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Determination of natural radioactivity levels and gamma radiation attenuation coefficients in propolis samples and the study of its antioxidant properties

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Abstract: Thanks to its rich content, propolis has been used to protect the hive from all kinds of external influences and for disinfection by bees. Furthermore, it is an important marker for monitoring environmental pollution because the main sources of propolis are plant and secretions. So, the present study aimed to research radiation attenuation capability and the natural radioactivity level of propolis samples. For this reason, both natural radioactivity concentrations (^{226}Ra, ^{232}Th and ^{40}K) and attenuation coefficients (Linear and Mass) in the propolis samples collected from 10 different points in Turkey were measured using high purity germanium detector (HPGe). The average natural radioactivity concentrations in samples were found to be 0.56 ± 0.19, 2.65 ± 0.31 and 70.08 ± 2.42 Bq/kg for ^{226}Ra, ^{232}Th and ^{40}K, respectively. These values were much lower than the average world values (35 Bq/kg for ^{226}Ra, 30 Bq/kg for ^{232}Th and 400 Bq/kg for ^{40}K) reported by United Nations Scientific Committee on Effects of Atomic Radiation (UNSCEAR) in foodstuff. The average linear attenuation coefficient, mass attenuation coefficients and half value layer values for gamma rays with 59.54 keV energy were determined as 0.1970 cm^{-1}, 0.1831 $cm^2 g^{-1}$ and 3.56 cm, respectively. In addition, antioxidant properties of the samples were measured using total phenolic content and ferric reducing antioxidant power. Their correlations with radioactivity were investigated.

***Corresponding author: Serdar Dizman**, Department of Physics, Faculty of Arts and Sciences, Recep Tayyip Erdogan University, Rize, 53100, Turkey, E-mail: serdar.dizman@erdogan.edu.tr
Oktay Yildiz: Department of Biochemistry, Basic Pharmaceutical Sciences, Faculty of Pharmacy, Karadeniz Technical University, Trabzon, 61750, Turkey, E-mail: oktayyildiz29@hotmail.com
Gülsüm Merve Boyraci: Department of Food Technology, Maçka Vocational High School, Karadeniz Technical University, Trabzon, 61750, Turkey, E-mail: merveturkut@ktu.edu.tr
Sevgi Kolayli: Department of Chemistry, Faculty of Sciences, Karadeniz Technical University, Trabzon, 61080, Turkey, E-mail: skolayli61@yahoo.com

Keywords: antioxidant capacity; attenuation coefficients; gamma radiation; propolis; radioactivity.

1 Introduction

Human beings are continuously exposed to ionizing radiation from cosmic rays and naturally occurring radioactive substances existing in a crust of the Earth. The naturally occurring radionuclides, especially potassium and the radionuclides of uranium and thorium series, are the major sources of natural radiation exposure to the human being [1]. Natural radionuclide concentrations in environmental samples can be very different due to geographical and geological factors [2]. Radioactive particles may accumulate in the soil and then they may transfer to the crops grown on it. Finally, they may contaminate bees and bee products [3, 4]. Therefore, it is very important to know the concentration levels of natural radionuclides received by the foodstuffs.

The studies of interactions of radiation with different materials are very important in basic physics. Today, radiation protection is used in a wide variety of areas like shielding materials for the radiation which are important in applied nuclear radiation fields such as radiation therapy and radiation health physics [5]. Scientists are look for natural and unnatural protective materials against radiation. Many experimental and theoretical works have been performed on radiation shielding with different materials (e.g., fabric, wood, polymer, alloy, colemanite, etc.) [5–8].

Some natural products are able to inhibit radiation effects. Propolis is a good candidate for it [9]. Propolis or bee glue is a kind of resinous substance collected from plant buds and shoots by honey bees. The actual meaning of propolis is a defense of hive in Greek and is responsible for the protection of bees and hives from many environmental pollutions, viruses, bacteria, insects, and provides hive isolation. It is also an important barrier in protecting the hives [10, 11]. Although the composition of propolis depends on varies conditions, like floral sources and geological characteristics, it generally contains many pharmaceutical compounds, such as aromatic acids (i.e., cinnamic acid, caffeic acid, ferulic acid), aromatic esters (caffeic acid phenyl esters), volatile compounds (geraniol, nerol, farnesol, β-eudesmol),

hydrocarbons (e.g., eicosane, tricosane, pentacosan), steroids (cholinasterol, stigmasterol), flavonoids (pinobanksin, pinocembrin, chrysin, galangin, apigenin, kaempferol) and macronutrients (Ca, K, Mg, Na, Zn, Fe, Mn, Al, Ba, Cl, etc.) [10, 12, 13]. It is known that propolis has high antioxidant and free radicals scavenging activities thanks to its high polyphenols content. For this reason, propolis is used as a food additive in food technology. Propolis is thought to protect the hives also against radioactive spills. However, it was reported that water-soluble propolis extracts protect mice from irradiation-induced damage and reduced tumor formation [9]. Bees and its products may be used in the monitoring of environmental pollution of radioactive elements due to its capacity to reflect the environmental condition [14].

Up to now, in Turkey, there is no study with propolis about radioisotope measurements and gamma radiation attenuation coefficients. The present study is aimed towards measuring three abundant radionuclides and gamma radiation attenuation capacities of propolis samples along with the study of its antioxidant properties. In addition, considering literature, it was found that there are no studies about color analysis of propolis extract. The findings can be used as baseline data for propolis samples and will also be a reference to future studies.

2 Materials and methods

2.1 Collection of samples

The studied propolis samples were collected from 10 different locations in Turkey in 2016 (Table 1). Samples were collected from the traps of propolis and stored in the freezer until the analysis stage.

2.2 Preparation of samples for radioactivity studies

Since the propolis samples were hard at room temperature, it was heated at 105 °C for about 1 h. The molten propolis samples were transferred into uncontaminated empty cylindrical plastic containers of uniform size (60 mL) and then weighed. The prepared samples were kept for four weeks before the analysis at air tight condition to allow secular equilibrium between radium and thorium and their decay products.

2.3 Preparation of samples for attenuation measurements

For the preparation of absorber samples, some of the melted propolis samples for radioactivity measurements were cast into a plastic mold to achieve desired thickness (0.50 cm) and diameter (2.14 cm).

2.4 Radioactivity measurements

The radiation levels of the samples were analyzed using gamma spectrometry which was equipped with a 55% efficiency high-purity germanium (ORTEC HPGe, Model No: GEM55P4-95) detector and a multichannel analyzer. The resolution (Full Width Half Maximum) of this detector system was 1.0 for 122 keV gamma ray of ^{57}Co and 1.9 for 1332 keV gamma ray of ^{60}Co. The detector was shielded by a cylindrical lead shield, which had a thickness of 10 cm to reduce the background level of the system. The efficiency of the detector was determined with a ^{152}Eu source with known activity. ^{152}Eu sources have been widely used for energy calibration and efficiency determination because of their large range of energies (122, 244, 344, 411, 443, 779, 964, 1112 and 1408 keV) with emission probabilities of 3%–29% [15]. An ideal measuring geometry of cylindrical source (a homogeneously distributed activity with constant volume and distance) was placed coaxially with the detector for the efficiency determination and the same procedure was applied for the sample measurements.

The activity concentration of ^{226}Ra was determined by averaging the measured concentrations for ^{214}Pb (295 and 351 keV gamma-ray energies) and ^{214}Bi (609 and 1120 keV gamma-ray energies). The activity concentration of ^{232}Th was determined by averaging the measured concentrations for ^{212}Pb (238 keV gamma-ray energy), ^{228}Ac (338 and 911 keV gamma-ray energies) and ^{208}Tl (583 keV gamma-ray energy) [16]. The activity concentration of ^{40}K was determined directly from the 1460 keV gamma-ray energy [16, 17]. The gamma spectra were analyzed using Gamma Vision, which is a data acquisition and analysis program.

Table 1: Total phenolic contents and antioxidant properties of the propolis samples.

Sample name (Code)	Total phenolic content (mg GAE/g)	FRAP antioxidant capacity (μmol $FeSO_4$*$7H_2O$/g)	DPPH radical scavenging activity (μg/mL) SC_{50}
Ardahan/Posof (P1)	202.90 ± 2.17^{i}	126.78 ± 1.48^{d}	72.10 ± 2.10^{b}
Ardahan/Çıldır (P2)	76.60 ± 0.36^{d}	84.27 ± 2.21^{c}	59.40 ± 0.20^{b}
Artvin/Murgul (P3)	14.76 ± 0.22^{b}	10.96 ± 0.96^{a}	384.30 ± 8.20^{d}
Trabzon (P4)	134.48 ± 4.71^{f}	176.89 ± 1.801^{g}	25.05 ± 0.20^{a}
Giresun/Keşap (P5)	201.37 ± 3.59^{i}	137.23 ± 4.43^{e}	61.00 ± 0.20^{b}
Artvin/Center (P6)	8.29 ± 0.20^{a}	5.48 ± 0.11^{a}	695.01 ± 38.30^{e}
Düzce (P7)	158.09 ± 1.68^{g}	194.37 ± 2.95^{h}	30.05 ± 2.05^{a}
Zonguldak (P8)	179.84 ± 4.35^{h}	128.88 ± 13.28^{d}	31.40 ± 2.20^{a}
Balıkesir (P9)	121.48 ± 3.22^{e}	161.23 ± 2.09^{f}	27.33 ± 0.20^{a}
Bursa (P10)	60.11 ± 3.78^{c}	63.39 ± 3.69^{b}	112.60 ± 0.80^{c}

Different letters (a–g) in the same columns are significantly different at the 5% level ($P < 0.05$).
Average values are expressed as mean±S.D. of three replicate determinations.

The samples were placed symmetrically on top of the detector and measured for a period of 80,000 s. After subtraction of the background from measurements, the activity concentrations of the radionuclides were calculated using Eq. (1).

$$A(Bq/kg) = \frac{C_s}{\varepsilon.P_\gamma.m.t} \quad (1)$$

where C_s is the peak area under the corresponding peak, $\mathcal{E}$ is the detector efficiency at the corresponding peak energy, P_γ is the absolute emission probability of the specific γ-ray at the corresponding peak energy, m is the mass of the sample (kg) and t is the counting time (s).

The minimum detectable activity (MDA) values for related radioisotopes in the present measurement system were calculated using Eq. (2) [18, 19].

$$MDA\ (Bq/kg) = \frac{2.71 + 4.65\sqrt{C_B}}{\varepsilon.P_\gamma.m.t} \quad (2)$$

where, C_B is the peak area under the background peak, $\mathcal{E}$ is the detector efficiency at the corresponding peak energy, P_γ is the absolute emission probability of the specific γ-ray at the corresponding peak energy, m is the mass of the sample (kg) and t is the counting time (s).

2.5 Attenuation measurements

The linear and mass attenuation coefficients of the propolis samples were measured by performing a gamma transmission experiment in the narrow beam geometry as shown in Figure 1. Since vegetable products like propolis have a low density, low energy rays should be used to absorb more beams. Thus, sufficient data can be obtained for absorption calculations. In this context, a cylindrical radioactive ^{241}Am point source with 370 MBq activity was used as the gamma-ray source. ^{241}Am emits gamma-ray at 59.54 keV (35.9%) and enables to study the attenuation at low energy. The radioactive point source is collimated by a lead collimator in order to obtain a photon beam perpendicular to the detector window. The propolis samples were irradiated with ^{241}Am radioactive gamma-ray source. The intensities of gamma-rays are measured using a High Purity Germanium Detector (HPGe). For measurements, each of the propolis samples was placed between gamma ray detector and ^{241}Am radioactive source. Gamma-ray intensity for each sample was counted three times for a fixed time period of 1200 s. The gamma spectra obtained as a result of measurements were analyzed by Gamma Vision software for determination of the photo-peak areas.

2.6 Calculation of total mass attenuation coefficient and linear attenuation coefficient

If an absorbent material is irradiated with gamma beam, the intensity of the beam will be attenuated by the absorbent material. In this case, the linear attenuation coefficients of the absorber material are calculated according to the Beer–Lambert's law Eq. (3) [20]:

$$I = I_0 e^{-\mu x} \quad (3)$$

where I_0 is the number of counts recorded in the detector without attenuation (with empty mold), I is the number of counts recorded in the detector when there is a absorber, μ is the linear attenuation coefficient (cm^{-1}), x is the thickness of the material in cm.

A more convenient parameter characterizing a given material is the mass attenuation coefficient (μ_m) and the relationship between μ and μ_m is given by the Eq. (4) [21]:

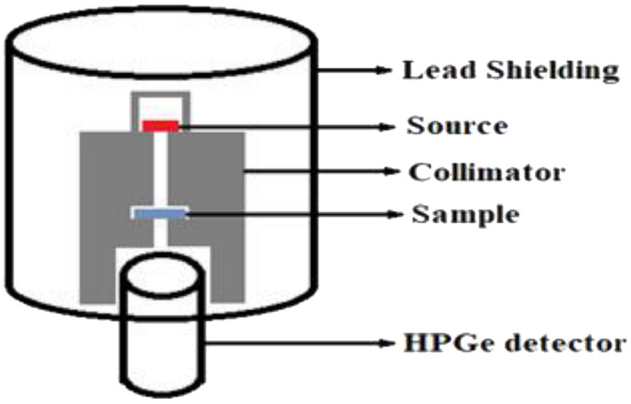

Figure 1: Experimental setup for measuring gamma-ray attenuation.

$$\mu_m = \mu/\rho \quad (4)$$

where μ_m is the mass attenuation coefficient of the material ($cm^2\ g^{-1}$); μ is the linear attenuation coefficient (cm^{-1}) and ρ is the density of the material (g/cm^3), which depends upon the physical state of the material.

Another parameter for the shielding materials is Half Value Layer (HVL). HVL is the thickness of the shielding materials necessary to reduce the intensity of the gamma-ray to half and it is calculated using Eq. (5) [22].

$$HVL\ (cm) = \frac{Ln\ 2}{\mu} \quad (5)$$

where μ (cm^{-1}) is the linear attenuation coefficient of the shielding material.

The experimental procedure used in this study was checked out by measuring the mass attenuation coefficient of pure cobalt of thickness 0.07 mm in the same gamma-ray energy (59.5 keV). The experimental mass attenuation coefficient of cobalt was found as 1.3329 $cm^2\ g^{-1}$. The theoretical mass attenuation coefficient of cobalt was calculated (1.3430 $cm^2\ g^{-1}$) using the NIST XCOM database [23]. It has been clearly seen that there is a very good concordance (relative error: 0.75%) between the experimental and theoretical mass attenuation coefficients.

2.7 Determination of antioxidant activities

2.7.1 Preparation of samples for biochemical analyses: The raw propolis samples were frozen then grounded to a fine powder and ethanolic extract solutions were prepared with powdered propolis/ethanol (70%) ratio of 1:10 (w/v). The mixtures were subsequently carried out in a shaker (HeidolphPromax 2020, Schwabach, Germany) at room temperature for 24 h. The suspensions were centrifuged (1000 rpm) for 10 min at room temperature. The supernatants were filtered through Whatman grade No.4 filter paper and the liquid filtrated was kept at 4 °C until the analysis.

2.7.2 Total phenolic content: The total phenolic content of the ethanolic extract was measured by Folin-Ciocalteu reagents using the gallic acid standard [24]. About 20 µL propolis extract was diluted to 680 mL with water in a plastic vial and 400 mL of 0.2 N Folin-Ciocalteu reagent was added and then vortexed. The mixture was allowed to react for 5 min. Then 400 mL Na_2CO_3 (7.5%) solution was added and

incubated for 1 h at room temperature in the dark. A absorbance was read at 760 nm using a UV spectrophotometer (Shimadzu UV-2450, Shimadzu Corporation, Kyoto, Japan). Results were expressed as mg of gallic acid equivalents per g samples.

2.7.3 Ferric reducing antioxidant power: Ferric reducing/antioxidant power (FRAP) method is based on the reduction of ferric-tripyridyltriazine (Fe(III)-TPTZ) complex to ferrous tripyridyltriazine (Fe(II)-TPTZ) [25]. The freshly prepared FRAP reagent included 300 mM acetate buffer, pH 3.6, 10 mM TPTZ in 40 mM HCl and 20 mM $FeCl_3$ $6H_2O$ solution in the ratio 10:1:1 (v/v/v). Next, the propolis extracts (100 µL) were allowed to react with 3 mL of the FRAP reagent for 4 min at 37 °C in the dark condition. The absorbance was measured at 595 nm against reagent blank containing distilled water. Results are expressed in µmol $FeSO_4*7H_2O$/g propolis. Higher the FRAP value means higher antioxidant capacity of the samples.

2.7.4 DPPH free radical-scavenging antioxidant activity: Free radical scavenging activity of DPPH (α,α-diphenyl-β-picrylhydrazyl) was measured by the method of Molyneux [26]. Briefly, 0.75 mL of 0.1 mM DPPH in ethanol was mixed with 0.75 mL propolis extracts, and incubated in the dark for 50 min. When DPPH reacts with an antioxidant compound, the color changes from deep violet to light yellow. After incubation, thus the change of the absorbance is monitored at 517 nm in the presence of different sample concentrations in order to obtain anti-radical curves for calculating the SC_{50} values. SC_{50} value represents the concentration of the extract (mg/mL) required to inhibit 50% of the free radicals. Higher SC_{50} value indicates lower radical scavenging activity.

2.7.5 Color analyses: The raw propolis color was measured using Hunter Lab Color Flex (Hunter Associates Inc., Reston, A., USA). The parameters calculated were L (black/white), a (redness/greenness) and b (yellowness/blueness). The L value ranges from 0 (black) to 100 (white), positive a and negative a value indicate the degree of redness and greenness, respectively. And also, positive b indicates yellowness, whereas negative b indicates blueness.

2.7.6 Statistical analysis: All analyses were performed in triplicate and the results were expressed as means ± standard deviation (SD). One-way analysis of variance (ANOVA) followed by post-hoc Duncan's test was used to determine the significant differences ($P \leq 0.05$) for all analyses (SPSS software version 21.0). Correlations between data were calculated using Pearson's correlation coefficient (r). The uncertainties of all measurements were calculated taking into account statistical fluctuations of the peak, the backgrounds and efficiency calibration. The radioactivity concentration of each sample was reported as means ± standard deviation. The minimum and maximum values for each examined radioisotope were also given.

3 Results

3.1 Total phenolic and antioxidant activity

Ten different regions of propolis samples from Turkey were used in this work and the total phenolic content of the samples was given in Table 1. Many studies indicate that hydroalcoholic solvent (ethanol) is the most suitable solvent for extraction of biologically active compounds in propolis. Also, ethanol is the best known solvent for bioactivity tests. It is assumed that it dissolves all the phenolic structures in the structure [10, 12, 27]. In this study, it was considered that 100% of the phenolic compounds were extracted into the ethanol so the total phenolic content was expressed as mg GAE/g propolis. Total phenolic content of the samples varied in a wide range from 8.29 to 202.9 mg GAE/g of raw sample. The statistically highest total phenolic contents were P1 (Ardahan/Posof) and P5 (Giresun/Kesap) and the lowest sample was P6 (Artvin/Center).

Antioxidant properties were measured by ferric-reducing antioxidant power and DPPH radical scavenging tests in the ethanolic extracts of the propolis. The results are presented in Table 1. The FRAP assay is one of the most widely used tests for total antioxidant capacity, and high FRAP shows high antioxidant capacity. The FRAP values of the propolis extracts are ranged from 5.5 to 194.4 µmol $FeSO_4*7H_2O$/g. According to the results, the highest FRAP value was detected in P7 (Düzce) sample, and the lowest values were detected P3 (Artvin/Murgul) and P6 (Artvin). The FRAP values demonstrated a significant difference as well as the total phenolic contents ($P < 0.05$).

DPPH radical scavenging test is used to define the antioxidant capacity of the samples that is another widely used assay for natural compounds, and values are expressed as SC_{50} (µg/mL) [28]. The SC_{50} value is the amount of extract that scavenges 50% of the radical [26]. DPPH results revealed that the SC_{50} value of the propolis extracts varied between 0.025 and 0.695 mg/mL. Samples of P4 (Trabzon), P7 (Düzce), P8 (Zonguldak) and P9 (Balıkesir) samples have the highest scavenging value statistically with lower SC_{50} values, indicating the higher antioxidant activity, and samples of P6 (Artvin) has the lowest scavenging value statistically.

The correlation between TPC and FRAP and TPC and DPPH were found very strong with correlation coefficients (r) of 0.808 and −0.740, respectively. The same situation was observed between FRAP and DPPH values with (r) -0.816. The correlation indicates that the high phenolic contents cause the high antioxidant capacity of propolis [29].

3.2 Radioactivity concentrations

The minimum detectable activity (MDA) values for ^{226}Ra, ^{232}Th and ^{40}K radionuclides in the present measurement system are calculated as 0.33, 0.38 and 2.36 Bq/kg, respectively. The natural radioactivity (^{226}Ra, ^{232}Th and ^{40}K) results of the propolis samples are given in Table 2. The

natural radioactivity levels found for these propolis samples are also shown graphically in Figure 2.

In the analyzed samples, ^{232}Th and ^{40}K radionuclides ranged from 1.42-3.89 and 35.93-122.54 Bq/kg, respectively. Among the 10 propolis samples studied, only three propolis samples (P5-P7) contain ^{226}Ra radionuclide. The others are below the MDA (P1–P4, P8–P10). The average activity concentrations of ^{226}Ra, ^{232}Th and ^{40}K radionuclides in the propolis samples were found as 0.56 ± 0.19, 2.65 ± 0.31 and 70.08 ± 2.42 Bq/kg, respectively.

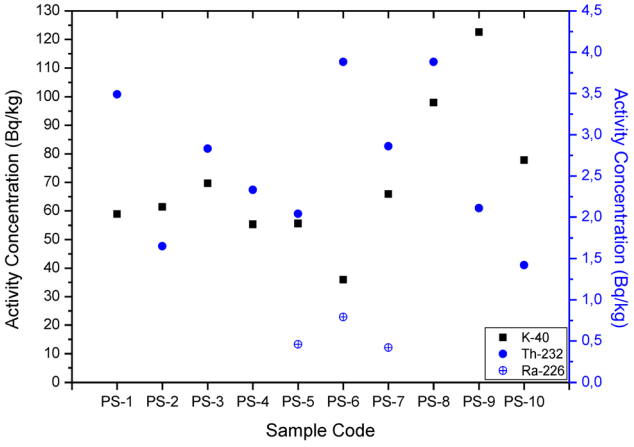

Figure 2: Natural radioactivity concentrations in the propolis samples (left axis: ^{40}K concentrations; right axis: ^{226}Ra and ^{232}Th concentrations).

3.3 Attenuation coefficients

The linear and mass attenuation coefficients of the samples were measured three times for photons with 59.5 keV energy by using the gamma transmission method. HVL values of the propolis samples were also calculated. The linear attenuation coefficients, mass attenuation coefficients and HVL values found for the propolis samples are given in Table 3. As shown in Table 3, values of LAC were ranged from 0.1627 to 0.2392 cm^{-1}, values of MAC were ranged from 0.1641 to 0.2138 cm^2 g^{-1} and value of HVL were ranged from 2.90 to 4.26 cm. The average values of the linear attenuation coefficients, mass attenuation coefficients and HVL values of the propolis samples were found as 0.1970 cm^{-1}, 0.1831 cm^2 g^{-1} and 3.56 cm, respectively. These differences in the samples are due to the change in the contents of propolis from region to region.

In this study, we also measured and compared correlations with antioxidant capacities and MAC and LAC values, and also propolis colors. The correlations are given in Table 4. There is a strong correlation among the three radioactivity parameters, correlation coefficient (r, $P < 0.05$) is 0.783 between MAC and LAC, –0.752 between MAC and HVL, and –0.992 between HVL and LAC. Although a high correlation is found between HLV and DPPH radical scavenging activity (r: 0.480, $P < 0.05$), moderately correlation is found between total phenolic contents and HVL (r: –0.364, $P < 0.05$). No significant correlations were found between FRAP and radioactivity parameters of LAC, MAC and HVL.

Table 2: Activity concentrations of ^{226}Ra, ^{232}Th and ^{40}K radionuclides in the propolis samples.

Sample code	^{226}Ra (Bq/kg)	^{232}Th (Bq/kg)	^{40}K (Bq/kg)
P1	<MDA	3.49 ± 0.18^{e}	58.84 ± 2.06^{bc}
P2	<MDA	1.65 ± 0.37^{ab}	61.39 ± 2.23^{c}
P3	<MDA	2.83 ± 0.27^{d}	69.63 ± 2.31^{d}
P4	<MDA	2.33 ± 0.16^{cd}	55.32 ± 1.99^{b}
P5	0.46 ± 0.15^{b}	2.04 ± 0.19^{bc}	55.56 ± 1.86^{b}
P6	0.79 ± 0.22^{c}	3.88 ± 0.37^{e}	35.93 ± 1.84^{a}
P7	0.42 ± 0.19^{a}	2.86 ± 0.39^{d}	65.85 ± 2.09^{d}
P8	<MDA	3.89 ± 0.51^{e}	97.96 ± 2.44^{f}
P9	<MDA	2.11 ± 0.37^{bc}	122.54 ± 2.99^{g}
P10	<MDA	1.42 ± 0.24^{a}	77.75 ± 4.35^{e}
Average	0.56 ± 0.19	2.65 ± 0.31	70.08 ± 2.42
Minimum	<MDA	1.42	35.93
Maximum	0.79	3.89	122.54

MDA: Minimum Detectable Activity. Different letters (a–g) in the same columns are significantly different at the 5% level ($P < 0.05$). Average values are expressed as mean±S.D. of three replicate measurement values.

The correlation among the phenolic content, antioxidant activity and color parameters of the propolis samples has also been investigated (Table 4). Color is an important physical parameter for propolis and honey. Mostly, high polyphenol contented natural products are darker [30]. For this reason, Hunter color parameters (Lab) were measured of the ingested propolis samples (Table 5). A strong correlation is found between total phenolic content and antioxidant activities. According to L (lightness) values, it is not seen a (redness/greenness) and b (yellowness/blueness). Furthermore, statistically significant correlations were found between b (yellowness/blueness) value and all radioactivity parameters.

4 Discussions

4.1 Total phenolic and antioxidant activity

One of the most important substances that exhibit biological activity among the components of propolis are the phenolic substances. Herein, the total phenolic content of propolis samples was investigated which are ranged

Table 3: The linear attenuation coefficients (LAC), mass attenuation coefficients (MAC) and half-value layer (HVL) of the propolis samples.

Sample Code	LAC (cm^{-1})	MAC ($cm^2\ g^{-1}$)	HVL (cm)
P1	0.2218 ± 0.0001^{i}	0.2138 ± 0.0001^{i}	3.12 ± 0.0050^{b}
P2	0.1792 ± 0.0001^{c}	0.1793 ± 0.0001^{e}	3.87 ± 0.0070^{g}
P3	0.2056 ± 0.0002^{g}	0.1863 ± 0.0002^{f}	3.37 ± 0.0020^{c}
P4	0.2392 ± 0.0002^{j}	0.1999 ± 0.0001^{h}	2.90 ± 0.0010^{a}
P5	0.1963 ± 0.0001^{f}	0.1751 ± 0.0001^{c}	3.53 ± 0.0060^{d}
P6	0.1627 ± 0.0001^{a}	0.1754 ± 0.0002^{d}	4.26 ± 0.0020^{i}
P7	0.1774 ± 0.0001^{b}	0.1655 ± 0.0002^{b}	3.91 ± 0.0060^{h}
P8	0.1935 ± 0.0002^{e}	0.1793 ± 0.0001^{e}	3.58 ± 0.0030^{e}
P9	0.1886 ± 0.0002^{d}	0.1641 ± 0.0001^{a}	3.67 ± 0.0010^{f}
P10	0.2059 ± 0.0002^{h}	0.1925 ± 0.0002^{g}	3.37 ± 0.0030^{c}
Mean	0.1970 ± 0.0002	0.1831 ± 0.0001	3.56 ± 0.0003
Min.	0.1627	0.1641	2.90
Max.	0.2392	0.2138	4.26

Different letters (a–h) in the same columns are significantly different at the 5% level ($P < 0.05$).
Average values are expressed as mean±S.D. of three replicate determinations.

Table 5: Hunter Lab values of ingested propolis samples.

Sample	L*	a*	b*
P1	71.04 ± 0.41^{b}	-0.26 ± 0.11^{a}	9.40 ± 0.04^{c}
P2	74.73 ± 0.41^{d}	10.28 ± 0.14^{h}	17.02 ± 0.31^{g}
P3	82.17 ± 0.02^{h}	8.65 ± 0.02^{g}	11.80 ± 0.02^{d}
P4	78.04 ± 0.13^{g}	1.18 ± 0.06^{c}	8.46 ± 0.31^{b}
P5	75.89 ± 0.01^{e}	1.50 ± 0.05^{d}	17.36 ± 0.17^{h}
P6	91.56 ± 0.02^{j}	1.00 ± 0.02^{b}	15.16 ± 0.02^{f}
P7	70.25 ± 0.33^{a}	1.25 ± 0.13^{c}	18.28 ± 0.03^{i}
P8	72.06 ± 0.14^{c}	2.94 ± 0.05^{e}	13.41 ± 0.13^{e}
P9	77.12 ± 0.14^{f}	1.12 ± 0.03^{bc}	6.66 ± 0.21^{a}
P10	87.06 ± 0.29^{i}	3.53 ± 0.11^{f}	8.30 ± 0.23^{b}

Different letters (a–h) in the same columns are significantly different at the 5% level ($P < 0.05$).
Average values are expressed as mean±S.D. of three replicate determinations.

from 8.29 to 202.90 mg GAE/g raw sample. When these results are compared with those for propolis samples from different countries, it is seen that total phenolic contents show a great difference depending on the collection region. The quality parameters of propolis have long been discussed and it has been reported that the total amount of phenolic compounds and total flavone and flavonol content, total flavanone and dihydroflavonol content that were related with the total phenolic contents should be taken as a reference [31]. However, in a Portugal propolis study, it is shown that total phenolic content ranged from 151 to 329 mg GAE/g [32], and in another group of Portugal samples, it ranged from 87 to 277 mg GAE/100 g [27], in Brazilian propolis, it ranged from 27 to 55 mg GAE/100 g [33], in Turkish propolis, it ranged from 115 to 210 mg GAE/g [34]. In general, polyphenols are generally responsible for the biological activities in bee products as well as natural products. Higher phenolic content indicates higher antioxidant, anti-inflammatory, antimicrobial and antitumoral activities [35, 36]. Therefore, polyphenol family is one of the active ingredients of propolis and its amount varies between 2% and 20% according to the propolis type [37].

In Table 1, ferric reducing antioxidant power (FRAP) and DPPH free radical-scavenging activity were summarised. Total phenolic content in the analyzed propolis extracts ranged from 5.479 to 194.367 μmol $FeSO_4*7H_2O$/g. Our FRAP values of the propolis extracts fall in the range reported in literature that changes from 1.57 to 1365 μmol $FeSO_4*7H_2O$/g [38–40]. Another determined antioxidant

Table 4: Pearson's correlation coefficients of antioxidant activities, total phenolics, Linear Attenuation coefficient (LAC), Mass Attenuation coefficient (MAC), Half Value Layer (HVL) and color parameters.

	TPC	FRAP	DPPH	LAC	MAC	HVL	L	a	b
TPC	1								
FRAP	0.808**	1							
DPPH	−0.740**	−0.816**	1						
LAC	0.328^{ns}	0.289^{ns}	−0.423*	1					
MAC	0.133^{ns}	-0.105^{ns}	-0.076^{ns}	0.783**	1				
HVL	−0.364*	-0.300^{ns}	0.480**	−0.992**	−0.752**	1			
L	−0.817**	−0.755**	0.775**	-0.198^{ns}	-0.014^{ns}	0.244^{ns}	1		
a	−0.523**	−0.489**	0.089^{ns}	-0.161^{ns}	-0.085^{ns}	0.127^{ns}	0.084^{ns}	1	
b	0.065^{ns}	-0.045^{ns}	0.151^{ns}	−0.620**	−0.464**	0.620**	-0.199^{ns}	0.239^{ns}	1

^{ns}non significant and *,** significant at $P < 0.05$ or 0.01, respectively.
Average values are expressed as mean±S.D. of three replicate determinations.

Table 6: Comparison of Linear Attenuation coefficient (LAC) and Mass Attenuation coefficient (MAC) in different materials with the propolis.

Sample	LAC (cm^{-1})	MAC ($cm^2 g^{-1}$)	References
Wood (*Teminalia alata*)	0.1360	0.193	[8]
Polypropylene		0.131	[53]
Polyamide		0.104	[53]
Polyethylene		0.116	[53]
Steel		1.230	[54]
Pearl		0.355	[54]
Mica		0.195	[54]
Wood (Aroeira)		0.179	[55]
Propolis samples	0.1971	0.183	This study

test was DPPH, the SC_{50} content of which ranged from 0.025 to 0.695 mg/mL. Some studies have stated that SC_{50} value ranged from 0.013 to 0.371 mg/mL for ethanolic propolis extracts, these findings are also related to our values [40–44]. In addition, both antioxidant results are found related to the total phenolic contents.

4.2 Radioactivity concentrations

In the literature, there is limited study on the determination of thorium and radium in propolis samples, but there are studies conducted in some different foodstuff [45–49]. Similar to our study, ^{137}Cs, ^{40}K and ^{7}Be radionuclides in Brazilian, Italian and Bulgarian propolis samples were measured. Among them, it is indicate that the ^{40}K radionuclide concentrations ranged from 94.56 to 176.91 Bq/kg [4]. In the same studies, while the ^{137}Cs radionuclide was not detected in the Brazilian propolis samples, it is detected as 14.33 Bq/kg in the Bulgaria-Sofia propolis samples [4]. The ^{40}K concentrations of the propolis samples in this study are lower than those of the propolis samples in Italy and Brazil. ^{40}K radionuclide concentrations range from 35.83 to 122.54 Bq/kg. The obtained radioactivity values for propolis samples are much lower than the limit values (35 Bq/kg for ^{226}Ra, 30 Bq/kg for ^{232}Th and 400 Bq/kg for ^{40}K) recommended by UNSCEAR in foodstuff [50]. The activity of ^{40}K radionuclide is seen to be higher than ^{226}Ra and ^{232}Th radionuclide activities in all the studied propolis samples. ^{40}K is the dominant gamma emitter and it is always present in foodstuff [4]. The ^{40}K radionuclide may have negative consequences for the health of humans with long-duration irradiation and exposure [51]. Therefore, its evaluation for human health is important. As shown in Table 2, the maximum activity concentrations were detected as 0.79 Bq/kg in Artvin for ^{226}Ra, 3.89 Bq/kg in Zonguldak for ^{232}Th and 122.54 Bq/kg in Balıkesir for ^{40}K. A study conducted radioactivity measurement in foods in Turkey, ^{226}Ra, ^{232}Th and ^{40}K concentrations in grape were reported as 8.04, 3.22 and 369.71 Bq/kg, respectively [48]. In a study conducted in Black Sea region of Turkey, ^{40}K radionuclide activity was measured as 348.8 Bq/kg in mussel, which is a kind of deep-sea fish [52]. When compared our results with the cited studies, radioactivity values of propolis are found to be much lower. The results indicate that propolis can be used as monitoring indicator for monitoring environmental radioactivity pollution.

4.3 Attenuation coefficients

The linear and mass attenuation coefficients of the samples are given in Table 3. LAC and MAC values were found between 0.1627 and 0.2392 cm^{-1}, 0.1641 and 0.2138 $cm^2 g^{-1}$, respectively. The density of the materials is very important in the absorption calculations. The density is also directly proportional to the mass of the material. The purpose of the absorption calculations is to identify materials that have less mass and are better absorbers. Therefore, propolis samples were compared to low-density materials (for gamma-ray with 59.5 keV) as shown in Table 6. The linear attenuation coefficient found for the propolis in the present study was found higher than those of the wood material sample (*Teminalia alata*) [8]. That means the propolis samples are better radiation absorber than the wood. In addition, MAC values the propolis samples were found higher than many materials such as polypropylene, polyamide, polyethylene and wood (Aroeira), but lower than materials such as wood (*Teminalia alata*), steel, pearl and mica. The results obtained from this study indicated that propolis is probably involved in the protection of beehives from radioactivity.

5 Conclusion

In conclusion, the natural (^{226}Ra, ^{232}Th and ^{40}K) radioactivity levels, gamma radiation attenuation coefficients and antioxidant properties of some Turkish propolis samples were studied in this study. The findings presented in this research are as follows;

- ^{226}Ra, ^{232}Th and ^{40}K radionuclide concentrations in the analyzed samples ranged from <MDA–0.79, 1.42–3.89 and 35.93–122.54 Bq/kg, respectively. All values of the

radionuclide concentrations are much lower than the limit values recommended in foodstuff.
- This study shows that the investigated propolis samples from Turkey can be consumed with peace of mind without any radiological hazards.
- Total phenolic content is the major factor in determined propolis quality. There is a moderate correlation between total phenolic contents, as well as total antioxidant capacity, and gamma radiation attenuation coefficients.
- Based on the linear attenuation coefficients determined, it has been concluded that propolis is better radiation absorber than some woods.
- As a result, it can be assumed that propolis is an important absorber for gamma radiations with low energy.

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