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Elemental composition and radionuclide content of inflorescences from *Sambucus nigra* L. from different regions of Bulgaria

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Abstract

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This study presents gamma-spectrometric data, elemental composition, and presence of radionuclides in inflorescences of *Sambucus nigra* L., collected from four different regions of Bulgaria. Radionuclides belonging to two radioactive families were detected: ^{232}Th, ^{226}Ra, ^{212}Pb, ^{214}Pb, ^{208}Tl, ^{214}Bi, ^{228}Ac and ^{40}K, together with the anthropogenic radionuclide ^{137}Cs. For all analyzed samples, the highest specific activity was observed for ^{40}K (750–1150 Bq/kg). Radionuclide doses to an adult, who consumed herbal tea made from these medicinal herbs ranged from 2.5 to 469.9 nSv for ^{137}Cs; 0.7 to 9.7 nSv for ^{238}U; 0.3 to 2.8 nSv for ^{232}Th and 7.64 to 11.7 µSv for ^{40}K. A correlation dependence was established between the intensity of the fluorescence maximum at an excitation wavelength of 498 nm and the specific activity of radium, thorium, and potassium. The content of macroelements in inflorescences follows the sequence K > Ca > Mg and coincides with results characteristic of the region.

Keywords: Sambucus nigra L.; gamma spectrometry; elemental composition; fluorescence spectroscopy

Introduction

Sambucus nigra L. is one of the nine species of the genus Sambucus, belonging to the Adoxaceae family (Bolli, 1994). Interest in the plant is growing because of its rich chemical composition and high antioxidant activity, and its wide application in food and pharmaceutical technologies (Imbrea et al., 2016; Mikulic-Petkovsek et al., 2015). Many authors have conducted extensive research on the phenolic compounds in the fruits and leaves of the plant (Senica et al., 2017; Thomas et al., 2006, Gardner, 2008). Inflorescences are often used to make syrups and teas, they are included in nutritional supplements. Due to their widespread use, they must be safe for human consumption and not be collected in a contaminated environment. Sometimes undesirable substances are present in the inflorescence of the plant, including natural and artificial radionuclides (WHO Guidelines, 2007; Kosalec et al., 2009). Gamma radiation emitted by naturally occurring radioisotopes like ^{40}K and radionuclides from the ^{232}Th and ^{238}U series, along with their decay products, constitutes the primary external source of radiation exposure to the human body. The body's content of ^{40}K is 0.18 % of the total potassium content in adults and around 0.2% for children (Mitrovi'c et al., 2009). The absorption of radionuclides tends to be highest in newborns. The results from animal studies suggest that passage through the intestines progressively decreases with age. For some radionuclides, a portion of the ingested activity enters through the small intestine, while the remaining part passes through the gastrointestinal tract without absorption. The activity in both the upper and lower parts of the large intestine, following the intake of radionuclides, is sometimes removed from the systemic circulation. The biokinetics of cesium has been extensively studied in humans and laboratory animals due to the significance of ^{137}Cs and ^{134}Cs as environmental pollutants. The most common forms of cesium are highly soluble and are almost completely absorbed into the bloodstream through the gastrointestinal or respiratory tract. As an anthropogenic radionuclide, ^{137}Cs is not naturally present in the environment. This specific isotope of cesium is a simultaneous source of beta and gamma rays. Its presence in the environment is primarily attributed to nuclear weapons tests conducted between the 1950s and 1970s, according to the Environmental Protection Agency. The Chernobyl nuclear power plant accident in 1986 led to increased radioactive contamination of the environment, with ^{137}Cs being one of the radionuclides found in deposits. Due to its long half-life, ^{137}Cs remains active in the environment for decades and poses a potential radiological risk (IAEA, 2010; Mitrović et al., 2014). Therefore, it is extremely important to evaluate the content of radionuclides in the different parts of medicinal plants used in dietary supplements. According to the World Health Organization, the health risk for individuals using dietary supplements from medicinal plants depends on the type and concentration in a case of accidental contamination with a specific radionuclide, as well as the duration of product use (WHO, 2007). Many elements included in food and herbal teas, can be more easily absorbed from the gastrointestinal tract than the inorganic forms of these elements (ICRP, 1898). Elderflowers contain relatively large amounts of potassium, calcium, magnesium and various other trace elements important for human health. In addition to macroelements and microelements such as Fe, Cu or Zn, they also play an important role in the proper functioning of various systems – circulatory, nervous, etc. (Młynarczyk et al., 2020).

Systematic studies on individual annual effective doses of radionuclide ingestion through the use of herbal decoctions, extracts, and dietary supplements are rare in the scientific literature.

The research aims to analyze the fluorescence spectra, the mineral content and the presence of radionuclides in the inflorescences of *Sambucus nigra* L., collected from four different regions of Bulgaria, and to calculate the equivalent annual effective dose from the absorption of radionuclides ^{137}Cs, ^{40}K, ^{228}Ac, ^{214}Bi, ^{208}Tl, ^{214}Pb, ^{212}Pb, ^{226}Ra and ^{232}Th present in the herbal infusions of *Sambucus nigra* L.

Materials and Methods

Samples

Fresh inflorescences of *Sambucus nigra* L. were collected from four different regions of Bulgaria, namely the regions of Burgas-Gramatikovo village, Plovdiv-Staro Zhelezare village, Western Rhodopes-Velingrad and Dobrich region. The inflorescences were cut during the flowering period depending on the altitude, in the range from May to July 2022 (Obretenov et al., 2002). Plant material identification was carried out at the Institute of Biodiversity and Ecosystem Research of the Bulgarian Academy of Sciences (IBER-BAS) in Sofia.

1. Western Rhodopes, Velingrad, 06.07. 2022;
2. Plovdiv, village Staro Zhelezare, 06.05. 2022;
3. Burgas, village Gramatikovo, 18.06. 2022;
4. Dobrich region, 09.05. 2022.

Drying

Freshly collected inflorescences from *Sambucus nigra* L. are dried in a thin layer and in the shade with periodic turning until a constant weight is obtained. The prepared samples were stored in cloth bags at room temperature in the dark.

Sample preparation for gamma spectrometric analysis

The dried samples were finely ground to a fraction size of 1–2 mm. They were placed in cylindrical containers with dimensions: 70 mm diameter and 20 mm height.

The annual effective dose of herbal tea intake is calculated by equation (1):

$$E_{ing} = C \times H \times DF_{ing}$$

where E_{ing} is the average annual effective dose from ingestion (Sv/y), C is the mass-specific activity in the product (Bq/kg), H is the consumption rate for the product (kg/y), and DF_{ing} is the dose conversion factor for the radionuclide in the body (Sv/Bq) (Kilic et al., 2009).

Fluorescence Spectroscopy

The fluorescence spectra of the ground samples of *Sambucus nigra* L. were obtained using a portable spectrometer

AvaSpec-ULS2048CL-EVO, Avantes-Apeldoorn (Apeldoorn, Netherlands), with the excitation light wavelength set at 385 nm. The measurement scheme is shown in Fig. 1, and the measurement methodology is the same as Ropelewska et al. (Ropelewska et al., 2022).

Fig. 1. Schematic diagram for measuring fluorescent spectra of opaque samples

Gamma Spectrometric Analysis

Each of the samples of *Sambucus nigra* L. with a mass of 0.012 g underwent gamma spectrometric analysis to determine the radionuclides present. The analysis was conducted at the Laboratory of Nuclear Physics and Radioecology at Shumen University. The gamma spectrometric setup includes a semiconductor detector with a relative efficiency of 4.5% for the ^{137}Cs line (E = 661.66 keV) and passive shielding to isolate the sample from the environment. Radionuclides were identified based on their gamma energy lines using the specialized software Anges (Mishev and Vidolov, 2020). The activity and specific activity of the radionuclides are calculated from the obtained data (Stefanova et al., 2017).

Decomposition of samples and determination of elemental composition

Weigh on an analytical balance of 0.5 g of sample, to which 6 ml of concentrated nitric acid and 2 ml of hydrogen peroxide were added. The thus prepared sample, located in the Teflon container of the microwave oven, was processed according to a set program of 15 minutes to raise the temperature to 180 °C and hold for 20 minutes. After cooling, the sample was quantitatively transferred into a 50 ml flask.

Concentrations of Ca, Mg, Fe, Mn, Cu, and Zn were measured using an ICP-OES iCAP 7000 SERIES (ThermoFisher Scientific) and of Na and K using a FAAS Thermo SOLAAR M5. Multi-Element Standard Solution IV for ICP (TraceCERT®, Merck, Germany) was used to prepare diluted working standard solutions to calibrate the instruments.

Statistical Analysis

Each of the investigated parameters was determined from three independent measurements. The mean values from the measurements ± standard deviation are presented. Statistical processing was performed using Microsoft Excel.

Results and Discussion

Table 1 presents the results of the determination of K, Ca, Mg, Na, Fe, Mn, Zn and Cu in the inflorescences of *Sambucus nigra* L. from the four regions in Bulgaria.

The concentrations of K are in the range of 31 – 18 g kg^{-1}, the content is the lowest in the samples from the Dobrich region, and the highest in the Rhodope region, Ca – from 6 to 8 g kg^{-1} and Mg – from 4 to 6 g kg $^{-1}$. Of the three macronutrients, potassium is dominant, and it and the other two are usually generated from the soil, their sequence being K > Ca > Mg and fully matching those reported by other researchers (Qazimi et al., 2024; Szymański and Szymański, 2022). Apart from the concentrations of the macroelements K, Ca, and Mg, the content of the other microelements corresponds to results obtained from samples from Kosovo and Poland, as well as

Table 1. Content of some elements in *Sambucus nigra* L. (RSD = 3–7 %, n = 3)

Element	Western Rhodopes, Velingrad	Plovdiv, village Staro Zhelezare	Burgas, village Gramatikovo	Dobrich region
K, mg kg^{-1}	31280	22950	23380	18320
Ca, mg kg^{-1}	5950	7930	6840	7290
Mg, mg kg^{-1}	4280	4150	5680	5830
Na, mg kg^{-1}	53.7	51.4	54.7	34.3
Fe, mg kg^{-1}	202	85.9	108	80.8
Mn, mg kg^{-1}	73.2	31.5	41.9	41.4
Zn, mg kg^{-1}	57.1	18.9	33.5	48.1
Cu, mg kg^{-1}	14.8	6.29	8.84	12.6

from wild black elderberry from Poland (Młynarczyk et al., 2020). Deviations in the compositions of the four samples are normal, as they grow in different regions of the country having different soil composition and climate.

The results from the gamma spectrometric analysis are presented in Table 2.

Unlike mosses and lichens, where radionuclides are deposited directly through ion-exchange processes, in the flowers of *Sambucus nigra L.,* they are adsorbed from the soil, on which the plant grows (Ugur et al., 2003).

Comparing the results in Table 2, radionuclides belonging to two radioactive families were found in the flower samples of *Sambucus nigra* L.: ^{232}Th, ^{226}Ra, ^{212}Pb, ^{214}Pb, ^{208}Tl, ^{214}Bi, ^{228}Ac, and ^{40}K. In the sample from the village of Staro Zhelezare, Plovdiv region, the technogenic radionuclide ^{137}Cs was also found, likely a consequence of the Chernobyl accident and still present in the soil and, respectively, in the plants. The lowest value for the specific activity of ^{232}Th, a member of the uranium-radium family, was measured in a sample from the Western Rhodopes, Velingrad, while the differences in the values of the specific activity measured in sample 2 (from the village of Staro Zhelezare, Plovdiv region) and sample 3 (from the village of Gramatikovo, Burgas region), are within the experimental error. No change is observed in the values for the specific activity obtained for the radionuclides ^{214}Pb, ^{208}Tl, and ^{228}Ac. A significant difference in the values of the specific activities reported for the radionuclide ^{226}Ra, belonging to the uranium-radium family, is observed. The lowest is in the sample from the Western Rhodopes 33.7 ± 5.1 Bq/kg, and the highest is in the sample from the village of Gramatikovo 58.0 ± 8.7 Bq/kg. The values obtained for the specific activity of ^{212}Pb and ^{214}Bi in samples from the Western Rhodopes and the village of Staro Zhelezare are close, within the experimental error. Those reported for the sample from the village of Gramatikovo, regarding ^{212}Pb is higher, while for ^{214}Bi, it is lower compared to the other two samples. ^{40}K is a radionuclide of natural origin but does not form a radioactive family. Its half-life is 1.277×10^9, and it is registered in all natural samples. The values for the specific activities of ^{40}K measured in our samples are in the range of 750 to 1160 Bq/kg. ^{137}Cs was found only in the sample from the village of Staro Zhelezare, Plovdiv region, with a specific activity of 1.2 ± 2 Bq/kg. The indicated value is higher than that reported in the work of I. Salamon and colleagues (1.100 ± 0.010 Bq/kg) (Salomon et al., 2015), and the data for the specific activity of ^{137}Cs, calculated after analyzing various plants widely used in medicine and pharmacy, are in the range of 0.3 to 10.7 Bq/kg (Desideri et al., 2010). According to Bulgarian legislation, the maximum permissible values for the specific activity of ^{137}Cs in the different types of food vary from 400 Bq/kg for baby food to 12 500 Bq/kg for other foods (Ordinance No. 11 of April 18, 2002).

For all samples analyzed, the highest specific activity was observed for ^{40}K. The experimental data of the studied samples are in accordance with those published by Jevremovic et al. (1199±26.2) Bq/kg. Regarding the content of the ^{232}Th, our results are higher than those reported by Jevremovic, but close to those of Djelic (Jevremovic et al., 2011; Djelic et al., 2016). The identified higher concentrations of the mentioned and other radionuclides can likely be explained by the geographical region. There are data indicating concentrations of ^{137}Cs that are 10 times higher in countries such as Ukraine, the Middle East, and Turkey Kilic et al., 2009; Handl et al., 2003).

For Bulgaria, the consumption rate of herbal infusions, expressed on an annual basis in kilograms, is 2.738 kg. Following these data and equation (1), Table 3 presents the annual effective dose of ingestion when consuming herbal tea from *Sambucus nigra* L. on an annual basis.

Table 2. Radionuclides and their specific activities obtained in samples of inflorescences from *Sambucus nigra* L.

Radionuclide	A_{sp}[Bq/kg]			
	Western Rhodopes, Velingrad	Plovdiv, village Staro Zhelezare	Burgas, village Gramatikovo	Dobrich region
^{232}Th	16.0±2.4	28.5±4.3	26.7±4.0	38.0±5.7
^{226}Ra	33.7±5.1	43.7±6.6	58.0±8.7	45.0±6.7
^{212}Pb	4.7±0.7	4.5±0.7	7.1±1.1	7.1±1.1
^{214}Pb	7.6±1.1	6.9±1.0	7.9±1.2	7.2±1.1
^{208}Tl	6.1±0.9	7.8±1.2	7.2±1.1	10.3±1.5
^{214}Bi	15.2±2.3	16.0±2.4	9.8±1.5	17.0±2.5
^{228}Ac	10.8±1.6	14.8±2.2	12.0±1.8	11.3±1.7
^{40}K	750±30	1100±50	1050±50	1150±50
^{137}Cs	MDA	1.2±0.2	MDA	MDA

Table 3. Specific effective dose obtained in consumption of tea from blossoms of *Sambucus nigra* L.

Radionuclide		E_{ing} [Sv/y]			
	[Bq/kg]	Western Rhodopes, Velingrad	Plovdiv, village Staro Zhelezare	Burgas, village Gramatikovo	Dobrich region
^{232}Th	3.40×10^{-9}	8.94×10^{-8}	1.59×10^{-7}	1.49×10^{-7}	2.12×10^{-7}
^{226}Ra	2.80×10^{-7}	1.55×10^{-5}	2.01×10^{-5}	2.67×10^{-5}	2.07×10^{-5}
^{212}Pb	6.00×10^{-9}	4.63×10^{-8}	4.44×10^{-8}	7.00×10^{-8}	7.00×10^{-8}
^{214}Pb	1.40×10^{-10}	1.75×10^{-9}	1.59×10^{-9}	1.82×10^{-9}	1.66×10^{-9}
^{208}Tl	1.20×10^{-9}	1.20×10^{-8}	1.54×10^{-8}	1.42×10^{-8}	2.03×10^{-8}
^{214}Bi	1.10 ×10^{-10}	2.75×10^{-9}	2.89×10^{-9}	1.77×10^{-9}	3.07×10^{-9}
^{228}Ac	4.30×10^{-10}	7.63×10^{-9}	1.05×10^{-8}	8.48×10^{-9}	7.98×10^{-9}
^{40}K	6.20×10^{-9}	7.64×10^{-6}	1.12×10^{-5}	1.07×10^{-5}	1.17×10^{-5}
^{137}Cs	1.30×10^{-8}	MDA	2.99×10^{-8}	MDA	MDA

In a fully grown individual, the accumulation of radium in the skeleton can decrease by a quarter within the first few days after ingestion to less than 10% after 1 month. Limited human data indicate that radium in soft tissues may represent 20% or more of the total body radium in the first few weeks after exposure (ICRP, 1989; Jee and Polig, 1989).

^{232}Th is primarily deposited on the surface of bones, where it persists for an extended period, either absorbed within their volume or transferred to the blood plasma and bone marrow through restructuring processes. In the spleen, thorium is deposited to a much lesser extent than americium and plutonium. It is assumed that 7% of the thorium leaving the blood plasma goes into urine, 0.5% goes into the gastrointestinal tract content, and subsequently, into feces, 70% is evenly distributed between cortical and trabecular bone surfaces, 4% is deposited in the spleen, 3% is deposited in the kidneys, and the remainder goes to other tissues. For individuals of all ages, it is assumed that thorium is excreted from the body through the kidneys with a half-life of 2 years and from the spleen and other soft tissues with a half-life of 5 years.

Soil contamination with ^{137}Cs is a primary source of plant contamination since ^{137}Cs deposited in the soil can accumulate in plants through their root system. The soil-plant-human sequence is a primary pathway for the transfer of radionuclides to humans (Calmon et al., 2009). Radionuclides from the soil can be transferred to plants together with nutrients and accumulate in various parts of the plant tissue. Plants relatively easily absorb cesium, which is present in its solution as a monovalent cation (Geras'kin et al., 2005). The content of natural and man-made radionuclides in the studied objects is due to natural sources, global atmospheric deposits and the accident in Chernobyl. Therefore, the study of the annual effective absorbed dose of radionuclides is of great importance.

The fluorescent spectra of the inflorescences of the four samples are presented in Figure 2

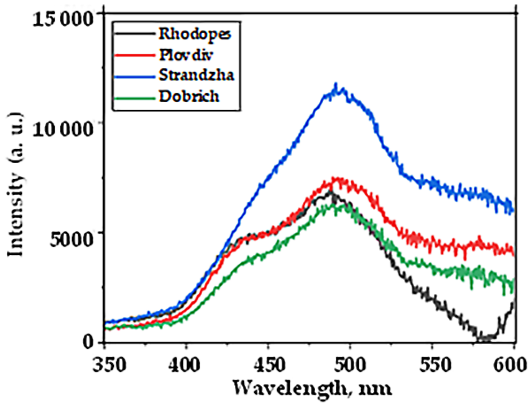

Fig. 2. Fluorescent spectra of inflorescences from *Sambucus nigra* L. at an excitation wavelength of 385 nm

In the fluorescent spectra, there is a pronounced peak at an emission wavelength of 498 nm and a weakly pronounced one around 428 nm. Since there are studies on other plant objects (Nikolova et al., 2021) showing existing correlations between the intensity of the fluorescent maximum and the mineral composition, the present study sought relationships between the activities of radionuclides and the intensity of the fluorescent maximum. The established correlation dependencies and correlation coefficients are presented in Table 4.

Table 4. Existing correlations between the specific activity of radionuclides and the intensity of the fluorescent maximum

Existing correlation dependence	Correlation coefficient
$I_{498\ nm} = 170.76\ Ra$	0.983
$I_{428\ nm} = -1.56\ K + 8022.5$	0.810
$I_{428\ nm} = -83.33\ Th + 6615.5$	0.820

Conclusions

The activity of the natural radionuclide ^{40}K ranges from 750 Bq/kg to 1150 Bq/kg. For the artificial ^{137}Cs only for the sample from Staro Zhelezare an activity of 1.2 Bq/kg was measured. The obtained results are within the limits of the natural background. Individual annual effective doses due to ingestion were estimated based on measured specific activities of 4 herbs from *Sambucus nigra* L. The best correlations were observed in the fluorescence spectra with a coefficient of determination $R^2 = 0.983$ for Ra. The content of macroelements in inflorescences follows this sequence K > Ca > Mg and coincides with the results characteristic of the area.

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Conflict of interest

The authors declare no conflicts of interest. The funders had no role in the design of the study; in the collection, analyses, or interpretation of data; in the writing of the manuscript; or in the decision to publish the results.

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