Original article

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Chemical and biological characteristics of propolis from *Apis mellifera caucasica* from the Ardahan and Erzurum provinces of Turkey: a comparative study

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The aim of this study was to compare the biological activities of ethanolic propolis extracts of *Apis mellifera caucasica* obtained from Ardahan and Erzurum provinces of Turkey. Samples were tested for antioxidant, anticytotoxic, anticarcinogenic, antibacterial, and antifungal potentials using different techniques. Propolis samples from the two provinces had different mineral and organic compositions related to their geographical origin. The ferric reducing antioxidant power (FRAP) test showed superiority of Ardahan propolis over the Erzurum. Regardless of origin and the presence of mitomycin C in the culture medium, propolis enhanced human peripheral lymphocyte viability, which depended on the duration and propolis concentration. Antiperoxidative activity on MCF-7 breast cancer cells was concentration-dependent. Erzurum propolis showed the highest anticarcinogenic activity at the concentrations of 62.5 µg/mL and 125 µg/mL, which dropped at higher concentrations. All propolis samples also showed antibacterial activity against the tested human pathogens similar to ampicillin and penicillin controls, except for *Pseudomonas aeruginosa*. However, they did not exert any antifungal activity against *Candida albicans* and *Yarrowia lipolytica*. In conclusion, propolis samples from both provinces showed promising biological activities, but further research should focus on finding the right concentrations for optimal effect and include the cell necrosis pathway to get a better idea of the anticarcinogenic effects.

KEY WORDS: anticarcinogen; antimicrobial; biological activity; mineral; mitomycin C; organic composition

Bees produce propolis from beeswax, vegetable balsam, pollens, and resins to strengthen and disinfect their beehives (1), and its biological properties have shown potential for human use from ancient times. Current research has established that its chemical composition and antimicrobial, antioxidant, anti-inflammatory, and anticarcinogenic properties vary from one location to another (2–8), as they stem from plant origin, phenolic compounds, flavonoids and their esters (9–11), climate, season, time of collection, and bee race (12, 13). Another important factor to consider is contamination of the beehive location (14).

Spanning over different climatic and geographic regions Turkey has different honeybee races and ecotypes. The Erzurum province accommodates smaller, yellow-coloured local bee ecotype, too aggressive for keeping, and the more common genotypes *Apis mellifera caucasica*, *A. m. carnica*, and *A. m. anatoliaca* (15). The last is the most preferred by beekeepers thanks to its wintering ability and honey yield (16). In contrast, the Ardahan and Artvin provinces are *A. m. caucasica* reserves, which means that no other bee races are kept and bred there.

Considering that an earlier study singled out antimicrobial activities of propolis from *A. m. caucasica* in the Erzurum province (17) and that little else is known about the bioactive properties of propolis of *A. m. caucasica* from its natural reserve (Posof District) in the Ardahan province, we wanted to compare the inorganic and organic composition, and biological activities of propolis samples of this race from the two adjacent, yet climatically different provinces.

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MATERIALS AND METHODS

Materials

All chemicals were purchased from Merck KGaA (Darmstadt, Germany) and its subsidiary Sigma-Aldrich (St. Louis, MO, USA) and included the following: certified reference material (CRM) BCR679 for mineral analysis, glucose, vanillic acid, sinapic acid, *trans*-ferulic acid, naringenin, sucrose, caffeic acid, *p*-coumaric acid, and sorbic acid standards (Sigma-Aldrich); vitamin C, benzoic acid, standard fatty acids CRM47885 (37 Component FAME Mix, Supelco, Bellefonte, PA), and standard fructose (Merck KGaA).

Propolis collection

All the propolis samples were collected in October 2016, placed in clean plastic pouches, and stored at -80 °C until processing. Ardahan propolis samples were obtained from *A. m. caucasica* beehives located in Posof in Ardahan province, and Erzurum propolis samples were obtained from *A. m. caucasica* beehives located at the apiculture units of the Atatürk University.

Preparation of ethanolic propolis extracts

To obtain biologically active compounds we used the ethanolic extraction method described elsewhere (18). Briefly, 2 g of solid propolis was mixed with 100 mL of 70 % ethanol. The mixture then sonicated in an ultrasonic bath (model 621.08.001, Isolab Laborgeräte GmbH, Eschau, Germany) at 300 W for 30 min. The solvent was removed in a rotary evaporator (Rotavapor R-210, Büchi Labortechnik AG, Flawil, Switzerland) at 16–17 kPa (160–170 mbar) and 60 °C. Extraction yields were 20.1 % (396.7/1973.6 w/w) and 23.75 % (486.7/2045.2 w/w) for the Ardahan and Erzurum samples, respectively. Dried matter was weighed and dissolved in 70 % ethanol to obtain a 20 mg/mL solution for further use, except for microbiological analysis (19). In our preliminary experiments, however, this ethanolic extract showed no antimicrobial activity against the tested microorganisms, which is why we used another extraction method to obtain propolis balsam for antimicrobial activity tests, as follows (20): 30 g of solid propolis was mixed with 300 mL of 95 % ethanol. The mixture was shaken in a shaker (SI-300, Lab Companion, Daejeon, South Korea) at 37 °C for 96 h, and the solvent evaporated in a rotary evaporator (RE100-Pro, SciLogex, Rocky Hill, CT, USA).

Determination of minerals in raw propolis

Because of lower mineral levels in the obtained propolis ethanolic extracts compared to raw samples reported elsewhere (21), we decided to determine the whole mineral content in raw material. Each raw propolis sample was divided in three samples, each analysed in triplicate to ensure statistical comparison. For this purpose, we used microwave-assisted digestion as described by Korn et al. (22) and analysed the samples for Co, Se, Li, Cd, As, Cr, Ni, Pb, Cu, Ca, Mg, K, Mn, Na, Zn, Fe, and Al content with inductively coupled plasma mass spectrometry (ICP-MS; NexION 350X, Perkin Elmer Inc., Waltham, MA, USA). Standard yttrium was also read to ensure the precision of the device (recovery interval was 97.8–119.3 %). Method accuracy was tested with the CRM BCR679 using the same protocol. Cd, Cu, and Ni were within the range of 95 % confidence interval (CI_{95}), while Zn was slightly above the CI_{95} reported for the CRM.

N, C, and S content was determined with an elemental analyser (Flash 2000, Thermo Fisher Scientific Inc., Waltham, MA, USA). Approximately 2 mg of raw propolis were digested with oxygen at 950 °C using helium as mobile phase. The results are given as the percentage of the total mass.

Determination of fatty acids in raw propolis

Fatty acid content in raw propolis was analysed in a gas chromatograph equipped with a flame ionisation detector (FID) (GC QP2010 Plus, Shimadzu Corp., Kyoto, Japan). Lipid extraction followed the method described by Hara and Radin (23) with a minor modification as follows: 5 g of raw propolis was homogenised in 6 mL of 3/2 (v/v) hexane/isopropanol mixture for 30 s and the homogenate centrifuged at 4500 *g* for 10 min. Methyl ester forms were derivatised according to the method described by Christie (24). Methyl ester derivatives were then injected into the Rx-2330 column (60 m x 0.25 mm inner diameter, 0.1 µm film thickness, Restek Corp., Bellefonte, PA, USA) in split mode at 250 °C using helium as carrier gas at 113.1 mL/min. The temperature program of the column was as follows: the initial temperature of 130 °C was held for 4 min, then increased at 3 °C/min to 230 °C and held for 10 min. The temperature of the FID was 255 °C. Standards were used to determine the retention time of fatty acids. The content of each fatty acid was calculated as the percentage of total peak area obtained from whole fatty acids in a propolis sample.

Determination of vitamin C and carbohydrate content in raw propolis

Here too we used raw propolis samples because of higher solubility of carbohydrates and vitamin C in water than in ethanol. 2 g of raw propolis was vortexed and then sonicated in a 2 mL of 95/05 methanol/water mixture (pH 3.0) for vitamin C analysis. 50 µL of this extract was injected into a high-performance liquid chromatograph (HPLC) (Prominence LC-20A, Shimadzu Corp.) equipped with a C18 ODS3 column (150 x 4.6 mm, 5 µm, Inertsil, GL Sciences Inc., Tokyo, Japan). The injection volume, pressure, flow rate in isocratic mode, and temperature were 50 µL, 200 bar, 1 mL/min, and 40 °C, respectively. Vitamin C content was determined with the photo-diode array

detector (SPD-M20A, Shimadzu Corp.) at 242 nm. The mobile phase was methanol/water mixture (5/95 v/v, pH 3). Vitamin C concentration in propolis was calculated using the standard curve.

For carbohydrate analysis, raw propolis (5 g) was mixed with 80 mL of ultra-pure water and then with 20 mL acetonitrile. Fructose, glucose, and sucrose in this extract were analysed with an HPLC (Prominence LC-20A, Shimadzu Corp.) equipped with an NH_2 column (250 x 4.6 mm, 5 µm, Inertsil, GL Sciences Inc.) and a refractive index detector (RID-20A, Shimadzu Corp.). The mobile phase was acetonitrile/water mixture (80/20 v/v). The injection volume, column pressure, flow rate in isocratic mode, and temperature were 20 µL, 200 bar, 1.3 mL/min, and 30 °C, respectively. Standard curves prepared with fructose, glucose, and sucrose were used to calculate the amount of these components in propolis samples.

Determination of alkaloid, organic acid, and flavonoid content in propolis extracts

Alkaloids, organic acid, and flavonoids were determined in ethanolic propolis extracts with a gas chromatograph (7890, Agilent Technologies Inc., Santa Clara, CA, USA) equipped with a mass spectrometer (5975C, Agilent Technologies Inc.). Silylation followed the method described by Proestos and Komaitis (25). The injection temperature was 280 °C, split ratio 40:1, flow rate 1 mL/min, and the run time 35 min. The composition of the eluates was matched with the NIST MS Search 2.0 library (National Institute of Standards and Technology, Gaithersburg, MD, USA).

Determination of phenolic acid content in propolis extracts

Phenolic acid content was analysed in ethanolic propolis extracts with an HPLC (Prominence LC-20A, Shimadzu Corp.) equipped with an ODS-3 column (250 x 4.6 mm, 5 µm, Inertsil, GL Sciences Inc.). The injection volume was 20 µL, column pressure 200 bar, flow rate in gradient mode 0.7 mL/min, and temperature 25 °C. Eluent A was a mixture of methanol, water, and acetic acid (10/89/1 v/v/v) and eluent B a mixture of methanol and acetic acid (99/1 v/v). The gradient program started with 100 % of solvent A to gradually reduce it to 95, 80, 75, 70, 60, 50, 40, and 0 % at 3, 18, 30, 35, 40, 55, 65, and 68 min, respectively. Diode array detector (SPD-M20A, Shimadzu Corp.) was used at 226 nm wavelength for benzoic acid and at 254 nm wavelength for vanillic acid, sinapic acid, *trans*-ferulic acid, naringenin, caffeic acid, *p*-coumaric acid, and sorbic acid. Standard curves were prepared to determine their amounts in the samples.

Determination of total phenolic and flavonoid content

Total phenolic and flavonoid contents were determined spectrophotometrically (S1205, Unico Science, Dayton, NJ, USA) in 20 mg/mL propolis extracts through total antioxidant capacity determination based on 2,2-diphenyl-1-picrylhydrazyl (DPPH) reducing potential and ferric reducing antioxidant power (FRAP).

For total phenolic content determination, we used the Folin-Ciocalteu method as described elsewhere (26). 0.5 mL of propolis extract was mixed with 2.5 mL of 0.2 eq/L Folin-Ciocalteu's reagent and 2 mL of 75 g/L sodium carbonate, and the mixture was incubated at room temperature for 2 h. The absorbance of the final solution was measured at 760 nm and converted to mass fraction using the standard graphic prepared with gallic acid (in the range of 0–250 mg/L). All the assays were done in triplicate, and the results presented as mg of gallic acid equivalent (GAE) per gram of propolis.

Total flavonoid content was spectrophotometrically determined following the methods described elsewhere (27, 28). 0.5 mL of the propolis extract was incubated with 1.5 mL of 95 % ethanol, 0.1 mL of 10 % of $AlCl_3$, 0.1 mL of 1 mol/L potassium acetate, and 2.8 mL of distilled water at room temperature for 30 min. Absorbance was measured at 415 nm and converted to mass fraction using the standard graphic prepared with quercetin (in the range of 0–700 mg/L). Data were presented as µg of quercetin equivalent (QE) per gram of propolis.

Total antioxidant capacity analysis

Total antioxidant capacity of ethanolic propolis extracts was determined with two methods: DPPH and FRAP. For the DPPH determination we mixed 1.5 mL of extract with 1.5 mL of 0.1 mmol/L DPPH and incubated the mixture in a dark place at room temperature for 50 min. Absorbance was measured at 517 nm and converted to concentration expressed as percentage of control using the formula provided by Molyneux (29).

For the FRAP analysis we mixed 100 µL of extract with 3 mL of freshly prepared FRAP solution and incubated it at 37 °C for 4 min. Absorbance was measured at 595 nm and converted to the concentration using the standard graphic prepared with $FeSO_4$ (in the range of 0.1–10 mmol/L). The analysis was done in triplicate and data presented as µmol/L of $FeSO_4$ equivalent per gram of propolis (30).

Determination of anticytotoxic potential of propolis extracts

For cytotoxicity tests we used human peripheral lymphocytes from blood samples donated by healthy volunteers (two men and two women), whose participation was approved by the Ethics Committee of the Kafkas University Faculty of Medicine (approval no. 80576354-050-99/158). 10 mL of whole blood was collected with a sterile syringe from each donor. Mitomycin C (MMC) was used a cytotoxic agent (positive control) in the concentration of 0.25 µg/mL (0.74 µmol/L), which was based on our

preliminary tests and an investigation by Kocaman and Topaktaş (31), who used mitotic index as toxicological endpoint. Lymphocyte viability was tested with 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT), which determines cellular metabolic activity (32). The lymphocytes were first cultured in a PB-MAX™ karyotyping medium (ThermoFisher Scientific Inc., Waltham, MA, USA) and then isolated from whole blood with a Histopaque®-1077 solution (Sigma-Aldrich). Isolated cells were counted, placed into the PB-MAX™ karyotyping medium (75 cells/µL of the medium), and incubated in a 5 % CO_2 Forma incubator (ThermoFisher Scientific Inc.) at 37 °C for 72 h. Propolis extracts were added at different concentrations either 24 h or 48 h after incubation started to see their 48 or 24 h effect on the lymphocytes. The concentrations used were based on preliminary 24 h LC_{50} test as follows: 500 µg/mL (LC_{50}), 250 µg/mL (1/2 LC_{50}), 125 µg/mL (1/4 LC_{50}), and 62.5 µg/mL (1/8 LC_{50}). They were added to cultures with or without 0.25 µg/mL (0.74 µmol/L) of MMC. Solvent control consisted of cultures with added ethanol as solvent (10 µL/mL) and negative control of untreated cultures in the medium. Four tubes were prepared for each propolis concentration and controls. After 24 h or 48 h of exposure to propolis, three measurements were done to evaluate cell viability using the MTT cell proliferation assay kit (Vybrant®, ThermoFisher Scientific Inc.) according to the kit protocol. The cells were seeded into wells of a microplate and 10 µL of 12 mmol/L MTT was added to each well. The microplate was incubated at 37 °C at 5 % CO_2 for 4 h. Absorbance was measured at 540 nm on a microplate reader (EON™, BioTek, Winooski, VT, USA), and cell viability calculated using the formula provided by Cheki et al. (33).

Determination of propolis antioxidant activity against model oxidant

MCF-7 human breast cancer cells [American Type Culture Collection (ATCC), Manassas, VA, USA] were cultured aseptically in Dulbecco's modified Eagle's medium (DMEM; Invitrogen, Thermo Fisher Scientific Inc.) containing 10 % foetal bovine serum (FBS; PAA Laboratories, GE Healthcare, Chicago, IL, USA), 1 % antibiotic solution (100 IU/mL penicillin and 0.1 mg/mL streptomycin) (Sigma-Aldrich) at 37 °C and 5 % CO_2. The medium was removed at 80 % cell growth, and trypsin-EDTA solution (Gibco, ThermoFisher Scientific Inc.) was added until it covered the plate surface (8–10 mL). Separated cells were collected and dissolved in complete-DMEM after removing the trypsin solution. Oxidative stress was tested in MCF-7 cells treated with 0.25 µg/mL of MMC (0.74 µmol/L) and propolis extracts from both provinces in one of the following concentrations: 32.5, 65, 125, 250, or 500 µg/mL. Antioxidative activity of propolis was established as reduction in respect to positive control treated with MMC alone. For negative control we used MCF-7 cells in culture and for solvent control MCF-7 cells treated with 10 µL/mL of ethanol. Oxidative stress was determined by measuring thiobarbituric acid reactive substances (TBARS) using a method described by Jain (34) and modified by Do et al. (35). 400 µL methanol containing 0.01 % butylated hydroxytoluene and 500 µL 1 % thiobarbituric acid dissolved in 1 % sulphuric acid was mixed with 100 µL supernatant. The mixture was vortexed and then incubated at 100 °C for 15 min. The absorbance of the resulting supernatant was obtained at 532 and 600 nm and converted to nmol/mg protein using a standard curve prepared with 1,1',3,3'-tetramethoxypropane. Total protein was measured using a modified Lowry method as described elsewhere (36).

Determination of propolis anticarcinogenic activity

MCF-7 cells were cultured and treated as described in the above subsection. Cells were counted in a Thoma counting chamber (Isolab Laborgeräte GmbH) under a microscope (Olympus Corp., Tokyo, Japan) at 10x magnification and seeded onto a 6-well plate (4500 cells per well). The plates were incubated at 37 °C and 5 % CO_2 for 24 h, treated and incubated for another 24 h.

Apoptotic cells were counted using the Roche *In Situ* Cell Death Detection Kit according to the producer's instructions (Roche Diagnostics GmbH, Mannheim, Germany). Treated cells were incubated with 0.002 % Triton X-100 for 10 min, added 50 µL of terminal deoxynucleotidyl transferase (dUTP) nick end labelling (TUNEL) master mix per well, and incubated at 37 °C and 5 % CO_2 for another 80 min. Red-stained TUNEL-positive cells were visualised under a fluorescent microscope (BX43 equipped with DP74 camera, Olympus Corp.) at 20x magnification and quantified with Image Processing and Analysis in Java version 1.51h software (ImageJ; National Institutes of Health, Bethesda, MD).

Determination of propolis antimicrobial activity

Propolis extracts were sterilised through 0.22 µm Millipore filters and their antimicrobial potential tested by disc diffusion or broth microdilution (37, 38) against known bacterial and fungal pathogens, cultured in Mueller-Hinton agar and 2 % Sabouraud dextrose agar, respectively. These included gram-positive bacteria *Bacillus megaterium* (Kahramanmaraş Sütçü İmam University Department of Biology, Kahramanmaraş, Turkey) and *Staphylococcus aureus* (ATCC 6538), gram-negative *Pseudomonas aeruginosa* (ATCC 9027) and *Klebsiella pneumoniae*, and fungi *Yarrowia lipolytica* and *Candida albicans* (Kahramanmaraş Sütçü İmam University). Gentamycin (Gen), ampicillin (Amp), erythromycin (Ery), and penicillin (Pen) were used as positive controls for the disc diffusion method. After 48 h of treatment, inhibition zones were measured with a digital calliper. All the tests were

triplicated, and the results presented as means ± standard deviations.

As the fungal species and *P. aeruginosa* showed no inhibition zone with broth microdilution method in our study, we tested antimicrobial activity of propolis against these species only with the disc diffusion method (39).

For broth microdilution method we diluted the propolis extracts to 0.67–173.5 μg/mL for the Erzurum and to 0.95–245.7 μg/mL for the Ardahan samples according to our preliminary experiments. After incubation at 37 °C for 18 h, 0.5 % 2,3,5-triphenyltetrazolium chloride (TTC) was added, and incubation continued at 37 °C for another 30 min. Plate wells without colour change were considered containing minimal inhibition concentrations (MIC) of propolis (40).

Statistical analyses

All statistical analyses were run on SPSS Statistics for Windows, Version 17.0 (SPSS Inc., Chicago, IL, USA). Normality of distribution was assessed with the Kolmogorov-Smirnov test, except for the MTT assay, for which we used the Shapiro-Wilk test. For the data that did not show normal distribution we ran the non-parametric Kruskal-Wallis test, followed by the *post-hoc* Mann-Whitney *U*-test. Data with normal distribution were analysed with one-way analysis of variance (ANOVA). Homogeneity of variance was tested with Levene's test. Duncan *post-hoc* test was applied for homogeneous subsets, while Dunnett's *C* test was used for non-homogeneous ones. All data are presented as means ± standard deviations. Significance (*P*-value) was set at 0.05.

Table 1 Comparison of mineral composition of *A. m. caucasica* propolis samples collected from the Ardahan and Erzurum provinces of Turkey

Element	Ardahan (mg/kg)	Erzurum (mg/kg)
Co	0.14±0.04[a]	0.25±0.06[b]
Se	0.038±0.016	0.025±0.006
Li	0.20±0.04	0.22±0.04
Cd	0.005±0.006	0.004±0.004
As	0.13±0.02[a]	0.22±0.049[b]
Cr	0.98±0.22[a]	1.39±0.18[b]
Ni	1.08±0.23	1.53±0.34
Pb	0.83±0.19	0.97±0.19
Cu	2.45±0.16	2.01±0.79
Ca	269.42±51.83[a]	428.97±76.28[b]
Mg	376.17±85.91	560.21±171.45
K	1156.06±278.58	2607.36±1468.87
Mn	5.297±0.71	7.47±3.27
Na	193.19±15.34	203.47±9.58
Zn	30.05±7.30	41.77±19.65
Fe	428.51±77.75	507.62±287.13
Al	408.46±88.17	406.86±202.20
	% of total mass	**% of total mass**
N	0.42±0.09	0.35±0.02
C	66.84±1.38	64.06±6.38
S	ND	ND

Data are given as mean ± standard deviation (*N*=3). Different superscript letters in the same row denote significant difference (*P*<0.05). ND – not detected

RESULTS AND DISCUSSION

Table 1 presents the mineral composition of propolis from both provinces. It was statistically similar except for Co, As, Cr, and Ca, which was higher in the Erzurum samples. The order of abundance in the Ardahan propolis samples was K>Fe=Al=Mg=Ca>Na>Zn>Mn>Cu>Ni=Cr=Pb>Li=Co=As>Se>Cd and in the Erzurum samples K > M g = F e = C a = A l > N a > Z n > M n > C u = C r ≥Ni=Pb>Li=Co=As>Se>Cd. For comparison, reports on propolis mineral composition from Croatia (21) and Spain (41) single out Ca, Mg, K, Al, Fe, Na, and Zn as the most abundant. Spanish propolis was reported similar or higher Cd, Ni, Fe, and Zn levels as did Polish (42).

According to Kruskal-Wallis *H* test, Ardahan propolis Fe, Al, Mg, and Ca levels (*P*=0.135) can be grouped in one cluster, Ni, Pb, and Cr (*P*=0.393) in another, and Li, Co, and As (*P*=0.148) in yet another cluster. Similar is true for the Erzurum samples with a minor exception for Cu in the second cluster. Fe, Al, Mg, and Ca formed one cluster (*P*=0.599), Cu, Ni, Pb, and Cr (*P*=0.090) the second, and Li, Co, and As (*P*=0.587) the third.

We believe that similarities in the mineral composition between the propolis from the two provinces is owed to their geographic vicinity. Similar reason may explain the highest Ca content in Turkey in propolis from these two adjacent provinces reported by Yozgat and Sivas (43).

Propolis from both our provinces had considerably lower Cd levels than reported in other Turkish provinces (44), most likely because these two provinces have little industry. However, Pb and Cr levels were much higher than in the rest of Turkey (44) and some regions of Croatia (21). Similar or lower levels than ours were reported in Polish and Spanish propolis (41, 42). While no data are available about soil mineral composition for both provinces, Erzurum is known for Cu, Pb, Zn, and Cr mining areas (44), especially near the Ardahan province border. This may partly explain the highest levels of Pb and Cr.

As and Al levels deserve special attention, those in the Erzurum propolis in particular. They are in the range reported in previous studies (21, 41) and are below the acceptable daily intake thresholds (300–1400 μg/day for Al, 20–514 μg/day for Pb, 20–250 μg/day for Cr, 10–60 μg/day for Cd, and 12–25 μg/day for As) (45).

We were surprised to see that propolis from neither province contained any sulphur, especially as it is an

Table 2 Comparison of fatty acid content in *A. m. caucasica* propolis samples collected from the Ardahan and Erzurum provinces of Turkey

Fatty Acid	Retention time (min)	Ardahan (%)	Erzurum (%)
Saturated			
Caproic acid	6.54	–	8.85
Caprylic acid	9.24	0.26	–
Decanoic acid (capric acid)	13.85	1.14–1.47	0.08
Undecanoic acid	17.80	0.67	–
Dodecanoic acid (lauric acid)	21.53	24.39–36.95	49.62–54.27
Tridecanoic acid	25.38	5.00–6.59	6.29–8.10
Tetradecanoic acid (myristic acid)	30.81	4.68–5.86	8.66
Pentadecanoic acid	34.13	6.97–8.85	–
Hexadecanoic acid (palmitic acid)	36.89	0.84–0.89	1.53
Heptadecanoic acid (margaric acid)	41.10	1.03–1.64	0.54–1.12
Octadecanoic acid (stearic acid)	44.19	1.86	1.48–2.12
Eicosanoic acid (arachidic acid)	49.47	–	3.13–4.43
Heneicosanoic acid	54.98	1.21	1.57–2.05
Docosanoic acid (behenic acid)	56.66	0.54	0.78–1.35
Tricosanoic acid	59.78	0.31–2.44	0.26
Lignoceric acid	62.16	0.45–0.57	0.22–0.45
TOTAL		**49.4–69.8**	**83.0–93.3**
Monounsaturated			
9-tetradecenoic acid (myristoleic acid)	32.80	–	0.49
cis-10-pentadecenoic acid	36.57	3.36–4.70	0.88–1.69
cis-9-hexadecenoic acid (palmitoleic acid)	39.52	1.48–2.61	0.32–2.83
cis-10-heptadecenoic acid	42.58	3.18–3.35	–
trans-9-octadecenoic acid (elaidic acid)	45.97	–	0.59–1.73
cis-9-octadecenoic acid (oleic acid)	46.98	1.75–1.92	0.56–1.15
cis-11-eicosenoic acid (gondoic acid)	51.98	1.14	1.61–2.28
cis-13-docosenoic acid (erucic acid)	57.64	0.66	–
cis-15-tetracosenoic acid (nervonic acid)	65.02	0.54–0.79	0.16–0.26
TOTAL		**12.1–15.2**	**4.6–10.4**
Polyunsaturated			
9,12-octadecadienoic acid (linolelaidic acid)	47.80	–	1.04–1.09
Linoleic acid	48.20	1.42–1.89	7.89–11.46
Gamma-linolenic acid	50.96	0.61–1.26	1.10–1.39
Linolenic acid	53.33	0.65–0.79	0.14–0.28
cis-11,14-eicosadienoic acid	55.09	1.44	0.26
cis-8,11,14-eicosatrienoic acid	57.22	16.30–22.14	0.16
cis-11,14,17-eicosatrienoic acid	58.10	2.00–2.14	1.04
all-cis-5,8,11,14-eicosatetraenoic acid (arachidonic acid)	59.28	0.94–2.69	0.66–0.92
cis-13, 16-docosadienoic acid	60.93	0.65–0.72	–
cis-5,8,11,14,17-eicosapentaenoic acid (timnodonic acid)	64.00	0.79–2.16	0.07–0.40
cis-4,7,10,13,16,19-docosahexaenoic acid (cervonic acid)	66.92	0.60–0.75	0.22–0.45
TOTAL		**25.4–36.0**	**12.6–17.5**

important component of various biological compounds. Similar absence of sulphur was reported in other Turkish provinces (43).

Table 2 shows that propolis fatty acid content differed between the provinces. Such variation has also been found in the rest of Turkey (46) and other countries of the world, such as Algeria (47), Brazil (48), and New Zealand (49).

Of the 36 fatty acids determined in the samples, six were found only in the Ardahan propolis and five only in the Erzurum propolis. The Ardahan propolis had higher mono- and polyunsaturated fatty acid content than the Erzurum propolis (12.1–15.2 % vs 4.6–10.4 % and 25.4–36.0 % vs 12.6–17.5 %, respectively). The Erzurum propolis, in turn, had much higher saturated fatty acid content (83.0–93.3 % vs 49.4–69.8 %).

While our findings show the highest content of lauric acid in the *A. m. caucasica* propolis from both provinces, Silici and Kutluca (17) reported only oleic and palmitic acid in Erzurum propolis collected from three bee races. Palmitic acid levels were the highest in *A. m. carnica*, followed by *A. m. anatoliaca*, and then *A. m. caucasica* propolis. It was also the most common in reports from Brasil (48), New Zealand (49), and Jordan (50).

The saturated fatty acid content of the Ardahan propolis was between the one reported for Algerian (41 %) (47) and Romanian (71 %) (51) propolis samples, while the Erzurum propolis had much higher saturated fatty acid content. Judging by earlier reports from Erzurum (17) and Brazil (48, 52), not only did forage on several botanical species but also genetic differences influence hydrocarbon chemistry of propolis samples.

Table 3 shows vitamin C and carbohydrate content in propolis from both provinces. Vitamin C content was higher in the Ardahan propolis and was comparable to the one reported in India (53). Ardahan propolis also had higher fructose and glucose but lower sucrose content than the Erzurum propolis. According to reports from Egypt (54), Canary Islands (55), and South East England (56), propolis fructose, glucose, and sucrose content very much depended on plant origin and geographic region.

Tables 4 and 5 show differences in alkaloid, organic acid, flavonoid, and phenolic content in propolis between the two provinces. The Ardahan samples had 17 and Erzurum 16 alkaloid, organic acid, and flavonoid compounds. Gallic acid, vanillic acid, benzoic acid, sorbic acid, naringenin, and myricetin were not found in samples from either province with HPLC analysis, but GC-MS revealed benzoic acid and naringenin peaks in the Ardahan propolis and naringenin peak in the Erzurum propolis. Ferulic acid in the Erzurum propolis was detected with both HPLC and GC-MS analysis, but only with HPLC in the Ardahan samples. This inconsistency points to the limitations of silylation, or characteristics of a particular column, as already observed in the study of García-Viguera et al. (57).

Table 3 Comparison of vitamin C and carbohydrate content in *A. m. caucasica* propolis samples collected from the Ardahan and Erzurum provinces of Turkey

	Ardahan (μg/g dry weight)	Erzurum (μg/g dry weight)
Vitamin C	40.31±2.97[a]	16.18±1.48[b]
Fructose	1.58±0.30[a]	0.86±0.18[b]
Glucose	0.98±0.20[a]	0.39±0.05[b]
Sucrose	0.15±0.04[a]	0.69±0.19[b]

Data are given as mean±standard deviation (N=3 for vitamin C, N=4 for carbohydrates). Letters a and b denote significant difference between parameters presented in the same row (P<0.001 for vitamin C, P<0.01 for carbohydrates)

The Erzurum propolis had higher pinostrobin chalcone, pinocembrin, tectochrysin, naringenin, chrysin, galangin, caffeic acid, and quercetin flavonoid and phenolic content than the Ardahan propolis. All these compounds are known anticarcinogens with or without antioxidant properties. Some have already been reported in the propolis of the three bee races from Erzurum studied earlier (17, 58), but our study is the first to report pyrocatechol, isoferulic acid, *p*-coumaric acid methyl ester, 4',5-dihydroxy-7-methoxyflavone, 5-methylisophthalic acid, and 1,2-dimethylcyclopropene. These, however, were not found in the Ardahan propolis.

Of the compounds with known antioxidant and anticarcinogenic potential Ardahan propolis had higher levels of sakuranetin, *p*-coumaric acid, and ferulic acid, while 2,3-dihydrobenzofuran levels were similar to the Erzurum propolis. Unlike the Erzurum propolis, however, it also had benzyl alcohol, guaiacol, benzoic acid, *p*-vinylguaiacol, vanillin, β-caryophyllene, benzylbenzoate, benzylcinnamate, corydaldine, 6-methoxypiperonal, benzyl 4-acetylbenzoate, 2, 5-bis dimethylamino-3, 9-dimethyl-3H-1,3,4,6-tetrasacyclopentazulene, and 3,4',7-trimethoxyflavone. Most of the compounds found in the Ardahan and Erzurum were already reported in the rest of Turkey and other countries of Europe. Judging by reports of poplar bud exudates from a number of locations (46, 56, 59–62), propolis from both our provinces may be categorised as poplar propolis.

Table 6 shows total flavonoid and phenolic content and total antioxidant capacity of propolis samples from both provinces. The Ardahan propolis had 2.18 times higher total phenolic and 1.79 times higher total flavonoid content. As a result, it had significantly higher antioxidant activity, as determined by the FRAP test. We think that the FRAP test is more convenient for this purpose, because the DPPH test showed no significant difference. Our flavonoid and phenolic content findings in the Ardahan propolis were similar to or higher than reported elsewhere for Ardahan and higher than reported for the Turkish province of Ankara (63). In addition, both Ardahan and Erzurum propolis had higher flavonoid and phenolic content than propolis collected from Greece (64), Ireland, Germany (59), and Argentina (65) but lower than Ethiopian propolis (66). Most

Table 4 Alkaloid, organic acid, and flavonoid content in ethanolic extracts of *A. m. caucasica* propolis obtained from the Ardahan and Erzurum provinces of Turkey

Peak	Retention Time (min)	Quantity in the sample (%)	Name	Retention Time (min.)	Quantity in the sample (%)	Name
			ARDAHAN			**ERZURUM**
1	7.41	0.70	Benzyl alcohol	8.43	1.59	2-Phenylethanol (Benzeneethanol)
2	8.13	1.18	Guaiacol	9.28	1.35	1,2-dihydroxybenzene (pyrocatechol)
3	9.02	3.85	Benzoic acid	9.52	0.62	2,3-dihydrobenzofuran (coumaran)
4	9.53	7.64	2, 3-dihydrobenzofuran (coumaran)	15.61	0.73	5-phenylpenta-2,4-dienoic acid (cinnamylidene acetic acid)
5	10.71	19.15	4-hydroxy-3-methoxystyrene (*p*-vinylguaiacol)	18.35	2.71	3-hydroxy-4-methoxycinnamic acid (isoferulic acid)
6	11.64	0.61	Iso-vanillin or vanillin	18.89	0.74	Palmitic acid
7	15.91	0.60	β-caryophyllene	20.41	1.02	2-nonadecanone
8	16.77	2.25	Benzylbenzoate	20.73	1.03	Elaidic acid
9	17.39	1.11	[1S,3S,(+)]-1-methyl-3-isopropenyl-4-cyclohexene	21.95	1.48	Methyl 4-hydroxycinnamate (*p*-coumaric acid methyl ester)
10	20.54	13.22	Benzyl cinnamate	22.08	0.82	4-hydroxy-3- methoxycinnamic acid (ferulic acid)
11	22.19	1.70	Tricosane or eicosane	22.19	2.14	Tricosane or eicosane or *n*-hexadecane
12	23.34	0.59	Corydaldine	23.11	2.89	5-methylisophthalic acid
13	23.57	0.79	6-methoxy-1,3-benzodioxole-5-carbaldehyde (6-methoxypiperonal)	23.62	1.73	1,2-dimethylcyclopropene
14	23.78	1.42	(E)-1-(2,6-dihydroxy-4-methoxyphenyl)-3-phenylprop-2-en-1-on (pinostrobin chalcone)	23.82	9.64	(E)-1-(2,6-dihydroxy-4-methoxyphenyl)-3-phenylprop-2-en-1-one (pinostrobin chalcone)
15	24.26	3.06	Pentacosane or eicosane	24.27	2.14	Pentacosane
16	24.43	5.29	Benzyl 4-acetylbenzoate	24.79	20.74	Pinocembrin
17	24.75	9.42	Pinocembrin	26.45	5.31	5-hydroxy-7-methoxyflavone (tectochrysin)
18	25.83	9.52	2,5-bis dimethylamino-3,9-dimethyl-3H-1,3,4,6-tetrasacyclopentazulene	27.02	3.38	Eicosane
19	26.40	0.72	5-hydroxy-7-methoxyflavone (tectochrysin)	27.34	10.02	Naringenin or chrysophanic acid
20	27.01	4.94	Eicosane or heptacosane	27.85	11.98	Chrysin
21	27.27	1.23	Naringenin or chrysophanic acid	28.44	0.93	4′,5-dihydroxy-7-methoxyflavone (an apigenin derivative)
22	27.74	2.68	Chrysin	28.76	6.92	Galangin
23	28.67	0.77	Galangin	29.20	0.94	Sakuranetin
24	29.18	1.11	Sakuranetin			
25	31.03	0.94	Nonacosane			
26	33.64	0.83	7,3',4'-trimethoxyflavone			

Table 5 Comparison of phenolic acid content in *A. m. caucasica* propolis samples collected from the Ardahan and Erzurum provinces of Turkey

Phenolic acids	Ardahan (mg/kg)	Erzurum (mg/kg)
3,4,5-trihydroxybenzoic acid (gallic acid)	ND	ND
4-hydroxy-3-methoxybenzoic acid (vanillic acid)	ND	ND
3,4-dihydroxycinnamic acid (caffeic acid)	0.033	0.046
4-hydroxycinnamic acid (*p*-coumaric acid)	0.042	0.008
4-hydroxy-3,5-dimethoxycinnamic acid (sinapic acid)	ND	ND
4-hydroxy-3-methoxycinnamic acid (*trans*-ferulic acid)	0.079	0.005
Quercetin	0.018	0.054
Benzoic acid	ND	ND
2,4-hexadienoic acid (sorbic acid)	ND	ND
Naringenin	ND	ND
Myricetin	ND	ND

ND – not detected

Table 6 Total flavonoid and phenolic content and antioxidant capacity of *A. m. caucasica* propolis samples obtained from the Ardahan and Erzurum provinces of Turkey

	Ardahan	Erzurum
Total flavonoid content (μg quercetin equivalent of total flavonoids/g)	591.5±26.2^{a}	271.7±2.9^{b}
Total phenolic content (mg gallic acid equivalent/g)	235.5±5.3^{a}	131.3±3.1^{b}
DPPH (% of control)	94.9±0.3^{a}	94.6±0.7^{a}
FRAP (μM $FeSO_4$ equivalent/g)	4017.7±16.4^{a}	3813.2±3.6^{b}

Data are presented as mean ± standard deviation (N=3). Different superscript letters in the same row denote significant difference (P<0.05). DPPH – 2,2-diphenyl-1-picrylhydrazyl; FRAP – ferric reducing antioxidant power

Table 7 Effects of different concentrations of propolis extracts obtained from Ardahan and Erzurum on the viability of human lymphocytes

	Cell viability (% of control)			
	Ardahan		Erzurum	
	24 h	48 h	24 h	48 h
Control	100.0±0.0^{a}	100.0±0.0	100.0±0.0	100.0±0.0^{ab}
Solvent control	97.3±1.3^{bd}	102.2±8.5	96.5±13.3	89.8±14.9^{a}
MMC (0.74 μmol/L)	97.3±2.6^{be}	97.7±1.2	92.4±13.4	89.1±14.0^{a}
Propolis extract				
62.5 μg/mL	98.0±2.8^{ade}	102.8±11.4	99.7±13.5	93.9±13.9^{a}
125 μg/mL	102.9±5.7^{acde}	105.2±7.9	104.0±19.6	100.7±19.5^{ab}
250 μg/mL	107.0±1.8^{c}	102.9±2.1	118.1±17.8	113.3±20.3^{abc}
500 μg/mL	116.5±6.6^{f}	108.8±3.9	139.9±22.5	127.0±20.0^{c}
Propolis + MMC				
62.5 μg/mL+MMC	99.5±3.2^{ab}	101.1±7.7	98.9±19.2	94.7±16.7^{ab}
125 μg/mL+MMC	99.6±1.9^{ab}	99.3±7.6	116.4±41.1	109.1±10.3^{abc}
250 μg/mL+MMC	100.5±6.1^{abc}	101.6±6.0	117.2±16.2	119.6±11.7^{bc}
500 μg/mL+MMC	112.5±2.8^{f}	113.0±5.5	138.9±23.8	133.5±19.6^{c}

Data are presented as mean ± standard deviation (N=12). Different superscript letters in the same column denote significant difference (P<0.05). MMC – mitomycin C

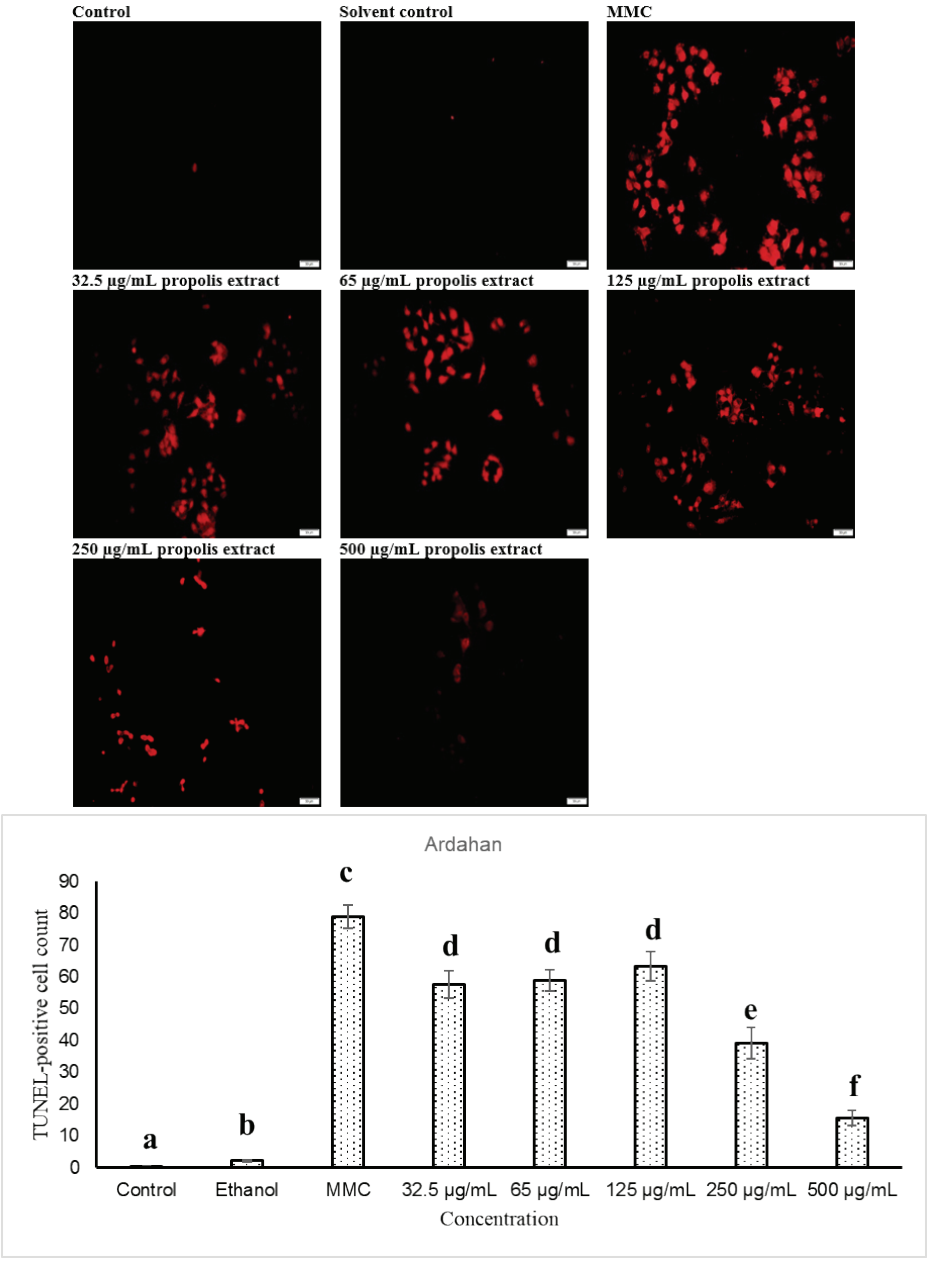

Figure 1 Apoptotic effects of *A. m. caucasica* propolis extracts from the Ardahan province of Turkey on MCF-7 cancer cells **A** (visualised with TUNEL, 20x magnification); **B** Apoptotic cell count (mean±standard error) (minimum 10, maximum 35 regions were photographed and scanned for their cell number). Columns marked with different letters denote significant difference (P<0.05). MMC – mitomycin C

of this variability may be depend on poplar forage (67) but also on the extraction method (68) and climate (62).

Our findings have also confirmed earlier reports (58, 64) that total phenolic and flavonoid content positively correlates with total antioxidant capacity, and propolis rich with phenols and flavonoids could replace commercial preparations of butylated hydroxytoluene and butylated hydroxyanisole used in food and medicinal preparations (58).

Table 7 shows the protective effects of ethanolic propolis extracts from the Ardahan and Erzurum provinces against MMC toxicity in human lymphocytes. Interestingly, the 0.74 µmol/L concentration of MMC used as positive control was cytotoxic only after the first 24 h of exposure to lymphocytes tested with the Ardahan propolis samples. Even this cytotoxicity might actually have originated from exposure to solvent and not MMC. We think that the difference in toxicological endpoints used, namely mitotic index and MTT, may explain this anomaly. We selected the MMC concentration based on our preliminary test with mitotic index, and a cytotoxicity report on MMC against human peripheral lymphocytes from an earlier study, also based on mitotic index (31). However, tested with the MTT test, MMC turned out not to be as cytotoxic. Similar was observed in other studies (69–71).

Table 8 Protective effects of the Ardahan and Erzurum propolis against oxidation in MCF-7 cells exposed to mitomycin C

	TBARS (nmol/mL)	
Control	0.40±0.02[a]	
Solvent control	0.41±0.02[a]	
MMC (0.74 μmol/L)	0.40±0.05[a]	
Propolis + MMC	**Ardahan**	**Erzurum**
32.5 μg/mL	0.03±0.02[b]	0.03±0.02[b]
65 μg/mL	0.10±0.02[bcd]	0.05±0.01[b]
125 μg/mL	0.06±0.02[bc]	0.07±0.02[bcd]
250 μg/mL	0.09±0.01[bcd]	0.10±0.03[bcd]
500 μg/mL	0.15±0.04[cd]	0.16±0.04[d]

Data are presented as mean ± standard error (N=12 for control, solvent control, and MMC groups; N=4–6 for propolis+MMC groups). Different superscript letters denote significant difference (P<0.05). MMC – mitomycin C

Even though MMC cytotoxicity was low, the Erzurum and Ardahan propolis extracts showed their protective effects against MMC and solvent (Table 7) by restoring cell viability to normal (control) there were it was reduced. Their protective (proliferative) effect was concentration-dependent and independent of the presence of MMC. Similar effects of propolis components were reported in a Brazilian green propolis water extract (72).

Table 8 shows the protective effects of propolis from both provinces against lipid peroxidation caused by MMC. Like with human peripheral lymphocytes, MMC did not cause lipid peroxidation in the MCF-7 breast cancer cell line, which confirms earlier reports that MMC may have a low oxidative potential against cancer cells (73) and erythrocytes in Sprague-Dawley rats (74).

Propolis extracts from both provinces were the most effective at lowering lipid peroxide levels in MCF-7 cells at their lowest concentrations, and this effect generally weakened as propolis concentrations increased, especially with the Erzurum propolis (Table 8). Similar was observed in a Croatian study (75) in which propolis at 100 mg/kg showed better effect at lowering lipid peroxide levels in female CBA/Hr mice plasma than at 300 mg/kg. Antioxidants in propolis such as phenols and especially flavonoids can become oxidants as their concentrations increase (76). For example, galangin, chrysin, and pinocembrin may start to behave as electron-carriers in the presence of metals such as iron and increase oxidative stress on the cell, as reported in human gastric and lung adenocarcinoma cell lines exposed to a New Zealand propolis extract (77).

Figures 1 and 2 show the anticarcinogenic potential of *A. m. caucasica* propolis extracts from both provinces on MCF-7 human breast cancer cell line. The most effective concentrations were 65 and 125 μg/mL, and the Erzurum propolis was significantly more potent at these concentrations than even MMC, let alone the Ardahan propolis. A similar pattern was reported for propolis from East Java, Indonesia (78), and Turkish propolis samples collected from the Sakarya, Kemaliye, Çanakkale, Van, Yalova, and Ankara

Table 9 Antimicrobial activities of the ethanolic extracts of *A. m. caucasica* propolis samples from the Ardahan and Erzurum provinces of Turkey

Microorganism	Ardahan (in millimetres)			Erzurum (in millimetres)			Gen	Amp	Ery	Pen
	50 μg	100 μg	150 μg	50 μg	100 μg	150 μg				
Klebsiella pneumoniae	8.07±1.22	9.00±1.29	9.38±1.28	7.94±0.36	8.52±0.47	9.56±0.97	24.25±1.20	9.22±0.67	31.64±1.56	9.62±0.36
Staphylococcus aureus ATCC 6538	8.10±0.23	10.84±2.36	12.46±3.42	-	8.90±1.41	9.80±1.11	23.81±0.15	10.68±1.03	30.99±0.70	10.54±1.22
Bacillus megaterium DSM 32	7.52±0.49	9.44±0.59	11.96±1.22	-	7.72±0.80	9.16±1.20	24.73±1.41	10.03±0.78	31.07±1.19	10.35±0.50
Pseudomonas aeruginosa ATCC 9027	-	-	-	-	-	-	28.02±0.11	13.92±0.68	26.83±0.79	13.92±0.94
Candida albicans	-	-	-	-	-	-	-	-	-	-
Yarrowia lipolytica	-	-	-	-	-	-	-	-	-	-

Data are presented as mean±standard deviation. Amp – ampicillin; Ery – erythromycin; Gen – gentamicin; Pen – penicillin

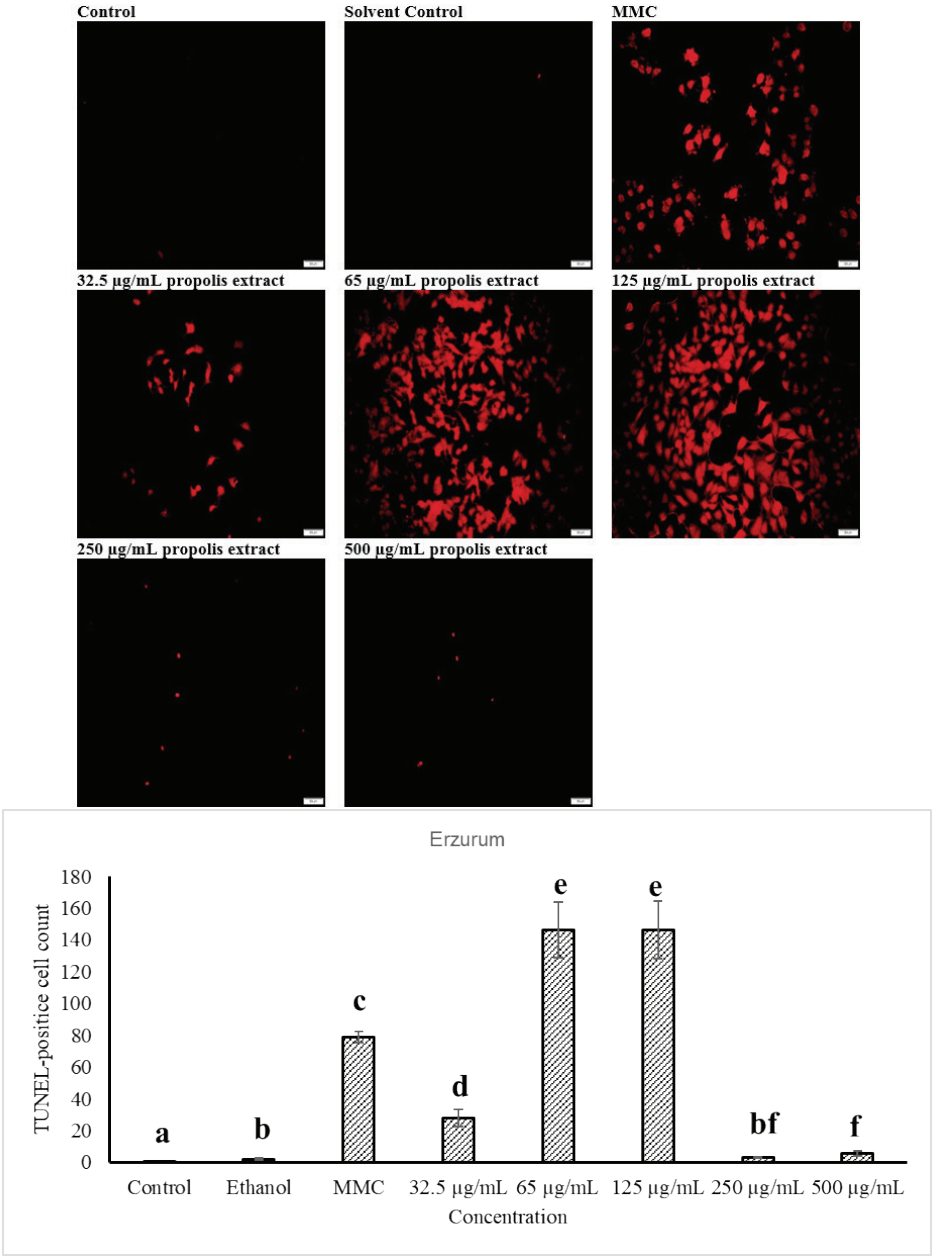

Figure 2 Apoptotic effects of *A. m. caucasica* propolis extracts from the Erzurum province of Turkey on MCF-7 cancer cells **A** (visualised with TUNEL, 20x magnification); **B** Apoptotic cell count (mean±standard error) (Minimum 10, maximum 35 regions were photographed and scanned for their cell number). Columns marked with different letters denote significant difference ($P<0.05$). MMC – mitomycin C

Table 10 Minimal inhibition concentrations of the ethanolic extracts of *A. m. caucasica* propolis samples from the Ardahan and Erzurum provinces of Turkey

Microorganism	Ardahan (µg/mL)	Erzurum (µg/mL)
Klebsiella pneumoniae	30.71	43.37
Staphylococcus aureus ATCC 6538	30.71	43.37
Bacillus megaterium DSM 32	30.71	43.37

provinces (79). The latter report suggests that lower propolis concentrations seem to trigger the extrinsic apoptotic pathway by inducing caspase-8 activity, while higher concentrations cause necrotic death, which cannot be detected by the TUNEL assay. Similarly, Szliszka and Krol (80) suggest that flavonoid and phenolic components in propolis cause apoptosis in cancer cells by increasing the TNF-related apoptosis-inducing ligand (TRAIL) activity, which is associated with caspase-8 activity. Another study (81) showed that caffeic acid phenethyl ester in propolis can trigger the extrinsic apoptotic pathway by inducing TRAIL activity in human hepatocellular carcinoma cells. There is also evidence that cinnamic acid derivatives found in propolis, such as artepillin C, baccharin, and drupanin, induced both extrinsic and intrinsic apoptotic death in human colon cancer cells (82).

Considering, however, that the TUNEL assay can only determine apoptotic cells, further mechanistic studies should determine the fate of cells exposed to propolis concentrations higher than 125 µg/mL, test our assumption of a necrosis pathway, and give a more comprehensive idea about the anticarcinogenic effects of propolis.

Tables 9 and 10 show that the Ardahan propolis was more potent against *K. pneumoniae*, *S. aureus*, and *B. megaterium* DSM 32 than the Erzurum propolis, but neither was effective against *P. aeruginosa* or the fungi *C. albicans* and *Y. lipolytica*. The higher antibacterial activity of the Ardahan propolis is probably related to its higher sugar content (83). Antimicrobial activity of propolis is also associated with its phenolic, flavonoid, and ester content, according to some reports (84, 85), which may explain the difference in antibacterial activity between the Ardahan and Erzurum propolis samples in our study but not the absence of antifungal effect in either, even though one Brazilian (86) and one Turkish study (87) reported a significant antifungal effect. One study from Chile (88) showed different phenolic/flavonoid content and antimicrobial activities of propolis from six locations but found no clear correlation between the two.

In conclusion, our findings confirm that geographical differences are important for the chemical composition of propolis and the related biological activity. Both the Ardahan and Erzurum propolis samples were produced by the same Caucasian bee race yet showed different fatty acid, phenolic, flavonoid, and other organic content. Higher sugar, flavonoid, and phenolic content of the Ardahan propolis may have contributed to its higher antioxidant and antibacterial properties. In turn, the Erzurum propolis showed higher anticarcinogenic potential, but this aspect requires further investigation to include the cell necrosis pathway.

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Conflict of interests

None to declare.

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Usporedba kemijskih i bioloških značajki propolisa pčelinje pasmine *Apis mellifera caucasica* iz turskih provincija Ardahana i Erzuruma

Cilj je ovoga istraživanja bio usporediti biološku aktivnost etanolnih ekstrakata propolisa pčelinje pasmine *Apis mellifera caucasica* iz dviju turskih provincija: Ardahana i Erzuruma. Testirana su njihova antioksidacijska, anticitotoksična, antikancerogena, antibakterijska i antifungalna svojstva. Uzorci iz tih dviju provincija razlikovali su se u mineralnom i organskom sastavu koji je odražavao njihovo zemljopisno podrijetlo. Test redukcije željeza/antioksidacijske snage (engl. *ferric reducing antioxidant power*, krat. FRAP) otkrio je superiornost ardahanskoga propolisa nad erzurumskim, no bez obzira na podrijetlo i prisutnost mitomicina C u mediju, oba su propolisa povećala vijabilnost ljudskih perifernih limfocita, a učinak je ovisio o koncentraciji i trajanju. Propolis iz Erzuruma iskazao je najveću antikancerogenu aktivnost u koncentracijama od 62,5 i 125 μg/mL, no ona se smanjila s višim koncentracijama. Oba su propolisa također iskazala antibakterijsku aktivnost sličnu ampicilinskoj i penicilinskoj kontroli, osim kad se radilo o bakteriji *Pseudomonas aeruginosa*. Međutim, oba su zakazala protiv plijesni *Candida albicans* i *Yarrowia lipolytica*. Može se zaključiti da uzorci propolisa iz obiju provincija pružaju obećavajuće biološke aktivnosti, no u daljnja istraživanja, koja se trebaju usmjeriti na traženje optimalnih koncentracija za postizanje željenog učinka, treba uključiti i nekrotični put u mehanizmu djelovanja kako bi se stekao bolji uvid u njihovo antikancerogeno djelovanje.

KLJUČNE RIJEČI: antikancerogeno djelovanje; antimikrobno djelovanje; biološka aktivnost; minerali; mitomicin C; organski sastav