The Total Phenolic and Flavonoid Amounts. The present study demonstrated high amounts of total phenolic content in the propolis extracts. The total phenolic contents of the extracts were determined as mg GAE/g. The total phenolic contents of the propolis extracts from Iğdır, Manisa, Muş, and Ordu cities were determined as 330.7 ± 9.2, 298.0 ± 7.4, 184.6 ± 2.7, and 492.3 ± 5.8 mg GAE/g, respectively. The Ordu propolis sample was found to have the richest phenolic content.

Also, total flavonoid contents of extracts were determined as mg QEq/g. The total flavonoid contents of the propolis extracts from Iğdır, Manisa, Muş, and Ordu cities were determined as 46.1 ± 1.2, 66.1 ± 2.3, 46.9 ± 1.6, and 96.1 ± 2.1 mg QE/g, respectively. The Ordu propolis sample was detected to have the richest flavonoid content as well. The total phenolic and flavonoid contents of the propolis samples are given in Table 1.

Table 1: Determination of the total phenolic and flavonoid contents of the propolis extracts.

Propolis extracts	Total phenolic contents (mg GAE/g)	Total flavonoid contents (mg QE/g)
Iğdır	330.7 ± 9.2	46.1 ± 1.2
Manisa	298.0 ± 7.4	66.1 ± 2.3
Muş	184.6 ± 2.7	46.9 ± 1.6
Ordu	**492.3 ± 5.8**	**96.1 ± 2.1**

Note: The values given are the three parallel measurements' means ± SD. Values in bold indicate the highest activity.
Abbreviations: GAE: gallic acid equivalents, QE: quercetin equivalents.

It was determined that both total phenolic and total flavonoid amounts of Ordu propolis were higher than other propolis samples. In the study in which Berdav propolis was investigated, it was found that total phenolic content was 53 mg GAE/g and total flavonoid content was 170.2 mg QE/g. When compared with this study, it was observed that all four propolis samples contained a higher total phenolic content than Berdav propolis, while the total flavonoid content was lower than Berdav propolis [30]. In the study in which Chinese propolis was investigated, the total phenolic substance was found to be 132.1 mg GAE/g. In similar studies, total phenolic matter in Brazilian propolis was 126.8 mg GAE/g, in Austrian propolis 142 mg GAE/g [31], in Lithuanian propolis 95–195 mg GAE/g [32], and in Portuguese propolis 151 and 329 mg GAE/g [33]. In the study in which Anatolian propolis was investigated, the total phenolic substance was found to be 147.2 mg GAE/g on average, and the total flavonoid substance was found to be 30.5 on average in 20 different propolis samples [34].

These studies once again determined that the total phenolic and flavonoid amounts of propolis samples from different geographies may be different. The main reasons for this difference were suggested to be flora, geography, climate, bee species, and bee health.

3.2. Antioxidant Potentials of the Propolis Samples. In the present study, five common in vitro spectrophotometric methods were utilized to evaluate the antioxidant activity of the propolis samples. For this purpose, CUPRAC, FRAP, and Fe^{3+} reducing ability methods were used for analyzing the reducing potentials, and ABTS and DPPH methods were used for the radical scavenging properties.

The FRAP method was used to evaluate the reducing potential of the propolis samples by measuring the reduction of Fe^{3+} to Fe^{2+} ions. According to the results, the ferric ion reducing potentials of the Ordu propolis were higher than the other three propolis samples, even higher than the standards. The results revealed that both Iğdır and Muş propolis samples that were gathered from natural flower locations had the lowest reducing abilities among the samples and standards (Figure 1(a)).

The CUPRAC method has been used for the determination of Cu^{2+} reducing ability. This method is based on measuring Cu^{2+}–Cu^{+} reduction. The results indicated that the Cu^{2+} reducing powers of the propolis extracts were

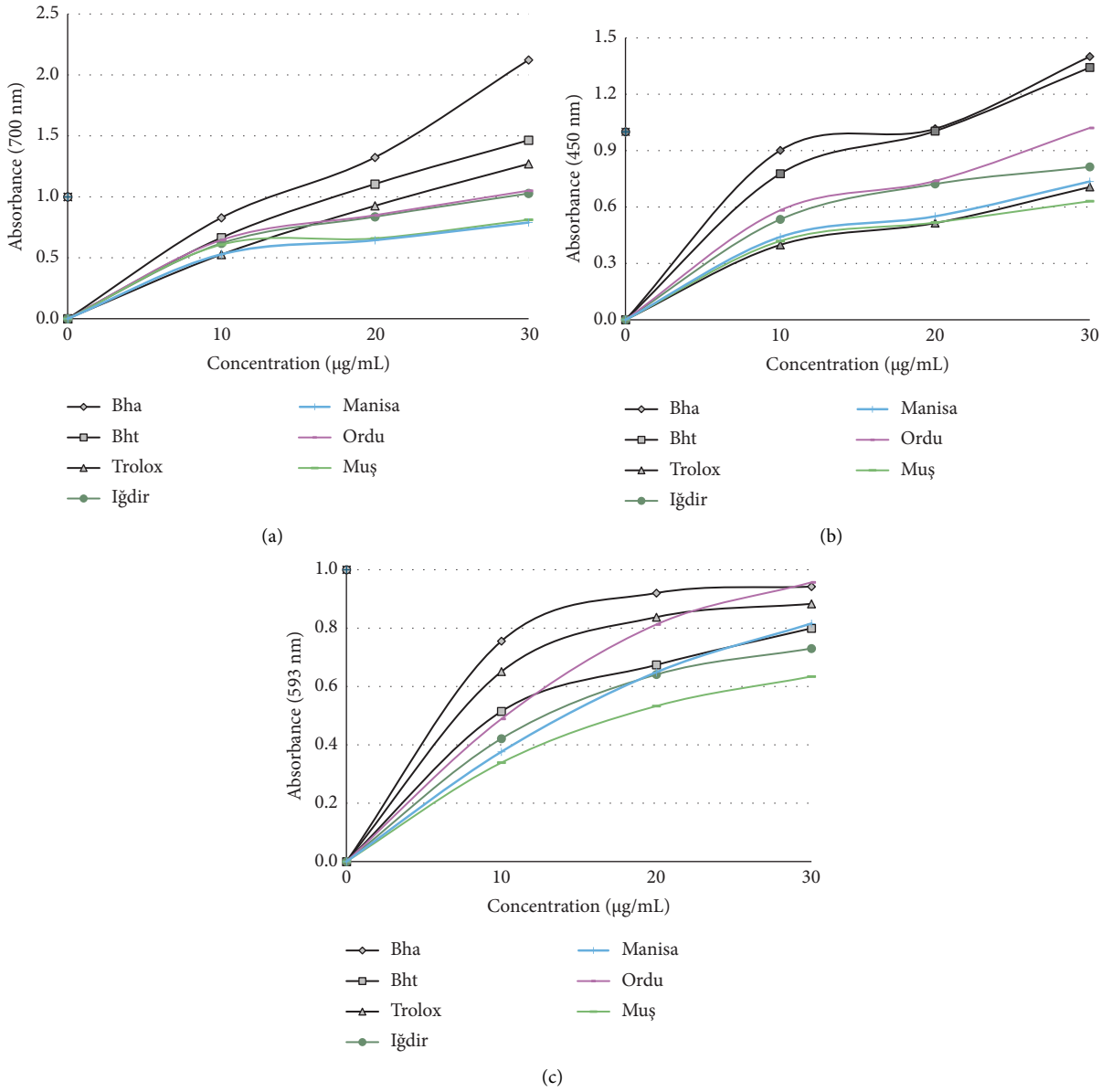

Figure 1: (a) FRAP, (b) CUPRAC, and (c) Fe^{3+}-TPTZ reducing ability methods for the reducing potentials of the propolis extracts and standards.

close to those of standard antioxidants. Again, the reducing potentials of the Ordu propolis were higher than all three propolis samples in the CUPRAC method. The reduction of Ordu propolis was measured to be higher than Trolox but lower than BHA and BHT standards (Figure 1(b)).

The Fe^{3+}-TPTZ reducing assay is the third method to determine the reducing power of the propolis extracts. According to this method, the reducing power of the samples and standards was ordered as BHA, BHT, Trolox, Ordu, Iğdır, Muş, and Manisa propolis extracts (Figure 1(c)).

The absorbance data of the extracts and standards for the three reducing methods are presented in Table 2. According to all three methods, the increasing absorbance values indicated high reducing abilities in the extracts of the samples. Ordu propolis was determined to be the most potent antioxidant in all three reducing methods. The effective antioxidant activity of Ordu propolis may be related to its environmental conditions, which include chestnut trees.

The DPPH· scavenging potentials of the extracts were determined by measuring and comparing the IC_{50} values of the extracts with standards. The lower IC_{50} value indicates a more effective radical scavenging potential. According to the results of the DPPH· study, the effectiveness of the propolis samples and the standards were ordered as Trolox (6.026 ± 0.10), Ordu (8.884 ± 0.84), Iğdır (9.240 ± 0.67), BHA (9.900 ± 0.47), Manisa (10.828 ± 0.71), BHT (14.140 ± 0.15), and Muş (15.750 ± 0.63), respectively. $ABTS^{\bullet+}$ scavenging method results of the propolis samples and the standards were ordered as BHA (4.331 ± 0.05), Ordu (4.589 ± 0.80), BHT (4.812 ± 0.52), Trolox (4.880 ± 0.71), Iğdır (5.500 ± 0.14), Manisa (7.786 ± 0.59), and Muş (13.075 ± 0.35), respectively. The IC_{50} values of the samples for both radical scavenging methods are presented in Table 3.

Table 2: Absorbance measurements of the propolis samples and standard compounds at 30 μg/mL concentration.

Standards and propolis extracts	FRAP		CUPRAC		Fe^{3+}-TPTZ	
	λ_{700}	r^2	λ_{450}	r^2	λ_{593}	r^2
BHA	2.123 ± 0.020	0.9914	1.400 ± 0.052	0.9472	0.942 ± 0.008	0.9830
BHT	1.464 ± 0.014	0.9797	1.342 ± 0.007	0.9775	0.799 ± 0.007	0.9860
Trolox	1.270 ± 0.015	0.9905	0.706 ± 0.017	0.9759	0.883 ± 0.021	0.9895
Iğdır	1.027 ± 0.005	0.9883	0.813 ± 0.010	0.9922	0.730 ± 0.018	0.9992
Manisa	0.789 ± 0.027	0.9728	0.736 ± 0.017	0.9718	0.816 ± 0.023	0.9713
Ordu	**1.051 ± 0.012**	0.9845	**1.021 ± 0.008**	0.9725	**0.957 ± 0.007**	0.9998
Muş	0.812 ± 0.065	0.9436	0.631 ± 0.008	0.9752	0.634 ± 0.013	0.9994

Note: The values given are the three parallel measurements' means ± SD ($p < 0.05$ is considered significant). Values in bold indicate the highest activity.

According to the results, the Ordu propolis extract was determined to be the most potent antioxidant in the DPPH and ABTS radical scavenging activity methods. The results of the radical scavenging antioxidant properties of the propolis extracts and reference standards at different concentrations (10–30 μg/mL) are graphically presented in Figure 2.

In the study in which the antioxidant activities of Berdav propolis were determined, $ABTS^{\bullet+}$ and $DPPH^{\bullet}$ scavenging activities, and Fe^{3+}, CUPRAC, and FRAP reducing capacities were found to be 8.15 (μg/mL), 20.55 (μg/mL), 1.545, 2.323, and 1.755, respectively [30]. In this study, the antioxidant activities of Ordu propolis with the highest results were found to be $ABTS^{\bullet+}$ 4.589 (μg/mL), $DPPH^{\bullet}$ 8.884 (μg/mL), Fe^{+3} reducing 0.957, CUPRAC 1.021, and FRAP 1.051 (μg/mL). Ordu propolis showed higher antioxidant activity than Berdav propolis according to ABTS and DPPH methods, while it showed lower activity according to other methods. The reason for this difference may be that phenolic compounds, which have an important effect on antioxidant activity, are present differently in the samples.

In the study in which brown, green, and dark Brazilian propolis were investigated, the samples showing the highest ABTS, FRAP, and DPPH activities were found to be brown (109.29 trolox μM/g), green (422.83 μM ferrous sulfate/g), and green (491.68 g of sample/g), respectively [35]. Although the antioxidant properties of Brazilian propolis were determined by 3 methods, 5 different in vitro spectrophotometric methods were used in this study.

In the study in which the antioxidant properties of propolis samples prepared with different extraction solvents were determined by FRAP and ABTS methods, alcoholic propolis extract (284.3 μg/mL) showed the highest activity in the FRAP method and aqueous propolis extract (202.8 μg/mL) in the ABTS method [36]. In this study, the ABTS (4.589 μg/mL) result of Ordu propolis showed much higher antioxidant activity than aqueous propolis extract, while the FRAP result showed low activity.

3.3. Phytochemical Content by LC–MS/MS. The phytochemical content of propolis extracts obtained from four different geographical regions of Anatolia was screened by 53 different phenolic, flavonoid, and organic acid components. While 35 components were quantitatively determined, other components were not determined because they were below the limit of determination. The phytochemical content results of propolis extracts are given in Table 4.

Table 3: Determination of half-maximal concentrations (IC_{50}) (μg/mL) of the propolis samples and standards for DPPH and ABTS radical scavenging activities.

Standards and propolis extracts	DPPH· scavenging		$ABTS^{\bullet+}$ scavenging	
	IC_{50}	r^2	IC_{50}	r^2
BHA	9.900 ± 0.47	0.9618	4.331 ± 0.05	0.9930
BHT	14.140 ± 0.15	0.9935	4.812 ± 0.52	0.9805
Trolox	6.026 ± 0.10	0.9429	4.880 ± 0.71	0.9349
Iğdır	9.240 ± 0.67	0.9879	5.500 ± 0.14	0.9631
Manisa	10.828 ± 0.71	0.9636	7.786 ± 0.59	0.9785
Ordu	**8.884 ± 0.84**	0.9872	**4.589 ± 0.80**	0.9991
Muş	15.750 ± 0.63	0.9752	13.075 ± 0.35	0.9893

Note: The values given are the three parallel measurements' means ± SD ($p < 0.05$ is considered significant). Values in bold indicate the highest activity.

LC–MS/MS chromatograms of Manisa and Iğdır propolis are given in Figure 3, and those of Muş and Ordu propolis are given in Figure 4.

In the LC–MS/MS results of propolis extracts, the highest concentration of the acacetin component (123.824 mg analyte/g extract) was determined in the Igdır sample. In addition, acacetin was determined to have the highest concentration in the other three propolis samples. According to these results, it was observed that especially Iğdır propolis could be a source of raw material for acacetin. Acacetin is a flavone-derived molecule (5,7-dihydroxy-40-methoxyflavone), a bioactive component, and has been reported to have strong anti-inflammatory, antioxidant, and anticancer activities [37, 38].

In Ordu propolis, *p*-coumaric acid 66.454 (mg analyte/g extract), caffeic acid 47.779 (mg analyte/g extract), ferulic acid 7.052 (mg analyte/g extract), isoquercitrin 2.855 (mg analytes/g extract), quercetin 8.673 (mg analytes/g extract), and naringenin 8.468 (mg analytes/g extract); in Iğdır propolis, chrysin 24.75 (mg analytes/g extract), apigenin 6.891 (mg analytes/g extract), luteolin 2.781 (mg analytes/g extract), in Muş propolis quinic acid 16.32 (mg analytes/g extract), kaempferol 6.395 (mg analytes/g extract), and quercitrin 3.498 (mg analytes/g extract); and in Manisa propolis, vanillic acid 5.682 (mg analytes/g extract) was founded.

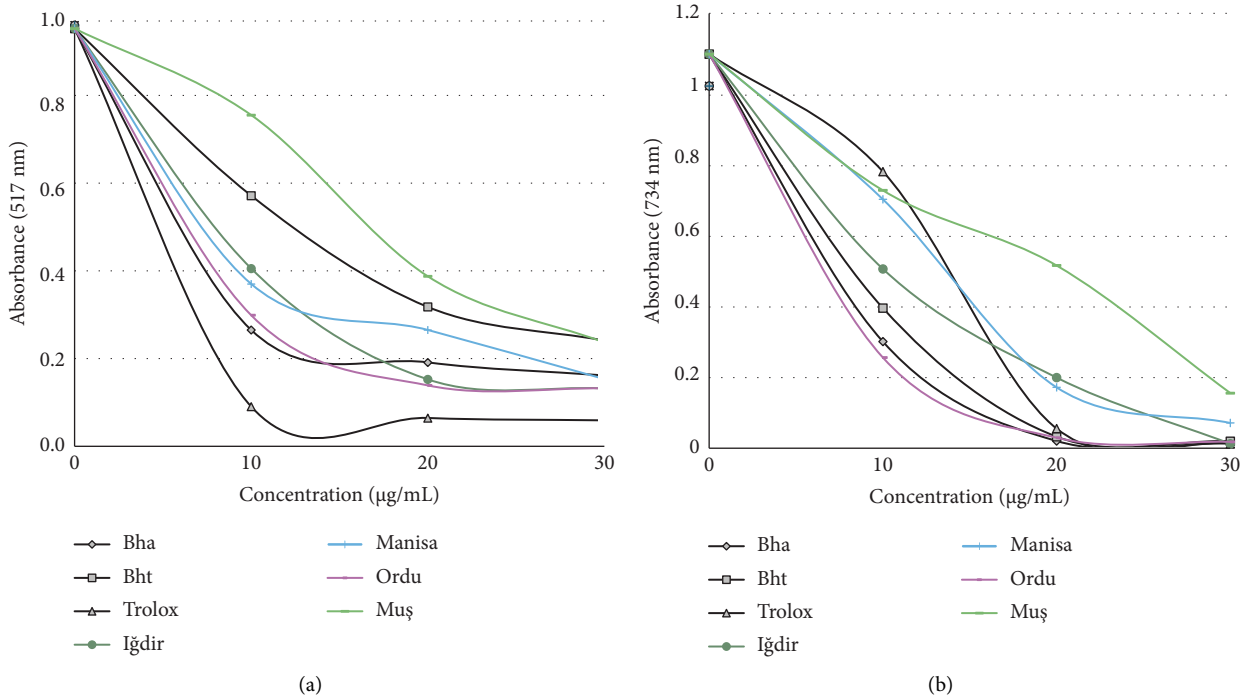

Figure 2: (a) DPPH and (b) ABTS radical scavenging activities of the propolis extracts and standards.

After acacetin, the highest concentration of *p*-coumaric acid was detected in propolis extracts. *p*-Coumaric acid is an essential bioactive phytochemical showing various biological activities such as antioxidant, anticancer, antivirus, anti-inflammatory, antimicrobial, antidiabetic, and anti-hyperlipaemia [39–41]. Caffeic acid is an important bioactive component. Its biological activities have been determined to be antitumor, antioxidant, antifibrotic, antihypertensive, and antivirus [42–45]. Chrysin (5,7-dihydroxyflavone) is a flavone compound found naturally in plants, honey, and propolis. Its biological activities include antioxidant, anti-inflammatory, anticancer, and antiviral effects. In addition, many studies have been conducted on its bioavailability [46–48]. Quinic acid, one of the phenolic acids, is an important phytochemical. Its biological activities include anticancer, antioxidant, anti-inflammatory, antihepatitis, antiviral, and antidiabetic effects [49, 50]. Quercetin is a flavonol component in the flavonoid class. It is found in natural products. It has a bitter flavor and is used as an ingredient in dietary supplements, beverages, and foods. It also has many different uses. It is widely recommended for its anti-inflammatory, antiobesity, antioxidant, and antiviral properties [51–54]. Naringenin (4′,5,7-trihydroxyflavonone) is a molecule in the flavanone structure, which is a subclass of the flavonoid class. The molecule has been reported to have antioxidant, antibacterial, anticancer, antiviral, antidiabetic, antimicrobial, antiobesity, gastroprotective, immunomodulatory, cardioprotective, nephroprotective, neuroprotective, and antifungal properties [55–57]. Apigenin (4′,5,7-trihydroxyflavone) is a component in the flavonoid class. It is a component that has a positive effect on human health and has anti-Alzheimer's disease potential as well as many biological activities. Antitumor, cardiovascular system, liver, respiratory system, endocrine system, central nervous system, bone, and joint on effects have been determined [58–60]. Kaempferol is a naturally occurring flavonol in flavonoids. Its biological activities include antioxidant, antimicrobial, anticancer, neuroprotective, hepatoprotective, and cardioprotective properties [61, 62]. Vanillic acid (4-hydroxy-3-methoxybenzoic acid) is a molecule in the phenolic acid class found in various food sources and medicinal plants. It has antioxidant, anticancer, antiobesity, antidiabetic, antibacterial, anti-inflammatory, neurodegenerative, cardiovascular, and hepatic effects [63, 64]. Quercitrin (quercetin-3-O-rhamnoside) is an important natural component in the flavonoid class. Bioactivity properties such as antibacterial, antioxidative stress, anti-inflammatory, immunomodulation, analgesia, wound healing, and vasodilatation have been determined [65–67].

The presence of these bioactive components in propolis samples showed that propolis may be a natural source of bioactive phenolic and flavonoid molecules. In addition, it was observed that the high antioxidant properties of propolis may be due to the bioactive components it contains.

The chemical content of propolis is the subject of research. In the study in which the chemical content of propolis was determined by HPLC, 38 phenolic compounds were detected. Among these compounds, ferulic acid, phloridzin, and myricetin were found at the highest concentrations [36]. In this study, ferulic acid was determined at the highest concentration (7.052 mg analyte/g extract) in Ordu propolis. The phytochemical content of Berdav propolis was determined using the same method as in this study. While 53 components were screened, 28 components

Table 4: Phytochemical content results of propolis extracts (mg analyte/g extract).

No	Analyte	RT	Manisa propolis	Iğdır propolis	Muş propolis	Ordu propolis
1	Quinic acid	3.0	4.316	0.312	16.32	5.517
2	Fumaric aid	3.9	0.743	—	—	0.102
3	Aconitic acid	4.0	—	—	—	—
4	Gallic acid	4.4	0.092	—	0.739	0.245
5	Epigallocatechin	6.7	—	—	—	—
6	Protocatechuic acid	6.8	0.994	0.121	1.086	1.003
7	Catechin	7.4	—	—	—	—
8	Gentisic acid	8.3	—	—	—	—
9	Chlorogenic acid	8.4	0.241	—	0.186	0.091
10	Protocatechuic aldehyde	8.5	1.374	0.498	0.858	1.972
11	Tannic acid	9.2	—	—	0.033	0.083
12	Epigallocatechin gallate	9.4	—	—	—	—
13	Cynarin	9.8	—	—	—	—
14	4-OH Benzoic acid	10.5	0.339	—	0.411	0.8
15	Epicatechin	11.6	—	—	—	—
16	Vanilic acid	11.8	5.682	1.399	5.381	5.675
17	Caffeic acid	12.1	17.241	7.578	6	47.779
18	Syringic acid	12.6	—	—	—	—
19	Vanillin	13.9	0.36	—	0.59	0.83
20	Syringic aldehyde	14.6	0.106	—	—	0.118
21	Daidzin	15.2	—	—	—	—
22	Epicatechin gallate	15.5	—	—	—	—
23	Piceid	17.2	—	—	—	—
24	*p*-Coumaric acid	17.8	8.198	4.799	7.453	66.454
25	Ferulic acid-D3-IS	18.8	NA	NA	NA	NA
26	Ferulic acid	18.8	1.425	1.887	6.393	7.052
27	Sinapic acid	18.9	—	—	—	—
28	Coumarin	20.9	—	—	—	—
29	Salicylic acid	21.8	—	—	—	0.026
30	Cyranoside	23.7	0.023	—	0.023	0.061
31	Miquelianin	24.1	—	—	0.849	0.601
32	Rutin-D3-IS	25.5	NA	NA	NA	NA
33	Rutin	25.6	1.303	—	0.907	1.382
34	Isoquercitrin	25.6	0.808	—	2.532	2.855
35	Hesperidin	25.8	0.758	0.029	0.385	0.51
36	*o*-Coumaric acid	26.1	—	—	—	—
37	Genistin	26.3	—	—	—	—
38	Rosmarinic acid	26.6	—	—	0.031	—
39	Ellagic acid	27.6	—	—	—	—
40	Cosmosiin	28.2	0.293	0.027	0.073	0.527
41	Quercitrin	29.8	0.378	0.033	3.498	0.97
42	Astragalin	30.4	0.35	—	1.467	0.63
43	Nicotiflorin	30.6	0.212	—	0.602	1.579
44	Fisetin	30.6	0.231	—	0.019	0.479
45	Daidzein	34.0	—	—	—	—
46	Quercetin-D3-IS	35.6	NA	NA	NA	NA
47	Quercetin	35.7	5.815	2.906	4.242	8.673
48	Naringenin	35.9	5.1	6.766	6.768	8.468
49	Hesperetin	36.7	0.296	0.546	0.47	0.598
50	Luteolin	36.7	0.977	2.781	0.441	1.345
51	Genistein	36.9	—	—	—	—
52	Kaempferol	37.9	4.168	3.772	6.395	5.727
53	Apigenin	38.2	2.718	6.891	1.931	4.032
54	Amentoflavone	39.7	0.012	—	—	—
55	Chrysin	40.5	6.635	24.759	7.135	6.029
56	Acacetin	40.7	12.68	**123.824**	55.179	10.865

Note: The value in bold indicates the highest concentration.
Abbreviations: —: not detected, NA: not applicable, RT: retention time.

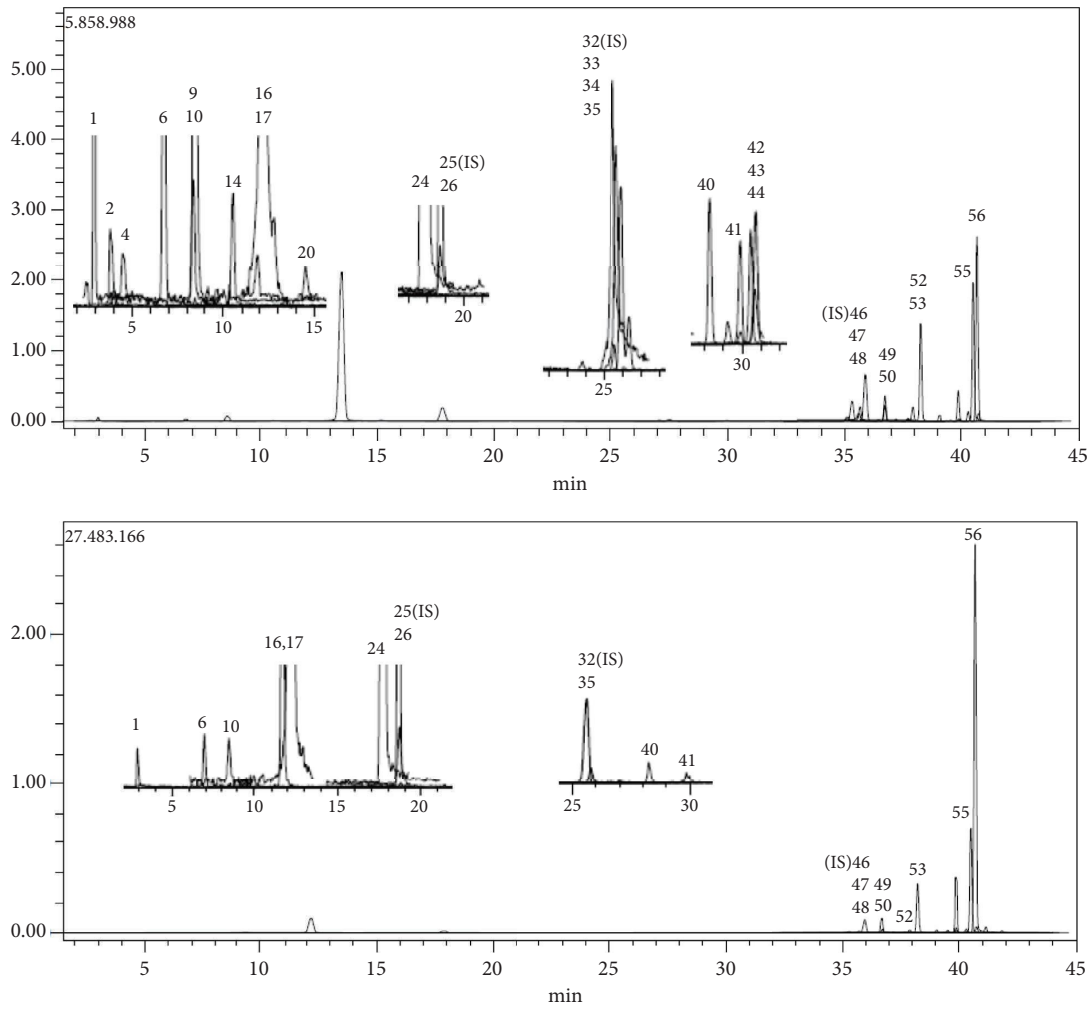

Figure 3: LC–MS/MS chromatograms of Manisa and Iğdır propolis samples.

were identified. In this study, 35 components were determined. It was determined that the phytochemical content of the propolis investigated in this study was higher than Berdav propolis. While the highest concentration of acacetin (76.359 mg/g) was detected in Berdav propolis, acacetin (123.824 mg/g) was also found in this study, but it was much higher. Tannic acid, syringic aldehyde, salicylic acid, cyranoside, rosmarinic acid, fisetin, and amentoflavone were not detected in Berdav propolis but were detected in the samples in this study. With these studies, it was determined again that the chemical content of propolis can vary according to different geography, climate, flora, bee species, and extraction methods.

3.4. Enzyme Inhibition Results. The antiglaucoma property of propolis samples was determined in relation to hCAII enzyme inhibition. The hCAII enzyme inhibition results of propolis are given in Table 5. The graphs of % activity and concentration from which IC_{50} values were calculated are presented in Figure 5.

Among the propolis samples, Muş propolis (IC_{50} [μg/mL]: 8.6) showed the highest inhibition against the hCAII enzyme. This value was higher than the standard acetazolamide (IC_{50} [μg/mL]: 8.98). Thus, it was determined that propolis samples can be recommended for the treatment of glaucoma patients. The antiglaucoma activity of propolis is very limited in the literature. While hCAII enzyme inhibition of Berdav propolis was found to be 19.6 (IC_{50} [μg/mL]) [30], it was found in the range of 8.6–13.7 (IC_{50} [μg/mL]) in this study. It was observed that the antiglaucoma activity of propolis in this study was higher than Berdav propolis. hCAII enzyme inhibition studies are of current interest [68]. Studies on propolis are also being researched with intense interest [69]. In addition, the studies were visualized in the graphical abstract.

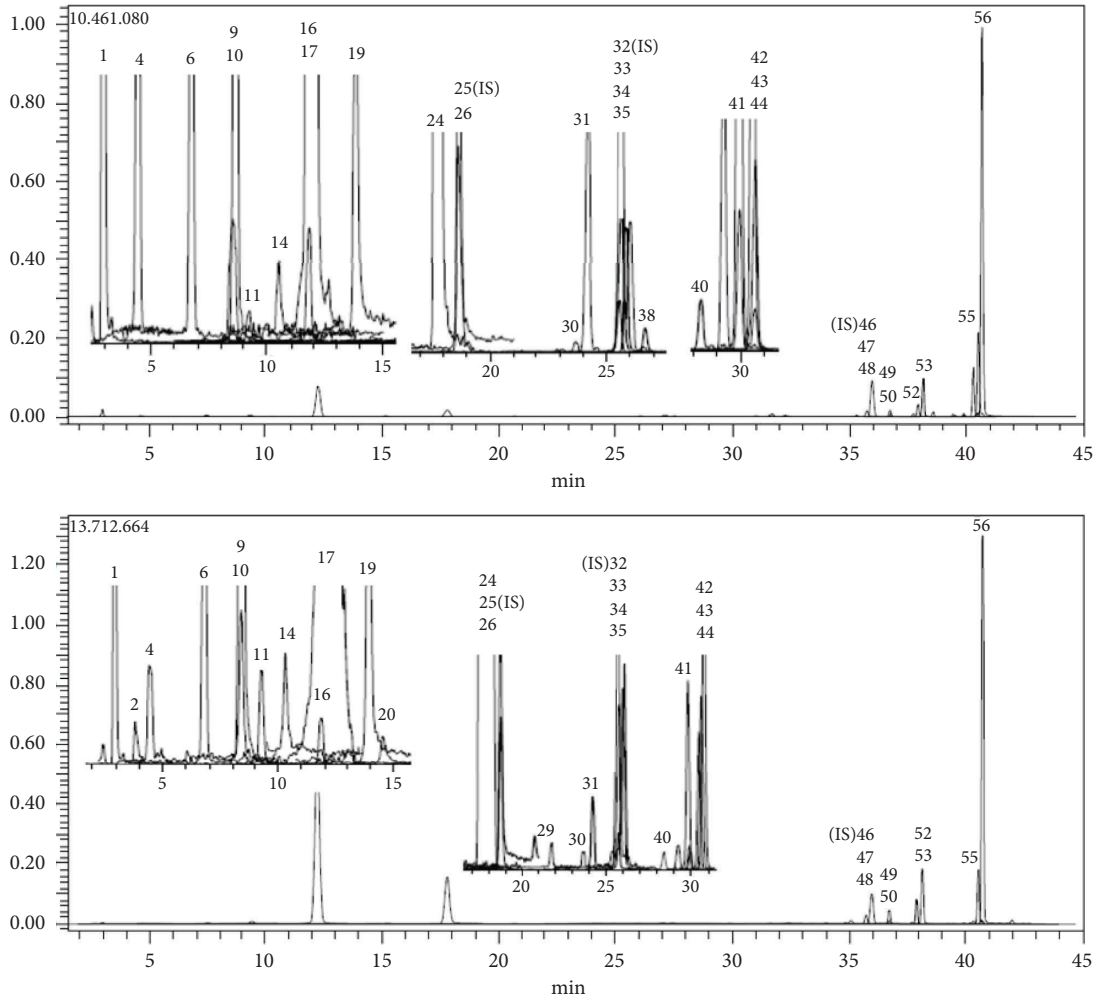

Figure 4: LC–MS/MS chromatograms of Muş and Ordu propolis samples.

Table 5: hCAII enzyme inhibition results of propolis samples.

Standard and propolis extracts	hCAII	
	IC_{50} (μg/mL)	r^2
Iğdır	13.7	0.9849
Manisa	13	0.9816
Ordu	12.7	0.9864
Muş	**8.6**	0.9942
Acetazolamide*	8.98	0.9957

Note: The value in bold indicates the highest activity.
*Acetazolamide was employed as a standard hCAII inhibitor.

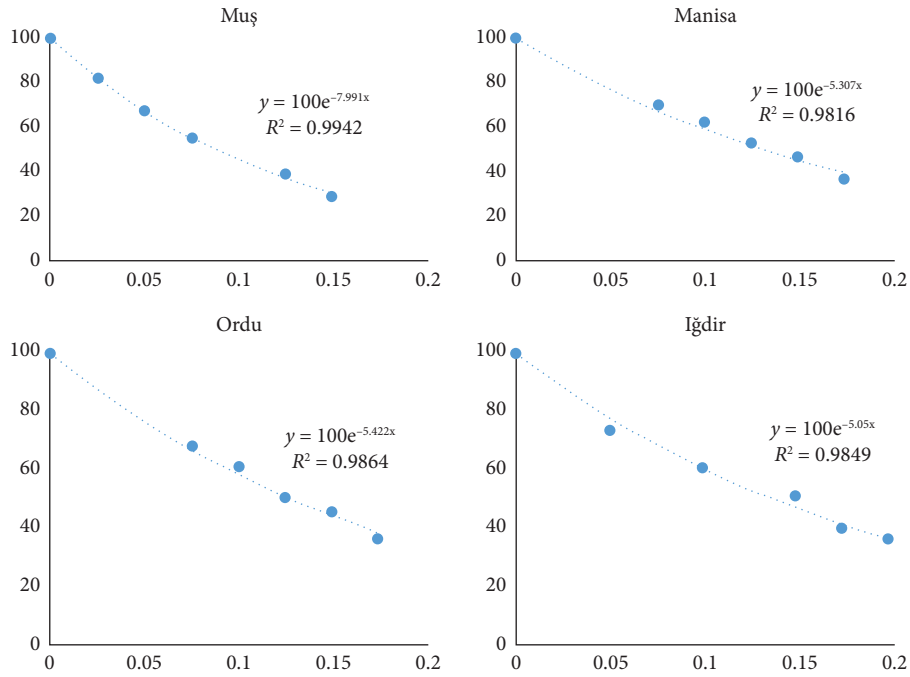

Figure 5: Concentration versus % activity plots of propolis samples for hCAII enzyme inhibition.

4. Conclusion

Propolis has been known for its various chemical compositions and biological properties. The effectiveness of different propolis samples varies depending on the climate, flora, soil structure, extraction method, bee species, and bee health. For this reason, the main idea and purpose of the study are to evaluate and compare the different propolis samples from four parts of Türkiye. The results supported that Ordu propolis, produced in chestnut forests, was the most effective antioxidant material, even more effective than the standards in all five in vitro antioxidant methods. In addition, it was determined that propolis samples can be raw materials for significant bioactive components such as acacetin, chrysin, naringenin, quercetin, caffeic acid, and quinic acid, which are rich in phenolic and flavonoid content. The antiglaucoma properties of propolis samples were found to be high. In this study, the antiglaucoma properties of propolis samples from four provinces were determined for the first time and included in the literature. In summary, in this study, the antioxidant, antiglaucoma, and chemical contents of propolis from different geographies were determined comparatively.

Data Availability Statement

The authors can provide data upon request.

Conflicts of Interest

The authors declare no conflicts of interest.

Author Contributions

Ercan Bursal, Ebubekir İzol, and İlhami Gülçin designed and conducted the study. İsa Yılmaz and Ercan Bursal provided the samples. Ebubekir İzol, İsa Yılmaz, İsmail Yapıcı, and Mustafa Abdullah Yilmaz performed the experiments. Ebubekir İzol wrote the manuscript. Ebubekir İzol, İlhami Gülçin and Ercan Bursal made the revisions. All authors read and approved the final manuscript.

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Supporting Information

Additional supporting information can be found online in the Supporting Information section. *(Supporting Information)*

In this study, the chemical content of propolis from four different provinces of Türkiye was determined by screening 53 different phytochemicals and 35 components were identified. In addition, the comprehensive biological activities of these propolis extracts were determined by 7 different antioxidant methods and antioxidant properties, hCAII enzyme inhibition, and antiglaucoma.

References

[1] M. S. Almuhayawi, "Propolis as a Novel Antibacterial Agent," *Saudi Journal of Biological Sciences* 27, no. 11 (2020): 3079–3086, https://doi.org/10.1016/j.sjbs.2020.09.016.

[2] M. Shahinozzaman, D. N. Obanda, and S. Tawata, "Chemical Composition and Pharmacological Properties of Macaranga-Type Pacific Propolis: A Review," *Phytotherapy Research* 35, no. 1 (2021): 207–222, https://doi.org/10.1002/ptr.6819.

[3] I. Gülçin, E. Bursal, M. H. Şehitoğlu, M. Bilsel, and A. C. Gören, "Polyphenol Contents and Antioxidant Activity of Lyophilized Aqueous Extract of Propolis from Erzurum,

Turkey," *Food and Chemical Toxicology* 48, no. 8-9 (2010): 2227–2238, https://doi.org/10.1016/j.fct.2010.05.053.

[4] E. İzol, "A Miraculous Bee Product:Propolis," in *Functional Medicine-Part 5*, eds. Y. K. Haspolat, A. Atabay, and F. Aşır (Orient Publications, 2023), 15–24.

[5] İ. Yapıcı, E. İzol, and A. Tarhan, "Significant Bioactive Components in Bee Products," in *Bee and Bee Products*, eds. E. İzol, M. Koçyiğit, and Y. K. Haspolat (Orient Publications, 2023).

[6] E. İzol, "The Place of Bee Products in Functional Medicine," in *Functional Medicine Part 2*, eds. Y. K. Haspolat, A. Atlı, and F. Aşır (Orient Publications, 2023), 11–16.

[7] D. Nicodemo, E. B. Malheiros, D. De Jong, and R. H. N. Couto, "Increased Brood Viability and Longer Lifespan of Honeybees Selected for Propolis Production," *Apidologie* 45, no. 2 (2014): 269–275, https://doi.org/10.1007/s13592-013-0249-y.

[8] F. Zulhendri, C. O. Perera, S. Tandean, et al., "The Potential Use of Propolis as a Primary or an Adjunctive Therapy in Respiratory Tract-Related Diseases and Disorders: A Systematic Scoping Review," *Biomedicine & Pharmacotherapy* 146 (2022): 112595, https://doi.org/10.1016/j.biopha.2021.112595.

[9] R. Lesmana, F. Zulhendri, J. Fearnley, et al., "The Suitability of Propolis as a Bioactive Component of Biomaterials," *Frontiers in Pharmacology* 13 (2022): 930515, https://doi.org/10.3389/fphar.2022.930515.

[10] İ. Gulcin, "Antioxidants and Antioxidant Methods: An Updated Overview," *Archives of Toxicology* 94, no. 3 (2020): 651–715, https://doi.org/10.1007/s00204-020-02689-3.

[11] İ. Gulcin and S. H. Alwasel, "DPPH Radical Scavenging Assay," *Processes* 11, no. 8 (2023): 2248, https://doi.org/10.3390/pr11082248.

[12] H. Karageçili, E. İzol, E. Kireçci, and İ. Gülçin, "Antioxidant, Antidiabetic, Antiglaucoma, and Anticholinergic Effects of Tayfi Grape (Vitis vinifera): A Phytochemical Screening by LC-MS/MS Analysis," *Open Chemistry* 21, no. 1 (2023): 20230120, https://doi.org/10.1515/chem-2023-0120.

[13] E. Bursal, M. A. Yılmaz, E. Izol, et al., "Enzyme Inhibitory Function and Phytochemical Profile of Inula Discoidea Using In Vitro and In Silico Methods," *Biophysical Chemistry* 277 (2021): 106629, https://doi.org/10.1016/j.bpc.2021.106629.

[14] H. Karagecili, E. Izol, E. Kirecci, and I. Gulcin, "Determination of Antioxidant, Anti-alzheimer, Antidiabetic, Antiglaucoma and Antimicrobial Effects of Zivzik Pomegranate (*Punica granatum*) —A Chemical Profiling by LC-MS/MS)," *Life* 13, no. 3 (2023): 735, https://doi.org/10.3390/life13030735.

[15] E. Izol, H. Temel, M. A. Yilmaz, et al., "A Detailed Chemical and Biological Investigation of Twelve Allium Species From Eastern Anatolia with Chemometric Studies," *Chemistry and Biodiversity* 18, no. 1 (2021): e2000560, https://doi.org/10.1002/cbdv.202000560.

[16] Ş. Çelik, G. Dervişoglu, E. İzol, et al., "Comprehensive Phytochemical Analysis of Salvia Hispanica L. Callus Extracts Using LC–MS/MS," *Biomedical Chromatography: Biomedical Chromatography* 38, no. 10 (2024): e5975, https://doi.org/10.1002/bmc.5975.

[17] E. İzol, M. Turhan, M. A. Yılmaz, C. Çağlayan, and İ Gülçin, "Determination of Antioxidant, Antidiabetic, Anticholinergic, Antiglaucoma Properties and Comprehensive Phytochemical Content by LC-MS/MS of Bingöl Honeybee Pollen," *Food Science and Nutrition* (2024).

[18] A. Aras, E. Bursal, F. Türkan, et al., "Phytochemical Content, Antidiabetic, Anticholinergic, and Antioxidant Activities of Endemic Lecokia Cretica Extracts," *Chemistry and Biodiversity* 16, no. 10 (2019): e1900341, https://doi.org/10.1002/cbdv.201900341.

[19] H. Inci, E. İzol, M. A. Yılmaz, M. İlkaya, Z. Bingöl, and İ. Gülçin, "Comprehensive Phytochemical Content by LC/MS/MS and Anticholinergic, Antiglaucoma, Antiepilepsy, and Antioxidant Activity of Apilarnil (Drone Larvae)," *Chemistry and Biodiversity* 20, no. 10 (2023): e202300654, https://doi.org/10.1002/cbdv.202300654.

[20] M. A. Yilmaz, O. Cakir, G. Zengin, E. Izol, and L. Behcet, "The Uprisal of a Lost Endemic Edible Species, Micromeria Cymuligera: Comprehensive Elucidation of its Biological Activities and Phytochemical Composition," *Food Bioscience* 104690 (2024).

[21] L. Durmaz, H. Kiziltas, H. Karagecili, S. Alwasel, and İ. Gulcin, "Potential Antioxidant, Anticholinergic, Antidiabetic and Antiglaucoma Activities and Molecular Docking of Spiraeoside as a Secondary Metabolite of Onion (Allium cepa)," *Saudi Pharmaceutical Journal* 31, no. 10 (2023): 101760, https://doi.org/10.1016/j.jsps.2023.101760.

[22] E. Bursal, P. Taslimi, A. C. Gören, and İ. Gülçin, "Assessments of Anticholinergic, Antidiabetic, Antioxidant Activities and Phenolic Content of Stachys Annua," *Biocatalysis and Agricultural Biotechnology* 28 (2020): 101711, https://doi.org/10.1016/j.bcab.2020.101711.

[23] R. Apak, K. Güçlü, M. Özyürek, S. Esin Karademir, and E. Erçağ, "The Cupric Ion Reducing Antioxidant Capacity and Polyphenolic Content of Some Herbal Teas," *International Journal of Food Sciences & Nutrition* 57, no. 5-6 (2006): 292–304, https://doi.org/10.1080/09637480600798132.

[24] M. Oyaizu, "Studies on Products of Browning Reaction--Antioxidative Activities of Products of Browning Reaction Prepared from Glucosamine," *The Japanese Journal of Nutrition and Dietetics* 44, no. 6 (1986): 307–315, https://doi.org/10.5264/eiyogakuzashi.44.307.

[25] M. A. Yılmaz, P. Taslimi, Ö. Kılıç, İ. Gülçin, A. Dey, and E. Bursal, "Unravelling the Phenolic Compound Reserves, Antioxidant and Enzyme Inhibitory Activities of an Endemic Plant Species, Achillea Pseudoaleppica," *Journal of Biomolecular Structure and Dynamics* 41, no. 2 (2023): 445–456, https://doi.org/10.1080/07391102.2021.2007792.

[26] M. S. Blois, "Antioxidant Determinations by the Use of a Stable Free Radical," *Nature* 181, no. 4617 (1958): 1199–1200, https://doi.org/10.1038/1811199a0.

[27] R. Re, N. Pellegrini, A. Proteggente, A. Pannala, M. Yang, and C. Rice-Evans, "Antioxidant Activity Applying an Improved ABTS Radical Cation Decolorization Assay," *Free Radical Biology and Medicine* 26, no. 9-10 (1999): 1231–1237, https://doi.org/10.1016/s0891-5849(98)00315-3.

[28] İ. Gulçin, P. Taslimi, A. Aygün, et al., "Antidiabetic and Antiparasitic Potentials: Inhibition Effects of Some Natural Antioxidant Compounds on α-Glycosidase, α-Amylase and Human Glutathione S-Transferase Enzymes," *International Journal of Biological Macromolecules* 119 (2018): 741–746, https://doi.org/10.1016/j.ijbiomac.2018.08.001.

[29] M. A. Yilmaz, "Simultaneous Quantitative Screening of 53 Phytochemicals in 33 Species of Medicinal and Aromatic Plants: A Detailed, Robust and Comprehensive LC–MS/MS Method Validation," *Industrial Crops and Products* 149 (2020): 112347, https://doi.org/10.1016/j.indcrop.2020.112347.

[30] H. Karagecili, M. A. Yılmaz, A. Ertürk, et al., "Comprehensive Metabolite Profiling of Berdav Propolis Using LC-MS/MS:

Determination of Antioxidant, Anticholinergic, Anti-glaucoma, and Antidiabetic Effects," *Molecules* 28, no. 4 (2023): 1739, https://doi.org/10.3390/molecules28041739.

[31] X. Wang, K. Sankarapandian, Y. Cheng, et al., "Relationship Between Total Phenolic Contents and Biological Properties of Propolis From 20 Different Regions in South Korea," *BMC Complementary and Alternative Medicine* 16 (2016): 65–12, https://doi.org/10.1186/s12906-016-1043-y.

[32] M. Stanciauskaite, M. Marksa, M. Liaudanskas, L. Ivanauskas, M. Ivaskiene, and K. Ramanauskiene, "Extracts of Poplar Buds (Populus Balsamifera L., Populus Nigra L.) and Lithuanian Propolis: Comparison of Their Composition and Biological Activities," *Plants* 10, no. 5 (2021): 828, https://doi.org/10.3390/plants10050828.

[33] L. Moreira, L. G. Dias, J. A. Pereira, and L. Estevinho, "Antioxidant Properties, Total Phenols and Pollen Analysis of Propolis Samples from Portugal," *Food and Chemical Toxicology* 46, no. 11 (2008): 3482–3485, https://doi.org/10.1016/j.fct.2008.08.025.

[34] S. Kolaylı, C. Birinci, Y. Kara, et al., "A Melissopalynological and Chemical Characterization of Anatolian Propolis and an Assessment of its Antioxidant Potential," *European Food Research and Technology* 249, no. 5 (2023): 1213–1233, https://doi.org/10.1007/s00217-023-04208-x.

[35] A. L. S. Vieira, V. T. D. V. Correia, A. L. C. C. Ramos, et al., "Evaluation of the Chemical Profile and Antioxidant Capacity of Green, Brown, and Dark Propolis," *Plants* 12, no. 18 (2023): 3204, https://doi.org/10.3390/plants12183204.

[36] A. M. Silva, B. Rocha, M. M. Moreira, C. Delerue-Matos, J. das Neves, and F. Rodrigues, "Biological Activity and Chemical Composition of Propolis Extracts With Potential Use in Vulvovaginal Candidiasis Management," *International Journal of Molecular Sciences* 25, no. 5 (2024): 2478, https://doi.org/10.3390/ijms25052478.

[37] S. Singh, P. Gupta, A. Meena, and S. Luqman, "Acacetin, a Flavone with Diverse Therapeutic Potential in Cancer, Inflammation, Infections and Other Metabolic Disorders," *Food and Chemical Toxicology* 145 (2020): 111708, https://doi.org/10.1016/j.fct.2020.111708.

[38] A. Ghanbari, C. Jalili, A. Abdolmaleki, and V. Shokri, "Effects of Cisplatin and Acacetin on Total Antioxidant Status, Apoptosis and Expression of OCTN3 in Mouse Testis," *Biotechnic & Histochemistry* 97, no. 3 (2022): 185–191, https://doi.org/10.1080/10520295.2021.1925347.

[39] K. Pei, J. Ou, J. Huang, and S. Ou, "p-Coumaric Acid and its Conjugates: Dietary Sources, Pharmacokinetic Properties and Biological Activities," *Journal of the Science of Food and Agriculture* 96, no. 9 (2016): 2952–2962, https://doi.org/10.1002/jsfa.7578.

[40] H. Boz, "p-Coumaric Acid in Cereals: Presence, Antioxidant and Antimicrobial Effects," *International Journal of Food Science & Technology* 50, no. 11 (2015): 2323–2328, https://doi.org/10.1111/ijfs.12898.

[41] Y. Shen, X. Song, L. Li, et al., "Protective Effects of P-Coumaric Acid against Oxidant and Hyperlipidemia-An In Vitro and In Vivo Evaluation," *Biomedicine & Pharmacotherapy* 111 (2019): 579–587, https://doi.org/10.1016/j.biopha.2018.12.074.

[42] R. W. Jiang, K. M. Lau, P. M. Hon, T. C. Mak, K. S. Woo, and K. P. Fung, "Chemistry and Biological Activities of Caffeic Acid Derivatives From Salvia Miltiorrhiza," *Current Medicinal Chemistry* 12, no. 2 (2005): 237–246, https://doi.org/10.2174/0929867053363397.

[43] İ. Gülçin, "Antioxidant Activity of Caffeic Acid (3, 4-dihydroxycinnamic Acid)," *Toxicology* 217, no. 2-3 (2006): 213–220, https://doi.org/10.1016/j.tox.2005.09.011.

[44] Y. Sato, S. Itagaki, T. Kurokawa, et al., "In Vitro And In Vivo Antioxidant Properties of Chlorogenic Acid and Caffeic Acid," *International Journal of Pharmaceutics* 403, no. 1-2 (2011): 136–138, https://doi.org/10.1016/j.ijpharm.2010.09.035.

[45] C. M. Spagnol, R. P. Assis, I. L. Brunetti, V. L. B. Isaac, H. R. N. Salgado, and M. A. Corrêa, "In Vitro Methods to Determine the Antioxidant Activity of Caffeic Acid," *Spectrochimica Acta Part A: Molecular and Biomolecular Spectroscopy* 219 (2019): 358–366, https://doi.org/10.1016/j.saa.2019.04.025.

[46] R. Mani and V. Natesan, "Chrysin: Sources, Beneficial Pharmacological Activities, and Molecular Mechanism of Action," *Phytochemistry* 145 (2018): 187–196, https://doi.org/10.1016/j.phytochem.2017.09.016.

[47] Y. Deldar, Y. Pilehvar-Soltanahmadi, M. Dadashpour, S. Montazer Saheb, M. Rahmati-Yamchi, and N. Zarghami, "An In Vitro Examination of the Antioxidant, Cytoprotective and Anti-inflammatory Properties of Chrysin-Loaded Nanofibrous Mats for Potential Wound Healing Applications," *Artificial Cells, Nanomedicine, and Biotechnology* 46, no. 4 (2018): 706–716, https://doi.org/10.1080/21691401.2017.1337022.

[48] G. Pushpavalli, P. Kalaiarasi, C. Veeramani, and K. V. Pugalendi, "Effect of Chrysin on Hepatoprotective and Antioxidant Status in D-Galactosamine-Induced Hepatitis in Rats," *European Journal of Pharmacology* 631, no. 1-3 (2010): 36–41, https://doi.org/10.1016/j.ejphar.2009.12.031.

[49] K. L. Ooi, T. S. T. Muhammad, M. L. Tan, and S. F. Sulaiman, "Cytotoxic, Apoptotic and Anti-α-Glucosidase Activities of 3, 4-Di-O-Caffeoyl Quinic Acid, an Antioxidant Isolated from the Polyphenolic-Rich Extract of Elephantopus Mollis Kunth," *Journal of Ethnopharmacology* 135, no. 3 (2011): 685–695, https://doi.org/10.1016/j.jep.2011.04.001.

[50] M. Karaman, K. Tesanovic, S. Gorjanovic, et al., "Polarography as a Technique of Choice for the Evaluation of Total Antioxidant Activity: The Case Study of Selected Coprinus Comatus Extracts and Quinic Acid, Their Antidiabetic Ingredient," *Natural Product Research* 35, no. 10 (2021): 1711–1716, https://doi.org/10.1080/14786419.2019.1628753.

[51] R. Kleemann, L. Verschuren, M. Morrison, et al., "Anti-Inflammatory, Anti-Proliferative and Anti-atherosclerotic Effects of Quercetin in Human In Vitro and In Vivo Models," *Atherosclerosis* 218, no. 1 (2011): 44–52, https://doi.org/10.1016/j.atherosclerosis.2011.04.023.

[52] S. Ganesan, A. N. Faris, A. T. Comstock, et al., "Quercetin Inhibits Rhinovirus Replication In Vitro and In Vivo," *Antiviral Research* 94, no. 3 (2012): 258–271, https://doi.org/10.1016/j.antiviral.2012.03.005.

[53] D. Xu, M. J. Hu, Y. Q. Wang, and Y. L. Cui, "Antioxidant Activities of Quercetin and Its Complexes for Medicinal Application," *Molecules* 24, no. 6 (2019): 1123, https://doi.org/10.3390/molecules24061123.

[54] M. Ay, A. Charli, H. Jin, V. Anantharam, A. Kanthasamy, and A. G. Kanthasamy, "Quercetin," in *Nutraceuticals* (Academic Press, 2021), 749–755.

[55] K. Uçar and Z. Göktaş, "Biological Activities of Naringenin: A Narrative Review Based on In Vitro and In Vivo Studies," *Nutrition Research* 119 (2023): 43–55, https://doi.org/10.1016/j.nutres.2023.08.006.

[56] B. Salehi, P. V. T. Fokou, M. Sharifi-Rad, et al., "The Therapeutic Potential of Naringenin: A Review of Clinical Trials," *Pharmaceuticals* 12, no. 1 (2019): 11, https://doi.org/10.3390/ph12010011.

[57] S. D. V. S. Kiran, P. Rohini, and P. Bhagyasree, "Flavonoid: A Review on Naringenin," *Journal of Pharmacognosy and Phytochemistry* 6, no. 5 (2017): 2778–2783.

[58] B. Salehi, A. Venditti, M. Sharifi-Rad, et al., "The Therapeutic Potential of Apigenin," *International Journal of Molecular Sciences* 20, no. 6 (2019): 1305, https://doi.org/10.3390/ijms20061305.

[59] J. S. Choi, M. Nurul Islam, M. Yousof Ali, E. J. Kim, Y. M. Kim, and H. A. Jung, "Effects of C-Glycosylation on Anti-diabetic, Anti-alzheimer's Disease and Anti-inflammatory Potential of Apigenin," *Food and Chemical Toxicology* 64 (2014): 27–33, https://doi.org/10.1016/j.fct.2013.11.020.

[60] X. Zhou, F. Wang, R. Zhou, X. Song, and M. Xie, "Apigenin: A Current Review on Its Beneficial Biological Activities," *Journal of Food Biochemistry* 41, no. 4 (2017): e12376, https://doi.org/10.1111/jfbc.12376.

[61] S. P. Bangar, V. Chaudhary, N. Sharma, V. Bansal, F. Ozogul, and J. M. Lorenzo, "Kaempferol: A Flavonoid With Wider Biological Activities and Its Applications," *Critical Reviews in Food Science and Nutrition* 63, no. 28 (2023): 9580–9604, https://doi.org/10.1080/10408398.2022.2067121.

[62] J. K. Kim and S. U. Park, "Recent Studies on Kaempferol and Its Biological and Pharmacological Activities," *EXCLI journal* 19 (2020): 627–634.

[63] J. Kaur, M. Gulati, S. K. Singh, et al., "Discovering Multifaceted Role of Vanillic Acid Beyond Flavours: Nutraceutical and Therapeutic Potential," *Trends in Food Science & Technology* 122 (2022): 187–200, https://doi.org/10.1016/j.tifs.2022.02.023.

[64] N. Sharma, N. Tiwari, M. Vyas, N. Khurana, A. Muthuraman, and P. Utreja, "An Overview of Therapeutic Effects of Vanillic Acid," *Plant Arch* 20, no. 2 (2020): 3053–3059.

[65] N. Uppugundla, A. Engelberth, S. Vandhana Ravindranath, et al., "Switchgrass Water Extracts: Extraction, Separation and Biological Activity of Rutin and Quercitrin," *Journal of Agricultural and Food Chemistry* 57, no. 17 (2009): 7763–7770, https://doi.org/10.1021/jf900998q.

[66] R. Hardiyanti, L. Marpaung, I. K. Adnyana, and P. Simanjuntak, "Isolation of Quercitrin From Dendrophthoe Pentandra (L.) Miq Leaves and It's Antioxidant and Antibacterial Activities," *Rasayan Journal of Chemistry* 12, no. 04 (2019): 1822–1827, https://doi.org/10.31788/rjc.2019.1235353.

[67] J. Chen, G. Li, C. Sun, et al., "Chemistry, Pharmacokinetics, Pharmacological Activities, and Toxicity of Quercitrin," *Phytotherapy Research* 36, no. 4 (2022): 1545–1575, https://doi.org/10.1002/ptr.7397.

[68] V. Farzaliyev, A. Ertürk, M. Abbasova, et al., "Synthesis and Inhibitor Effect Novel Alkoxymethyl Derivatives of Dihetero Cycloalkanes on Carbonic Anhydrase and Acetylcholinesterase," *Chemistry and Biodiversity* 21, no. 6 (2024): e202400296, https://doi.org/10.1002/cbdv.202400296.

[69] A. Aboulghazi, M. Fadil, S. Touzani, L. Hibaoui, C. Hano, and B. Lyoussi, "Phenolic Screening and Mixture Design Optimization for In Vitro Assessment of Antioxidant and Antimicrobial Activities of Honey, Propolis, and Bee Pollen," *Journal of Food Biochemistry* 2024, no. 1 (2024): 8246224, https://doi.org/10.1155/2024/8246224.