Check for updates

Chemistry & Biodiversity

CHEMISTRY & BIODIVERSITY

RESEARCH ARTICLE

Chemical Composition and Biological Activities of Propolis From Eastern Anatolia: Antioxidant, Enzyme Inhibitory, and Antimicrobial Activities

Mehmet Çağlar Fırat[1] | Zeynep Akşit[1] | Samed Şimşek[2] | Sevgi Altın[3] | İlyas Yıldız[4] | Hüseyin Akşit[5]

[1]Department of Hotel, Restaurant and Service, Tourism and Hospitality Vocational School, Erzincan Binali Yıldırım University, Erzincan, Türkiye | [2]Department of Medical Services and Techniques, Çayırlı Vocational School, Erzincan Binali Yıldırım University, Erzincan, Türkiye | [3]Institute of Science and Technology, Erzincan Binali Yıldırım University, Erzincan, Türkiye | [4]Department of Molecular Biology and Genetics, Faculty of Arts and Science, Gaziosmanpaşa University, Tokat, Türkiye | [5]Department of Analytical Chemistry, Faculty of Pharmacy, Erzincan Binali Yıldırım University, Erzincan, Türkiye

Correspondence: Samed Şimşek (samed.simsek@erzincan.edu.tr)

Received: 24 January 2026 | **Revised:** 4 July 2026 | **Accepted:** 6 July 2026

Keywords: antimicrobial activity | antioxidant activity | bioactive compounds | chemical composition | enzyme inhibition | propolis

ABSTRACT

Propolis is recognized for its biological activities; however, its chemical composition varies according to environmental conditions, which presents challenges for quality standardization and biological evaluation. Although propolis has been widely investigated, information regarding variation among samples collected from geographically close locations within the same region is limited. Therefore, this study investigated whether propolis samples obtained from different locations within Erzincan Province exhibit differences in chemical composition and biological activities and evaluated the relationship between environmental conditions and propolis characteristics. Eleven propolis samples collected from Erzincan, Türkiye, were analyzed for phenolic composition, antioxidant capacity, antimicrobial activity, acetylcholinesterase (AChE), butyrylcholinesterase (BChE), and α-glucosidase inhibitory activities. Twenty-eight compounds were identified, with gallic acid, protocatechuic acid, chlorogenic acid, hydroxybenzaldehyde, caffeic acid, and vanillin detected as major constituents. AChE inhibitory activity varied between 1.28 and 6.44 μg/mL, while BChE inhibitory activity varied between 1.85 and 9.66 μg/mL. All samples exhibited antibacterial activity. Total phenolic and flavonoid contents were 141.70–255.04 mg GAE/g extract and 30.48–93.81 mg QE/g extract, respectively. The KMH sample showed the antioxidant activity. The results demonstrated variation in phytochemical composition and biological activities among propolis samples collected within the same province, indicating associations between flora, environmental conditions, propolis quality, and bioactivity.

1 | Introduction

Propolis is a complex resinous material produced by honeybees (*Apis mellifera* L.) from plant resins collected from buds, leaves, bark, and other plant parts and mixed with beeswax and salivary secretions [1, 2]. Within the hive, propolis serves as a structural sealant and a component of social immunity, providing protection against environmental stress factors and microbial contamination [2]. Propolis has been widely investigated as a source of bioactive natural products because of its ecological functions and phytochemical composition. To date, more than 300 compounds have been identified in propolis, including phenolic acids, flavonoids, phenolic acid esters, terpenoids, aromatic aldehydes, amino acids, vitamins, and plant-derived secondary metabolites [1, 3]. The chemical composition of propolis reflects the diversity of botanical sources used by honeybees and is associated with a wide range of biological activities [4].

All rights reserved, including rights for text and data mining and training of artificial intelligence technologies or similar technologies.
© 2026 Wiley-VHCA AG.

The biological activities of propolis are largely attributed to its phenolic constituents. Antioxidant, antimicrobial, anti-inflammatory, antiviral, antidiabetic, immunomodulatory, and anticancer properties have been widely reported [1, 3]. Phenolic acids such as caffeic, chlorogenic, and p-coumaric acids, together with flavonoids including quercetin, kaempferol, chrysin, luteolin, and pinocembrin, are major contributors to these biological effects [3]. The capacity of these compounds to scavenge reactive oxygen species, modulate cellular signaling pathways, and interact with microbial and enzymatic targets is associated with the pharmacological relevance of propolis in pharmaceutical and nutraceutical applications [5, 6]. These properties, in combination, position propolis as a promising candidate for the development of functional foods and plant-derived therapeutic agents.

Propolis exhibits considerable chemical variability, although its biological importance is well recognized. In contrast to many plant-derived natural products obtained from a single botanical source, propolis originates from plant resins collected from multiple plant species and is characterized by considerable chemical diversity [4]. The phytochemical profile of propolis is associated with botanical origin, climatic conditions, altitude, seasonal variation, geographic location, and honeybee foraging behavior, and variation in these factors influences both the qualitative and quantitative composition of propolis as well as its biological properties [6, 7]. This chemical variability represents a major challenge for the standardization and quality control of propolis.

Botanical origin is widely considered a major determinant of propolis chemistry, as the phytochemical profile of the final product directly reflects the plant species available within the foraging territory of the colony [2, 3]. Samples collected from different geographic regions exhibit differences in phenolic composition and biological activity associated with variation in plant diversity and ecological conditions [7, 8]. Comparative studies on propolis from different geographic areas have shown that differences in surrounding vegetation are more strongly associated with chemical composition than geographic classification [9, 10]. Propolis samples have commonly been compared according to country, region, or administrative province, although the relationship between these classifications and propolis composition has been questioned.

Macro-geographical classifications may not reflect the chemical variability observed in propolis because honeybees collect plant resins within a limited area around the hive, and propolis phytochemistry is closely associated with local botanical composition [2]. Within the area used by honeybees for resin collection, local vegetation patterns, environmental variation, and microclimatic conditions may be more strongly associated with propolis composition than geographic classifications [3]. The well-documented effects of altitude on plant secondary metabolism [11, 12], together with evidence linking geographic origin, plant source, and polyphenolic composition to the antimicrobial properties of propolis [10], support the hypothesis that micro-regional ecological dynamics contribute to propolis chemical diversity. Investigations targeting intra-provincial or micro-regional variability may provide additional information on propolis quality and bioactivity compared with geographic classifications.

Türkiye constitutes one of the most botanically diverse countries in the Mediterranean basin and represents an important center of global plant biodiversity. The intersection of the Euro-Siberian, Mediterranean, and Irano-Turanian phytogeographical regions contributes to the high plant diversity and endemism observed in Türkiye [13]. Altitudinal gradients, geological heterogeneity, and the coexistence of continental and Mediterranean climate regimes contribute to this diversity [14], resulting in a mosaic of habitats with different resin-producing plant species available to honeybees and contributing to the chemical diversity observed in Turkish propolis. Regional variation in flora composition is associated with differences in phenolic profiles and biological activities of propolis samples [15], highlighting the need for fine-scale investigations of the ecological factors associated with propolis chemistry in Türkiye.

Erzincan Province, located in Eastern Anatolia, is an important beekeeping region characterized by ecological heterogeneity. The province includes lowland valleys, mountainous areas, and high-altitude plateaus with variation in vegetation structure, climatic conditions, and floristic composition [16]. Asteraceae and Lamiaceae species are common in lowland areas [17, 18], and these families are well-recognized sources of phenolic acids and flavonoids, including caffeic acid derivatives [19, 20]. Highland areas contain alpine flora and Apiaceae species associated with different phytochemical profiles. In addition, Erzincan contains numerous endemic and regionally distributed plant species, indicating the high floristic diversity of the area [14]. This ecological heterogeneity can be used to investigate whether micro-regional conditions contribute to chemical and biological variation among propolis samples collected within a restricted geographic area.

Neurodegenerative diseases, particularly Alzheimer's disease, constitute a major global public health burden, and reinforcement of cholinergic neurotransmission is a central pharmacological strategy in their management. The AChE and BChE are enzymes responsible for the hydrolysis of acetylcholine, and their inhibition is one of the main therapeutic approaches used to slow cognitive impairment associated with dementia [21, 22]. Similarly, type 2 diabetes mellitus is a major global health concern, and inhibition of α-glucosidase, an enzyme involved in the conversion of oligosaccharides to absorbable monosaccharides in the small intestine, is a widely used approach for reducing postprandial hyperglycemia and supporting glycemic control [23]. Natural products with inhibitory activity against these enzymes have been investigated as potential sources of bioactive compounds for the development of functional foods and therapeutic agents [24, 25]. Phenolic compounds, including caffeic acid and flavonoids such as luteolin, quercetin, and kaempferol, have previously been reported as inhibitors of cholinesterase enzymes [26–28], while caffeic acid and related phenolic acids have also been associated with α-glucosidase inhibitory activity [29, 30]. However, information on micro-regional variability in these enzyme inhibitory activities among propolis samples is limited.

Numerous studies have investigated the chemical composition and biological activities of propolis; however, information regarding phytochemical and bioactivity variation among samples collected from locations within the same province is limited. Most previous studies have classified propolis samples according to geographic origin, including country, region, or province.

16121880, 2026, 7, Downloaded from https://onlinelibrary.wiley.com/doi/10.1002/cbdv.71533 by Samed MEK - Erzincan Universitesi , Wiley Online Library on [26/07/2026]. See the Terms and Conditions (https://onlinelibrary.wiley.com/terms-and-conditions) on Wiley Online Library for rules of use; OA articles are governed by the applicable Creative Commons License

Because honeybees collect plant resins within a limited foraging area, micro-regional ecological differences may contribute to variation in propolis chemistry and biological activity. Such micro-regional variability is essential for propolis standardization, quality control, and the identification of environmental factors associated with the distribution of bioactive compounds. Therefore, the present study aimed to investigate whether micro-regional differences in the phytochemical composition of propolis collected from different locations within Erzincan Province, Türkiye, are reflected in multiple biologically relevant activities. Phenolic constituents were characterized by LC-ESI-MS/MS, and hierarchical cluster analysis was used to evaluate phytochemical relationships among samples. Antioxidant, antimicrobial, cholinesterase (AChE and BChE), and α-glucosidase inhibitory activities were evaluated as complementary biological assays to determine whether variations in phenolic composition are associated with antioxidant potential, antimicrobial activity, and enzyme inhibitory properties relevant to oxidative stress, microbial defense, neurodegenerative disorders, and type 2 diabetes mellitus. This integrated approach was designed to provide a comprehensive evaluation of the biological significance of micro-regional phytochemical variation and to identify relationships between chemical composition and multiple bioactivities. The findings contribute to the understanding of micro-regional variability in propolis and highlight the association of altitude and local botanical composition with propolis chemistry and bioactivity.

2 | Results and Discussion

2.1 | Chemical Composition

A total of 28 phenolic compounds were identified in the propolis extracts out of the 43 compounds analyzed (Table 1). Gallic acid, protocatechuic acid, chlorogenic acid, hydroxybenzaldehyde, caffeic acid, vanillin, o-coumaric acid, salicylic acid, polydatin, sinapic acid, *p*-coumaric acid, kaempferol-3-glucoside, fisetin, baicalin, chrysin, hesperetin, kaempferol, baicalein, and luteolin were consistently detected in all samples. Among these, caffeic acid was the major compound in CVZ2, KMH, YYLB, TRC, RFY, and ESS, while syringic acid was the major constituent in KLY1, KLY2, ERG, CVZ1, and CYR. Statistical analysis ($p < 0.05$) indicated significant differences among samples for these compounds, especially caffeic acid. Caffeic acid exhibits a significantly higher concentration (140 times) in the propolis of the lowland sample (CVZ2), compared to the highland sample (CVZ1), which are both products of the same beekeeper. CVZ2 formed a distinct statistical group with the highest value (2777.34 μg/g), while YYLB, TRC, and RFY followed at significantly lower levels but still higher than the remaining samples. The observed variation in caffeic acid contents of CVZ1 and CVZ2 samples may be attributed to differences in regional flora and environmental conditions. Lowland areas in the studied region contain a high proportion of plant species belonging to Asteraceae and Lamiaceae [17, 18], both of which are recognized as major sources of phenolic compounds, including caffeic acid and its derivatives [19, 20]. Plants in these families often contribute significantly to the resin and nectar pool accessible to honeybees. In addition, ecological factors associated with low altitude, such as higher average temperatures, reduced UV stress, and longer vegetative periods, may contribute to the biosynthesis of caffeic acid derivatives in these plants. The presence of such species in lowland environments may contribute to the higher caffeic acid concentrations observed in propolis samples from these areas [10, 19, 20]. Similarly, Altuntaş et al. (2023) reported that the chemical composition of propolis obtained from geographically close regions may not be similar to each other [31]. In the study, although Tekirdağ was grouped in the same cluster with Gaziantep province, which are 1265 km far from each other, it was conversely grouped in a different cluster with Kırklareli province, which are 83 km far from each other. Similar observations have been reported for Karabük and Samsun provinces. These findings support our results.

2.2 | Hierarchical Clustering Analysis

Hierarchical Clustering Analysis provides information on the similarity and dissimilarity among samples according to their phytochemical composition. Figure 1a presents the HCA dendrogram, in which samples connected earlier in the clustering process show higher similarity in their chemical composition. Samples connected later in the clustering process show lower similarity in their chemical composition. Figure 1b complements the dendrogram by showing the cluster membership of each propolis sample and the cluster level at which each sample is grouped, providing a clear view of sample clustering. The HCA dendrogram and cluster analysis (Figure 1a,b) showed no association between geographical location and the chemical composition of the propolis samples. While KLY1 and KLY2, collected from the same area, were grouped within the same cluster, CVZ1 and CVZ2, obtained by the same beekeeper from different locations within the same region, were assigned to different clusters. This observation suggests a stronger association between local ecological conditions and propolis composition than between geographical location and propolis composition. The comparison between CVZ1 and CVZ2 supports this observation. Although both samples originated from locations within the same region, caffeic acid concentration was about 140-fold higher in CVZ2 than in CVZ1. Such differences may be associated with variation in the plant species available to honeybees. Previous studies have shown that the chemical composition of propolis is associated with the botanical sources from which bees collect resinous materials and not with geographical location [8, 9].

Field observations indicated that lowland areas in the study region contained more Asteraceae and Lamiaceae species, while highland locations contained more alpine vegetation and Apiaceae species. Members of the Asteraceae and Lamiaceae families are recognized as important sources of phenolic acids and flavonoids, including caffeic acid derivatives, which may be associated with the higher caffeic acid concentrations observed in lowland samples such as CVZ2. Similarly, differences in local flora influence the phenolic profile of propolis and may contribute to chemical variation within relatively small geographical areas [3].

The observed phytochemical variation may also be associated with variation in elevation among sampling locations. Environmental conditions associated with elevation, including temperature, ultraviolet radiation, soil characteristics, and water

16121880, 2026, 7, Downloaded from https://onlinelibrary.wiley.com/doi/10.1002/cbdv.71533 by Samed MEK - Erzincan Universitesi , Wiley Online Library on [26/07/2026]. See the Terms and Conditions (https://onlinelibrary.wiley.com/terms-and-conditions) on Wiley Online Library for rules of use; OA articles are governed by the applicable Creative Commons License

TABLE 1 | Chemical composition of propolis samples (μg analyte/g extract).

Compound*	KLY1	KLY2	ERG	CVZ1	ESS	CYR	CVZ2	RFY	TRC	YYLB	KMH
Gallic acid	23.18±0.82^{d}	17.51±0.69^{c}	9.77±0.42^{a}	18.89±0.68^{c}	16.93±0.60^{c}	28.94±1.35^{e}	26.87±0.67^{e}	17.75±0.72^{c}	16.81±0.40^{c}	19.32±0.78^{c}	11.98±0.47^{b}
Protocatechuic acid	22.81±1.01^{f}	13.20±0.54^{c}	11.59±0.33^{c}	3.37±0.14^{b}	12.19±0.56^{c}	19.38±0.45^{e}	49.09±1.80^{g}	16.81±0.73^{d}	3.54±0.10^{b}	13.44±0.53^{c}	1.66±0.04^{a}
Chlorogenic acid	5.04±0.16^{a}	4.48±0.12^{a}	4.24±0.16^{a}	9.39±0.34^{c}	4.53±0.22^{a}	4.42±0.15^{a}	4.24±0.17^{a}	4.62±0.23^{a}	4.19±0.14^{a}	7.10±0.17^{b}	4.49±0.19^{a}
Hydroxybenzaldehyde	6.10±0.13^{b}	8.58±0.34^{c}	8.27±0.30^{c}	40.38±0.85^{g}	5.09±0.20^{a}	5.72±0.16^{b}	9.72±0.31^{d}	23.50±0.54^{f}	15.83±0.65^{e}	10.61±0.42^{d}	9.81±0.31^{d}
Caffeic acid	4.53±0.11^{a}	4.84±0.19^{a}	4.39±0.21^{a}	19.76±0.55^{b}	747.81±24.95^{c}	4.56±0.10^{a}	2777.34±105.45^{f}	828.66±31.99^{d}	894.31±18.60^{d}	1387.13±54.10^{e}	711.32±15.43^{c}
Syringic acid	188.98±9.16^{b}	201.75±8.87^{b}	205.78±9.96^{b}	194.68±4.96^{b}	162.49±7.47^{a}	202.56±4.55^{b}	204.55±6.23^{b}	210.22±5.47^{b}	225.80±11.03^{b}	193.88±5.35^{b}	ND
Vanillin	8.02±0.31^{b}	7.24±0.22^{a}	14.70±0.39^{d}	16.09±0.66^{d}	8.06±0.30^{b}	18.94±0.90^{e}	19.21±0.91^{e}	14.25±0.64^{d}	24.15±1.07^{f}	11.19±0.41^{c}	14.05±0.56^{d}
o-Coumaric acid	11.09±0.25^{d}	8.20±0.28^{b}	18.09±0.61^{f}	13.09±0.65^{e}	11.20±0.45^{d}	9.32±0.28^{c}	18.14±0.84^{f}	7.39±0.18^{a}	8.14±0.28^{b}	18.29±0.74^{f}	11.09±0.31^{d}
Salicylic acid	64.02±2.07^{a}	58.69±1.33^{a}	77.37±2.96^{a}	73.25±2.06^{a}	86.94±2.39^{a}	69.77±3.04^{a}	71.46±3.21^{a}	79.20±2.54^{a}	88.15±3.88^{a}	86.74±1.74^{a}	80.80±1.65^{a}
Polydatin	0.92±0.03^{a}	0.94±0.03^{a}	0.93±0.02^{a}	0.92±0.03^{a}	0.92±0.02^{a}	0.93±0.02^{a}	0.92±0.04^{a}	0.92±0.03^{a}	0.92±0.04^{a}	0.92±0.03^{a}	0.92±0.02^{a}
trans-Ferulic acid	18.91±0.69^{a}	17.18±0.60^{a}	17.62±0.77^{a}	18.07±0.74^{a}	18.53±0.67^{a}	17.24±0.50^{a}	17.24±0.39^{a}	19.20±0.83^{a}	18.82±0.61^{a}	17.25±0.72^{a}	19.77±0.70^{a}
Sinapic acid	4.19±0.12^{d}	2.55±0.06^{a}	4.07±0.09^{d}	4.54±0.15^{d}	3.92±0.09^{d}	2.56±0.10^{a}	3.86±0.18^{d}	3.03±0.08^{b}	5.79±0.17^{e}	3.37±0.09^{c}	2.64±0.12^{a}
p-Coumaric acid	5.37±0.12^{d}	6.94±0.34^{e}	4.43±0.11^{c}	5.86±0.16^{d}	3.96±0.13^{b}	3.34±0.12^{a}	3.56±0.16^{a}	4.02±0.14^{b}	8.01±0.34^{f}	13.59±0.64^{g}	8.50±0.20^{f}
Isoquercetin	ND	ND	ND	59.29±1.52^{a}	ND	ND	ND	ND	ND	ND	ND

(Continues)

16121880, 2026, 7, Downloaded from https://onlinelibrary.wiley.com/doi/10.1002/cbdv.71533 by Samed MEK - Erzincan Universitesi , Wiley Online Library on [26/07/2026]. See the Terms and Conditions (https://onlinelibrary.wiley.com/terms-and-conditions) on Wiley Online Library for rules of use; OA articles are governed by the applicable Creative Commons License

TABLE 1 | (Continued)

Compound*	KLY1	KLY2	ERG	CVZ1	ESS	CYR	CVZ2	RFY	TRC	YYLB	KMH
Rutin	22.84±1.08^{d}	154.55±3.46^{g}	ND	55.79±2.50^{e}	92.09±3.15^{f}	3.94±0.12^{a}	7.84±0.28^{b}	157.36±5.27^{g}	ND	12.93±0.34c	ND
Kaempferol-3-glucoside	9.41±0.28^{a}	32.41±0.74^{e}	15.41±0.36^{c}	18.79±0.64^{d}	12.42±0.57^{b}	12.51±0.25^{b}	12.43±0.52^{b}	12.39±0.34^{b}	15.46±0.57^{c}	14.43±0.51^{c}	18.43±0.73^{d}
Fisetin	9.80±0.42^{a}	9.80±0.45^{a}	9.80±0.29^{a}	13.28±0.44^{b}	9.81±0.22^{a}	9.80±0.32^{a}	9.80±0.20^{a}	9.82±0.47^{a}	9.81±0.23^{a}	9.80±0.37^{a}	9.79±0.20^{a}
Baicalin	2.59±0.12^{a}	2.70±0.11^{a}	2.59±0.11^{a}	2.64±0.08^{a}	2.62±0.11^{a}	2.61±0.06^{a}	2.61±0.06^{a}	2.59±0.11^{a}	2.65±0.13^{a}	2.59±0.06^{a}	2.59±0.11^{a}
Chrysin	0.19±0.01^{a}	0.19±0.01^{a}	0.18±0.01^{a}	0.19±0.01^{a}	0.20±0.01^{a}	0.19±0.00^{a}	0.19±0.00^{a}	0.19±0.01^{a}	0.18±0.00^{a}	0.18±0.01^{a}	0.19±0.01^{a}
Daidzein	ND	ND	ND	ND	ND	ND	ND	ND	ND	42.86±0.97^{a}	ND
Quercetin	ND	ND	17.66±0.65^{a}	17.56±0.75^{a}	ND	17.60±0.73^{a}	17.58±0.53^{a}	17.56±0.43^{a}	ND	17.59±0.74^{a}	17.56±0.55^{a}
Naringenin	15.18±0.72^{a}	15.10±0.57^{a}	15.25±0.55^{a}	14.91±0.52^{a}	14.94±0.43^{a}	ND	15.25±0.32^{a}	15.32±0.52^{a}	15.37±0.37^{a}	15.41±0.72^{a}	15.42±0.76^{a}
Silibinin	0.35±0.01^{e}	0.13±0.00^{d}	1.20±0.06^{h}	0.09±0.00^{c}	0.63±0.02^{f}	0.08±0.00^{b}	0.09±0.00^{c}	0.07±0.00^{a}	0.70±0.02^{g}	0.12±0.00^{d}	0.12±0.00^{d}
Hesperetin	48.63±1.98^{a}	52.99±1.64^{a}	48.31±0.98^{a}	47.65±1.33^{a}	58.68±2.42^{a}	61.32±2.03^{a}	49.02±1.15^{a}	53.66±1.71^{a}	51.08±1.50^{a}	53.31±2.30^{a}	85.40±2.59^{b}
Kaempferol	34.03±1.26^{f}	36.99±1.14^{f}	18.84±0.71^{d}	12.46±0.52^{b}	28.24±0.65^{e}	40.62±1.29^{f}	13.99±0.43^{c}	50.60±1.44^{g}	19.47±0.48^{d}	5.10±0.19^{a}	20.02±0.97^{d}
Baicalein	0.17±0.01^{c}	0.11±0.00^{a}	0.22±0.01^{d}	0.36±0.01^{f}	0.21±0.01^{d}	0.28±0.01^{e}	0.18±0.01^{c}	0.13±0.01^{b}	0.17±0.00^{c}	0.82±0.02^{h}	0.43±0.02^{g}
Luteolin	20.16±1.00^{e}	14.16±0.60^{c}	8.16±0.38^{a}	32.16±0.90^{g}	15.16±0.75^{c}	11.16±0.39^{b}	8.16±0.32^{a}	25.16±0.88^{f}	8.16±0.35^{a}	14.16±0.55^{c}	18.16±0.60^{d}
Diosgenin	7.37±0.16^{c}	3.57±0.09^{a}	ND	3.84±0.15^{a}	3.60±0.17^{a}	3.71±0.18^{a}	8.83±0.31^{d}	13.76±0.62^{e}	8.68±0.35^{d}	15.67±0.55^{f}	6.57±0.14^{b}

*Shikimic acid, epigallocatechin, catechin, vanillic acid, caffeine, resveratrol, taxifolin, scutellarin, coumarin, protocatechuic acid ethyl ester, hesperidin, quercetin-3-xyloside, *trans*-cinnamic acid, morin, and biochanin A were analyzed but not detected in any of the samples.

**$^{a\text{-}g}$Different letters in the same row indicate statistical differences ($p < 0.05$).

16121880, 2026, 7, Downloaded from https://onlinelibrary.wiley.com/doi/10.1002/cbdv.71533 by Sanea MEK - Erzincan Universitesi , Wiley Online Library on [26/07/2026]. See the Terms and Conditions (https://onlinelibrary.wiley.com/terms-and-conditions) on Wiley Online Library for rules of use; OA articles are governed by the applicable Creative Commons License

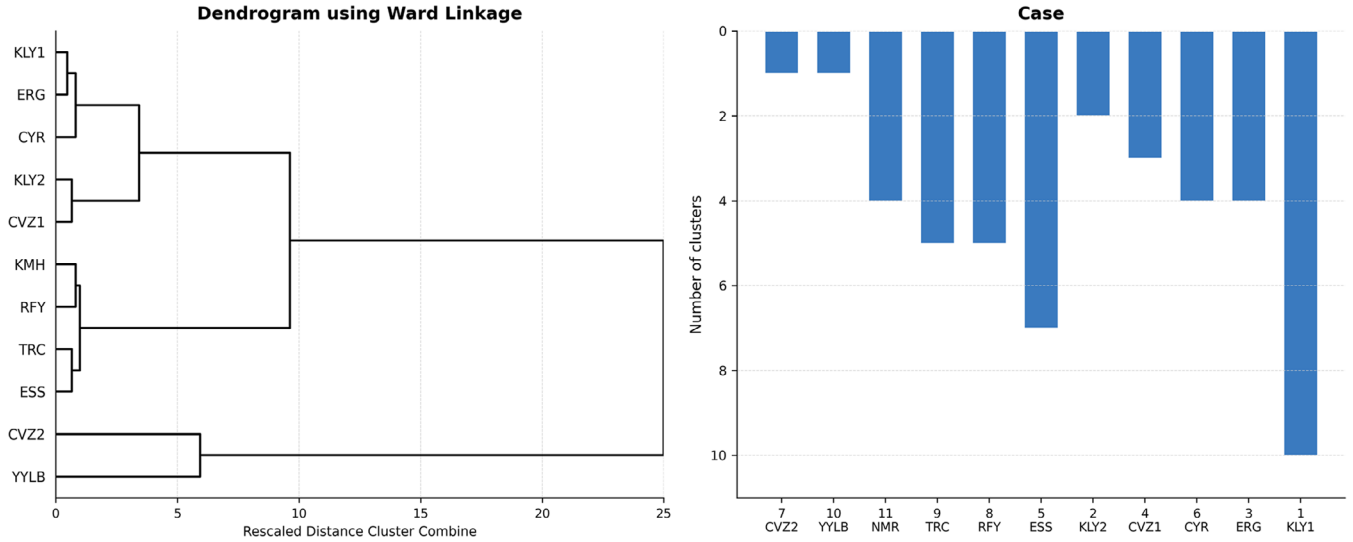

FIGURE 1 | HCA of propolis samples based on their chemical composition: (a) dendrogram generated using Ward's linkage method and (b) cluster membership plot showing the grouping of individual samples.

availability, can influence plant secondary metabolism and contribute to variation in phenolic compound concentrations. Plant biochemical composition may vary along elevation gradients in association with ecological and physiological adaptations [11]. Similarly, phenolic profiles may vary along elevation gradients in association with environmental conditions associated with plant populations [12].

The present findings are consistent with previous investigations showing that geographical location is not always associated with chemical similarity in propolis. Turkish propolis samples collected from different provinces were not always grouped according to their phytochemical composition, and samples from different regions showed similar chemical characteristics [31]. Similar observations have been reported, indicating that local botanical diversity is one of the main factors associated with chemical variation in propolis [8]. Therefore, the clustering pattern observed in the present study supports the hypothesis that micro-regional floral composition and altitude-related environmental conditions are important factors associated with propolis phytochemistry and may be associated with the variation observed among samples collected within the same province.

TABLE 2 | AChE, BChE, and α-glycosidase enzyme inhibition values (IC_{50}) for propolis samples (μg/mL).

Samples	AChE*	BChE*	α-glycosidase*
KLY1	5.17±0.06^{e}	9.66±0.24^{e}	3.81±0.15^{f}
KLY2	6.44±0.13^{e}	2.81±0.19$^{a.b}$	3.35±0.14$^{d.e}$
ERG	3.3±0.28^{b}	2.21±0.07$^{a.b}$	2.45±0.24^{c}
CVZ1	4.67±0.14^{d}	6.53±0.12^{d}	4.38±0.18^{g}
ESS	4.53±0.39^{d}	2.99±0.13^{c}	4.82±0.32^{h}
CYR	3.13±0.13^{b}	2.34±0.34$^{a.b}$	3.56±0.25$^{e.f}$
CVZ2	3.22±0.09^{c}	2.37±0.09$^{a.b}$	2.75±0.27^{c}
RFY	1.77±0.29^{a}	2.13±0.11$^{a.b}$	2.06±0.17^{b}
TRC	3.49±0.33^{b}	4.23±1.74^{d}	3.73±0.27$^{e.f}$
YYLB	3.15±0.15^{b}	1.85±0.24^{a}	1.12±0.14^{a}
KMH	1.28±0.08^{a}	2.99±0.12^{c}	3.16±0.17^{d}
Tacrine	3.18±0.17^{b}	2.52±0.14$^{a.b}$	—
Acarbose	—	—	3.49±0.17$^{d.e}$

*Inhibitions were expressed as mean ± SD of three independent measurements, μg/mL.

[a-h]Different letters in the same column indicate statistical difference ($p < 0.05$).

2.3 | Enzyme Inhibition Activity

The inhibitory effects of propolis samples on AChE, BChE, and α-glucosidase were evaluated, and the IC_{50} values are presented in Table 2. The IC_{50} values for AChE inhibition among the propolis samples were between 1.28 ± 0.08 μg/mL (KMH) and 6.44 ± 0.13 μg/mL (KLY2), while tacrine exhibited an IC_{50} of 3.18 ± 0.17 μg/mL. According to Tukey's HSD test ($p < 0.05$), KMH and RFY showed significantly stronger inhibition than tacrine, while CYR and YYLB exhibited comparable activity. CVZ2, ERG, and TRC also showed IC_{50} values similar to tacrine ($p > 0.05$), whereas KLY1, KLY2, ESS, and CVZ1 showed significantly lower inhibitory activity. The overall mean AChE IC_{50} value for the tested propolis was 3.65 μg/mL.

BChE inhibition showed variation, with IC_{50} values between 1.85 ± 0.24 μg/mL (YYLB) and 9.66 ± 0.24 μg/mL (KLY1), compared to 2.52 ± 0.14 μg/mL for tacrine. Samples YYLB, RFY, ERG, CYR, and CVZ2 inhibited BChE more effectively than tacrine ($p < 0.05$), while KLY2, KMH, and ESS demonstrated comparable activity. In contrast, TRC, CVZ1, and KLY1 exhibited significantly lower inhibitory activity.

α-glucosidase IC_{50} values were between 1.12 ± 0.14 μg/mL (YYLB) and 4.82 ± 0.32 μg/mL (ESS), while acarbose exhibited an IC_{50} of 3.49 ± 0.17 μg/mL. YYLB demonstrated the strongest inhibition and exhibited significantly lower IC_{50} values than acarbose ($p <$

16121880, 2026, 7, Downloaded from https://onlinelibrary.wiley.com/doi/10.1002/cbdv.71533 by Samet MEK - Erzincan Universitesi , Wiley Online Library on [26/07/2026]. See the Terms and Conditions (https://onlinelibrary.wiley.com/terms-and-conditions) on Wiley Online Library for rules of use; OA articles are governed by the applicable Creative Commons License

0.05), followed by RFY and ERG, which also exhibited lower IC_{50} values than acarbose.

When compared with the literature, the AChE IC_{50} values obtained in the present study (1.28–6.44 μg/mL) were lower than those reported for several other propolis samples, indicating a comparatively potent inhibitory capacity. Previous studies reported AChE IC_{50} values of 5.17 μg/mL for Bitlis propolis, 3.4 μg/mL for Berdav propolis, and 16.98–54.36 μg/mL for Ankara propolis, while tacrine exhibited IC_{50} values between 5.97 and 8.28 μg/mL [32–34]. In a recent study, Şiran propolis showed an AChE IC_{50} of 2.15 μg/mL compared with 8.82 μg/mL for tacrine, which is consistent with the high inhibitory activity observed for KMH and RFY samples in the present study [34]. In contrast, Algerian propolis exhibited lower AChE inhibitory activity, with IC_{50} values between 16.06 and 180.80 μg/mL, while galantamine exhibited an IC_{50} of 6.27 μg/mL [35]. AChE inhibition for Turkish propolis has previously been reported with IC_{50} values between 0.081 and 1.353 mg/mL, Sudanese propolis showing inhibition rates between 25.5% and 91.7%, and Moroccan propolis with IC_{50} values between 0.002 and 3.555 mg/mL [35, 36]. These comparisons highlight that the Erzincan propolis samples investigated here are among the more potent AChE inhibitors reported for propolis in the literature. The mean AChE IC_{50} of 3.65 μg/mL across all samples is comparable to the tacrine reference value of 3.18 μg/mL obtained in this study; notably, five samples (KMH, RFY, YYLB, CYR, and CVZ2) exhibited IC_{50} values comparable to or lower than that of tacrine, indicating that these extracts may exhibit inhibitory activity similar to the reference drug within the tested concentration range.

The BChE inhibitory activity observed in the present study was within the lower IC_{50} range reported for propolis samples in the literature. Bitlis propolis, a previously characterized Turkish propolis, exhibited a BChE IC_{50} of 7.50 μg/mL against a tacrine reference of 8.28 μg/mL [33], and Berdav propolis demonstrated a BChE IC_{50} of about 3.4 μg/mL [32]. Algerian propolis extracts from four geographic regions showed BChE IC_{50} values of 16.06 μg/mL, with all extracts exhibiting higher selectivity for BChE than AChE [35]. In a comprehensive study of 12 Iranian propolis samples, BChE IC_{50} values were between 13.05 and 155.34 μg/mL, and most samples showed higher inhibitory activity against BChE than AChE, a selectivity pattern consistent with findings for several samples in the present study [37]. The BChE IC_{50} values observed for YYLB, RFY, ERG, CYR, and CVZ2 were lower than those reported in these studies, indicating comparatively strong BChE inhibitory activity. This observation is therapeutically relevant, as BChE activity increases relative to AChE during the progression of Alzheimer's disease, and BChE inhibition is considered an important therapeutic target [21]. The flavonoid constituents identified in the present samples, including kaempferol, luteolin, quercetin, fisetin, and rutin, have been associated with BChE inhibitory activity. Structural studies have shown that these flavonoids are associated with BChE inhibition through hydrogen bonds and π–π interactions involving the enzyme active region, and that the number and position of hydroxyl groups are important factors associated with inhibitory potency [38].

Regarding α-glucosidase, nine of the eleven samples exhibited significant inhibitory activity, with YYLB, TRC, CVZ2, ERG, and CYR showing the strongest inhibitory effects. YYLB exhibited higher α-glucosidase inhibitory activity than acarbose, which is consistent with previous studies reporting α-glucosidase inhibitory activity of propolis extracts comparable to or higher than that of acarbose. Previous investigations on Turkish propolis reported IC_{50} values of 3.7 μg/mL for Berdav propolis and 5.72 μg/mL for Siirt propolis [32, 39]. Chinese propolis extracts have been reported to show α-glucosidase IC_{50} values between 7.24 and 20.01 μg/mL, while acarbose exhibited an IC_{50} value of 177.5 μg/mL in the same assay system [40]. Similarly, Moroccan propolis exhibited α-glucosidase IC_{50} values between 90.99 and 876.24 μg/mL, indicating considerable variation in inhibitory activity associated with the botanical origin and composition of the samples [41]. These comparisons suggest that the activity observed in the present study, particularly for YYLB and RFY, is among the stronger inhibitory activities reported for propolis in comparable assay systems. The superior activity may be associated with the high caffeic acid content observed in samples such as YYLB and CVZ2, as caffeic acid and protocatechuic acid have been identified as potent α-glucosidase inhibitors among phenolic acids with multiple hydroxyl groups. The inhibitory activity of phenolic acids has been associated with their structural characteristics, and differences in hydroxylation patterns have been reported to influence α-glucosidase inhibition [42, 43]. The observed α-glucosidase inhibitory activity suggests that the tested propolis samples may possess antihyperglycemic properties, which may be associated with the presence of phenolic acids, including caffeic acid, vanillin, and protocatechuic acid, previously reported as α-glucosidase inhibitors [29, 30]. However, further in vitro and in vivo studies are required to evaluate their potential role in diabetes management.

The correlation analysis between phenolic/flavonoid compounds and enzyme inhibitory activities (AChE, BChE, and α-glucosidase) revealed additional relationships regarding the potential bioactive constituents of the propolis samples. Principal component analysis (PCA) was conducted to investigate the associations between phenolic composition and enzyme inhibitory activities. The first two principal components accounted for 39% of the total variance (PC1: 20.7%; PC2: 18.4%). The PCA biplot (Figure 2) showed a distinct distribution of propolis samples according to their phenolic profiles. Samples such as RFY and KMH were related to vectors indicating higher caffeic acid and rutin content, while CYR and CVZ2 showed association with kaempferol, luteolin, and gallic acid. The orientation of enzyme inhibition vectors toward certain compounds suggested the potential contribution of these phenolics to the observed biological activities.

Overall, the findings suggest that specific phenolic acids (particularly caffeic acid) and flavonoids (such as luteolin and kaempferol) may contribute to the enzyme inhibitory activities of propolis. PCA analysis supports the interpretation of phytochemical–bioactivity associations and indicates that the biological activities of propolis result from interactions among multiple compounds rather than from a single constituent.

2.4 | Antibacterial Activity and MIC Analysis

In this study, nine foodborne pathogens were tested to ascertain the antibacterial activity of the samples shown in Table 3. All

16121880, 2026, 7, Downloaded from https://onlinelibrary.wiley.com/doi/10.1002/cbdv.71533 by Samed MEK - Erzincan Universitesi , Wiley Online Library on [26/07/2026]. See the Terms and Conditions (https://onlinelibrary.wiley.com/terms-and-conditions) on Wiley Online Library for rules of use; OA articles are governed by the applicable Creative Commons License

TABLE 3 | Antibacterial properties of propolis samples.

	Gram (+)				Gram (−)				
Samples	***B. cereus* (mm)**	***L. monocytogenes* (mm)**	***C. perfringens* (mm)**	***E. faecium* (mm)**	***E. coli* (mm)**	***P. aeruginosa* (mm)**	***P. fluorescens* (mm)**	***S.* Enteritidis (mm)**	***S. enterica* (mm)**
KLY1	13	12	15	10	12	13	10	11	14
KLY2	15	14	11	13	13	10	10	10	13
ERG	16	16	14	15	8	10	12	11	12
CVZ1	12	12	9	9	10	9	9	12	11
KMH	12	12	17	15	14	12	12	11	10
ESS	14	13	13	15	16	11	9	11	12
CYR	12	13	14	15	12	14	15	10	10
CVZ2	15	10	13	14	11	15	14	10	12
RFY	10	10	12	13	11	10	12	7	16
TRC	15	18	16	13	14	16	17	9	22
YYLB	15	11	15	12	9	14	14	10	13
İmipenem	26	21	24	26	28	25	24	28	35

*The inhibition zones are shown in mm. < 13 mm resistant, 13-15 intermediate, ≥16 susceptible.

16121880, 2026, 7, Downloaded from https://onlinelibrary.wiley.com/doi/10.1002/cbdv.71533 by Saned MEK - Erzincan Universitesi , Wiley Online Library on [26/07/2026]. See the Terms and Conditions (https://onlinelibrary.wiley.com/terms-and-conditions) on Wiley Online Library for rules of use; OA articles are governed by the applicable Creative Commons License

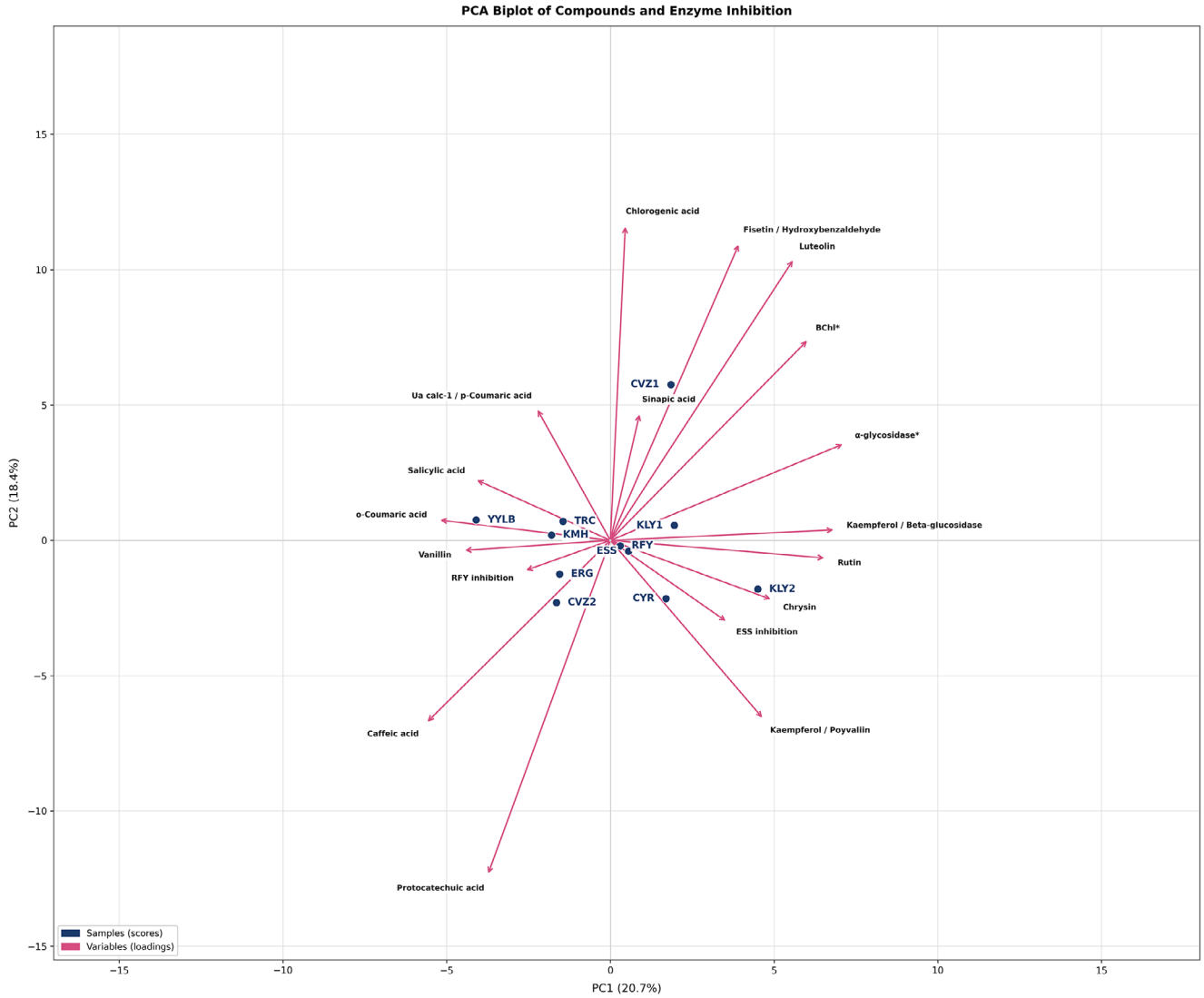

FIGURE 2 | PCA biplot of phenolic compounds and enzyme inhibitory activities. PCA was performed with standardized data; PC1 and PC2 explained 39% of the total variance.

propolis samples showed antibacterial activity against the nine tested ATCC pathogens. The TRC sample showed antibacterial activity against *L. monocytogenes*, *C. perfringens*, *P. aeruginosa*, *P. fluorescens*, and *S. enterica*, intermediate activity against *B. cereus*, *E. faecium*, and *E. coli*, and no activity against *S. Enteritidis*. In addition, antibacterial activity was observed for ERG against *B. cereus* and *L. monocytogenes*, KMH against *C. perfringens*, ESS against *E. coli*, and RFY against *S. enterica*. The lowland CVZ2 sample had a more considerable antibacterial effect than the highland CVZ1 sample. The antibacterial effects of propolis are significantly influenced by the region. Propolis inhibits bacteria by disrupting bacterial cell walls and cytoplasmic membranes; inhibiting energy metabolism, enzyme activity, nucleic acid synthesis, and protein synthesis; and inducing the release of intracellular contents [5, 44, 45]. The chemical composition of propolis, including flavonoids, phenolic acids, terpenes, and other bioactive compounds, is associated with its antimicrobial activity. As mentioned above, the main components of the samples are caffeic acid and syringic acid. Caffeic acid exhibits antibacterial activity by affecting membrane permeability, damaging DNA and protein structures, and inhibiting enzyme function [46]. Syringic acid also exhibits an antibacterial effect by changing the shape of the cell and changing the intracellular pH, ATP levels, membrane polarization, permeability of the cell membrane, and concentrations of internal anions [47].

Among the bacteria tested, Gram-negative strains were more resistant to the propolis samples and exhibited smaller inhibition zones than Gram-positive strains. This finding is consistent with previous reports indicating that propolis generally exhibits stronger activity against Gram (+) bacteria, since the outer membrane of Gram (−) bacteria limits compound penetration [5, 48, 49].

Based on zone size, MIC analysis was performed on all samples since they were shown to be highly efficient against four Gram (+) bacteria and four Gram (−) bacteria (Table 4). The MIC values of these samples were between < 16 and 250 μg/mL. The antibacterial activity of the extracts was considered to be high if their MIC was < 100 μg/mL; moderate if it was 100–500 μg/mL; low if it was 500–1000 μg/mL; and inactive if it was >1000 μg/mL. KLY2 and CYR samples have MIC values lower than 16 μg/mL

16121880, 2026, 7, Downloaded from https://onlinelibrary.wiley.com/doi/10.1002/cbdv.71533 by Samed MEK - Erzincan Universitesi , Wiley Online Library on [26/07/2026]. See the Terms and Conditions (https://onlinelibrary.wiley.com/terms-and-conditions) on Wiley Online Library for rules of use; OA articles are governed by the applicable Creative Commons License

TABLE 4 | MIC values of propolis samples.

Samples	Gram (+)				Gram (−)			
	B. cereus (µg/mL)	L. monocytogenes (µg/mL)	C. perfringens (µg/mL)	E. faecium (µg/mL)	E. coli (µg/mL)	P. aeruginosa (µg/mL)	P. fluorescens (µg/mL)	S. enterica (µg/mL)
KLY1	> 1000	> 1000	250	> 1000	> 1000	> 1000	> 1000	> 1000
KLY2	< 16	> 1000	> 1000	> 1000	> 1000	> 1000	> 1000	> 1000
ERG	63	63	> 1000	125	> 1000	> 1000	> 1000	> 1000
CVZ1	> 1000	> 1000	> 1000	> 1000	> 1000	> 1000	> 1000	> 1000
KMH	> 1000	> 1000	250	63	> 1000	> 1000	> 1000	125
ESS	> 1000	> 1000	> 1000	125	125	> 1000	> 1000	250
CYR	> 1000	> 1000	> 1000	63	> 1000	> 1000	< 16	> 1000
CVZ2	250	> 1000	> 1000	> 1000	> 1000	125	> 1000	> 1000
RFY	> 1000	> 1000	> 1000	> 1000	> 1000	> 1000	> 1000	> 1000
TRC	125	125	250	> 1000	> 1000	125	250	> 1000
YYLB	250	> 1000	250	> 1000	> 1000	> 1000	> 1000	> 1000

for *B. cereus* and *P. fluorescens*, respectively, which indicates high antibacterial activity.

PCA was applied to phenolic compound profiles and antimicrobial activity data to investigate the associations between chemical composition and antibacterial effects. The first two principal components (PC1 and PC2) accounted for 38.2% of the total variance (PC1: 21.4%; PC2: 16.9%). The biplot (Figure 3) showed that some samples, such as TRC and RFY, were associated with vectors representing stronger inhibition against Gram-positive bacteria (e.g., *B. cereus*, *C. perfringens*), while others were related to variables corresponding to Gram-negative bacteria (*E. coli*, *P. aeruginosa*). In addition, several phenolic acids (e.g., caffeic acid, syringic acid, o-coumaric acid) showed association with antibacterial activity vectors, suggesting their potential role in antimicrobial activity. These results suggest that the antibacterial activities of propolis samples are not attributable to a single compound but rather to complex interactions among multiple phenolics and specific microbial targets.

2.5 | Antioxidant Potential of Samples

The antioxidant properties of propolis samples varied significantly according to DPPH radical scavenging, ferric reducing power (FRAP), total phenolic content (TPC), and total flavonoid content (TFC) assays (Table 5). The IC_{50} values for DPPH scavenging activity were between 27.78 ± 2.89 µg/mL (KMH) and 72.69 ± 0.17 µg/mL (KLY1), indicating variation in antioxidant potential. KMH exhibited the strongest radical-scavenging activity, followed by ERG and CYR, while KLY1 and CVZ1 showed the lowest activity ($p < 0.05$).

A similar pattern was observed in the FRAP assay, where reducing power values were between 95.23 ± 1.89 mg TE/g extract (KLY1) and 254.33 ± 0.75 mg TE/g extract (KMH). KMH and ERG demonstrated significantly higher reducing capacity compared to KLY1, with CYR, YYLB, and TRC also showing strong reducing ability.

The TPC analysis revealed significant differences among the samples, with values between 141.70 ± 2.80 mg GAE/g extract (KLY1) and 255.04 ± 3.39 mg GAE/g extract (KMH). Samples with higher phenolic content, such as KMH, ERG, and CYR, corresponded to higher antioxidant activity, supporting the strong correlation between TPC and antioxidant performance. In contrast, KLY1 exhibited the lowest TPC and the lowest antioxidant potential. Although KLY1 exhibited the highest total flavonoid content (93.81 ± 2.18 mg QE/g extract), it showed the lowest antioxidant activity in both DPPH and FRAP assays. This observation indicates that antioxidant activity is not determined only by the total concentration of flavonoids. In contrast to its elevated TFC value, KLY1 exhibited the lowest total phenolic content among all samples and contained lower concentrations of caffeic acid than the samples showing the strongest antioxidant activity. Previous structure–activity studies have demonstrated that the antioxidant activity of phenolic compounds is strongly influenced by the number and position of hydroxyl groups, which are associated with electron-donating and radical-scavenging properties [50]. In particular, caffeic acid and related hydroxycinnamic acid derivatives contain a catechol structure associated with free-

16121880, 2026, 7, Downloaded from https://onlinelibrary.wiley.com/doi/10.1002/cbdv.71533 by Samed MEK - Erzincan Universitesi , Wiley Online Library on [26/07/2026]. See the Terms and Conditions (https://onlinelibrary.wiley.com/terms-and-conditions) on Wiley Online Library for rules of use; OA articles are governed by the applicable Creative Commons License

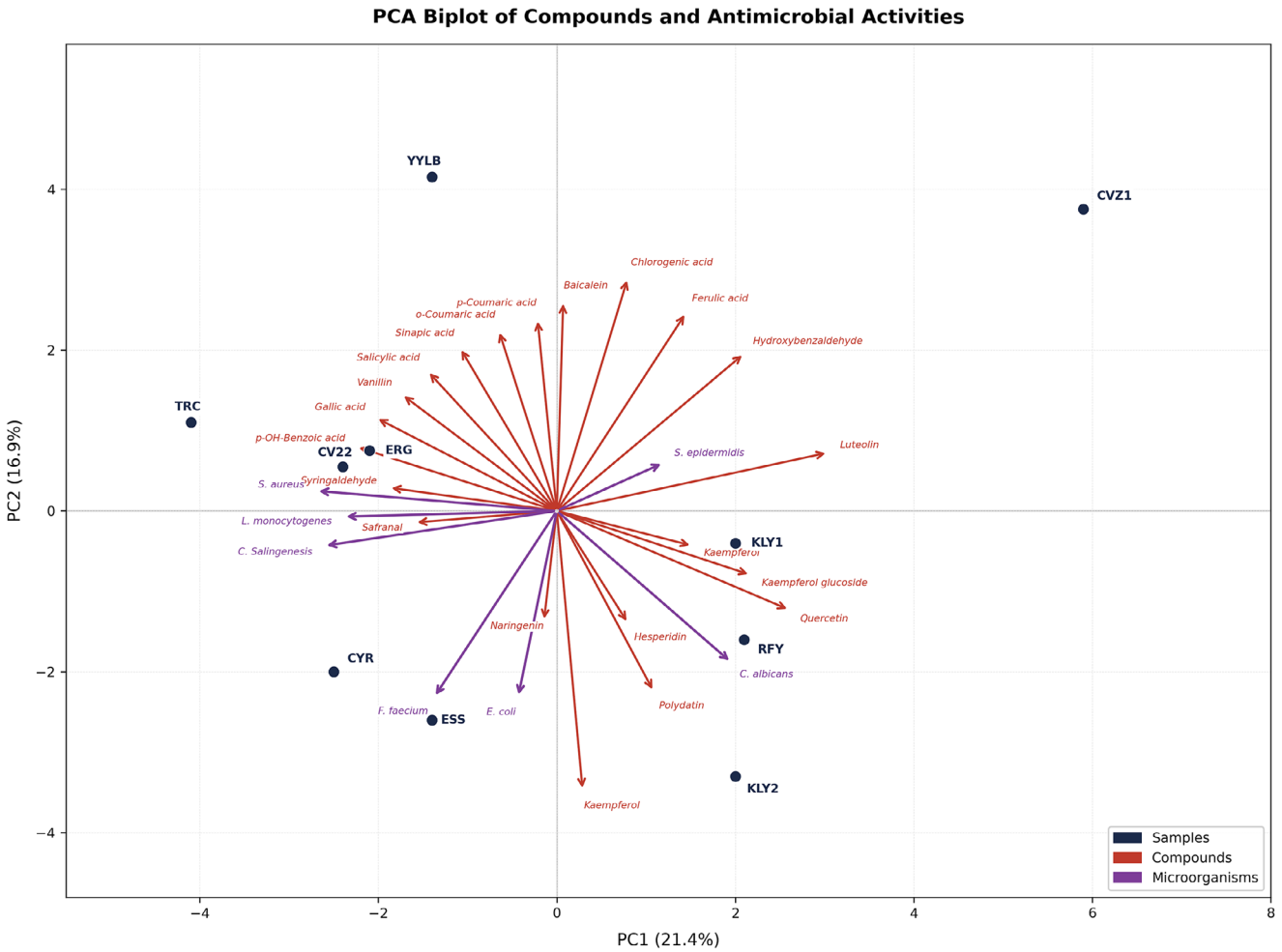

FIGURE 3 | PCA biplot of phenolic compounds and antimicrobial activities. PCA was performed using standardized variables; PC1 and PC2 together explained 38.2% of the total variance.

TABLE 5 | Antioxidant properties of propolis.

Samples	DPPH˙ IC_{50} (μg mL^{-1})	Total phenolic (mg GAE g^{-1} Extract)	Total flavonoid (mg QE g^{-1} Extract)	Reducing power (mg TE g^{-1} Extract)
KLY1	72.69 ± 0.17^{d}	141.70 ± 2.80^{a}	93.81 ± 2.18^{g}	95.23 ± 1.89^{a}
KLY2	50.25 ± 1.15^{b}	186.52 ± 2.31^{c}	57.24 ± 2.72^{e}	184.41 ± 1.36^{c}
ERG	28.86 ± 1.31^{a}	245.78 ± 1.11^{f}	52.86 ± 1.43^{d}	234.98 ± 1.20^{e}
CVZ1	61.23 ± 0.34^{c}	169.85 ± 2.31^{b}	30.48 ± 3.60^{a}	123.85 ± 0.61^{b}
KMH	27.78 ± 2.89^{a}	255.04 ± 3.39^{f}	45.71 ± 1.43^{c}	254.33 ± 0.75^{f}
ESS	59.57 ± 2.63^{c}	182.81 ± 3.90^{c}	63.81 ± 2.97^{f}	124.58 ± 0.37^{b}
CYR	29.76 ± 1.55^{a}	229.11 ± 2.94^{e}	66.67 ± 2.18^{f}	223.20 ± 0.65^{d}
CVZ2	30.99 ± 0.51^{a}	197.26 ± 1.70^{c}	58.57 ± 2.86^{e}	194.58 ± 0.37^{c}
RFY	31.06 ± 1.30^{a}	189.11 ± 3.33^{c}	37.67 ± 2.26^{b}	192.22 ± 0.73^{c}
TRC	30.70 ± 0.67^{a}	204.30 ± 3.57^{c}	62.38 ± 3.60^{f}	203.28 ± 1.02^{c}
YYLB	29.92 ± 2.24^{a}	215.04 ± 3.90^{d}	46.67 ± 1.65^{c}	215.39 ± 0.49^{d}
Trolox	8.25 ± 1.16^{a}	—	—	—
Ascorbic acid	—	—	—	383.66 ± 0.98^{g}

*[a-g]Different letters in the same column indicate statistical difference ($p < 0.05$).

16121880, 2026, 7, Downloaded from https://onlinelibrary.wiley.com/doi/10.1002/cbdv.71533 by Samed MEK - Erzincan Universitesi , Wiley Online Library on [26/07/2026]. See the Terms and Conditions (https://onlinelibrary.wiley.com/terms-and-conditions) on Wiley Online Library for rules of use; OA articles are governed by the applicable Creative Commons License

radical stabilization and reducing capacity [50]. Therefore, the comparatively low antioxidant activity of KLY1 may be associated with lower concentrations of these highly active phenolic constituents in comparison with its flavonoid content. These findings indicate that the antioxidant properties of propolis are associated with the composition and relative concentrations of individual phenolic compounds rather than total flavonoid content.

High total antioxidant activity in propolis has been reported, as determined by both DPPH and FRAP assays [51]. Similar findings were obtained using DPPH, FRAP, and ABTS assays, confirming the strong antioxidant potential of propolis [19]. DPPH radical scavenging activity has been reported in propolis extracts [52]. Overall, the literature provides evidence that propolis is a natural source of antioxidants, with potential applications in medical and biological fields. These antioxidant properties have been associated with phenolic compounds.

The total phenolic content reported for Turkish propolis samples was between 34.53 and 259.4 mg GAE/g [53]. Significant variations in TPC values have also been documented in propolis from stingless bees in Southeastern Mexico [54], and in Greek and Cypriot propolis, where values were between 80 and 338 mg/g [55]. Variation between 31 and 299 mg/g has been reported for propolis samples collected from different regions of the world [56].

The TFC in propolis also shows considerable variability. Previous reports indicated TFC values of 8.3–162 mg/g [19] and 2.5–176 mg/g [56], while samples from Guanajuato, Mexico, exhibited values from 13 to 379 mg QE/g [57]. The values obtained in the present study were within the values reported in previous studies, indicating that the antioxidant properties and phenolic composition of the tested propolis are consistent with findings from different regions of the world. However, the variations observed even within a relatively small sampling area highlight the influence of local factors, including floral diversity, microclimatic conditions, and regional vegetation patterns, on the chemical profile of propolis. These ecological variables contribute to phenolic composition and, consequently, antioxidant activity.

PCA analysis integrating phenolic compound composition with antioxidant activity parameters indicated associations between the chemical profiles of the propolis samples and their antioxidant properties. The first two principal components represented 39.2% of the total variance, with contributions of 20.8% for PC1 and 18.4% for PC2. As shown in Figure 4, ERG, CVZ2, KMH, and YYLB were positioned in the negative region of PC1, close to the vectors representing TPC and reducing power, indicating an association with higher antioxidant capacity. KLY1 and KLY2 were positioned in the positive region of PC1 together with the DPPH IC_{50} vector, suggesting lower radical-scavenging activity. CVZ1 was positioned in the positive region of PC2 and was associated with compounds such as chlorogenic acid, hydroxybenzaldehyde, fisetin, isoquercetin, and luteolin, indicating a different phenolic profile from the other samples. The TFC vector was located in the positive PC1 and negative PC2 region, together with kaempferol, chrysin, baicalin, and polydatin, suggesting a contribution of these constituents to flavonoid-related variation among samples.

Among the phenolic compounds, caffeic acid, syringic acid, and rutin were associated with antioxidant activity parameters, suggesting their potential contribution to the antioxidant activity of propolis. In contrast, some compounds, such as vanillin and o-coumaric acid, were negatively associated with the antioxidant parameters. These findings suggest that antioxidant activities result from the combined effects of multiple phenolics, with certain compounds showing stronger contributions.

The variations in antibacterial, antioxidant, and enzyme inhibitory activities observed among the samples may be associated with the diversity of plant species present in the foraging areas of the honeybees. Türkiye has high floristic diversity because it is located at the intersection of the Euro-Siberian, Irano-Turanian, and Mediterranean phytogeographical regions, resulting in diverse habitats and microclimatic conditions [58]. Altitudinal gradients (0–4500 m), geological heterogeneity, and the presence of both continental and Mediterranean climate regimes contribute to this diversity and are associated with the rich natural resources investigated in natural product research [14]. Within this context, Erzincan Province reflects these characteristics, featuring both lowland valleys and high-altitude zones, which contribute to distinct botanical profiles. Observations during sample collection indicated that lowland regions such as CVZ2 and YYLB contained numerous nectar- and resin-producing plant species [20, 59], particularly members of the Asteraceae and Lamiaceae families [17, 18], which are recognized sources of caffeic acid and flavonoids [19, 20]. In contrast, highland regions such as CVZ1 and KMH contained more alpine flora and Apiaceae species, resulting in different phytochemical contributions.

3 | Conclusions

The present study demonstrated variation in the phytochemical composition and biological activities of propolis samples collected from different locations within the same province. Twenty-eight phenolic compounds were identified, and significant differences were observed among samples in phenolic profiles, antioxidant capacity, enzyme inhibitory activity, and antibacterial effects. The most important finding of this study is that altitude and local floral composition are more strongly associated with propolis phytochemical composition and biological activity than geographic classification based on province or region. Samples collected from lowland environments, where Asteraceae and Lamiaceae species were observed more frequently, showed higher caffeic acid concentrations and different biological activity profiles, while samples collected from highland areas containing Apiaceae species and alpine vegetation showed different phytochemical and biological characteristics. An important observation was that differences were observed between CVZ1 and CVZ2, two samples obtained by the same beekeeper from locations within the same region, indicating an association between micro-regional ecological conditions and propolis composition. HCA and PCA analyses further supported this conclusion, showing that phytochemical similarity was not associated with geographic location and was more strongly related to local flora and altitude. These findings demonstrate that micro-regional environmental conditions are major factors associated with propolis quality and bioactivity and indicate that classification based on geographic origin may not

16121880, 2026, 7, Downloaded from https://onlinelibrary.wiley.com/doi/10.1002/cbdv.71533 by Samed MEK - Erzincan Universitesi , Wiley Online Library on [26/07/2026]. See the Terms and Conditions (https://onlinelibrary.wiley.com/terms-and-conditions) on Wiley Online Library for rules of use; OA articles are governed by the applicable Creative Commons License

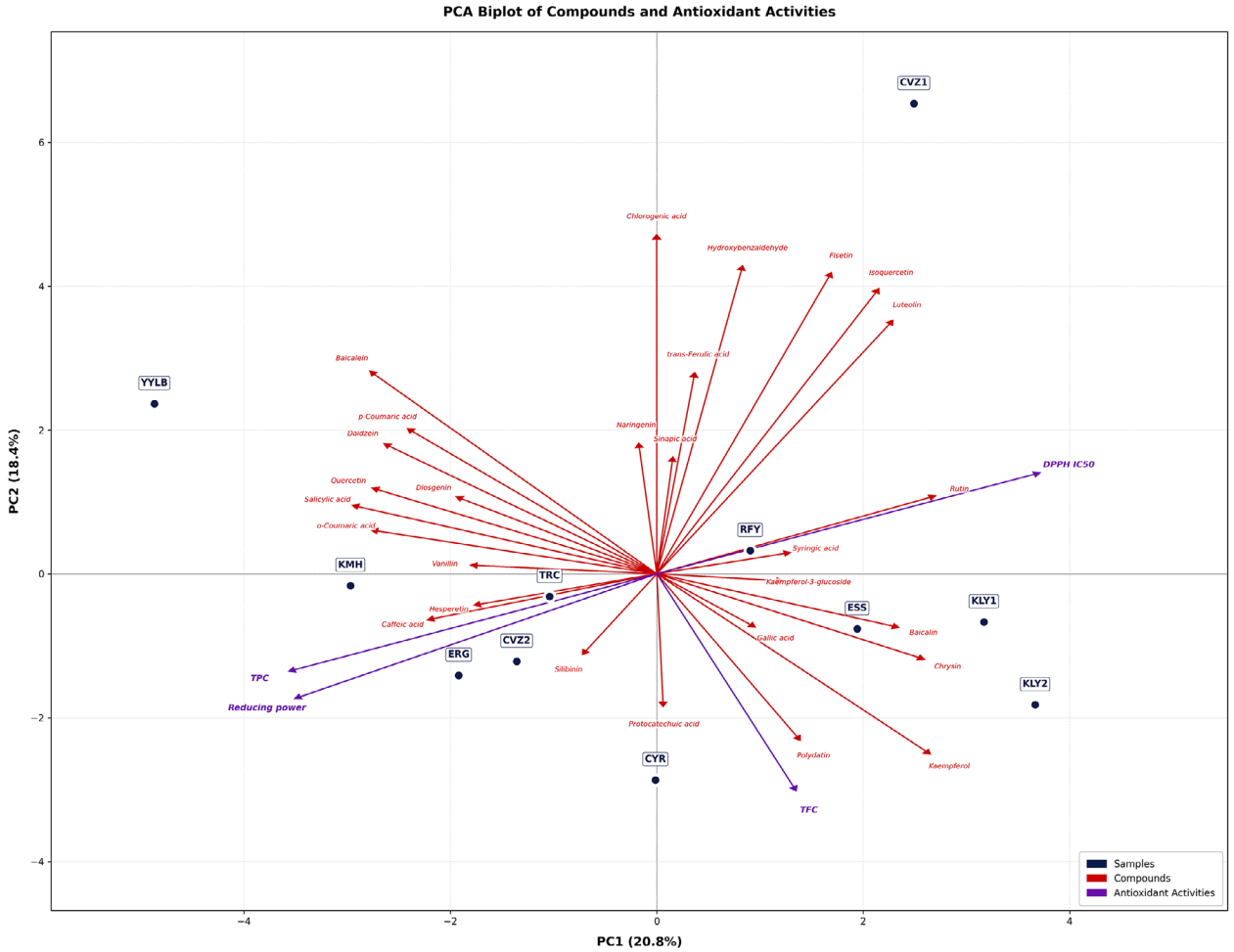

FIGURE 4 | PCA biplot of phenolic compounds and antioxidant activities. PCA was performed using standardized variables; PC1 and PC2 together explained 41.3% of the total variance.

reflect the chemical composition and biological characteristics of propolis. Overall, the results highlight the importance of considering micro-regional environmental conditions and local botanical diversity in propolis characterization, standardization, and quality evaluation.

4 | Experimental Section

4.1 | Propolis Sampling

A total of 11 propolis samples were collected in May–June 2023 from different locations in Erzincan, Türkiye, where commercial beekeeping operations were present. The sampling locations differed in plant composition because of geographic and climatic variation (Figure 5). The traditional scraping technique was used to collect the samples, which were kept in dark bottles at -20°C until analysis.

4.2 | Extraction

Propolis samples were initially defatted with n-hexane (Supelco, USA) using a biomass-to-solvent ratio of 1:20 (w/v). The mixture was homogenized with an Ultra-Turrax for 5 min, followed by ultrasound-assisted extraction for 15 min. This procedure was repeated three to six times until a clear solution was obtained, and the fat fractions (FFs) were discarded. The defatted propolis residues were subsequently extracted with methanol (Merck, Germany) using the same procedure to give defatted propolis methanol extract (DFPME).

4.3 | LC-MS/MS Conditions for the Detection and Quantification of Phytochemicals

The phytochemical profiling of the propolis samples was conducted using a previously developed and validated LC–ESI–MS/MS method [60, 61]. For the LC-MS/MS analysis, 10 mg of DFPMEs were dissolved in a solution containing 1 mL of methanol and 1 mL of n-hexane. The mixture was vortexed for 2 min and centrifuged at 9000 rpm for 10 min at 4°C. After discarding the n-hexane layer, the methanol phase was diluted with distilled water at a ratio of 1/10. The final mixture was filtered through a 0.45 µm syringe filter. 5 µL volume was directly injected into an Agilent 1260 Infinity II LC-MS/MS instrument.

The flow rate was set at 0.5 mL/min, while the oven temperature was maintained at 40°C. The chromatographic separation was performed using an Agilent Poroshell 120 EC-C18 reversed-phase

16121880, 2026, 7, Downloaded from https://onlinelibrary.wiley.com/doi/10.1002/cbdv.71533 by Samed MEK - Erzincan Universitesi , Wiley Online Library on [26/07/2026]. See the Terms and Conditions (https://onlinelibrary.wiley.com/terms-and-conditions) on Wiley Online Library for rules of use; OA articles are governed by the applicable Creative Commons License

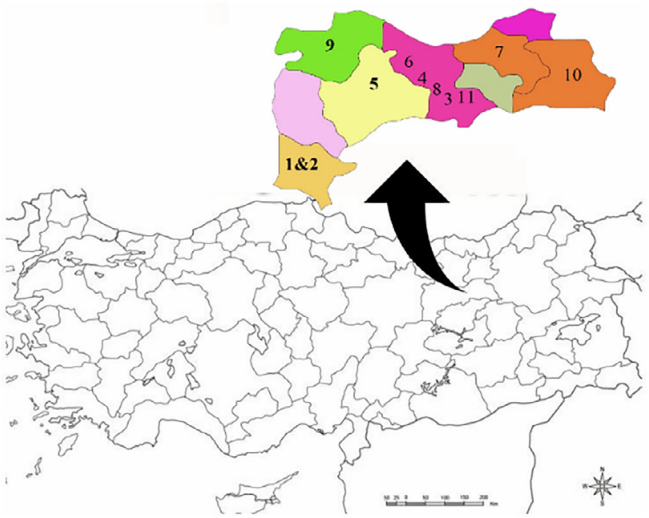

FIGURE 5 | The regions where propolis samples were collected. (1) KLY1, (2) KLY2, (9) RFY, (5) KMH, (7) CYR, (10) TRC, (3) ERG, (4) CVZ1, (8) CVZ2, (6) ESS, (11) YYLB. Two of them are lowland (CVZ2) and highland (CVZ1) products from the same beekeeper.

analytical column (100 mm × 3.0 mm, 2.7 μm). The mobile phase had two components: (A) an aqueous solution containing 5 mM ammonium formate, and (B) methanol containing 0.01% formic acid. The gradient elution program was conducted as follows:

An initial isocratic flow consisting of 97.5% A and 2.5% B was maintained for 3 min. This was followed by a linear gradient to 75% A and 25% B over a period of 9 min. The gradient was changed to 50% A and 50% B over 4 min. A further gradient was applied to obtain 10% A and 90% B over 5 min, and this composition was maintained for 5 min. Finally, the system was returned to the initial condition over a period of 5 min.

An Agilent 6460 Triple Quadrupole Mass Spectrometry system equipped with electrospray ionization (LC-ESI-MS/MS) was used to conduct the mass spectrometry analysis. The acquisition was performed utilizing both positive and negative ionization modes. Data analysis was performed using Agilent Mass Hunter software. Phytochemicals were identified and quantified with extreme precision utilizing a multiple reaction monitoring (MRM) strategy. The collision energies (CE) were optimized for the fragmentation and transmission of the target ions. The nitrogen (N_2) drying gas flow rate of 15 mL/min, the atomizing gas flow rate of 8 mL/min, the capillary voltage of 4000 V, and the gas temperature of 300°C were all set in the mass spectrometer.

Compound identification and quantification were performed using a targeted multiple reaction monitoring (MRM) approach. The quantification was based on authentic reference standards analyzed under identical chromatographic and mass spectrometric conditions. Calibration curves were constructed for each compound using standards supplied by Sigma-Aldrich and Cayman Chemicals. Retention times and MRM transitions of sample peaks were matched with those of the standards for compound identification.

4.4 | Anti-Cholinesterase Activity

The inhibitory effects of propolis samples on AChE and BChE were determined spectrophotometrically using a previously described method with minor modifications [62–64]. 10 mg of each DFPME was dissolved in 1 mL of DMSO. The stock solution was diluted with deionized water to obtain different concentrations. To assess the inhibitory activity of cholinesterase, the following components were added to the solution: 100 μL of buffer (1 M, pH 8.0; Tris-HCl buffer for AChE and phosphate buffer for BChE), 10 μL of enzyme (0.385 U/mL for BChE and 0.880 U/mL for AChE). Then, 50 μL (10 mM) of substrate solution (acetylthiocholine iodide/butyryl thiocholine iodide) was added to start the reaction. After the initiation of the reaction, the absorbance of the reaction mixture at 412 nm was recorded using a spectrophotometer (Unico, S1205) within a time frame of 5 min. The IC_{50} values were determined from the relationship between activity (%) and concentration. Tacrine was used as a positive control.

4.5 | α-glycosidase Inhibition Activity

A previously described procedure with minor modifications was used to determine α-glucosidase inhibitory activity [65]. 20 μL of an enzyme solution (11.1 U in pH 7.4 phosphate buffer) and 200 μL of phosphate buffer (0.1 M, pH 7.4) were combined with 10 μL of a propolis stock solution prepared at various concentrations. The mixture was incubated at 37°C for 10 min. Following the incubation period, 20 μL of p-NPG (5 mM in phosphate buffer, pH 7.4) was added and incubated at 37°C. Acarbose was used as the positive control. The absorbance of the final mixture at 405 nm was recorded using spectrophotometry. IC_{50} values were calculated from the relationship between concentration and activity (%).

4.6 | Antibacterial Activity and MIC Analyses

A total of nine strains of food-borne pathogens from the ATCC collection were used to assess the antibacterial activity, including *Listeria monocytogenes* (ATCC 51774), *Enterococcus faecium* (ATCC 8459), *Clostridium perfringens* (ATCC 13124), *Bacillus cereus* (ATCC 10876) as gram-positives, and *Pseudomonas aeruginosa* (ATCC 15442), *Pseudomonas fluorescens* (ATCC 13225), *Escherichia coli* (ATCC 25922), *Salmonella Enteritidis* (*Salmonella enterica* subsp. enterica serovar Enteritidis) (ATCC 13076), and *Salmonella enterica* (ATCC 35664) as gram-negatives. The antibacterial activity of the samples was evaluated using the disc diffusion technique following previously described procedures [66–69]. The samples were dissolved in a 10% (v/v) DMSO (Sigma-Aldrich, USA) solution at a concentration of 1 mg/mL. Sterile paper discs (6 mm) were impregnated with 20 μL of the sample solution. Imipenem discs (10 μg; Oxoid, UK) were used as a positive control, while 10% DMSO served as the negative control. Plates were incubated for 24 h at 37°C. A digital caliper was used to measure the inhibition zones in mm.

Samples with inhibition zones ≥15 mm were subject to MIC (minimum inhibitory concentration) analysis. The MIC study

16121880, 2026, 7, Downloaded from https://onlinelibrary.wiley.com/doi/10.1002/cbdv.71533 by Samed MEK - Erzincan Universitesi , Wiley Online Library on [26/07/2026]. See the Terms and Conditions (https://onlinelibrary.wiley.com/terms-and-conditions) on Wiley Online Library for rules of use; OA articles are governed by the applicable Creative Commons License

was conducted using the Resazurin-based 96-well plate microdilution technique, as described by Akşit et al. [70], with a minor modification. 50 microliters of sterile Mueller–Hinton Broth (Oxoid, UK) was dispensed into the wells of rows 2–7 of the plate. Initially, 100 µL of the test sample was added to the first row. Serial dilutions were prepared by transferring 50 µL from one well to the next. The concentrations of the serial dilutions were between 1000 and 16 µg/mL across rows 1–7. 10 µL of bacterial solution was added to each of these wells. The eighth row was used as the negative control and contained 10 µL of bacterial solution, 25 µL of DMSO (10% v/v), and 25 µL of broth. The 12th column was used for broth sterility testing, containing 50 µL of a 10% v/v DMSO solution, and 10 µL of sterile broth was added. Each of the 96 wells on the plate was supplemented with 30 µL of resazurin (Sigma-Aldrich, USA) solution at a concentration of 0.02%. A volume of 10 µL of bacterial solution containing 1×10^8 CFU/mL was inoculated into each well of the 96-well plate (Corning, USA). The plate was then incubated at a temperature of 37°C for a duration of 6 h. The transition from purple to pink signifies the presence of bacterial activity. The well before the initial color alteration was noted as the minimum inhibitory concentration (MIC) value of the sample.

4.7 | DPPH Radical Scavenging Activity

The DPPH radical scavenging capacity of the extracts was determined following a previously described procedure [71–75]. First, a 0.26 mM $DPPH^{\bullet}$ solution was prepared in methanol, followed by extract stock solutions (1 mg/mL) for testing. The stock solutions were added to test tubes in different amounts (20–1000 µL) and diluted to a final volume of 3 mL with methanol. Following, 1.0 mL of 0.26 mM $DPPH^{\bullet}$ solution was added to each tube. The mixture was then vortexed and incubated at room temperature for 30 min. Each mixture's absorbance was measured at 517 nm, and the results were converted to % $DPPH^{\bullet}$ scavenging activity. IC_{50} (µg/mL) of the extracts was calculated and compared with the standard antioxidant, Trolox. All analyses were conducted in triplicate (n = 3), and the results are presented as mean ± standard deviation.

4.8 | Ferric Reducing Antioxidant Power (FRAP)

The reducing power activity of the propolis samples was evaluated using previously described procedures [76–80]. For testing, stock solutions (1 mg/mL) were first prepared from extracts. After preparing the stock solutions, 100 µL was added to test tubes and diluted with 1.25 mL of phosphate buffer (0.2 M, pH 6.6). Following the addition of 1.25 mL of $K_3[Fe(CN)_6]$ solution, the mixture was incubated for 20 min at 50°C. Finally, 1.25 mL of TCA (10%) and 0.25 mL of $FeCl_3$ (0.1%) were added to the reaction medium, and the absorbance of the mixture was measured at 700 nm. A calibration curve was constructed with Trolox ($y = 0.0041x + 0.1289$, $r^2 = 0.9992$), and the results were expressed as mg TE/g extract. Ascorbic acid was used as a reference standard for comparison. All tests were performed in triplicate (n = 3).

4.9 | Total Phenolic Content (TPC)

The Folin–Ciocalteu colorimetric method was used to determine the total phenolic content of each propolis extract [67, 81–83]. Extracts were dissolved in methanol, and 1 mg/mL stock solutions were prepared. 100 µl Folin-Ciocalteu, 4.5 mL distilled water, and 300 µl Na_2CO_3 were added to 100 µl of each sample solution. The total phenolic content was measured with a spectrophotometer at 760 nm after 120 min of incubation at room temperature. The total phenolic content of the samples was determined by creating a calibration curve with the regression equation $y = 0.0009x + 0.0208$ and $r^2 = 0.999$ using various concentrations (1–1000 µg/mL) of gallic acid as a standard. The results were expressed as mg GAE/g extract. All tests were performed in triplicate (n = 3).

4.10 | Total Flavonoid Content (TFC)

The total flavonoid content of the propolis samples was determined following a previously described procedure [68, 84–86]. First, to the test tubes containing 100 µL of sample stock solutions prepared at a concentration of 1 mg/mL, 4.7 mL of methanol was added. Following this, 100 µL of a 10% $AlCl_3$ solution and 100 µL of a 1 M NH_4CH_3COO solution were added to the mixture, vortexed, and kept under dark conditions for 45 min. At the end of the incubation period, the total flavonoid content of the mixture was measured by a spectrophotometer at 415 nm. Using different concentrations of quercetin as standards, a calibration curve was created to determine the total flavonoid content, and the results were expressed as mg QE/g extract. All tests were performed in triplicate (n = 3).

4.11 | Statistical Analysis

Statistical analysis was performed using SPSS Version 20.0 software. One-way analysis of variance (ANOVA) was used to evaluate differences among groups, followed by Tukey's HSD post hoc test for multiple comparisons ($p < 0.05$). HCA dendrograms and clusters were generated using Ward's linkage method and Rescaled Distance Cluster Combine.

Author Contribution

Mehmet Çağlar Fırat: writing original draft, formal analysis, writing review editing. **Zeynep Akşit:** writing original draft, formal analysis, investigation. **Samed Şimşek:** investigation, writing review editing, methodology, formal analysis, writing original draft. **Sevgi Altın:** investigation, formal analysis, writing review editing. **İlyas Yıldız:** investigation, writing review editing, formal analysis. **Hüseyin Akşit:** supervision, conceptualization, methodology, writing review editing, writing original draft.

Conflicts of Interest

The authors declare no conflicts of interest.

Funding

No funding was received for conducting this study.

Data Availability Statement

The data that supports the findings of this study are available in the Supporting Information of this article.

16121880, 2026, 7, Downloaded from https://onlinelibrary.wiley.com/doi/10.1002/cbdv.71533 by Samed MEK - Erzincan Universitesi , Wiley Online Library on [26/07/2026]. See the Terms and Conditions (https://onlinelibrary.wiley.com/terms-and-conditions) on Wiley Online Library for rules of use; OA articles are governed by the applicable Creative Commons License

References

1. S. I. Anjum, A. Ullah, K. A. Khan, et al., "Composition and Functional Properties of propolis (bee glue): A Review," *Saudi Journal of Biological Sciences* 26 (2019): 1695–1703, https://doi.org/10.1016/j.sjbs.2018.08.013.

2. M. Simone-Finstrom and M. Spivak, "Propolis and Bee Health: The Natural History and Significance of Resin Use by Honey Bees," *Apidologie* 41 (2010): 295–311, https://doi.org/10.1051/apido/2010016.

3. V. Bankova, M. Popova, and B. Trusheva, "The Phytochemistry of the Honeybee," *Phytochemistry* 155 (2018): 1–11, https://doi.org/10.1016/j.phytochem.2018.07.007.

4. A. Sarapa, A. Peter, A. Buettner, and H. M. Loos, "Organoleptic and Chemical Properties of Propolis: A Review," *European Food Research and Technology* 251 (2025): 1331–1352, https://doi.org/10.1007/s00217-025-04708-y.

5. R. Silva-Carvalho, F. Baltazar, and C. Almeida-Aguiar, "Propolis: A Complex Natural Product With a Plethora of Biological Activities That Can be Explored for Drug Development," *Evidence-Based Complementary Alternative Medicine* (2015): 206439, https://doi.org/10.1155/2015/206439, 2015.

6. R. Hossain, C. Quispe, R. A. Khan, et al., "Propolis: An Update on Its Chemistry and Pharmacological Applications," *Chinese Medicine* 17 (2022): 100, https://doi.org/10.1186/s13020-022-00651-2.

7. M. Alvear, E. Santos, F. Cabezas, A. Pérez-SanMartín, M. Lespinasse, and J. Veloz, "Geographic Area of Collection Determines the Chemical Composition and Antimicrobial Potential of Three Extracts of Chilean Propolis," *Plants* 10 (2021): 1543, https://doi.org/10.3390/plants10081543.

8. A. Salatino, É. W. Teixeira, G. Negri, and D. Message, "Origin and Chemical Variation of Brazilian Propolis," *Evidence-Based Complementary and Alternative Medicine* 2 (2005): 33–38, https://doi.org/10.1093/ecam/neh060.

9. V. Bankova, "Recent Trends and Important Developments in Propolis Research," *Evidence-Based Complementary and Alternative Medicine* 2 (2005): 29–32, https://doi.org/10.1093/ecam/neh059.

10. D. S. Dezmirean, L. A. Mărghitaş, F. Chirilă, et al., "Influence of Geographic Origin, Plant Source and Polyphenolic Substances on Antimicrobial Properties of Propolis Against Human and Honey Bee Pathogens," *Journal of Apicultural Research* 56 (2017): 588–597, https://doi.org/10.1080/00218839.2017.1356205.

11. C. Körner, "The Use of 'Altitude'in Ecological Research," *Trends in Ecology Evolution* 22 (2007): 569, https://doi.org/10.1016/j.tree.2007.09.006.

12. C. Zidorn, "Altitudinal Variation of Secondary Metabolites in Flowering Heads of the Asteraceae: Trends and Causes," *Phytochemistry Reviews* 9 (2010): 197–203, https://doi.org/10.1007/s11101-009-9143-7.

13. J. Noroozi, G. Zare, M. Sherafati, et al., "Patterns of Endemism in Turkey, the Meeting Point of Three Global Biodiversity Hotspots, Based on Three Diverse Families of Vascular Plants," *Frontiers in Ecology and Evolution* 7 (2019): 159, https://doi.org/10.3389/fevo.2019.00159.

14. A. Kandemir, H. İ. Türkoğlu, and F. Yıldız, "Erzincan (Türkiye) Florasına Dair Bazı Gözlemler Ve Öneriler," *Bağbahçe Bilim Dergisi* 9 (2022): 34, https://doi.org/10.35163/bagbahce.1022593.

15. S. Silici and S. Kutluca, "Chemical Composition and Antibacterial Activity of Propolis Collected by Three Different Races of Honeybees in the Same Region," *Journal of Ethnopharmacology* 99 (2005): 69–73, https://doi.org/10.1016/j.jep.2005.01.046.

16. F. Yıldız, A. Kandemir, E. Kılıç, N. Y. Doğan, and H. İ. Türkoğlu, "Functional Ecology of Floral Traits and Plant Pollinator Interactions in Two Endemic Scrophularia L. Species From Erzincan, Türkiye," *Turkish Journal of Science Technology* 20 (2025): 603, https://doi.org/10.55525/tjst.1705635.

17. A. Kandemir and Z. Türkmen, "The Flora of Üzümlü-Sakaltutan (Erzincan-Gümüşhane)," *Turkish Journal of Botany* 32 (2008): 265.

18. Y. Kaya, "The Flora of Tercan Environments and of Sengül (Erzincan) and Bagirbaba (Tunceli) Mountains," *Turkish Journal of Botany* 20 (1996): 75–98, https://doi.org/10.55730/1300-008X.2665.

19. M. R. Ahn, S. Kumazawa, Y. Usui, et al., "Antioxidant Activity and Constituents of Propolis Collected in Various Areas of China," *Food chemistry* 101 (2007): 1383–1392, https://doi.org/10.1016/j.foodchem.2006.03.045.

20. G. Burdock, "Review of the Biological Properties and Toxicity of Bee Propolis (propolis)," *Food and Chemical Toxicology* 36 (1998): 347–363, https://doi.org/10.1016/S0278-6915(97)00145-2.

21. R. M. Lane, S. G. Potkin, and A. Enz, "Targeting Acetylcholinesterase and Butyrylcholinesterase in Dementia," *International Journal of Neuropsychopharmacology* 9 (2006): 101, https://doi.org/10.1017/S1461145705005833.

22. M. B. Čolović, D. Z. Krstić, T. D. Lazarević-Pašti, A. M. Bondžić, and V. M. Vasić, "Acetylcholinesterase Inhibitors: Pharmacology and Toxicology," *Current Neuropharmacology* 11 (2013): 315, https://doi.org/10.2174/1570159X11311030006.

23. F. A. Van de Laar, "Alpha-glucosidase Inhibitors in the Early Treatment of Type 2 Diabetes," *Vascular Health Risk Management* 4 (2008): 1189, https://doi.org/10.2147/vhrm.s3119.

24. A. P. Murray, M. B. Faraoni, M. J. Castro, N. P. Alza, and V. Cavallaro, *Current Neuropharmacology* 11 (2013): 388–413, https://doi.org/10.2174/1570159X11311040004.

25. R. Tundis, M. R. Loizzo, and F. Menichini, *Mini-Reviews in Medicinal Chemistry* 10 (2010): 315–331, https://doi.org/10.2174/138955710791331007.

26. H. Khan, S. Amin, M. A. Kamal, and S. Patel, "Flavonoids as Acetylcholinesterase Inhibitors: Current Therapeutic Standing and Future Prospects," *Biomedicine & Pharmacotherapy* 101 (2018): 860–870, https://doi.org/10.1016/j.biopha.2018.03.007.

27. I. Orhan, M. Kartal, F. Tosun, and B. Şener, "Screening of Various Phenolic Acids and Flavonoid Derivatives for Their Anticholinesterase Potential," *Zeitschrift für Naturforschung C* 62 (2007): 829–832, https://doi.org/10.1515/znc-2007-11-1210.

28. D. Szwajgier, "Anticholinesterase Activities of Selected Polyphenols-a Short Report," *Polish Journal of Food Nutrition Sciences* 64 (2014): 59–64, https://doi.org/10.2478/v10222-012-0089-x.

29. L. Tang, Q. Guan, L. Zhang, M. Xu, M. Zhang, and M. S. Khan, "Synergistic Interaction of Cu(II) With Caffeic Acid and Chlorogenic Acid in α -Glucosidase Inhibition," *Journal of the Science of Food and Agriculture* 104 (2024): 518–529, https://doi.org/10.1002/jsfa.12955.

30. M. Yu, S. Zhu, D. Huang, X. Tao, and Y. Li, "Inhibition of Starch Digestion by Phenolic Acids With a Cinnamic Acid Backbone: Structural Requirements for the Inhibition of α-amylase and α-glucosidase," *Food chemistry* 435 (2024): 137499, https://doi.org/10.1016/j.foodchem.2023.137499.

31. Ü. Altuntaş, İ. Güzel, and B. Özçelik, "Phenolic Constituents, Antioxidant and Antimicrobial Activity and Clustering Analysis of Propolis Samples Based on PCA From Different Regions of anatolia," *Molecules* 28 (2023): 1121, https://doi.org/10.3390/molecules28031121.

32. H. Karagecili, M. A. Yılmaz, A. Ertürk, et al., "Comprehensive Metabolite Profiling of Berdav propolis Using LC-MS/MS: Determination of Antioxidant, Anticholinergic, Antiglaucoma, and Antidiabetic Effects," *Molecules* 28 (2023): 1739, https://doi.org/10.3390/molecules28041739.

33. E. İzol and M. Turhan, "In-Depth Phytochemical Profile by LC-MS/MS, Mineral Content by ICP-MS, and in Vitro Antioxidant, Antidiabetic, Antiepilepsy, Anticholinergic, and Antiglaucoma Properties of Bitlis Propolis," *Life Sciences* 14 (2024): 1389, https://doi.org/10.3390/life14111389.

34. B. Neslihan, A. Kubra, E. Adem, and G. Ilhami, "Evaluation of Antioxidant and Enzyme Inhibition Properties of Siran Propolis:

16121880, 2026, 7, Downloaded from https://onlinelibrary.wiley.com/doi/10.1002/cbdv.71533 by Samet MEK - Erzincan Universitesi , Wiley Online Library on [26/07/2026]. See the Terms and Conditions (https://onlinelibrary.wiley.com/terms-and-conditions) on Wiley Online Library for rules of use; OA articles are governed by the applicable Creative Commons License

Correlations with Phenolic Content Determined by LC-MS/MS," *Food Science & Nutrition* 13 (2025): e70654, https://doi.org/10.1002/fsn3.70654.

35. S. Boulechfar, A. Zellagui, C. Bensouici, M. Asan-Ozusaglam, S. Tacer, and D. J. Hanene, "Anticholinesterase, Anti-α-glucosidase, Antioxidant and Antimicrobial Effects of Four Algerian Propolis," *Journal of Food Measurement and Characterization* 16 (2022): 793–803, https://doi.org/10.1007/s11694-021-01203-2.

36. S. El-Guendouz, S. Aazza, B. Lyoussi, M. D. Antunes, M. L. Faleiro, and M. G. Miguel, "Anti-Acetylcholinesterase, Antidiabetic, Anti-Inflammatory, Antityrosinase and Antixanthine Oxidase Activities of Moroccan Propolis," *International Journal of Food Science & Technology* 51 (2016): 1762–1773, https://doi.org/10.1111/ijfs.13133.

37. S. Fathi Hafshejani, S. Lotfi, E. Rezvannejad, M. Mortazavi, and A. Riahi-Madvar, "Correlation Between Total Phenolic and Flavonoid Contents and Biological Activities of 12 Ethanolic Extracts of Iranian Propolis," *Food Science & Nutrition* 11 (2023): 4308–4325, https://doi.org/10.1002/fsn3.3356.

38. M. Katalinić, G. Rusak, J. Domaćinović Barović, et al., "Structural Aspects of Flavonoids as Inhibitors of human Butyrylcholinesterase," *European Journal of Medicinal Chemistry* 45 (2010): 186–192, https://doi.org/10.1016/j.ejmech.2009.09.041.

39. E. Ören, S. Tuncay, Y. E. Toprak, et al., "Antioxidant, Antidiabetic Effects and Polyphenolic Contents of Propolis From Siirt, Turkey," *Food Science Nutrition Cancer* 12 (2024): 2772, https://doi.org/10.1002/fsn3.3958.

40. H. Zhang, G. Wang, T. Beta, and J. Dong, "Inhibitory Properties of Aqueous Ethanol Extracts of Propolis on Alpha-glucosidase," *Evidence-Based Complementary Alternative Medicine* (2015): 587383, https://doi.org/10.1155/2015/587383, 2015.

41. H. Laaroussi, P. Ferreira-Santos, Z. Genisheva, et al., "Unraveling the Chemical Composition, Antioxidant, α-amylase and α-glucosidase Inhibition of Moroccan Propolis," *Food Bioscience* 42 (2021): 101160, https://doi.org/10.1016/j.fbio.2021.101160.

42. D. Şöhretoğlu, G. Renda, R. Arroo, J. Xiao, and S. Sari, "Advances in the Natural α-glucosidase Inhibitors," *Efood* 4 (2023): e112, https://doi.org/10.1002/efd2.112.

43. A. Aleixandre, J. V. Gil, J. Sineiro, and C. M. Rosell, "Understanding Phenolic Acids Inhibition of α-amylase and α-glucosidase and Influence of Reaction Conditions," *Food Chemistry* 372 (2022): 131231, https://doi.org/10.1016/j.foodchem.2021.131231.

44. O. Mirzoeva, R. Grishanin, and P. Calder, "Antimicrobial Action of Propolis and Some of Its Components: The Effects on Growth, Membrane Potential and Motility of Bacteria," *Microbiological Research* 152 (1997): 239–246, https://doi.org/10.1016/S0944-5013(97)80034-1.

45. X. Zheng, Y. Al Naggar, Y. Wu, et al., "Untargeted Metabolomics Description of Propolis's in Vitro Antibacterial Mechanisms Against *Clostridium Perfringens*," *Food Chemistry* 406 (2023): 135061, https://doi.org/10.1016/j.foodchem.2022.135061.

46. F. Khan, N. I. Bamunuarachchi, N. Tabassum, and Y.-M. Kim, "Caffeic Acid and Its Derivatives: Antimicrobial Drugs Toward Microbial Pathogens," *Journal of Agricultural and Food Chemistry* 69 (2021): 2979–3004, https://doi.org/10.1021/acs.jafc.0c07579.

47. C. Srinivasulu, M. Ramgopal, G. Ramanjaneyulu, C. M. Anuradha, and C. Suresh Kumar, "Syringic Acid (SA)–a Review of Its Occurrence, Biosynthesis, Pharmacological and Industrial Importance," *Biomedicine & Pharmacotherapy* 108 (2018): 547–557, https://doi.org/10.1016/j.biopha.2018.09.069.

48. H. Fokt, A. Pereira, A. Ferreira, A. Cunha, and C. Aguiar, "How Do Bees Prevent Hive Infections? The Antimicrobial Properties of Propolis," *Current Research, Technology and Education Topics in Applied Microbiology and Microbial Biotechnology* 1 (2010): 481.

49. A. Kujumgiev, I. Tsvetkova, Y. Serkedjieva, V. Bankova, R. Christov, and S. Popov, "Antibacterial, Antifungal and Antiviral Activity of Propolis of Different Geographic Origin," *Journal of Ethnopharmacology* 64 (1999): 235–240, https://doi.org/10.1016/S0378-8741(98)00131-7.

50. J. Chen, J. Yang, L. Ma, J. Li, N. Shahzad, and C. K. Kim, "Structure-antioxidant Activity Relationship of Methoxy, Phenolic Hydroxyl, and Carboxylic Acid Groups of Phenolic Acids," *Scientific Reports* 10 (2020): 2611, https://doi.org/10.1038/s41598-020-59451-z.

51. J. Kocot, M. Kiełczykowska, D. Luchowska-Kocot, J. Kurzepa, and I. Musik, "Antioxidant Potential of Propolis, Bee Pollen, and Royal Jelly: Possible Medical Application," *Oxidative Medicine and Cellular Longevity* 2018 (2018): 7074209, https://doi.org/10.1155/2018/7074209.

52. N. L. Rosli, H. Roslan, E. A. Omar, N. Mokhtar, N. H. A. Hapit, and N. Asem, *Phytochemical Analysis and Antioxidant Activities of Trigona Apicalis Propolis Extract* (AIP Publishing LLC, 2016), https://doi.org/10.1063/1.4968873.

53. A. Özkök, M. Keskin, A. E. Tanuğur Samancı, E. Yorulmaz Önder, and Ç. Takma, "Determination of Antioxidant Activity and Phenolic Compounds for Basic Standardization of Turkish Propolis," *Applied Biological Chemistry* 64 (2021): 37, https://doi.org/10.1186/s13765-021-00608-3.

54. M. Esfandiarifard, "Study on the Antioxidant Activityof Propolis Extract and Its Effect on the Oxidation of Sunflower Oil," *Journal of Food Science and Technology* 17 (2021): 119.

55. N. Kalogeropoulos, S. J. Konteles, E. Troullidou, I. Mourtzinos, and V. T. Karathanos, "Chemical Composition, Antioxidant Activity and Antimicrobial Properties of Propolis Extracts From Greece and Cyprus," *Food Chemistry* 116 (2009): 452–461, https://doi.org/10.1016/j.foodchem.2009.02.060.

56. S. Kumazawa, T. Hamasaka, and T. Nakayama, "Antioxidant Activity of Propolis of Various Geographic Origins," *Food Chemistry* 84 (2004): 329–339, https://doi.org/10.1016/S0308-8146(03)00216-4.

57. M. S. Hernández Zarate, M. D. R. Abraham Juárez, A. Cerón García, et al., "Flavonoids, Phenolic Content, and Antioxidant Activity of Propolis From Various Areas of Guanajuato, Mexico," *Food Science and Technology* 38 (2018): 210–215, https://doi.org/10.1590/fst.29916.

58. G. Savcı, A. İlçim, and F. Karahan, "Hatay'da Yayılış Gösteren Bazı *Geranium* L.(Geraniaceae) Türlerinin Palinolojisi Ve Tohum Mikromorfolojisi," *Commagene Journal of Biology* 2 (2018): 28, https://doi.org/10.31594/commagene.482198.

59. V. Bankova, "Chemical Diversity of Propolis and the Problem of Standardization," *Journal of Ethnopharmacology* 100 (2005): 114–117, https://doi.org/10.1016/j.jep.2005.05.004.

60. S. Şimşek, "Integrated Analysis of Volatile and Phenolic Profiles and Biological Activities of *Satureja Hortensis* From Erzincan," *New Zealand Journal of Botany* 64 (2026): e70032, https://doi.org/10.1002/nzb2.70032.

61. T. C. Akman, S. Simsek, Z. Aksit, et al., "Comparative Liquid Chromatography–electrospray Ionization–tandem Mass Spectrometry Profiling and Bioactivity Evaluation of Ebenus Macrophylla Jaub. & Spach Leaf, Stalk and Root Extracts: An in Vitro and in Silico Approach," *Journal of the Science of Food and Agriculture* 106 (2026): 2637–2647, https://doi.org/10.1002/jsfa.70362.

62. A. R. Tüfekçi, H. Akşit, S. Şimşek, et al., "Evaluation of Insecticidal and Enzyme Activity Potentials of Essential Oils and Extracts of *Chenopodium Botrys* Against Storage Products Pests," *Bulletin of Biotechnology* 4 (2023): 7, https://doi.org/10.51539/biotech.1213740.

63. T. C. Akman, S. Simsek, İ. N. Erken, et al., "Phytochemical Profile, Antioxidant, Anticholinesterase, and Antiproliferative Activities of Endemic Onosma Discedens: In Vitro and in Silico Approaches," *Chemistry & Biodiversity* 22 (2025): e02147, https://doi.org/10.1002/cbdv.202502147.

64. E. Köksal, A. Altay, S. Altın, et al., "Screening of Chemical Profile by LC–MS/MS and Assessment of Biological Properties of *Achillea sintenisii* Hub. Mor. From Turkey," *Chemistry Biodiversity* 22 (2025): e202500026, https://doi.org/10.1002/cbdv.202500026.

65. T. Cagri Akman, S. Simsek, Ö. Kayir, Z. Aksit, H. Aksit, and N. Genc, "LC-ESI-MS/MS Chemical Characterization, Antioxidant and Antidiabetic Properties of Propolis Extracted With Organic Solvents From

16121880, 2026, 7, Downloaded from https://onlinelibrary.wiley.com/doi/10.1002/cbdv.71533 by Sanet MEK - Erzincan Universitesi , Wiley Online Library on [26/07/2026]. See the Terms and Conditions (https://onlinelibrary.wiley.com/terms-and-conditions) on Wiley Online Library for rules of use; OA articles are governed by the applicable Creative Commons License

Eastern Anatolia Region," *Chemistry & Biodiversity* 20 (2023): e202201189, https://doi.org/10.1002/cbdv.202201189.

66. S. Gözcü, Z. Akşit, S. Şimşek, and H. Akşit, "Chemical Composition and Antimicrobial Activity of *Hesperis Isatidea* (Boiss.) DA German & Al-Shehbaz Essential Oil," *Chemistry Biodiversity* (2025): e00455, https://doi.org/10.1002/cbdv.202500455.

67. T. Ç. Akman, S. Şimşek, Z. Akşit, A. Aydın, and M. A. Yılmaz, "Exploring the Potential of *Psephellus Huber-Morathi* (Wagenitz) Wagenitz: A Comprehensive UHPLC-MS/MS Analysis of Phytochemical Composition and Evaluation of Antioxidant, Antimicrobial, and Antiproliferative Activities," *Kahramanmaraş Sütçü İmam Üniversitesi Tarım ve Doğa Dergisi* 27 (2024): 782, https://doi.org/10.18016/ksutarimdoga.vi.1339345.

68. T. Ç. Akman, S. Şimşek, Z. Akşit, et al., "Liquid Chromatography–tandem Mass Spectrometry Profile and Antioxidant, Antimicrobial, Antiproliferative, and Enzyme Activities of Thymus Pectinatus and Thymus Convolutus: In Vitro and in Silico Approach," *Journal of the Science of Food and Agriculture* 104 (2024): 4039–4049, https://doi.org/10.1002/jsfa.13286.

69. S. Gözcü, Z. Akşit, H. Akşit, S. Şimşek, and M. Korkmaz, "Chemical Composition and Antibacterial Activity of *Rosa villosa* subsp. Mollis R. Keller & Gams., *Rosa Hemisphaerica* J. Herrm. And Natural Hybrid Rosa ozcelikii Korkmaz & Kandemir Essential Oil," *Flavour Fragrance Journal* 41 (2026): 58, https://doi.org/10.1002/ffj.70028.

70. Z. Akşit, M. Ç. Fırat, H. Akşit, Y. Bayar, M. Alkan, and S. Şimşek, "Chemical Composition, Antifungal, Antibacterial, and Insecticidal Activity of *Echinophora Chrysantha* Essential Oil," *Journal of Essential Oil Bearing Plants* 27 (2024): 374, https://doi.org/10.1080/0972060X.2024.2302969.

71. H. Akşit and S. Şimşek, "Assessment of Antioxidant Potential, Verbascoside, and Sugar Composition of the Floral Parts of *Verbascum Sinuatum*," *Turkish Journal of Analytical Chemistry* 7 (2025): 370, https://doi.org/10.51435/turkjac.1729453.

72. S. Şimşek, "Green Synthesis, Characterization, and Multifunctional Bioactivity of *Thymus Pectinatus*-Derived Silver Nanoparticles," *ChemistrySelect* 10 (2025): e03764, https://doi.org/10.1002/slct.202503764.

73. F. R. Alkan, B. Çetiner, H. Akşit, and S. Şimşek, "Phenolic Content, Antioxidant Activity and Quality Characteristics of Hulled Wheats Originating From Türkiye," *Turkish Journal of Agriculture—Food Science and Technology* 13 (2025): 1937–1944, https://doi.org/10.24925/turjaf.v13i7.1937-1944.7764.

74. S. Şimşek, "Comprehensive Analysis of Trace Elements, Amino Acids, and Antioxidant Potential of *Verbascum Orientale*," *Erzincan University Journal of Science Technology* 18 (2025): 391, https://doi.org/10.18185/erzifbed.1666259.

75. A. Kiliç, T. Ç. Akman, and S. Şimşek, "Antioxidant Activity and Phenolic–flavonoid Content of Phlomis Pungens Var. Pungens Collected in Erzincan (Türkiye)," *Pharmata* 5 (2025): 52, https://doi.org/10.62425/pharmata.1758776.

76. S. Şimşek, T. Varol, Z. Akşit, M. Elveren, and E. Osma, "Antioxidant and Antimicrobial Activities of Plant Flowers Growing in Distinct Geological Habitats (serpentine, gypsum, and limestone)," *New Zealand Journal of Botany* 63 (2025): 609, https://doi.org/10.1080/0028825X.2025.2454591.

77. S. Şimşek, H. Akşit, A. Aydın, and E. Köksal, "Ferruginoside D: a Novel Phenylethanoid From *Verbascum Leiocarpum*," *Chemistry Biodiversity* 20 (2023): e202301200, https://doi.org/10.1002/cbdv.202301200.

78. Z. Akşit, H. Akşit, S. Şimşek, et al., "LC-MS/MS Profiling Phytochemical Content of *Echinophora Chrysantha* (Apiaceae) and Antiproliferative, Antioxidant Activity," *Pharmacy & Pharmacology International Journal* 10 (2022): 190, https://doi.org/10.15406/ppij.2022.10.00384.

79. S. Şimşek, H. Akşit, E. Köksal, and A. Altay, "Antioxidant, Antidiabetic, Antiproliferative Activities, and Verbascoside Content of Four Endemic Verbascum Species: *V. leiocarpum*, *V. ozturkii, V. alyssifolium*, and *V. tuna-ekimii*," *New Zealand Journal of Botany* 64 (2026): e70023, https://doi.org/10.1002/nzb2.70023.

80. N. Kaya, S. Şimşek, and S. Gözcü, "Isolation, NMR Characterization, and Comparative in Vitro Antioxidant Evaluation of Resveratrol From Grape Seeds (Vitis vinifera L.)," *Recent Trends in Pharmacology* 4 (2026): 24–28, https://doi.org/10.62425/rtpharma.1887856.

81. G. Adıgüzelli, E. Osma, T. Varol, S. Şimşek, and A. Kandemir, "Erzincan İli-Ergan Dağı'nın Farklı Rakımlarında Yayılış Gösteren Bazı Taksonların Antioksidan Kapasiteleri," *Osmaniye Korkut Ata Üniversitesi Fen Bilimleri Enstitüsü Dergisi* 7 (2024): 2315, https://doi.org/10.47495/okufbed.1455270.

82. S. Gözcü, Z. Akşit, S. Şimşek, et al., "LC-MS/MS Characterization and Biological Activities of *Morina persica* L.(Caprifoliaceae)," *Journal of Research in Pharmacy* 28 (2024): 961, https://doi.org/10.29228/jrp.779.

83. E. N. Albayrak, S. Şimşek, A. B. Musatat, Z. Akşit, H. Akşit, and A. Atahan, "Antioxidant Activity and Theoretical Profile of Novel 2, 4, 6-triarylpyridine Derivatives Based on Syringaldehyde," *Düzce Univ Sci Technol J* 12 (2024): 981, https://doi.org/10.29130/dubited.1320385.

84. S. Gözcü, Z. Akşit, A. Aydın, M. A. Yılmaz, and S. Şimşek, "Comprehensive Phenolic Profiling and Biological Evaluation of *Centaurea Glastifolia* L.(Asteraceae)," *Natural Product Research* 39 (2025): 633, https://doi.org/10.1080/14786419.2024.2403028.

85. T. Varol, E. Osma, S. ŞİmŞek, and M. Elveren, "Antioxidant Activities of Plant Species Growing in Different Habitats (serpentine, gypsum and limestone)," *Frontiers in Life Sciences and Related Technologies* 4 (2023): 150–156, https://doi.org/10.51753/flsrt.1357325.

86. S. Gözcü, Z. Akşit, S. Şimşek, et al., "Phytochemical Analysis and Biological Evaluation of *Ferulago setifolia* K. Koch," *Journal of the Science of Food Agriculture* 104 (2024): 1382, https://doi.org/10.1002/jsfa.13017.

16121880, 2026, 7, Downloaded from https://onlinelibrary.wiley.com/doi/10.1002/cbdv.71533 by Samed MEK - Erzincan Universitesi , Wiley Online Library on [26/07/2026]. See the Terms and Conditions (https://onlinelibrary.wiley.com/terms-and-conditions) on Wiley Online Library for rules of use; OA articles are governed by the applicable Creative Commons License