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RESEARCH

Elemental and Radiological Characterisation of *Arbutus unedo* L. Leaves and Tea: Impact of Preparation Method on Nutritional Risk/Benefit

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Abstract
The aim of this study was to characterise the elemental and radiological composition of strawberry tree (*Arbutus unedo L.*) leaves and tea preparations and compare it with commercial Uvin H herbal mixture, widely used in treatment of urinary tract infections. The concentration of 17 elements and the activity concentration of selected radionuclides were measured in strawberry tree leaves/Uvin H herbal mixture, as well as in herbal tea prepared by infusion or decoction of leaves for 5 or 10 min. In both leaves and tea preparations, Ca, K, Mg, and Na were the most abundant elements, while the lowest levels were measured for As, Co, Mo, and Se. Only ^{137}Cs and ^{40}K were detected in analysed leaves/herbal mixture, while the activity of radionuclides in tea preparations was below the detection limit. The maximum possible health benefits can be obtained by a 10-min decoction of leaves, which resulted in the highest total phenolic content and antioxidant activity and levels of K, Ca, Mg, Na, Fe, Mn, and Se in comparison to the other preparation methods evaluated in this study. The calculated intake of potentially toxic elements and radionuclides does not represent a health risk to consumers.

Keywords *Arbutus unedo* L. · Tea · Elements · Radionuclides · Antioxidant activity · Exposure assessment

Introduction

Arbutus unedo L., commonly known as strawberry tree, is an evergreen shrub or small tree from the Ericaceae family. It is native to the Mediterranean region and western Europe: north to western France, Ireland, Portugal, and Turkey. Almost all parts of the plant (leaves, fruits, bark, and roots) are used in traditional medicine for the treatment of different diseases, such as kidney, gastrointestinal, dermatological, urological, cardiovascular and hypertensive diseases, and diabetes [1–3]. The medicinal characteristics of strawberry tree are in general related to phytochemicals, mainly phenolic compounds, produced in different parts of the plant as a defence mechanism against challenging environmental

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conditions. The leaves, for instance, are rich in phenolic substances such as tannins, flavonoids, iridoids, anthocyanins, carotenoids, terpenoids, and fatty acids as major classes of bioactive constituents [4–6]. These compounds are responsible for the strong antioxidant activity of leaves and their anti-diabetic, anti-inflammatory, astringent, and urinary antiseptic properties [6]. Their mechanism of action against urinary tract pathogens is associated with a high content of arbutin [7], present also in the Ericaceae species *Arctostaphylos uva ursi* (L.) Spreng. (bearberry). Bearberry leaves (*Uvae ursi folium*) are well known in herbal medicine as a urinary antiseptic and astringent [8, 9] thereby widely used as the main herb in tea mixtures. Since arbutin undergoes metabolic conversion into toxic hydroquinone, a safe daily dose for human consumption corresponds to up to 800 mg of arbutin, during a maximum period of 2 weeks, while extended use is generally not recommended [7, 9].

While phenolics have been extensively studied in the strawberry tree raw material (e.g. strawberry tree leaves, fruits, and honey) considered for the development of functional foods and nutraceuticals [5, 6, 10, 11], there is limited data on the content of essential and potentially toxic elements and bioactive compounds in infusions and/or decoctions of leaves used for medicinal purposes. Several authors have reported reduced content of plant nutritional components after its extraction with boiling water [12, 13]. Nevertheless, besides phenolics, the regular consumption of herbal tea could contribute to the daily dietary requirements of essential elements, such as Ca, Cu, Fe, K, Mg, Mn, Mo, and Zn. The amount of elements extracted into the infusion depends principally on their chemical and biological behaviour in plant leaves and their solubility in herbal infusions, as well as on brewing conditions [14]. The latter primarily includes the preparation method (infusion or decoction) and the preparation time [14, 15].

Besides the nutritional benefits, a herbal preparation intended for therapy has to be safe and nonharmful to human health. The determination of toxic elements, such as As, Cd, Ni and Pb in this respect, is of utmost relevance for consumer safety. Due to their cumulative properties and high toxicity, the ingested dose of toxic elements could reach threshold levels and potentially trigger adverse health effects [6, 14]. Alongside toxic elements, radionuclides such as ^{137}Cs, ^{40}K, members of ^{238}U decay chain (^{234}Th, ^{214}Pb, ^{214}Bi, ^{210}Pb), and ^{232}Th decay chain (^{212}Pb, ^{212}Bi, ^{208}Tl, ^{228}Ac) accumulate in plants and may influence human health directly or indirectly [16–18]. Radioactivity in the environment may originate from natural, terrestrial, extra-terrestrial, and anthropogenic sources (e.g. nuclear accidents, the normal operation of nuclear power plants, or recycling of spent nuclear fuel). Among the aforementioned radionuclides, ^{137}Cs is the only anthropogenic radionuclide that has similar chemical and metabolic characteristics to ^{40}K and can thus accumulate in human muscles upon the ingestion of contaminated food [19]. With the exception of the studies reporting levels of Na, Mg, K, Ca, and Fe in strawberry tree leaves from Algeria [20], and Al, Fe, Zn, and Cu from Turkey [6], to the best of the authors' knowledge, there have been no published data about the stabile multi-elemental composition and radionuclide activities in strawberry tree leaves and tea infusions/decoctions.

The aim of this study was therefore to assess the risks/benefits of chemical parameters in strawberry tree leaves and brewed tea using different preparation conditions (infusion or decoction for 5 or 10 min) as a final step of the quality control of this food supplement. To back up the commercialisation of this valuable herb in treating urinary tract infections, we compared its chemical profile with the most commonly used herbal mixture (Uvin H with bearberry as the predominant herb) bearing the same mechanism of action.

Materials and Methods

Sample Collection and Storage

Strawberry tree leaf samples (500 g) were randomly collected at three locations in four time periods on the Adriatic coast in October 2013–2016. Leaves were sampled from adult wild plants on the islands of Ugljan ($N = 1$; 44°03′29″ N, 15°12′14″ E; 16 m a.s.l.), Koločep ($N = 2$; 42°40′34″ N, 18°00′35″ E; 34 m a.s.l.), and Lošinj ($N = 4$; 44°31′50″ N, 14°28′06″ E; 14 m a.s.l.). The surrounding environment of the sampling sites could be considered pristine, far from roads with any significant traffic. Upon transfer to the laboratory, leaves were dried in a dark place at room temperature, ground in a laboratory mill, and stored in plastic containers at room temperature until analysis. An herbal mixture commercially available for treating urinary tract inflammation (Uvin H) was bought in the pharmacy shop. Uvin H is a mixture of six herbs containing bearberry leaves (30%), aerial parts of heather (*Calluna vulgaris*; 20%), birch leaves (*Betulla* spp.; 15%), restharrow root (*Ononis spinosa*; 15%), aerial parts of horsetail (*Equisetum arvense*; 10%), and corn silk (*Zea mays*; 10%).

Herbal Tea Preparation (Infusion/Decoction)

Tea infusions were prepared by adding 200 mL of boiled ultrapure water to 2 g of minced strawberry tree leaves/Uvin H herbal mixture. Infusions were allowed to stand for 5 and 10 min and then filtered through filter paper. Decoction extracts were prepared by boiling 2 g of minced leaves/herbal mixture in 200 mL of ultrapure water for 5 or 10 min and then filtered through filter paper. After cooling, the aliquoted filtrates intended for element analyses were acidified with purified nitric acid (0.1 mL HNO_3/200 mL herbal tea) and stored in plastic tubes.

Multi-elemental Composition in Leaves and Herbal Teas Prepared by Different Methods

Minced leaves of strawberry tree and Uvin H herbal mixture (0.2 g) were weighted to Teflon tubes and digested in an UltraCLAVE IV microwave digestion system (Milestone, Sorisole, Italy) after addition of 2 mL of ultrapure water (18 MΩ cm; GenPure system, TKA, Germany) and 2 mL of purified (duoPUR, Milestone, Italy) nitric acid (p.a. 65%; Merck, Germany).

Elements (Al, As, Ca, Cd, Co, Cr, Cu, Fe, K, Mg, Mn, Mo, Na, Ni, Pb, Se, and Zn) in digested leaves/herbal mixture and diluted teas (v/v 1:2) were quantified using an Agilent 7500cx (Agilent Technologies, Japan) inductively coupled plasma mass spectrometer following a previously described procedure [10]. All measurements were done in triplicate. To monitor the analytical quality of digestion and measurement, duplicate samples of standard reference materials (SRM) 1570a spinach, 1573a tomato leaves, and 1571 orchard leaves (National Institute of Standards and Technology, USA) were included in all steps of sample preparation (Table S.1).

Overall recoveries were from 94 to 104% of the assigned analytical values. Detection (MDL) and quantification limits of the method (MQL) were calculated as the concentration corresponding to three and 10 times the standard deviation of 10 blank samples, respectively.

Total Phenolic Content (TPC) in Herbal Teas Prepared by Different Methods

TPC in teas was determined with a slightly modified Folin-Ciocalteu's method described by Brčić Karačonji et al. [5]. Fifty microliters of infusion/decoction was mixed with 1.4 mL of water and 100 µL of Folin-Ciocalteu reagent (Kemika, Croatia). The reaction mixture was incubated at room temperature for 5 min and mixed with 1.5 mL of Na_2CO_3 solution (6% w/v; Kemika, Croatia). Absorbance was measured using spectrophotometer Cary 50 (Varian, Mulgrave, Australia) at 765 nm after 30 min at 40 °C and the results were expressed as mg of gallic acid equivalents (GAE) per g of dried leaves. All measurements were done in triplicate.

Antioxidant Activity of Herbal Teas Prepared by Different Methods

Radical scavenging activity of the prepared infusion/decoction was measured with a slightly modified 1,1-diphenyl-2-picrylhydrazyl (DPPH) method described by Brčić Karačonji et al. [5]. Fifty microliters of infusion/decoction was mixed with 1.95 mL of methanol (Merck, Germany). Then, 1.5 mL of DPPH (Sigma-Aldrich, Germany) methanolic solution (0.18 mmol/L) was added and vortexed vigorously. The mixture was incubated in the dark for 30 min at 25 °C. The absorbance was measured at 517 nm. The results were expressed as µmol of the Trolox (Fluka, Germany) equivalent (TE) antioxidant capacity per g of dried leaves. All measurements were done in triplicate.

Radionuclide Activity Concentration in Leaves and Herbal Teas Prepared by Decoction

Minced leaves of strawberry tree and Uvin H herbal mixture (40–50 g) were tightly packed in 100-mL Marinelli beakers and left undisturbed to reach secular equilibrium. The measurement of radionuclide activity concentration (^{40}K, ^{137}Cs, ^{234}Th, ^{214}Pb, ^{214}Bi, ^{210}Pb, ^{212}Pb, ^{212}Bi, ^{208}Tl, and ^{228}Ac) were performed in the accredited laboratory using high-resolution gamma spectrometry in energy range (40–2000 keV) according to the HRN EN ISO/IEC 17025:2017 standard. High-purity Ge Mirion Technologies (Canberra), Inc. GC5019 photon detector system of a 54% relative efficiency and resolution of 1.9 MeV, all at 1.33 MeV ^{60}Co, was used to quantify the radionuclide activity concentration in dry herbal samples by measuring at least 250,000 s. Samples were then aliquoted and tea (10 g/1 L of water) was brewed by 10-min decoction. Cooled and filtered tea was packed in 1-L Marinelli beaker and measured for 320,000 s on a high-purity Ge photon detector system ORTEC HP GMX of a 74% relative efficiency and resolution of 2.26 MeV, all at 1.33 MeV ^{60}Co. Radionuclides were quantified only in tea brewed by a 10-min decoction method (highest transfer rate of elements from leaves to tea; EMA [9]) due to the long measurement time. Calibrations were performed using calibration standards acquired from Czech Metrology Institute. Quality control was assured through regular participation in interlaboratory comparisons and correction for coincidence summing.

The annual effective dose (D; Sv/y) received by the person drinking herbal tea was calculated via the following equation:

$$D = A \times In \times e$$

where A is the activity concentration of radionuclide (Bq/L), In is the annual intake of the herbal tea (L), and e is the effective dose coefficient for ingestion for adults (1.3×10^{-8} Sv/Bq for ^{137}Cs [21]).

Estimation of Dietary Intake and Health Risk Assessment for Consumers of Herbal Teas

We compared the nutritional and risk estimation of the daily intake of elements by consumption of strawberry tree or Uvin H tea brewed by 10-min decoction. These calculations present the "worst case scenario", considering that the transfer rate of elements from leaves to the tea is generally highest when tea is prepared in such a manner (Table S.2). The transfer rate *T* (%) of the analysed element from strawberry tree leaves/Uvin H herbal mixture into tea infusion/decoction is calculated via the following equation:

$$T(\%) = \left(C_d \times V_d / C_t \times M_t\right) \times 100\%$$

where C_d (mg/L or μg/L) and C_t (mg/kg or μg/kg) are the concentration of each element in the tea brewed by decoction and tea leaves, respectively, V_d is the volume of prepared tea (1 L), and M_t refers to the quantity of herbal sample used for brewing (10 g) [15].

The estimated daily intake (EDI) of each element is calculated via the following equation:

$$\text{EDI} = C \times D \times T / \text{BW}$$

where *C* refers to the concentration (mg/kg or μg/kg) of the analysed element in the herbal tea, *D* represents the amount of tea consumed daily (10 g, recommended daily amount of Uvin H herbal mixture), *T* is the transfer rate for each element, and BW is the approximate body weight of an adult person (70 kg).

We performed a nutritional assessment for tea consumers regarding the intake of certain essential elements (Ca, K, Mg, Cu, Fe, Mn, Mo, Se, Zn), as well as an assessment of intake of elements considered toxic (Al, Cr, Ni, As, Cd, Pb). Adequate daily amounts of essential elements taken up by food are defined by dietary reference values (DRV) [22], while tolerable intake (TI) data refer to the allowed intake of non-essential elements. The estimation of daily intake of elements was presented as a % DRV or % TI defined for adults (> 18 years). DRV is an umbrella term that includes population reference intake (PRI), average requirement (AR), and adequate intake (AI), while TI includes tolerable weekly intake (TWI) defined for Al and Cd, tolerable daily intake (TDI) defined for Cr and Ni or experimental benchmark response of 1% extra risk (BMDL_{01}) for elements considered genotoxic and/or carcinogenic (As and Pb). Since these values may be defined differently for males and females from the general population, we used the highest DRV value or the lowest TI value defined for adults in our calculations. Calculation was based on the following equations:

$$\%\text{DRV} = (\text{EDI}/\text{DRV}) \times 100$$

$$\%\text{TI} = (\text{EDI}/\text{TI}) \times 100$$

where EDI is the estimated daily intake of each element, DRV is the dietary reference value of the respective essential element [22, 23], and TI is the tolerable intake value of the respective non-essential element [24–29].

Statistical Analysis

Statistical analyses were performed using statistical software Dell™ Statistica™ Version 14.0.0.15 (TIBCO Software Inc., Palo Alto, CA, USA) and MedCalc® Statistical Software version 22.006 (MedCalc Software Ltd, Ostend, Belgium). The level of statistical significance was set at $p < 0.05$ for all analyses. Normality of data distribution was tested with Shapiro-Wilk test. With respect to the data distribution, Friedman ANOVA and post hoc Wilcoxon matched pairs test were used for testing differences in element levels, TPC, and antioxidant activity between herbal tea samples prepared using different methods and brewing time (infusion or decoction for 5 or 10 min). Natural clustering of the element levels in strawberry tree leaves was explored by principal component analysis (PCA). PCA is used to represent a multivariate data table as smaller set of variables—principal components (PCs) in order to observe trends and uncover the relationships between observations and variables, and among the variables.

Results and Discussion

Multi-elemental Composition of Strawberry Tree Leaves

The mean concentrations of the 17 elements measured in the seven samples of strawberry tree leaves from the Croatian Adriatic and in Uvin H herbal mixture are shown in Table 1. The most abundant elements found in all of the samples analysed in this study were Mg, K, and Ca, followed by Na, Fe, Al, and Zn. The lowest concentrations were measured for As, Mn, and Se.

To the best of our knowledge, the data on total concentrations of elements in leaves are available only for strawberry trees from Algeria [20] and Turkey [6]. The mean concentration of Na, Al, Mg, K, Ca, Fe, Zn, and Cu in strawberry tree leaves from Croatia (Table 1) was lower than the concentration measured in samples from Algeria, as reported by Asmaa et al. [20] (Na 247.0 mg/kg, Mg 1860 mg/kg, K 17,430 mg/kg, Ca 12,980 mg/kg, Fe 268.0 mg/kg), and Turkey, as reported by Erdoğan and Uysal [6] (Al 631.2 mg/kg, Fe 550.1 mg/kg, Zn 422.0 mg/kg, Cu 98050 μg/kg).

Table 1 Concentration of elements in strawberry tree leaves and Uvin H herbal mixture. The results are presented as mean ± standard deviation (n = 3)

Element	Uvin H herbal mixture (2020)	Strawberry tree							
		All locations	Location (year)						
			Lošinj (2013)	Lošinj (2014)	Lošinj (2015)	Lošinj (2016)	Koločep (2013)	Koločep (2015)	Ugljan (2016)
Na* (mg/kg)	56.31 ± 0.97	145.5 ± 54.5	106.0 ± 0.4	178.3 ± 0.4	120.7 ± 0.8	199.1 ± 0.8	57.00 ± 0.36	149.5 ± 1.4	207.6 ± 0.5
Mg* (mg/kg)	2434 ± 5	1387 ± 501	1020 ± 1	965 ± 3	1229 ± 15	1165 ± 5	1353 ± 15	1550 ± 9	2431 ± 13
Al* (mg/kg)	328.0 ± 1.1	70.86 ± 23.95	91.95 ± 0.18	85.14 ± 0.04	28.11 ± 0.42	70.51 ± 0.29	57.25 ± 0.02	98.17 ± 0.05	64.91 ± 0.42
K* (mg/kg)	9516 ± 37	7185 ± 1697	9279 ± 11	8766 ± 22	6802 ± 73	5956 ± 6	8077 ± 57	7045 ± 15	4369 ± 50
Ca (mg/kg)	8665 ± 45	10,347 ± 2522	6930 ± 25	8654 ± 6	10,007 ± 71	12,124 ± 26	8332 ± 81	13,431 ± 59	12,951 ± 178
Cr* (µg/kg)	1422 ± 15	172.1 ± 38.4	174.5 ± 1.7	220.1 ± 2.7	111.9 ± 4.5	164.4 ± 7.9	143.5 ± 0.7	217.2 ± 2.2	173.1 ± 2.6
Mn* (mg/kg)	223.6 ± 1.5	10.61 ± 3.28	13.68 ± 0.02	13.93 ± 0.07	13.65 ± 0.12	10.12 ± 0.04	6.839 ± 0.055	6.151 ± 0.029	9.904 ± 0.072
Fe* (mg/kg)	224.1 ± 0.6	65.51 ± 30.24	129.1 ± 0.4	66.12 ± 0.21	34.92 ± 0.47	56.64 ± 0.31	49.71 ± 0.53	69.36 ± 0.34	52.69 ± 0.57
Co* (µg/kg)	258.9 ± 0.9	46.11 ± 7.17	55.77 ± 0.10	50.51 ± 0.77	36.88 ± 0.83	49.57 ± 0.05	36.74 ± 0.41	48.89 ± 0.21	44.44 ± 0.82
Ni* (µg/kg)	6257 ± 14	411.6 ± 229.3	776.1 ± 7.1	544.8 ± 0.9	156.7 ± 3.8	203.4 ± 3.0	572.4 ± 1.8	237.5 ± 0.4	390.7 ± 2.8
Cu (µg/kg)	4452 ± 20	3661 ± 855	4520 ± 121	4419 ± 15	4551 ± 70	2956 ± 8	3701 ± 4	2534 ± 22	2945 ± 33
Zn* (mg/kg)	43.59 ± 0.13	25.53 ± 4.24	27.01 ± 0.43	27.75 ± 0.02	32.43 ± 0.39	20.81 ± 0.25	21.94 ± 0.09	27.18 ± 0.29	21.60 ± 0.14
As* (µg/kg)	72.21 ± 2.21	23.27 ± 7.48	27.64 ± 0.56	23.55 ± 2.18	14.25 ± 0.19	19.47 ± 0.51	19.55 ± 1.03	37.47 ± 1.47	20.98 ± 0.32
Se* (µg/kg)	208.9 ± 2.3	50.75 ± 40.10	45.26 ± 0.37	75.17 ± 0.44	24.49 ± 1.75	28.06 ± 2.09	30.97 ± 1.38	20.02 ± 0.60	131.2 ± 3.2
Mo* (µg/kg)	194.2 ± 1.3	55.88 ± 25.09	85.62 ± 0.94	90.96 ± 5.70	51.75 ± 1.00	40.67 ± 1.24	34.03 ± 1.45	25.94 ± 0.28	62.22 ± 0.17
Cd (µg/kg)	100.3 ± 1.8	187.1 ± 71.6	274.9 ± 1.5	262.7 ± 1.4	236.5 ± 0.6	162.5 ± 3.2	153.7 ± 0.4	133.7 ± 0.4	85.6 ± 0.7
Pb* (µg/kg)	464.1 ± 1.5	128.8 ± 28.0	162.6 ± 10.7	163.3 ± 0.6	127.2 ± 10.2	96.80 ± 6.91	139.4 ± 0.1	116.7 ± 2.3	95.62 ± 0.65

*Significantly different concentration between Uvin H herbal mixture and strawberry tree leaves (Mann-Whitney U test, $p < 0.05$)

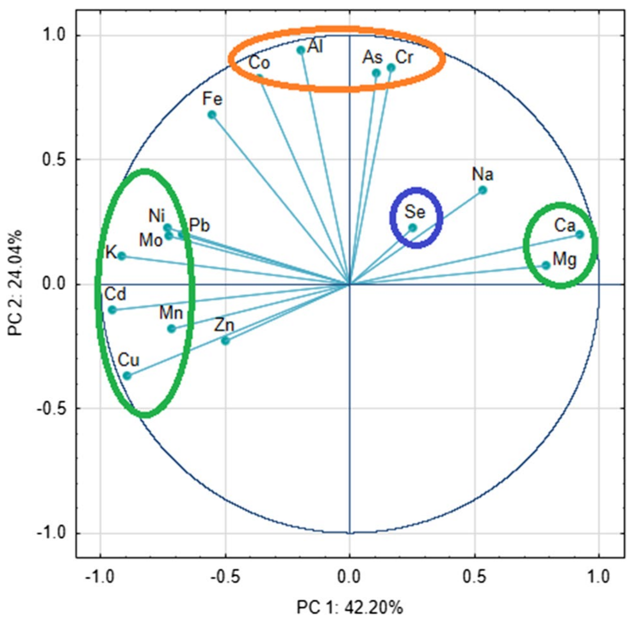

Fig. 1 Loading/variable plot of first two principal components (PC) for element concentration measured in leaves of strawberry trees from Croatia (elements representing the individual factor are grouped within the oval shapes of the same colour)

The mean concentration of Mn in strawberry tree leaves from all Croatian locations was similar to that measured in leaves from Turkey (13.78 mg/kg) and Algeria (11.0 mg/kg). Erdoğan and Uysal [6] reported concentrations of Cr, Cd, and Pb lower than MDL, while in strawberry tree leaves from Croatia we were able to quantify those elements. In order to protect consumers from contaminants in food and food supplements, the World Health Organization [30] has set maximum permissible levels in plant materials for As, Cd, and Pb of 1.0, 0.3, and 10 mg/kg, respectively. Although Cd levels in some samples were close to 0.3 mg/kg, none of the analysed leaves from the Croatian strawberry trees exceeded the maximum permissible levels of As, Cd, and Pb.

It is interesting to notice that significantly higher concentrations of potentially toxic elements Al, Cr, Ni, As, and Pb were measured in the commercially available Uvin H herbal mixture in comparison to strawberry tree leaves. On the other hand, Uvin H contained significantly higher concentrations of essential elements Mn, Fe, Zn, Co, Se, and Mo in comparison to strawberry tree leaves. We cannot say with certainty that Uvin H herbal mixtures generally have such levels, as our sample was bought at one time point and the differences in the aforementioned elements compared to strawberry tree are probably the result of processing and storage conditions as well as different plant species reflecting the soil, air, and water element levels of the surrounding area.

In an attempt to identify the sources of elements in strawberry tree leaves, the natural clustering of the data (element concentrations) was explored with PCA. PCA produced four important PCs covering 94.86% of the total data variance (Table S.3). Figure 1 shows variable/loading plot constructed using the first two PCs (62.59% of total variance) allowing for a visualisation of the grouping of parameters. Elements Ca, Mg, K, Mo, Ni, Cd, Mn, and Cu were the main variables in the most important PC1, which was primarily linked to natural sources. Major elements Mg, K, Ca, and Na and trace elements Mo, Mn, and Cu are commonly found in many medicinal plants [14]. The content of these elements in plants is usually affected by the physical and chemical properties of the surrounding soil and the ability of the plant to selectively accumulate certain elements. They are essential for a wide range of physiological processes in living organisms and have certain nutritional benefits [10, 31]. However, there is a specific range of intake for these elements that is safe and adequate for the body, while insufficient, as well as excessive intake, may lead to both acute and chronic toxicity [22].

As opposed to essential elements, elements grouped under PC2 (Al, As, Co, and Cr) are considered to be toxic as they tend to accumulate in an organism and interfere with normal biological functions. The content of toxic elements in the plant mainly depends on anthropogenic factors such as pollution from busy highways, industrial pollution, and application of pesticides and fertilisers containing As [31]. In plants used for consumption, levels of toxic elements may also be associated with contamination of the sample during harvest and/or technological processes [14]. PC3 was represented by essential element Se. Its uptake, translocation, and distribution from the surrounding soil into a plant mainly depend upon the chemical form and concentration of Se and physiological conditions of soil (salinity and soil pH) [32]. In addition, levels of Se in strawberry tree leaves were rather low, which may be the reason for its grouping away from other essential elements.

Influence of Preparation Process on the Concentration of Elements in Herbal Tea

All of the measured elements were detected in the final herbal tea infusions and decoctions. For elements Al, Cr, Zn, Mo, and Pb, there was no statistically significant difference between the measured concentrations regarding the preparation method and its duration. For other elements, the preparation method had an effect on the final concentration in a way that decoction or longer infusion (10 min) resulted in a more efficient transfer of elements from leaves into the tea. The concentration was significantly lower after the 5-min infusion in comparison to herbal teas prepared by other preparation methods (Fig. 2). A notable separation of

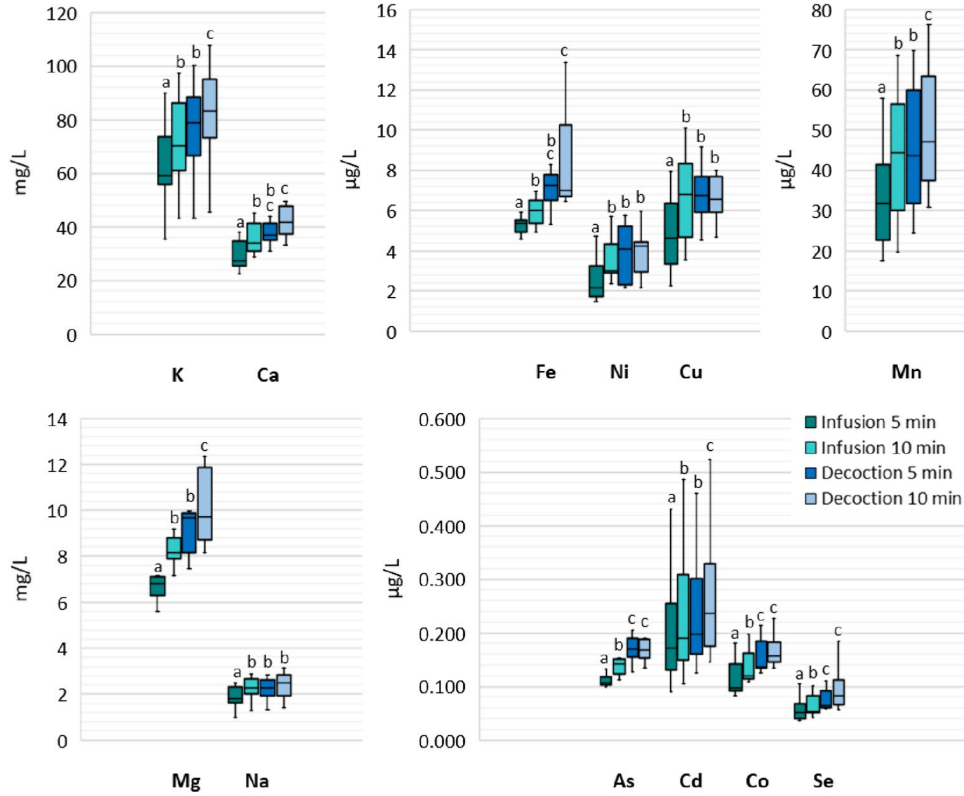

Fig. 2 Concentration of elements in tea prepared by infusion or decoction of strawberry tree leaves ($n = 7$) for 5 or 10 min. Only the elements that significantly differed between groups are shown [$p < 0.05$; Friedman ANOVA followed by post hoc Wilcoxon matched pairs test; values (box and whisker column) marked with different lowercase letters (a, b, c) are significantly different]. Results are presented as median (line), 25th and 75th percentile (box), and range (whisker)

these preparation methods (10-min infusion and 5- or 10-min decoction) from a 5-min infusion of herbal tea can be seen on a score plot of the first two principal components for element concentrations measured in herbal tea (Fig. S.1). For most elements, the time of decoction was not shown to affect the final concentration. When tea was prepared by decoction, significantly higher concentrations of K, Mg, Mn, and Cd were measured in herbal tea whose preparation lasted for 10 min in comparison to 5 min. In addition, for elements K, Ca, Mg, Fe, Mn, As, Cd, Co, and Se, significantly higher concentrations were measured in herbal tea prepared by 10-min decoction in comparison to 10-min infusion of leaves.

Contrary to our results, Zhang et al. [15] reported that the levels of Cu and Mn in black tea infusion were not dependent on the brewing time. The same authors, however, reported that levels of Ni and Cr increased with the brewing time, which was also the case for Ni in our samples. A similar increase in element concentrations in infusions of linden leaves by increasing brewing time was reported by Pytlakowska et al. [14] for Al, B, Ba, Cu, Fe, K, Mn, P, Sr, and Zn, but the reported results were not confirmed statistically.

Nutritional and Risk Assessment of Herbal Tea Consumption

Based on the obtained concentration of elements in the prepared herbal teas, we conducted a comparative nutritional and risk estimation of consumption between strawberry tree and Uvin H tea (Table 2). The estimations relied on the most recent EFSA data on DRV for essential elements, TI data, and BMDL for non-essential elements, and the recommended daily amount of Uvin H tea consumption in cases of urinary tract infection treatment. However, it has to be noted that the bioavailability (percentage of intestinal absorption) of each element was not considered in the calculations. Therefore, the total concentration of elements measured in leaves and in tea may have overestimated the actual health risk because the amount of absorbed element can be a relatively small fraction of the total amount present in the consumed foodstuff.

Their bioavailability aside, the nutritional contribution of elements from strawberry tree tea brewed by decoction of leaves was generally low and ranged from 0.001% of DRV for Fe and Mo to 0.06% of DRV for Ca (Table 2). Values obtained for Uvin H tea were somewhat higher and ranged from 0.003% of DRV for Fe to 0.5% of DRV for Mn. Thus, consumption of the recommended amount of Uvin H tea moved the intake of mentioned essential elements closer to reference values compared to consumption of strawberry tree tea, for the average consumer. Regarding the exposure assessment to non-essential elements, regular consumption of tea from strawberry tree leaves will contribute negligibly (Al, Cr, Pb) or very little (Ni, As, Cd) to the total dietary intake of respective elements and is proposed to be safe for human health. The % TI values calculated for Ni, As, and Pb

Table 2 Nutritional and risk estimation of daily consumption of herbal tea made of strawberry tree leaves/Uvin H herbal mixture regarding elements and EFSA's dietary recommendations (DRV for essential and TI for non-essential elements)

Element	DRV (mg/day)	Strawberry tree			Uvin H		
		Mean conc. in tea* (mg/kg)	EDI# (mg/kg b.w.)	% DRV	Mean conc. in tea* (mg/kg)	EDI# (mg/kg b.w.)	% DRV
Mg	300–**350**[a]	10.95	0.158	**0.05**	10.00	0.286	**0.08**
K	**3500**[a]	85.15	0.970	**0.03**	86.96	1.238	**0.04**
Ca	750[a]–**1000**[b]	41.31	0.626	**0.06**	15.17	0.551	**0.06**
Mn	**3**[a]	0.054	7.69×10^{-4}	**0.03**	0.636	0.016	**0.5**
Fe	6[c]–**16**[b]	8.39×10^{-3}	1.35×10^{-4}	**0.001**	0.021	4.60×10^{-4}	**0.003**
Cu	1.3–**1.6**[a]	6.82×10^{-3}	9.93×10^{-5}	**0.006**	0.010	1.17×10^{-4}	**0.007**
Zn	7.5–**16.3**[a]	0.117	1.69×10^{-3}	**0.01**	9.13×10^{-2}	2.87×10^{-3}	**0.02**
Se	**0.07**[a]	9.61×10^{-5}	1.61×10^{-6}	**0.002**	1.40×10^{-3}	6.85×10^{-6}	**0.01**
Mo	**0.065**[a]	6.73×10^{-5}	8.19×10^{-7}	**0.001**	2.82×10^{-4}	2.72×10^{-6}	**0.004**
	TI (μg/kg b.w.)	**Mean conc. in tea*** (μg/kg)	**EDI** (μg/kg b.w.)	% TI	**Mean conc. in tea*** (μg/kg)	**EDI** (μg/kg b.w.)	% TI
Al	**1000**[d]	7.786	0.115	**0.01**	35.06	0.525	**0.05**
Cr	**300**[e]	0.172	2.55×10^{-3}	$\mathbf{8.51 \times 10^{-4}}$	0.510	0.022	**0.01**
Ni	**13**[e]	4.189	0.054	**0.4**	15.64	0.737	**5.7**
As	**0.3**–8[f]	0.187	2.67×10^{-3}	**0.9**	0.217	8.50×10^{-3}	**2.8**
Cd	**2.5**[d]	0.305	4.27×10^{-3}	**0.2**	0.073	2.16×10^{-3}	**0.09**
Pb	**0.5**[f]	0.398	6.16×10^{-3}	$\mathbf{1.23 \times 10^{-4}}$	0.830	0.023	**4.6**

DRV, dietary reference values (for persons over 18 years of age); *TI*, tolerable intake; bold values are taken in calculation of % DRV/TI; *EDI*, estimated daily intake

*Tea was prepared by 10 min decoction of strawberry tree leaves/Uvin H herbal mixture

[#] For consumption of 1 L of tea/day (10 g of strawberry tree/Uvin H herbal mixture)

[a] *AI*, adequate intake [33–40]

[b] *PRI*, population reference intakes [43, 49]

[c] *AR*, average requirement [41]

[d] *TWI*, tolerable weekly intake [24, 27]

[e] *TDI*, tolerable daily intake [25, 26]

[f] *$BMDL_{01}$*, benchmark dose lower confidence limit at 1% extra risk: $BMDL_{01}$ for As for increased risk of cancer of the lung, skin and bladder, skin lesions [29]; developmental neurotoxicity $BMDL_{01}$ for Pb [28]

in Uvin H herbal tea (5.7%, 2.8%, and 4.6%, respectively) were noticeably higher in comparison to those obtained for these elements in tea from strawberry tree leaves. However, the obtained data did not exceed the proposed 10% cut-off limit previously suggested for content of toxic elements in dietary supplements [42]. The results of the study by Abreu et al. [43] indicated that the consumption of infusion made of leaves and twigs of strawberry tree growing on soils with high concentrations of As, Cd, Cu, and Zn would not represent a significant additional risk for consumers with respect to overall dietary exposure. The chemical exposure dose for the intake of an infusion made with leaves and twigs was calculated considering that 0.3 kg of leaves and twigs per year is used for infusions consumed for 96 days/year at the most. Similar studies of other herbal teas also report a marginal intake of both essential and non-essential elements from herbal tea infusions [15, 42, 44–49].

Total Phenolic Content (TPC) and Antioxidant Activity of Herbal Tea

The TPC content and antioxidant activity of the strawberry tree leaf and Uvin H infusions and decoctions brewed for 5 or 10 min are shown in Table 3. Although TPC slightly increased with infusion time, the difference was not statistically significantly. This finding is in accordance with Labbé et al. [50], who reported that the concentration of phenolics in green tea increased up to a certain level, and then remained constant after a further increase of infusion time. On the contrary, several studies reported that an increase in preparation time favours the extraction of phenolics by increasing their solubility and the diffusion coefficient [51]. The preparation method (infusion vs. decoction) had a significant effect on the level of the phenolics: TPC content in decoctions was ~ 1.2- to 1.3-fold higher than that of the

Table 3 Total phenolic content and antioxidant activity of the strawberry tree leaves from three Croatian locations in different time periods and Uvin H herbal mixture infusions and decoctions

Herbal preparation	Total phenolic content (TPC) (mg GAE/g dried leaf)		Antioxidant activity (DPPH) (µmol TE/g dried leaf)	
	Strawberry tree leaf (*N* = 7)	Uvin H# herbal mixture (*N* = 1)	Strawberry tree leaf (*N* = 7)	Uvin H# herbal mixture (*N* = 1)
	Median (range)	Mean ± SD	Median (range)	Mean ± SD
Infusion 5 min	42.1[a] (32.9–55.7)	16.8 ± 0.9	141.5[a] (137.8–146.9)	100.7 ± 0.2
Infusion 10 min	45.8[a] (36.2–60.7)	28.1 ± 4.4	152.6[b] (147.7–158.0)	151.6 ± 0.3
Decoction 5 min	55.2[b] (40.7–66.8)	29.4 ± 0.5	157.1[c] (155.2–160.4)	155.3 ± 1.5
Decoction 10 min	54.0[b] (43.1–71.6)	30.1 ± 0.0	160.2[d] (159.1–164.0)	156.5 ± 4.5

Mixture of bearberry leaves (30%), aerial parts of heather (20%), birch leaves (15%), restharrow root (15%), aerial parts of horsetail (10%), and corn silk (10%). The results for single sample are presented as mean ± standard deviation ($n = 3$)

Values within the same column marked with different lowercase letters are significantly different (Friedman test, $p < 0.05$)

Samples are measured in triplicate

GAE, gallic acid equivalents; *TE*, trolox equivalents

herbal tea infusions. In accordance with our results, Erkekoglou et al. [12] reported an ~ 1.4-fold higher TPC in strawberry tree leaf decoction than in infusion.

Despite of the lowest value of TPC in 5-min strawberry tree leaf infusion (84.2 mg GAE) compared to other preparation methods, it ensured a much higher amount of phenolics per 200-mL cup compared to the amount per serving cup reported for infusions from other herbal materials, e.g. rosemary (8.5 mg GAE), oolong tea (30.7 mg GAE), green tea (32.4 mg GAE), black tea (34.1 mg GAE), sage (34.5 mg GAE), thyme, (58.7 mg GAE), and Greek mountain tea (73.2 mg GAE), although they were prepared using higher amount of herbal material per serving cup (2.4–2.5 g) [52–54].

The preparation method (infusion *vs*. decoction) and time had a significant influence on the antioxidant activity of strawberry tree leaf teas. Previously published reports on herbal teas also showed that both the TPC and antioxidant capacity of different herbal tea extracts correlated well with extraction time [55]. A moderate correlation between TPC and antioxidant activity ($r = 0.592$; $p < 0.001$) could point to antagonism/synergism among diverse bioactive compounds, including phenolics. Contribution of other bioactive compounds was reported by Erkekoglou et al. [12], who found that up to 7% of the radical scavenging efficiency was attributed to herbal tea constituents other than phenolics, e.g. vitamins (C, E) and carotenoids.

To achieve the maximum possible health benefits by consuming strawberry tree tea with the highest TPC and antioxidant activity, we recommend decoction for 10 min as the preparation method of choice. The decoction method for functional tea preparation was already proposed by several authors [12, 56].

Table 4 Activity concentrations (Bq/kg) of ^{137}Cs and ^{40}K in dry strawberry tree leaves from three Croatian locations in different time periods and Uvin H herbal mixture expressed as a mean value with 2σ measurement uncertainty

Radionuclide	MDL*	Uvin H herbal mixture	Strawberry tree leaves Location (year)						
			Lošinj (2013)	Lošinj (2014)	Lošinj (2015)	Lošinj (2016)	Koločep (2013)	Koločep (2015)	Ugljan (2016)
^{137}Cs	0.6	1.3 ± 0.7	2 ± 1	1.3 ± 0.7	0.9 ± 0.7	1.9 ± 0.9	1.3 ± 0.8	1.5 ± 0.6	1.2 ± 0.7
^{40}K	6	244 ± 5	279 ± 5	258 ± 5	188 ± 3	170 ± 2	238 ± 4	209 ± 4	103 ± 2

*Method detection limit (Bq/kg)

Table 5 Radionuclide activity concentrations (Bq/kg) in dry herbal samples used for tea preparation reported in the literature

Plant	Location	^{137}Cs	^{40}K	^{226}Ra	^{228}Ra	Reference
Strawberry tree	Croatia	0.9–2	103–279	< MDL	< MDL	This study
Medicinal herbs	Serbia	< 0.2–24.7	103–1100	N/A	N/A	[57]
Camellia sinensis	Turkey	< MDL –112	355–570	< MDL –23.4	0.42–7.38	[58]
Camellia sinensis	Korea	0.076–0.372	212–589	N/A	N/A	[19]
Camellia sinensis	Bangladesh	< MDL	151–1243	2.4–18.93	1.4–27.22	[59]
Camellia sinensis	Bangladesh	< 0.4	132–258	3.6–5.7	2.4–5.8	[60]
Camellia sinensis	Vietnam	0.24–0.66	171–220	17.1–27.6	12.7–18.9	[61]
Various herbs	Vietnam	0.3–3.5	414–704	< 0.9–6.2	2.0–29.8	[17]
Various herbs	Brazil	N/A	421–732	< 27	3.0–27.0	[62]
Various herbs	Iraq	N/A	630–1354	2.87–22.03	5.80–64.74	[63]

N/A, not analysed

Radionuclide Activity Concentrations in Leaves and Herbal Teas

Only ^{137}Cs and ^{40}K were detected in strawberry tree leaves and Uvin H herbal mixture (Table 4). Radionuclides from ^{238}U and ^{232}Th decay chains were below the MDL; therefore, ^{238}U and ^{232}Th decay chains were not considered in further analyses. All radionuclides, except for ^{137}Cs, come from natural sources. ^{137}Cs activity concentrations were similar in all dry herbal samples with a 95% confidence interval overlap. Only the sample with the highest value and two with the lowest values did not overlap at the 68% confidence level. There was no statistical difference between mean ^{137}Cs in Uvin H herbal mixture and the average ^{137}Cs in samples of strawberry tree leaves collected from three Croatian locations in different time periods.

Due to the lack of any data on radionuclide activity concentrations in strawberry tree herbal samples used for tea preparation, we compared our results with available data for other herbal plants from the literature (Table 5). We noticed that our results were at the lower end of levels reported worldwide. This is especially evident for radionuclides from the ^{238}U and ^{232}Th decay chains, which were below the MDL for all our samples and were detected in other studies. The highest levels of ^{137}Cs and ^{226}Ra were measured in samples of *Camellia sinensis* from Turkey [58] and Vietnam [61], respectively, while the highest levels of ^{40}K and ^{228}Ra were measured in herbal samples from Iraq [63]. Levels of ^{137}Cs in tea cultivated in Argentina were from non-detectable to 10.3 Bq/kg and authors observed correlation between the amount of ^{137}Cs contamination in the tea leaves and the fertilisers used in their cultivation [64]. However, the authors report that the values of hazard indices were low for all analysed tea samples, indicating that moderate consumption of tea made from analysed herbs does not pose a health risk to consumers [58, 61, 63].

From a radiation protection point of view, ^{40}K is of less significance compared to ^{137}Cs. Traces of ^{40}K (around 0.012%) are found naturally in the Earth's total K content. Since the human body balances K content to maintain homeostasis, excess K, including ^{40}K, will be excreted after ingestion. The annual effective dose due to the presence of ^{40}K in the body is typically about 0.165 mSv for adults [65].

Unlike ^{40}K, ^{137}Cs presents a serious threat to human health due to its long half-life (30.1 years) and chemical resemblance to K. Today's ^{137}Cs activity concentrations found in the European environment are the result of the Chernobyl accident in 1986 and atmospheric testing of nuclear weapons in the 1950s and 1960s, with a small amount resulting from the Fukushima accident. Once ^{137}Cs enters the human body, it will present toxic effects for decades. Due to the general chemical similarity to K, Cs can replace K in the cell and change its biochemical role within the human body [66]. Once ingested or inhaled, ^{137}Cs can ionise atoms within the body not just through 661.62 keV gamma rays but also through beta particles.

In herbal teas prepared by decoction in this study, the activity concentrations of ^{137}Cs were below the MDL (Table S.4). Zehringer et al. [67] reported that around 80% of ^{137}Cs is transferred from the leaf into the tea during the brewing. The range of ^{210}Po (11–26%) transferred from the tea leaves (*Camellia sinensis*) marketed in Syria to the aqueous extract reached maximum at the highest temperature of infusion (100 °C) [68]. Since the activity of ^{137}Cs is too low to be detected in the tea, a 100% transfer rate can be conservatively assumed. Assuming a 100% transfer rate of ^{137}Cs from leaves into herbal tea, and that 10 g of leaves was used to make 1 L of tea, concentration A for herbal tea made from the Uvin H herbal mixture is 13 ± 7 mBq/L of ^{137}Cs. The recommended intake of Uvin H tea is 1 L per day for 14 days, repeated no more than five times throughout a year. The resulting calculation showed that a person drinking Uvin H herbal tea once a year, for a 14-day period, would receive an effective dose of 2.4 ± 1.3 nSv, while in the case of an annual five-time repetition of a 14-day treatment, a person would receive an effective dose of 11.8 ± 6.4

nSv. Maximum exposure while consuming strawberry tree leaves tea was calculated with the maximum measured ^{137}Cs value, at the maximum of the confidence interval ($A + 2\sigma$). In the case of one annual 14-day consumption of this tea, an adult person would receive an effective dose of 5.5 nSv. If the therapy were repeated for five times in a year, the total effective dose received would be 27.3 nSv.

According to the United Nations Scientific Committee on the Effects of Atomic Radiation [65], the average annual effective dose from natural sources (cosmic radiation, external terrestrial radiation, inhalation, and ingestion of naturally occurring radionuclides) is 2.4 mSv, and a typical range is between 1 and 13 mSv. The average annual effective dose from the intake of ^{40}K is 0.165 mSv. The dose received by drinking the maximum recommended yearly amount of Uvin H tea or strawberry tree leaf decoction, considering the highest measured value of ^{137}Cs, was almost four orders of magnitude lower than the dose from the ^{40}K in the human body and close to five orders of magnitude lower than the average annual effective dose from natural sources. Therefore, the analysed Uvin H tea and strawberry tree leaf decoction cannot be considered a threat to human health from a radiation protection point of view.

This is the first study that reports the activity concentration of radionuclides and a larger number of elements in strawberry tree leaves from Croatia and its tea infusions/decoctions. The preparation method as well as preparation time had a significant influence on the levels of certain elements and the antioxidant activity of strawberry tree leaf tea. The maximum possible health benefits were shown to be obtained by a 10-min decoction of strawberry tree leaves, which resulted in the highest TPC and antioxidant activity and levels of essential elements (K, Ca, Mg, Na, Fe, Mn, and Se). Regarding toxic elements (e.g. As, Cd, and Pb), their EDI values were well below the tolerable intake value and therefore safe for human consumption. In addition, the estimated radiological risk from the prepared strawberry tree leaves and Uvin H tea consumption was negligible. According to the obtained results, herbal tea prepared from strawberry tree leaves may be considered a good dietary source of natural antioxidants and a worthy replacement for the Uvin H herbal mixture. High levels of K, Ca, Mg, and Na justify its use as a nutritional supplement, especially when herbal tea is used for diuretic purposes that may result in increased excretion of mineral elements.

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Declarations

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