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ORIGINAL PAPER

Determination of phenolic content and bioactive characterization of Anatolian propolis

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Abstract

In this study, propolis referred to as of Türkiye Anatolian propolis was utilized. This work was aim to prepare a 70% ethanolic extract of propolis and subsequently determine certain biochemical parameters, total phenolic content (TPC), total flavonoid content (TFC), phenolic and flavonoid composition, inhibitory effects on certain enzymes [acetylcholinesterase (AChE), urease, and alpha-amylase], along with assessments of antiviral activity against Vero cells, lung cancer cells (A549), and breast cancer cells (MDA-MB-231). The richness of phenolic content in Anatolian propolis clearly indicates a high inhibitory effect on these enzymes. Additionally, it was observed to exert significant effects on cancer cells and demonstrated effective antiviral activity. It can be contemplated that the Anatolian propolis might serve as a potential source for novel drugs, and the isolation of its compounds could offer opportunities for utilization in traditional medicine.

Keywords Anatolian propolis · Phenolic · Flavonoid · Enzyme inhibition · Antiviral activity

Introduction

Propolis is a substance created by bees through the combination of salivary enzymes (specifically, β-glucosidase), wax, pollen, and natural resins gathered from the environment. Bees use this resinous material to fill gaps and seal various parts of their hive [1–3]. The therapeutic activity of propolis has been utilized for medicinal purposes across various fields in traditional medicine, spanning from ancient times to the present day [4]. One of the earliest methods of application was observed among the Egyptians, who employed propolis in the mummification of cadavers. Propolis was preferred for its ability to impede the proliferation and decomposition of bacteria and fungi [5]. Numerous studies have substantiated the extraordinary pharmacological and biological attributes of propolis, including its proven antibacterial, antitumor, antifungal, anti-protozoal, antiviral, antioxidant, anti-inflammatory, hepatoprotective, cardioprotective, anti-neurodegenerative, antitubercular, local anesthetic, immune-stimulating, cytotoxic, and anti-aging properties [6–8]. The complex chemical composition of propolis presents a considerable challenge in its transformation into a pharmaceutical product [8, 9]. It has been proven in numerous studies that the composition of propolis varies according to the flora, geographic location, and bee species [10, 11]. Propolis is known to encompass around 800 identified compounds, with the possibility of discovering new compounds in the future [12]. The bioactive attributes of propolis are attributed to phenolic acids (such as cinnamic and caffeic acids) and their esters, along with flavonoids (comprising flavones, flavanones, flavonols, and dihydroflavonols) and terpenes [1, 13].

In this research endeavor was used propolis samples sourced from Anatolian propolis producer in Türkiye. A

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comprehensive investigation was conducted to explore some the bioactive properties of this Anatolian propolis sample, encompassing aspects such as TPC, TFC, phenolic profile, acetylcholinesterase (AChE), urease, alpha-amylase, enzyme inhibition, cytotoxic activity, and antiviral activity. There are studies in the literature that determine the biochemical properties of various propolis samples in Türkiye [14, 15]. Nevertheless, in this investigation, we focused on delineating the phenolic profile of Anatolian propolis and assessing its inhibitory effects on key enzymes, including (AChE), urease, and Alpha-amylase. Additionally, we evaluated its antiviral activity against Vero cells, as well as lung cancer cells (A549) and breast cancer cells (MDA-MB-231). Many bioactive properties of Anatolian propolis have been identified, we may suggested that potential avenues for further exploration in future studies.

Materials and methods

Propolis samples

In 2022, a single propolis sample was created under the name of Anatolian propolis by taking equal amounts of propolis obtained from experienced beekeepers from different regions of Turkey (Bursa, Ardahan, Yalova, Trabzon, Çorum and Sivas).

Raw propolis was powdered using a blender for study. Raw propolis sample 3 g was extracted with 30 mL 70% ethanol was stirred at ambient temperature for 24 h using a shaker (Heidolph PROMAX). At the end of the time, it filtered through the filter paper and was subsequently stored at + 4° C for future experiments to be done.

Determination of TPC and TFC of Anatolian propolis

The determination of total phenolic content in propolis extract prepared in 70% ethanol was carried out using the Slinkard and Singleton [16] method. First, 680 µL of distilled water was added both sample and standards, followed by the addition of 0.2 N 400 µL Folin–Ciocalteu's reagent. Then, 20 µL of was added both sample and each standard diluted gallic acid vortexed, and incubated at room temperature for 3 min. Finally, 400 µL of 10% Na_2CO_3 was added. After 2 h of incubation, readings were made on a spectrophotometer at 760 nm. Gallic acid is preferred as the standard to determine the total phenolic content in the propolis sample. The results were expressed as milligrams of gallic acid equivalent per mL of sample.

The determination of total flavonoid content in propolis extract prepared in 70% ethanol was carried out using the Ghasemi et al. [17] method. 250 µL propolis sample was added to 1.25 mL methanol, 0.05 mL 10% $Al(NO_3)_3$ and 0.05 mL of 1 M $NH_4.CH_3COO$. After incubation at room temperature for 50 min. readings were made on a spectrophotometer at 415 nm. The results were expressed as milligrams of quercetin equivalent per mL of sample.

In vitro acetylcholinesterase, urease and alpha-amylase inhibition study of Anatolian propolis

The inhibition of acetylcholinesterase (AChE) was determined using Ellman's method [18] and reported with slight modification as reported by Kantar et al. [19]. Initially, 50 µL of 2.5U/mL enzyme, 50 µL of the sample, 3 ml of pH:8 100 mM phosphate buffer was mixed and left for 5 min. Subsequently, the reaction was initiated by the addition of 100 µL of a 10 mM solution of 5,5-dithiobis(2-nitrobenzoic) acid (DTNB) and, 20 µL of a 75 mM solution of acetyl thiocholine chloride (ATCl). After 30 min, absorbance values were recorded at 412 nm. Donepezil was used as the standard.

Urease is an enzyme that helps break down urea into carbon dioxide and ammonia. To determine urease inhibition activity, ammonia production was measured using the indophenol method [20, 21]. The experiment involved studying the interaction between the enzyme and substrate, leading to the formation of ammonium ions. This interaction was carried out using urea in a buffer solution with a pH of 8.2. The resulting mixture was then combined with a phenol reagent (1% phenol + 0.005% sodium nitroprusside) and an alkaline reagent (0.5% NaOH + 0.1% sodium hypochlorite) to produce a blue-navy color.

The inhibition of alpha-amylase activity was carried out following slight modifications to the method described earlier [22, 23]. Alpha-amylase (porcine pancreatic alpha-amylase) was prepared including 0.02 M sodium phosphate buffer (pH 6.9). Briefly, 250 µL each of the sample and enzyme were taken and incubated for 10 min. At the end of the incubation period, 250 µL of 1% starch solution in 0.02 M sodium phosphate buffer (pH 6.9 with 0.006 M sodium chloride) was added to each tube at 5-s intervals. The mixture was incubated at 25 °C for 10 min. To stop the reaction, 500 µL of dinitrosalicylic acid color reagent was added. The tubes were incubated in a boiling water bath for 5 min and subsequently cooled to room temperature. Finally, the reaction mixture was further diluted by adding 2 mL of distilled water, and absorbance was measured at 540 nm.

RP-HPLC–PDA analysis of Anatolian propolis

25 phenolic acid standards were used in determining the phenolic content of Anatolian propolis. These are p-OH benzoic acid, m-OH benzoic acid, protocatechuic acid, gallic acid, chlorogenic acid, syringic acid, ellagic acid, t-cinnamic

acid, ferulic acid, p-coumaric acid, caffeic acid, caffeic acid phenethyl ester (CAPE), rhamnetin, quercetin, rutin, myricetin, epicatechin, chrysin, daidzein, apigenin, luteolin, pinocembrin, hesperetin, curcumin, resveratrol.

The analysis of the phenolic composition in Anatolian propolis sample was conducted using a modified method as detailed in the study by Can et al. [24]. High-performance liquid chromatography (HPLC) was employed, and the analysis was carried out using a Shimadzu Liquid Corporation LC 20AT HPLC system, equipped with a photodiode array (PDA) detector. The analytical column used had dimensions of 250 mm × 4.6 mm with a particle size of 5 µm, supplied by GL Sciences [25]. The elution process involved a gradient program consisting of two mobile phases. Mobile phase A was a 70% acetonitrile-ultrapure water solution, while mobile phase B was a 2% acetic acid in water solution. The flow rate during the analysis was set at 1 mL/min, and the injection volume was 20 µL for both the samples and standards. The column temperature was maintained at 30 °C throughout the analysis. The detection range spanned from 250 to 360 nm, with specific wavelengths monitored at 250, 280, 320, and 360 nm.

Cytotoxicity activity of Anatolian propolis

The cytotoxic activity of the extract was investigated on healthy epithelial cells (Vero), lung cancer cells (A549), and breast cancer cells (MDA-MB-231) using the MTT ([3-(4,5-dimethylhiazol-2-yl)-2,5-diphenyltetrazoliumbromide) method. Vero and A549 cells were grown in DMEM (Dulbecco's modified Eagle's medium) medium containing 10% FBS and 1% antibiotics, MDA-MB-231 cells were grown in RPMI (Roswell Park Memorial Institute) medium containing 10% FBS (fetal bovine serum) and 1% antibiotics, and at 37˚C in an environment containing 5% CO_2.Cells were placed in a flat-bottomed 96-well plate at a rate of 1×10^4.3.12–400 µg/mL concentrations of the extract were added to the wells, three wells of each concentration. The plates were incubated at 37 °C containing 5% CO_2 for 72 h. Wells containing only cells were used as controls. At the end of the period, 10 µL MTT (0.5 mg mL^{-1}) was added to all wells. The plates were incubated for 3.5 h at 37 °C containing 5% CO_2. The wells were emptied and 100 µL DMSO was added to each well. The plates were incubated in a dark environment until the formazan crystals dissolved. Absorbance values in the wells were measured at 570 nm. The viability of the cells in the wells was calculated as a percentage using the control wells as reference. The experiment was repeated twice. Using the graphs drawn with these data, the IC_{50} values and selectivity indices of the extract were calculated [26, 27].

Antiviral activity of Anatolian propolis

HSV-1 Wal strain was used in the experiment. Vero cells were placed at a rate of 1×10^4 in a flat-bottomed 96-well plate and the cells were infected with the virus at a rate of $1TCID_{50}$.

Concentrations of 6.25–25 µg/mL extract, which did not affect the on Vero cells, were added to the cells, three wells of each concentration. Acyclovir at a concentration of 50 µg/mL was used as a positive control, and wells containing only virus were used as a negative control. The plates were incubated for three days at 37 °C in a containing 5% CO_2. At the end of the period, 10 µL of MTT was added to the wells and incubated for 3.5 h at 37˚C in an oven containing 5% CO_2. At the end of the incubation, the medium in the wells was emptied and 100 µL DMSO was added to the wells. The plates were incubated in a dark environment until the formazan crystals dissolved. Absorbance values in the wells were measured at 570 nm. The viability of the cells in the wells was calculated as a percentage, using only the wells containing cells as reference. The experiment was repeated twice [15].

Statistical analysis

All experiments results were expressed as mean values and standard deviations (mean ± SD) for three replicates.

Results and discussion

Determination of TPC and TFC of Anatolian propolis

In this study, propolis obtained from beekeeper in the Anatolian region from Türkiye. The 70% ethanolic extract of this propolis sample was prepared and utilized in the study. The pharmacological characteristics of propolis generally arise from the presence of its phenolic compounds [28]. The total phenolic content of Anatolian propolis in the study was found to be 77.85 mg GAE/mL, while the flavonoid content was found to be 19.34 mg QE/mL (Table 1).

Saroğlu et al. [29] quantified the total phenolic content (TPC) of propolis samples different region of Bayburt province, determining it to be within the range of 10.283–7264.5 mg GAE/100 g. The TPC results of Anatolian propolis and Bayburt propolis in our study indicate a close resemblance. In another study, it was reported that propolis samples obtained from various regions of Turkey had total phenolic content (TPC) ranging from 44.19 to 166.91 mg GAE/g and total flavonoid content (TFC) ranging from 12.50 to 41.58 mg QE/g [15]. Özkök et al. [30] reported that Propolis samples were collected from 23 different cities in Turkey in 2019, and these propolis samples TPC

Table 1 Total phenolic content, total flavonoid content, inhibition of acetylcholinesterase, urease and a-amylase

Sample	TP (mg GAE/mL)	TF (mg QE/mL)	Inhibition of acetylcholinesterase IC_{50} (mg/mL)	Inhibition of urease IC_{50} (mg/mL)	Inhibition of *a*-amylase IC_{50} (mg/mL)
Anatolian Propolis	77.85 ± 1.08	19.34 ± 0.47	0.15 ± 0.002	0.05 ± 0.001	0.91 ± 0.003
Donepezil			16.60 ± 0.001		
Acetohydroxamic Acid				25.10 ± 0.100	
Acarbose					6.46 ± 0.040

All standards expressed as µg/mL

results 34.53–259.4 mg GAE/g while, TFC results of 21.28 and 152.56 mg CE/g. Mohtar et al. [31] found that propolis samples from Venezuela, Argentina, and Brazil exhibited total phenolic content ranging from 22.9 to 182 mg GAE/g and flavonoid content ranging from 2.8 to 60.2 mg QE/g. Some differences in the content of flavonoids and phenolic compounds were observed in Anatolian propolis sample compared with propolis samples in this study. We can say this difference to be in geographical origins and vegetation type. Some studies suggest that the composition of propolis varies significantly depending on the flora [32]. Therefore, the variance in the chemical composition of our sample compared to propolis samples other countries' propolis is to be expected.

In vitro acetylcholinesterase, urease, and alpha-amylase inhibition of Anatolian propolis

In this study, the inhibitory effects on three enzymes critically important for humans (acetylcholinesterase, urease, and alpha-amylase), were investigated in the propolis sample. The evaluation aimed to assess the potential impact of the propolis sample on the inhibition of these enzymes, which play a crucial role in human physiological processes. In the acetylcholinesterase enzyme inhibition study, donepezil was employed as a positive control (Table 1). The AChE enzyme inhibition effect of the propolis sample was determined to be 0.15 mg/mL. Baltas et al. [30] was reported that the IC_{50} values for AChE enzyme inhibition in Turkish, Nakhchivan, and Brazilian red propolis samples as 0.081, 1.353, and 0.221 mg/mL, respectively, in their conducted study. The results obtained in our study are observed to be in alignment with the findings for Turkish propolis reported in the reference investigation. We can say to assert that the effectiveness of Turkish propolis in AChE enzyme inhibition can be attributed to its flora origin. According to the literature and our current study, we can assert a relationship between the phenolic content of propolis and the outcomes of AChE inhibition. Based on our findings, it is plausible to state that the phenolic acids present in propolis contribute to the inhibition of the enzyme.

In the study, urease enzyme was worked as the second enzymatic inhibition. Based on the result of our propolis sample, it is possible to assert that the inhibition effect on urease enzyme is notably strong (Table 1). Baltas et al. [33] was reported that the IC_{50} values of propolis samples against the urease enzyme exhibited a range of 0.080–1.560 mg/mL. In another study, Turkish propolis samples demonstrated urease enzyme inhibitory IC_{50} values ranging from 0.260 to 1.525 mg/mL [34]. Our findings are observed to be in accordance with existing studies in the literature. One of the enzymes responsible for carbohydrate digestion in humans is *a*-amylase [35]. The inhibition of this enzyme plays a crucial role in the prevention of type 2 diabetes (T2D) through the stabilization of postprandial blood sugar levels [36].

Finally, the *a*-amylase enzyme inhibition effect was studied on the propolis sample, founded that the IC_{50} value of 0.91 mg/mL. Acarbose was used as the positive control in this study. The *a*-amylase enzyme inhibition values, as determined from propolis samples sourced from various regions of Morocco, were reported to exhibit a range of IC_{50} values from 195.09 to 963.79 µg/mL [37]. Our findings are observed to be consistent with the obtained results in literature. In our conducted study, it is evident that Anatolian propolis exhibits significant effectiveness on three enzymes. It is plausible to attribute this efficacy to the phenolic acids present in the propolis composition.

RP-HPLC–PDA analysis of Anatolian propolis

A total of 25 phenolic compounds were identified by RP-HPLC–PDA in the studied propolis samples. Major components such as pinocembrin, chrysin, CAPE, and hesperetin were identified, while other constituents were also detected in varying proportions (Table 2).

In several studies, certain phenolic acids (such as caffeic acid and caffeic acid phenethyl ester (CAPE), quercetin, kaempferol, *p*-coumaric acid, galangin, chrysin) have been identified as significant components with anti-inflammatory and immune-regulatory effects. It is conceivable that these compounds may play a crucial role in the treatment of numerous viral diseases, including COVID 19 [38]. In

Table 2 Phenolic content of Anatolian propolis

Phenolic content (µg phenolic/mL sample)		
Phenolic acids	*Hydroxybenzoic acids*	
	p-OH Benzoic acid	–
	m-OH Benzoic acid	–
	Protocatechuic acid	–
	Gallic acid	–
	Chlorogenic acid	–
	Syringic acid	–
	Ellagic acid	–
	Hydroxycinnamic acids	
	t-Cinnamic acid	10.99
	Ferulic acid	476.36
	p-Coumaric acid	165.53
	Caffeic acid	641.39
	Caffeic acid phenethyl ester (CAPE)	879.24
Flavanoids	*Flavonol*	
	Rhamnetin	279.44
	Quercetin	113,83
	Rutin	-
	Myricetin	-
	Flavan-3-ols	
	Epicatechin	-
	Flavones	
	Chrysin	1401.73
	Daidzein	-
	Apigenin	262.53
	Luteolin	37.58
	Flavanones	
	Pinocembrin	1638.89
	Hesperetin	603.09
	Other polyphenols	
	Curcumin	–
	Resveratrol	–

– not detected

our study, significant proportions of pinocembrin, chrysin, and CAPE were identified in the Anatolian propolis under investigation. This observation suggests the potential therapeutic utility of the Anatolian propolis in the treatment of various viral infections. In a conducted study, pinocembrin was identified as the major component in propolis samples obtained from African and Asian regions [39]. The HPLC analysis results of our current study are consistent with these previous findings. In another study, were reported that the phenolic acids ferulic and caffeic acid, along with the flavonoids ladanein and pectolinarigenin in Bayburt propolis [29]. Differences are observed when comparing our study with others, underscoring the regional variability in the characteristic compounds of propolis.

In addition, apigenin was found to be present at a concentration of 262.53 µg/mL in our propolis sample. Studies have reported that apigenin can be utilized in the treatment of diabetes by stimulating insulin secretion and promoting hepatic glycogen storage. Based on our research, it can be suggested that the propolis sample in our study may serve as an alternative for diabetes therapy [37, 40]. In other study reported that identified *p*-coumaric, ferulic, caffeic, galangin, pinobanksin, vanillin, and apigenin in poplar buds and Lithuanian propolis [41]. We may say to be accordance with this literature study the phenolic acids identified in Anatolian propolis. The other conducted study, the flavonoid content of poplar type propolis was investigated, revealing pinobanksin, pinocembrin, pinobanksin-3-acetate, and chrysin as predominant flavonoids identified in the propolis samples [42]. In our study, it can be asserted that Anatolian propolis is quite enriched with the flavonoids' pinocembrin and chrysin.

Cytotoxicity activity of Anatolian propolis

Cancer ranks as the second leading cause of death globally and in our country, following cardiovascular diseases, with an incidence rate of 22%. Among the most common types of cancer are breast and lung cancer. Many chemotherapeutic drugs are used in cancer. However, these drugs have many side effects. Apitherapeutic approaches, a significant facet of complementary medicine, are increasingly gaining prevalence to mitigate drug side effects and enhance overall quality of life. Propolis is one of the leading apitherapeutic products.

Propolis has been used for a wide variety of purposes since ancient times. With the determination of its anticancer properties, propolis and its components have been used successfully in a wide variety of cancer types and stages. It is also known that cancer patients are satisfied with using propolis, which is a natural and well-known product [43]. Clinical studies report that propolis is generally well tolerated by cancer patients. It is preferred to reduce the negative effects of radiotherapy and chemotherapy [44]. In our study, the cytotoxic effects of Anatolian propolis were investigated on Vero, A549, and MDA-MB-231 cells (Tables 3, 4, 5, 6; Fig. 1).

It was observed that the extract had a cytotoxic effect on Vero cells at concentrations of 50 µg/mL and above, on A549 cells at concentrations of 100 µg/mL and above, and on MDA-MB-231 cells at concentrations of 50 µg/mL and above. The IC_{50} value of the extract was found to be 313.1 µg/mL, 298.4 µg/mL and 264.3 µg/mL for Vero, A549 and MDA-MB-231 cells, respectively. The selectivity index of the extract was calculated as 1.1 and 1.2 for A549 and MDA-MB-231 cells, respectively. It

Table 3 Vero cell cytotoxicity of Anatolian propolis

	Control	Extract concentrations							
		400	200	100	50	25	12.5	6.25	3.12
% of living cells in the wells	98.94	35.70	67.71	90.08	102.21	120.77	139.68	134.46	136.28
	97.46	45.33	70.26	84.72	83.30	142.23	141.70	136.83	157.31
	103.60	42.93	54.78	89.75	88.17	119.50	139.67	136.83	134.86
Average	100	41.32	64.25	88.19	91.22	127.50	140.34	136.04	142.82
Standard deviation	3.20	5.01	8.31	3.00	9.82	12.77	1.17	1.37	12.57

Table 4 A549 cell cytotoxicity of Anatolian propolis

	Control	Extract concentrations							
		400	200	100	50	25	12.5	6.25	3.12
% of living cells in the wells	100.00	41.28	50.16	72.13	121.92	151.03	174.86	198.91	202.62
	99.45	53.83	54.05	71.48	136.76	147.33	187.99	195.48	200.20
	99.55	37.35	53.63	67.81	123.80	149.65	224.92	193.43	200.20
Average	100	44.16	52.61	70.47	127.49	149.34	195.92	195.94	201.01
Standard deviation	0.87	8.61	2.13	2.33	8.08	1.87	25.95	2.77	1.39

Table 5 MDA-MB-231 cell cytotoxicity of Anatolian propolis

	Control	Extract concentrations							
		400	200	100	50	25	12.5	6.25	3.12
% of living cells in the wells	102.32	44.20	41.90	47.13	91.29	117.02	140.38	123.65	133.51
	99.18	39.64	38.73	53.77	41.65	107.50	146.96	136.98	122.16
	98.51	39.64	52.38	53.77	77.22	75.16	85.93	123.65	133.51
Average	100	41.16	44.33	51.56	70.06	99.89	124.42	128.09	129.73
Standard deviation	2.04	2.64	7.14	3.83	25.58	21.94	33.50	7.70	6.55

Table 6 Cytotoxicity results of the extract (edited for graphics) of Anatolian propolis

	Control	Extract concentrations							
		400	200	100	50	25	12.5	6.25	3.12
Vero	100	41.32	64.25	88.18	91.22	127.50	140.34	136.04	142.82
A549	100	44.16	52.61	70.47	127.49	149.34	195.92	195.94	201.00
MDA-MB-231	100	41.16	44.33	51.56	70.06	99.89	124.42	128.09	129.73

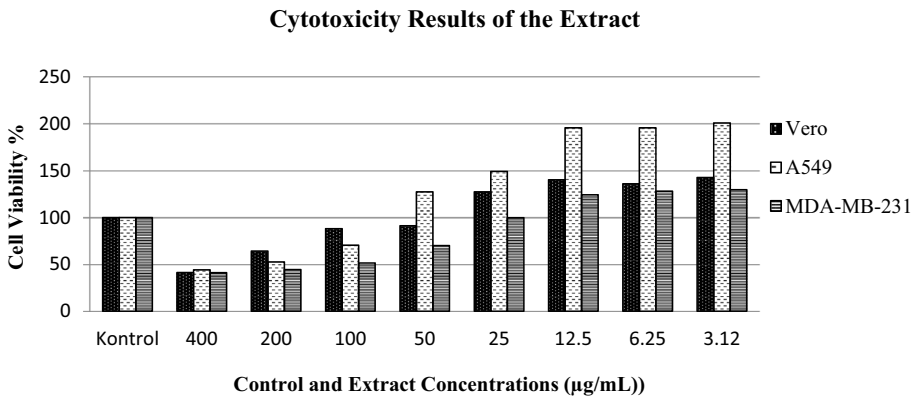

Fig. 1 Cytotoxicity results of Anatolian propolis

was determined that the extract had a cytotoxic effect on healthy and cancer cells at similar concentrations.

Demir et al. [27] investigated the cytotoxic impact of the ethanolic extract of Turkish propolis (EEP) on the A549 cell line, representative of human lung cancer cells. The results of the study indicated that EEP exhibited a discernible level of selective toxicity against A549 cells in comparison to normal fibroblast cells. In a study conducted in Lebanon, ethanol extracts of propolis were prepared and their anticancer activities on MDA-MB-231 (human breast cancer) and HCT-116 (human colorectal cancer) cells were investigated. The IC_{50} value of the extracts is 22.3 and 61.7 µg/mL for MDA-MB-231 and 33.3 and 50.9 µg/mL for HCT-116 [45]. Studies containing propolis samples are included in the literature, in countries such as Cyprus, Algeria and Egypt. It is reported to be more effective especially in breast adenocarcinoma (MDA-MB-231) [46, 47]. However, it is reported that propolis may also be allergenic and cause stomach problems [48]. The particular limitation in the use of propolis is its highly variable chemical composition, which depends on botanical origin and extraction methods. As a result, different propolis extracts are characterized by different biological activities. Therefore, it is necessary to develop standardization methods that will allow to combine the presence of specific compounds with biological activity and develop recommendations for the use of different types of propolis [49]. These studies will increase the clinical importance of propolis and make it a potential supportive product.

Antiviral activity of Anatolian propolis

Viruses can be reactivated from time to time and may require long-term antiviral treatment in high doses, especially in people with suppressed immune systems. This situation can often lead to the emergence of drug-resistant strains [50]. Therefore, there is a great need for new antiviral effective and non-toxic products. Nowadays, research on natural ingredients as new candidates for chemopreventive agents and various cancer chemotherapies is increasing. Propolis has great potential as a natural product with its rich phenolic content and various biological and chemical activities reported in vitro and in vivo. Propolis shows great potential with its natural resinous structure that shows anticancer potential [51]. In our antiviral activity test, concentrations of propolis extract that did not affect Vero cells were employed. It was determined that wells treated with propolis extract had a higher number of viable cells compared to wells infected only with the virus (Table 7, Fig. 2).

HSV-1 and HSV-2 infection is common worldwide [52, 53] stated in their study that propolis samples collected especially from autumn to spring showed significant antiproliferative activity in human and mouse cancer cells.

Huleihel and Isanu [54] stated in their studies that the antiviral properties of propolis are probably due to the prevention

Table 7 Antiviral activity test measurement of Anatolian propolis

	Cell	Virus	Aciclovir (50 µg/mL)	25 µg/mL	12.5 µg/mL	6.25 µg/mL
% of living cells in the wells	103.99	55.22	89.01	71.41	64.95	65.50
	95.24	55.88	88.14	73.81	68.89	61.35
	90.33	58.61	92.07	64.41	63.31	60.03
Average	96.52	56.57	89.74	69.87	65.72	62.29
Standard deviation	6.92	1.80	2.07	4.89	2.87	2.85

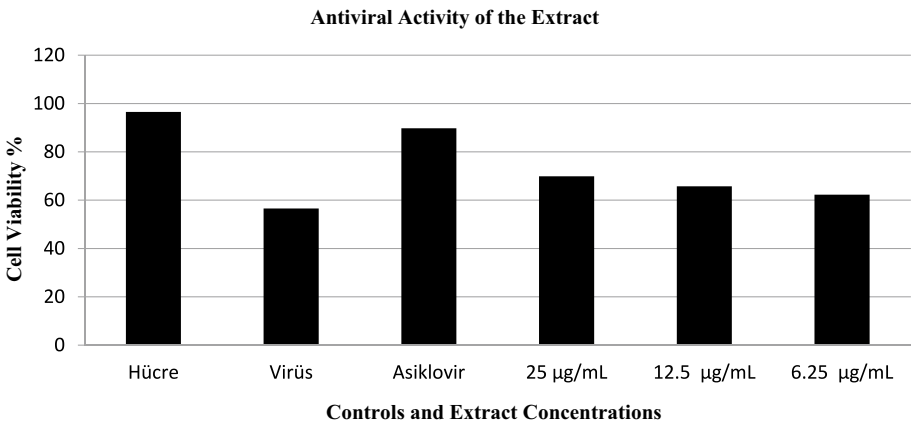

Fig. 2 Antiviral activity of Anatolian propolis. Cell; wells containing only Vero cells, Virus; wells containing virus-infected cells, Acyclovir; positive control

of viral adsorption. Schnitzler et al. [55] reported that aqueous and ethanolic extracts of propolis have high antiviral activity against HSV-2. In their study, Yıldırım et al. [56] investigated the antiviral activity of propolis collected from Hatay province on HSV-1 and HSV-2. Study findings show that the effectiveness of propolis against these two virus types is extremely high, and Hatay propolis has been reported to have a higher level of antiviral activity than acyclovir.

Conclusion

In our study, a single sample named Anatolian propolis was created by taking equal proportions from propolis samples obtained from various regions of Turkey. A 70% ethanolic extract of this propolis was prepared to investigate certain bioactive properties. When evaluating the findings of the study, it can be concluded that Anatolian propolis possesses a rich phenolic profile, exhibits potential as an inhibitor for certain enzymes from a clinical perspective and well as, Anatolian propolis demonstrates the ability to inhibit cancer cell proliferation, may have suggesting a promising role in the potential development of novel anticancer drugs in the future. To fully evaluate the potential of Anatolian propolis in preventing and/or treating cancer, it is essential to conduct dedicated research using animal models and clinical investigations. It will be provide a comprehensive understanding of the efficacy of Anatolian propolis in the context of cancer prevention and treatment.

Author contributions ZC: responsible for enzyme inhibition, phenolic profile, and writing the article in the study. CB: antioxidant activity and phenolic compound analysis. YK: phenolic compounds analysis and enzyme inhibition. ÜZÜS: determining cytotoxic activity and viral activity. SK designed the experimental plans. All authors have read and approved the final manuscript.

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Data availability Data will be made available on request.

Declarations

Conflict of interest The authors assert that they have no conflicts of interest to declare with regard to the publication of this manuscript. This means that there are no personal, professional, or financial relationships or circumstances that could potentially influence the findings or conclusions of the research in a biased manner.

Compliance with ethics requirements This study does not contain any studies with human participants or animals performed by any of the authors.

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