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Element composition of propolis tinctures prepared from Hungarian raw propolis

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ABSTRACT

Although the element composition of raw propolis gets more and more attention, their products, like tincture are barely analyzed. In this study the element content of tinctures prepared by 80% (v/v) ethanol is measured parallel to the initial raw propolis. Most of the essential elements had relatively high transfer coefficients to the tincture in the range of 14.2–67.3% by median. In contrast, most of the potentially toxic elements (Al, Ba, Cd, Cs, Sr, V) had transferred at a rate usually below 10%. Transfer coefficients of Ni and Cr were the highest: 7.02–62.6% and 1.20–30.4%, respectively. When considering the consumption of tinctures, potentially toxic elements are taken into the body in just a nanogram per day magnitude or less. The quantity of bioactive compounds in tincture are higher in several orders of magnitude, than potentially toxic elements. Nevertheless, raw propolis containing a high concentration of potentially toxic elements, especially Ni and Cr, is to be avoided. The transfer coefficient was applied for the prediction of the mineral composition in raw propolis due to the mineral content of tincture. Generally, even an overestimation by two or an equal level of underestimation should be expected, even if the same extraction process was used.

1. Introduction

Propolis is a resinous material collected by *Apis mellifera* bees from the buds of trees. The bee colony practically uses raw propolis for covering the wall of the hive and, with it, also fill in the cracks and holes (Pierini et al., 2013). Furthermore, there is a microbial inhibitor effect of raw propolis in the bee colony (Borba, Wilson, & Spivak, 2017). Propolis is often present in the practice of human healing as an anti-inflammatory (Inui, Hosoya, Yoshizumi, Sato, & Kumazawa, 2021), antimicrobial (Okińczyc et al., 2020) and antiviral (Silva-Beltrán, Balderrama-Carmona, Umsza-Guez, & Souza Machado, 2020) agent. Following the first step, extraction, raw propolis is usually consumed or applied in processed forms. Extracted propolis can be encapsulated (Irigoiti, Yamul, & Navarro, 2021), placed in creams (Arenberger, Arenbergerova, Hladíková, Holcova, & Ottillinger, 2018), in honey (Osés, Pascual-Maté, Fernández-Muiño, López-Díaz, & Sancho, 2016) or can be used as a tincture. Tincture is the extract of raw propolis along with different extractants harmless to the human body, just like the ethanol/water mixture. The ratio of the aforementioned two compounds is not defined, usually 70 or 80% (v/v) ethanol is used (Bankova, Trusheva, & Popova, 2021).

The organic composition of propolis after extraction is often evaluated. Propolis contains alcohols, aliphatic acids, aromatic acids, esters, flavonoids, anthraquinones, ketones, sugars, terpenes, and other organic compounds (Kalogeropoulos, Konteles, Troullidou, Mourtzinos, & Karathanos, 2009). The extraction process is frequently optimized for maximizing the bioactive compounds in tincture (Cvek et al., 2007; Oldoni et al., 2015; Zhang, Cai, Chen, Ji, & Sun, 2020).

The mineral composition of raw propolis is also increasingly analyzed. Mineral composition of raw propolis is used for geographical authentication in several studies (Cantarelli, Camiña, Pettenati, Marchevsky, & Pellerano, 2011; Gong, Luo, Gong, Gao, & Xie, 2012; Siqueira et al., 2020; Soós, Bódi, Várallyay, Molnár, & Kovács, 2021). It is also utilized as an environmental indicator for metals and metalloids (Conti & Botrè, 2001; Finger, Filho, Torres, & Quináia, 2014; Golubkina, Sheshnitsan, Kapitalchuk, & Erdenotsogt, 2016). Toxic elements are also checked in raw propolis from the physiological point of view (González-Martín et al., 2015; Roman, Madras-Majewska, & Popiela-Pleban, 2011). However, the element content of propolis tinctures and other propolis-containing products is rarely analyzed (González Rodríguez, Blázquez Abellán, & Orzáez Villanueva, 1999; Cvek et al., 2008; González-Martín, Revilla, Betances-Salcedo, &

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Vivar-Quintana, 2018).

In our previous study we have checked the effect of the different extraction parameters (0, 50, 80 and 100% (v/v) ethanol for 1 h, 1 day, 1 week and 1 month) in the case of a sole raw propolis. We have proved that the ethanol content in an extraction solvent has notable effects on the element composition of the tincture. Several elements dissolved better in water than in ethanol-content solvents. Most of the essential elements were extracted well, also in up to an 80% (v/v) ethanol content extractant. The concentration of most of the elements did not change greatly after 1 week of extraction (Soós, Bódi, Várallyay, Molnár, & Kovács, 2019).

In this article the element content of tinctures is evaluated, prepared from Hungarian raw propolis by using an 80% (v/v) ethanol content extractant. Important from the physiological standpoint, we would like to increase the available data for the mineral composition of propolis tinctures. Moreover, we have checked the transfer coefficient of minerals from raw propolis to their tinctures. Finally, the estimation of the mineral content of raw propolis is performed based on the transfer coefficient and the mineral composition of its tincture.

2. Materials and methods

2.1. Chemicals

High purity deionized water (18.2 MΩ cm^{-1}) was used from MilliQ system (MilliQ, Millipore Corp., Bedford, MA, USA) for the whole analytical process. High purity anhydrous ethanol (≥99.8% (v/v)) was utilized from VWR International (Fontenay-sous-Bois, France) for the extraction in 80% (v/v) concentration. Nitric acid (65% w/w) and hydrogen peroxide (30% w/w) from Scharlab S.L. Sentmenat, Spain were applied for the digestion. Nitric acid was distilled with a Milestone subPUR sub-boiling distillation system (Milestone Srl, Sorisole, Italy) for further purification. Calibration standards were prepared from 1000 mg L^{-1} monoelement standards (Scharlau, Barcelona, Spain) except for rare earth elements, where a multielement standard solution was used with 100 mg L^{-1} concentration (Teknolab A/S, Drøbak, Norway). Rhodium was applied as an internal standard diluted from 1000 mg L^{-1} monoelement stock solution (Fluka, Buchs, Switzerland) for ICP-MS analysis.

2.2. Samples

Raw propolis samples were collected in autumn of 2014 by the beekeepers from Hungarian apiaries in fixed location, and the collection was controlled by the National Beekeepers' Association of Hungary. In total, 252 raw propolis samples were collected, the element composition together with their geographical authentication of which was evaluated in our previous work (Soós et al., 2021), 27 samples then being selected randomly from these for the extraction process. Samples originated from Western Transdanubia (5 samples; P1–P5), Central Transdanubia (5 samples; P6–P10), Southern Transdanubia (3 samples; P11–P13), Central Hungary (2 samples; P14–P15), Northern Hungary (5 samples; P16–P20), the Northern Great Plain (3 samples; P21–P23) and the Southern Great Plain (4 samples; P24–P27), respectively.

2.3. Extraction process

The extraction process is similar to that described in our previous study (Soós et al., 2019). The applied extraction conditions are not adopted from other authors in its entire form, but are similar to the most often used conditions in the literature (extractant, duration, solid-liquid ratio, Bankova et al., 2021). Briefly, about 0.50 ± 0.01 g from about 5 to 10 g of ground and homogenized raw propolis was weighed into plastic centrifuge tubes, then 5 mL of 80% (v/v) ethanol as an extractant was added. The extraction took place at room temperature (23 ± 2 °C) in triplicate. The tubes with the raw propolis and the extraction solvents were intensively mixed by Biosan Vortex V-1 at the beginning of the extraction process, then twice every working day. Extraction was finished after 1 week. After the extraction period the samples were mixed again and centrifuged at 1600×*g* for 10 min at 21 ± 2 °C. The supernatant was filtered with Filtrak 388 filter paper and kept at room temperature in a dark place in closed centrifuge tubes until digestion.

2.4. Sample preparation

The digestion process of propolis tinctures and raw propolis is described in detail in Soós et al. (2019) and Soós et al. (2021). Briefly, after shaking the samples, 2 mL of the tinctures were pipetted into quartz tubes (80 mm × 16 mm, total volume is approx. 11 mL), and dried in an oven at 45 °C to their constant weight. For raw propolis approximately 0.1 g homogenized samples were weighted into quartz tubes. The following steps were concordant, namely 2.0 mL nitric acid were pipetted and were left overnight. The next day 0.6 mL H_2O_2 were added and the quartz tubes were sealed with household Teflon tape. A maximum of three closed quartz tubes were placed into polytetrafluoroethylene (PTFE) vessels of the microwave digestion system. Ten mL portions of distilled water were added between the quartz and the PTFE containers, which were then closed smoothly and were placed into the SK-10 high pressure segmented rotor then into the microwave digestion system (Milestone Start D, Milestone Srl, Sorisole, Italy). The samples were heated up to 180 °C in 15 min, were kept at a constant temperature at 180 °C for 20 min, finally cooled down to room temperature at about 60 min. Samples were washed into 15 mL centrifuge tubes and filled up to 9.5–10.5 mL with MilliQ water. A volumetric flask was not used in order to avoid cross-contamination and reaching the lowest limit of detections. The accurate volumes of the digested samples were calculated in relation to the mass of the digested samples multiplied by the densities of the solutions. Digestion of raw propolis samples were done in triplicate, while each of the three parallel tinctures were digested once.

2.5. Apparatus

Digested samples were measured by inductively coupled plasma optical emission spectrometry (ICP-OES; Thermo Scientific iCAP 6300 Dual view) and inductively coupled plasma mass spectrometry (ICP-MS, Thermo Scientific X-Series II). The instrument parameters together with the performance characteristics (limit of detection, accuracy, repeatability, stability) were described in detail in Soós et al. (2019) and Soós et al. (2021). The following elements were measured by ICP-OES (with their detected wavelengths in nm): Al (394.401), B (208.959), Ba (455.403), Ca (315.887), Fe (238.204), K (766.490), Mg (279.079), Na (818.326), P (213.618), S (182.034), Sr (407.771) and Zn (213.856), while the following isotopes were measured by ICP-MS: ^{51}V, ^{52}Cr, ^{55}Mn, ^{59}Co, ^{60}Ni, ^{65}Cu, ^{95}Mo, ^{111}Cd, ^{133}Cs, ^{139}La, ^{140}Ce, ^{141}Pr, ^{146}Nd, ^{147}Sm, ^{153}Eu, ^{157}Gd, ^{159}Tb, ^{163}Dy, ^{165}Ho, ^{166}Er, ^{169}Tm, ^{172}Yb, ^{175}Lu and ^{238}U.

2.6. Statistical analysis, calculation of the transfer coefficient and similarity

The graphs were made by using Microsoft Excel 2013. SPSS 25.0 for Pearson's correlation was applied with a $p < 0.01$ and $p < 0.05$ significance level. The transfer coefficient (TC) was calculated by the concentration of element in tinctures divided by the concentration of element in raw propolis, multiplied by 10 as the dilution factor, then multiplied by 100. Similarity of estimated and actual mineral content is calculated by the transfer coefficient of individual samples divided by the median of the transfer coefficient.

3. Results and discussion

3.1. Element content of raw propolis

We have measured and evaluated all 252 Hungarian raw propolis samples, which were described in detail elsewhere (Soós et al., 2021). However, the mineral content of 27 raw propolis specimens that were used for the preparation of tinctures are present in Table S1 (Supplementary material). The most important statements about the characteristics of raw propolis in the aforementioned article are also proper for this smaller batch of samples. The elements were arranged by descending order of median concentration in raw propolis in Table S1.

3.2. Element content of propolis tinctures

Beyond analysis of raw propolis, the element content of propolis tinctures prepared by 80% (v/v) ethanol as an extractant was also measured. The median, the lowest and the highest concentrations of elements are indicated in Table 1, while the element content of individual tinctures parallel to the element content of initial raw propolis is shown in Table S1 (Supplementary material). The highest concentration in tinctures belongs to potassium with a median of 50.6 mg L^{-1}. Potassium is followed by P, Mg and S with 10.7 mg L^{-1}, 4.71 mg L^{-1} and 4.47 mg L^{-1} concentrations, respectively. The median concentrations of Ca, Na and Zn were 1.96 mg L^{-1}, 1.92 mg L^{-1} and 1.28 mg L^{-1}, respectively. Median content of Fe and B were in the range of 0.1–1 mg L^{-1}. The median concentrations of Mn, Cu, Al, Ni, Cr, Co, Sr and Mo were between 1 and 100 μg L^{-1}. Some of the samples by their element concentrations were out of the last range. Four tinctures contained 200–300 μg L^{-1} of Cu, besides 736 μg L^{-1} of Cu measured in one sample. Aluminum is present in two samples with 0.446 mg L^{-1} and 1.15 mg L^{-1} concentration. The two highest Ni contents in tinctures were 23.1 and 202 μg L^{-1}. All the tinctures had 1.44–91.5 μg L^{-1} of Cr, except one (117 μg L^{-1}). Cobalt content did not reach the 1–100 μg L^{-1} range in one sample (0.290 μg L^{-1}). Vanadium, caesium and uranium were in the range of 0.197–2.88 μg L^{-1}, 17.6–278 ng L^{-1} and <5.17–56.7 ng L^{-1}, respectively. Cadmium concentration was under the limit of detection in 16 tinctures, and the remaining samples barely reached 1.05 μg L^{-1}. Barium was below the LOD, except in one tincture. The contents of lanthanides were presented under the limit of detection in at least half of the tinctures; therefore, the medians were also below the LOD. However, the highest content of the lanthanides in tinctures did not reach 60 ng L^{-1}. The concentrations of those element in tinctures, which were reached the LOD in at least half of the samples were illustrated on a box plot graph in Fig. 1.

There are just a few articles about the element composition of propolis tinctures. Cvek et al. (2008) measured the element composition of ten tinctures. Comparing our data with the median values of the mentioned authors, potassium, sodium and boron contents were similar to ours, but copper, iron, and manganese contents were a little higher in Croatian tinctures. However, it should be highlighted that calcium, aluminum, barium, strontium, and zinc were more than ten times higher in their samples. Only Mg was lower, while Cr and Ni could not be measured. The deviation can be explained by the differences in the element content of initial raw propolis (Soós et al., 2021). Despite the solid:liquid ratio and the ethanol content of the extractant being the same, there are differences in the extraction process, because a magnetic stirrer was applied for 30 min in their experiment. It should be also

Table 1
The element content of propolis tinctures and the transfer coefficient (TC) between raw propolis and propolis tinctures.

Element	Concentration in tinctures				Transfer coefficient (%)		
	Unit	Median	Minimum	Maximum	Median	Minimum	Maximum
K	mg L^{-1}	50.6	27.8	101	67.3	36.1	81.2
P	mg L^{-1}	10.7	1.80	31.5	47.4	8.53	70.2
Mg	mg L^{-1}	4.71	1.52	9.89	32.8	12.3	62.3
S	mg L^{-1}	4.47	1.04	10.6	17.7	10.4	36.3
Ca	mg L^{-1}	1.96	0.516	5.59	4.14	0.85	13.3
Na	mg L^{-1}	1.92	1.05	9.21	56.8	19.6	85.1
Zn	mg L^{-1}	1.28	0.217	15.9	18.0	6.13	64.7
Fe	mg L^{-1}	0.441	<0.0153	3.54	2.46	n.c.[a]	15.1
B	mg L^{-1}	0.259	0.115	0.416	42.6	8.64	70.3
Mn	μg L^{-1}	96.0	7.16	258	18.5	4.79	34.6
Cu	μg L^{-1}	90.6	20.8	736	47.6	23.1	68.2
Al	μg L^{-1}	81.0	<0.0111	1150	0.56	n.c.[a]	2.24
Ni	μg L^{-1}	9.62	1.25	202	25.4	7.02	62.6
Cr	μg L^{-1}	6.52	1.44	117	8.73	1.20	30.4
Co	μg L^{-1}	3.76	0.290	55.2	36.1	8.36	97.1
Sr	μg L^{-1}	2.73	0.637	11.7	1.53	0.25	8.51
Mo	μg L^{-1}	1.42	0.138	7.20	14.2	4.76	34.6
V	μg L^{-1}	0.854	0.197	2.88	3.42	1.11	11.2
Cs	ng L^{-1}	70.1	17.6	278	3.82	0.20	18.0
U	ng L^{-1}	20.1	<5.17	56.7	1.42	n.c.[a]	7.40
Cd	μg L^{-1}	<0.0904	<0.0904	1.05	n.c.[a]	n.c.[a]	11.8
Ba	μg L^{-1}	<3.40	<3.40	3.84	n.c.[a]	n.c.[a]	0.06
Ce	ng L^{-1}	<15.3	<15.3	59.1	n.c.[a]	n.c.[a]	0.22
La	ng L^{-1}	<9.87	<9.87	23.2	n.c.[a]	n.c.[a]	0.24
Dy	ng L^{-1}	<6.99	<6.99	15.1	n.c.[a]	n.c.[a]	0.94
Gd	ng L^{-1}	<4.35	<4.35	13.3	n.c.[a]	n.c.[a]	2.08
Eu	ng L^{-1}	<1.86	<1.86	12.7	n.c.[a]	n.c.[a]	2.35
Er	ng L^{-1}	<2.21	<2.21	9.59	n.c.[a]	n.c.[a]	1.29
Pr	ng L^{-1}	<6.74	<6.74	9.08	n.c.[a]	n.c.[a]	0.34
Yb	ng L^{-1}	<4.29	<4.29	6.04	n.c.[a]	n.c.[a]	0.93
Tb	ng L^{-1}	<2.34	<2.34	3.14	n.c.[a]	n.c.[a]	0.24
Ho	ng L^{-1}	<1.17	<1.17	2.97	n.c.[a]	n.c.[a]	1.06
Lu	ng L^{-1}	<1.36	<1.36	1.59	n.c.[a]	n.c.[a]	1.50
Tm	ng L^{-1}	<2.05	<2.05	<2.05	n.c.[a]	n.c.[a]	n.c.[a]
Nd	ng L^{-1}	<46.3	<46.3	<46.3	n.c.[a]	n.c.[a]	n.c.[a]
Sm	ng L^{-1}	<18.5	<18.5	<18.5	n.c.[a]	n.c.[a]	n.c.[a]

[a] Not calculated.

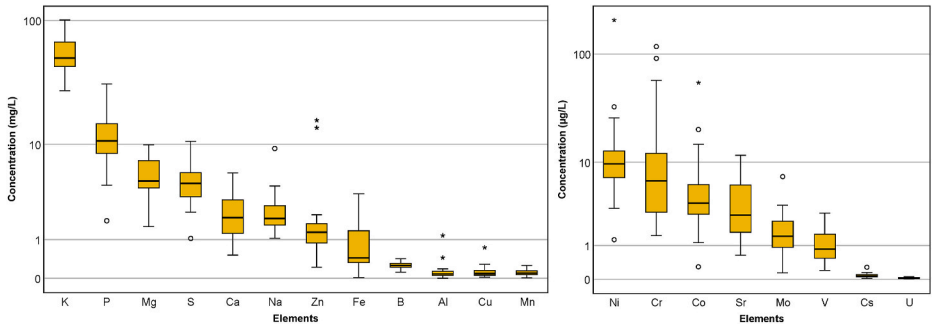

Fig. 1. Element concentrations in propolis tinctures illustrated on box plot graphs.

noted, that the season or the collection procedure may affect the element content of raw propolis (Souza, Zaluski, Veiga, & Orsi. 2016) and even the extractability of them into the tincture in the experiments. González-Martín et al. (2018) measured four commercially available tinctures. Copper content there (<0.01–0.70 mg kg^{-1}) was similar to our results. Nickel content (0.01–0.04 mg kg^{-1}) of commercial products had a narrower range than ours. There was an outlier in our samples with a 202 mg L^{-1} concentration of Ni. Zinc was present in 0.20–0.71 mg kg^{-1} in commercially available tinctures, but our sample batch contained some tinctures, which were 20 times higher than the highest result of González-Martín et al. (2018). However, our lowest and highest Cr contents were lower by one order of magnitude, than the lowest and highest results (0.10–0.87 mg kg^{-1}) of González-Martín et al. (2018).

3.3. Transfer coefficient (TC) of the elements from raw propolis to their tinctures and the possible risk of the tinctures

We have checked the transfer coefficient (TC) of 36 elements from raw propolis to their tinctures, extracted for 1 week by an 80% (v/v) ethanol content extractant. The median, the minimum and the maximum value of transfer coefficient are present in Table 1, moreover the TC of individual samples are illustrated with circles and the medians are marked with black rectangles in Fig. 2. The same color of the circles means the transfer coefficients calculated by same raw propolis-tincture pairs. In general, the transfer coefficient varies extremely by checking the same element. For example, TC of P, Mg, Mo and Cr was in the range of 8.53–70.2%, 12.3–62.3%, 4.76–34.6% and 1.2–30.4%, respectively. The range is narrower in the case of elements with lower TC, but relative difference is also high. For instance, TC of Ca and Sr were in the range of 0.85–13.3% and 0.25–8.51%, respectively, which are 15.6-fold and 34.0-fold differences.

The transfer coefficient of most of the essential elements was relatively high: 14.2%, 17.7%, 18.0%, 18.5%, 32.8%, 36.1%, 42.6%, 47.4%, 56.8% and 67.3% of Mo, S, Zn, Mn, Mg, Co, B, P, Na and K contents were transferred from raw propolis into their tincture by considering the median value. Potassium had the highest TC by median, but in two individual cases Na and Co had higher TC (85.1% and 97.1%) than the highest TC of potassium. The high transfer coefficient of these elements can be explained by the relatively high extractability in the 80% (v/v) ethanol content extractant. The listed elements, except Cu and Mo belong to that group, based on our previous classification, which are extracted the best in 0 and 50% (v/v) ethanol content extractant, but are extracted also well in 80% (v/v) ethanol content tinctures (Soós el al., 2019). Calcium and iron are also essential for humans, but considering the relatively low TC (4.14% and 2.46% by median) and also low concentration in raw propolis, it is not sufficient for the physiological needs.

Chromium was considered to be either essential or carcinogenic, depending on the oxidation state of the element, but the essentiality is ambiguous in relation to recent studies (Pavesi & Moreira, 2020). Propolis may accumulate chromium by industrial activity (Golubkina et al., 2016), and Cr may be undesirable, especially in the absence of a speciation analysis. The transfer coefficient of Cr was 8.73% by the median, but was up as high as 30.4%. Two separate groups can be observed in relation to the TC: 1.20–13.6% (20 samples) and 22.6–30.4% (7 samples). High Cr content in raw propolis goes together with high TC in a few cases. Based on our previous study, Cr is extracted in the highest amount by 80% (v/v) ethanol content extractant (Soós et al., 2019).

Nickel is a potentially toxic element (Genchi, Carocci, Lauria, Sinicropi, & Catalano, 2020), but its transfer coefficient was closer to the essential elements mentioned above. Median value was 25.4%, while the range was 7.02–62.6%. Although the highest Ni content of tincture (202 µg L^{-1}) was prepared from the raw propolis with the highest Ni content (28.8 mg kg^{-1}), nevertheless TC was the lowest in that case (7.02%). However, the highest TC belongs to a raw propolis with an intermediate level of Ni concentration. It demonstrates that raw propolis with high Ni content (González-Martín et al., 2015; Soós et al., 2021) may give not as high Ni content in tincture. However, generally about one quarter of the Ni content transfers into the tincture, which may cause a health risk.

Strontium, caesium and barium are also potentially toxic elements,

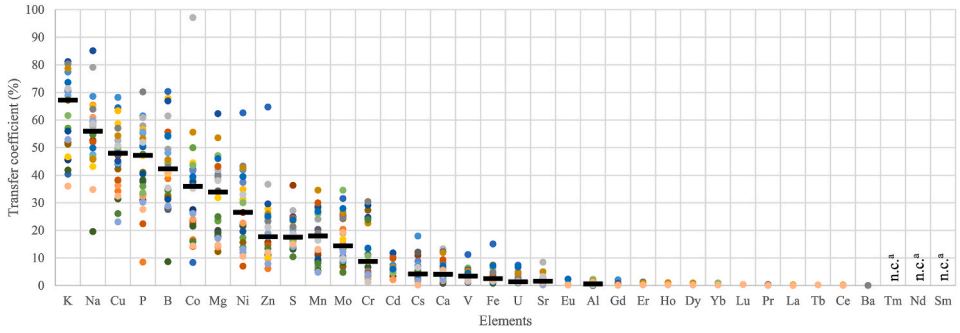

Fig. 2. The transfer coefficient of elements from raw propolis to their tinctures, extracted for 1 week by 80% (v/v) ethanol content extractant
[a] not calculated.

but had low TC into the tinctures. Transfer coefficients of the first two elements were in the range of 0.25–8.51% and 0.20–18.0%, respectively, while Ba was under the LOD in all tinctures except one, namely 0.06% of TC in that case. Other toxic elements, like Cd, Al and V also had low transfer coefficients into propolis tinctures. The TC of cadmium was not able to be determined in 16 out of 27 cases, however the highest value was 11.8%. Translocation of Al was determined in all the tinctures, except one, but did not exceed 2.24%. Vanadium content was transferred into the tinctures in the range of 1.11–11.2%, but the second highest TC was just 6.42%. Uranium was transferred into tinctures in 1.42% by median. The TC of lanthanides was very low. The concentration of these elements was below the LOD in more than half of the tinctures; therefore TC was not calculated in those cases. Furthermore, Nd, Sm and Tm were under the LOD in all the tinctures. The transfer coefficient of lanthanides was up to 2.35%. Taking into account the lanthanide concentration in raw propolis and the transfer coefficient to tinctures, no physiological risk is expectable (Rucki et al., 2021).

Comparing our transfer coefficients with the results of Cvek et al. (2008) it is apparent, that the range of TCs was a little wider, nevertheless they measured 10 samples. For example, the transfer coefficient of Na was between 5.64 and 62.64%, the TC of boron between 4.62 and 94.36%, while manganese had between 1.85 and 49.52%. The difference between the lowest and the highest value was higher than 10-fold in all three cases. However, by comparing the median values, the transfer coefficient was similar in the case of K, B, Cu, Mn, Zn and Fe. Higher differences were found in the case of Na, Mg and Al, because our values were 55.3%, 33.8% and 0.55, while Cvek et al. (2008) found 29.53%, 8.21% and 3.28%, respectively. Difference was remarkable in the case of Ca, Sr and Ba, because Cvek et al. (2008) measured 48.24%, 27.91% and 15.28%, respectively, while in our samples by contrast 4.81%, <0.07% and 2.02% was measured. Croatia is a neighboring country to Hungary, therefore there are no huge difference in botanical origin of propolis. Mediterranean type of propolis may present in some parts of Croatia (Saftić, Peršurić, Fornal, Pavlešić, & Pavelić, 2019). Deviation of the TC could be mainly explained by differences in the extraction process, possibly in the season or in the collection procedure, as mentioned before.

Besides propolis, the transfer coefficient is used for other types of samples. Malandrino et al. (2015) extracted buds, which are partially similar to propolis, by the mixture of water/ethanol/glycerol (50/20/30 by weight). They found the following order in TC: Ca < Mn < P < Mg < K < Na, which is in good agreement with our results. The highest TC of Ca and Mn were 22.9% and 49.7%, respectively, while they measured the foregoing elements under the limit of detection in some samples. Transfer coefficients of P, Mg and K were in the range of 26.8–75.8%, 27.4–61.5% and 59.8–88.4%, respectively. The TC of sodium was not published in this article. Other plant parts were extracted by water/-ethanol (60/40 by weight), which results in lower TCs, compared to the values in buds. The order of the transfer coefficient is as follows: Ca < Mn < Mg < P < K < Na.

In conclusion, by using 80% (v/v) ethanol content extractant, the transfer coefficient of potentially toxic elements (e.g. Al, Ba, Cd, Cs, Sr and V) is generally low from raw propolis into propolis tinctures. In spite of the fact that Al, Ba or Cd may have high concentrations in raw propolis (Bonvehí & Bermejo, 2013; Cvek et al., 2008; Gong et al., 2012; Soós et al., 2021; Tosic, Stojanovic, Mitic, Pavlovic, & Alagic, 2017), these elements may be present in tinctures at a much smaller level. The highest TC of potentially toxic elements belongs to Ni and Cr (25.4% and 8.73%, by median), which reaches up to 202 μg L^{-1} and 117 μg L^{-1} concentrations in tinctures. However, the typical consumption of a propolis tincture should be also considered. Generally, a few milliliters or even less of tincture is recommended daily. This means that the potentially toxic elements are taken into the body in nanogram per day magnitude or less. Besides much higher quantities of several bioactive compounds in the tincture (e.g. total polyphenol and flavonoid contents), even in a percentage order of magnitude (Kumazawa, Hamasaka, & Nakayama, 2004; Ahn et al., 2007; Kalogeropoulos et al., 2009), the amount of potentially toxic elements is almost negligible. Nevertheless, raw propolis contains high concentrations of potentially toxic element, especially Ni and Cr, is to be avoided.

3.4. Estimation of the element content of raw propolis by the element content of their tinctures

In regard to the knowledge of the transfer coefficient, the estimation of the mineral content of raw propolis may arise by the element content of its tincture e.g. for geographical authentication. We have checked the possibility of the determination of minerals in raw propolis samples based on the median of the transfer coefficient (n = 27) and the mineral composition of its tincture. Firstly, we have evaluated the correlation between the element content of tincture and the initial raw propolis. Other circumstances which influence the element content of tinctures (e.g. ethanol content of extractant or extraction time) were bypassed during the evaluation. Pearson's correlation coefficients are shown in Table 2.

Pearson's correlation was indicated for those elements which reached the limit of detection in at least half of the tinctures. Therefore, the lanthanides, Ba and Cd were excluded from evaluation. Some outlier data in the case of Al, B, Cu, Na, Co, Ni and Zn, which could distort the correlation, were excluded before calculation. Statistically significant correlation is observed for Na, Cr, S, Cu, P, Co, K, Mo, V, Fe, Ni and B ($p < 0.01$), as well as for Mn ($p < 0.05$). The relationship of Na, P, Cu, K, Cr and Co content in raw propolis and their tinctures is visualized in Fig. 3. The R^2 is around 0.60 or higher for all the aforementioned elements. However, the scatter plots of Cr and Co still contain raw propolis and tincture pairs, which increased R-squared values. Nevertheless, several points do not fit the line. Fig. S1 (Supplementary material) presents the relationship of V, Mo, Fe, Ni, B, Mn content in raw propolis and their tinctures which have significant Pearson's correlation ($p < 0.05$ or $p < 0.01$), but strong relationship is not absolutely obvious on a scatter plot. A positive relationship is observed, but R^2 is below 0.50. In particular, B and Mn content of tinctures barely depended on the concentration of the same elements in initial raw propolis. The U, Mg, Zn, Al, Cs, Ca and Sr concentrations of tincture have no significant correlation with their contents in raw propolis.

Finally, we have estimated the element content of raw propolis considering the element content of their tinctures and the median of the transfer coefficient. Similarity of the prediction is shown in Fig. 4

Table 2
Pearson's correlation between the element content of raw propolis and its tinctures (80% (v/v) ethanol content extractant, 1 week extraction time). *$p < 0.05$; **$p < 0.01$

Element	Pearson's correlation coefficient	Significance level	Nr. of samples
Na	0.909**	<0.0005	25
Cr	0.873**	<0.0005	26
S	0.834**	<0.0005	27
Cu	0.827**	<0.0005	26
P	0.821**	<0.0005	27
Co	0.774**	<0.0005	26
K	0.769**	<0.0005	27
Mo	0.680**	<0.0005	26
V	0.676**	<0.0005	27
Fe	0.630**	0.001	25
Ni	0.571**	0.002	26
B	0.513**	0.007	26
Mn	0.462*	0.015	27
U	0.410	0.065	21
Mg	0.342	0.081	27
Zn	0.339	0.098	25
Al	0.330	0.100	26
Cs	−0.093	0.644	27
Ca	−0.056	0.781	27
Sr	−0.016	0.937	27

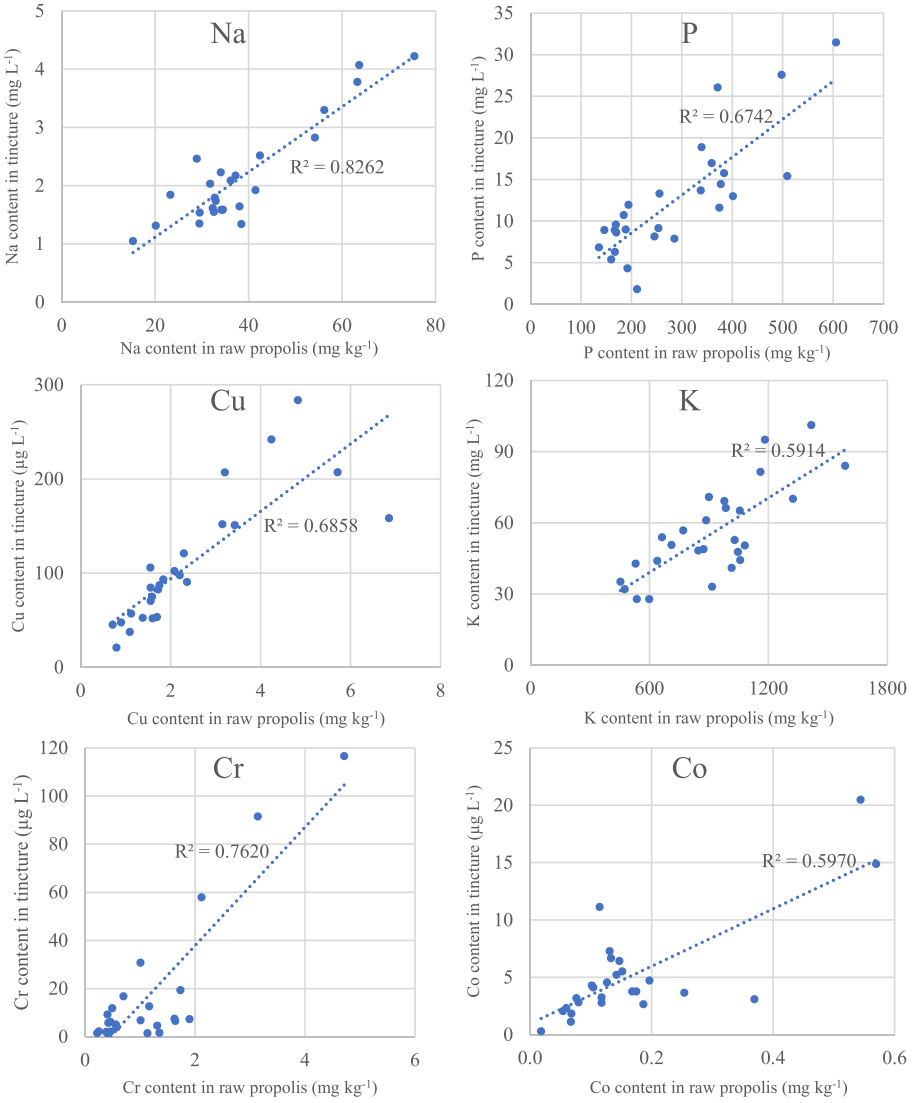

Fig. 3. Na, P, Cu, K, Cr and Co content in propolis tincture (80% (v/v) ethanol content extractant, 1 week extraction time) in relation to the element content of the initial raw propolis.

illustrated on a base 2 logarithmic scale. Individual samples are presented with circles, as well as the medians shown with black rectangles. The same color of the circles means the similarities belong to the same raw propolis. While the median of TC is determined by the same raw and extracted propolis which is used for the estimation, the median of the similarity is 100% for each element. The level of the error of estimation was demonstrated by the distance from the actual value. Potassium content of raw propolis was estimated the most accurately, since the similarity is between 53.6% and 120.8%. This means, that potassium content was overestimated by 20% in some of the raw propolis, but underestimated by 50% in certain cases. In the latter case only half of the potassium concentration was predicted in concerned raw propolis. The similarity is in the 50–200% range for Cu, P, Na, B and S, except a few cases. Accordingly, the mineral content of raw propolis may be overestimated by even two or underestimated to half of the actual content. The estimation of Mg, Mn, Ni, Mo, Co, V, Zn, Al, Cs and Fe contents was in the aforementioned range in most cases, however, either overestimation up to six times or underestimation of less than a twentieth occurred. The rest of the elements (Ca, Cr, Sr, U) had similar prediction efficiency, though higher number of samples were out of the 50–200% range. The concentration of those elements in raw propolis, which were below the LOD in the majority of tinctures (the lanthanides, Ba and Cd) were not able to be estimated.

Geographical authentication of raw propolis samples is often performed by their element composition (Cantarelli et al., 2011; Gong et al., 2012; Soós et al., 2021). However, propolis is consumed in processed form, like tinctures. The element composition of tinctures is affected by e.g. the ethanol content of the extractant and by the extraction time (Soós et al., 2019). Our data mentioned above describes another uncertainty, namely the difference of the transfer coefficient for the individual samples. This is the case even if the conditions are the same (ethanol content of the extractant, extraction time, solid:liquid ratio). Although the correlation is significant and the relationship was obvious between raw propolis and its tincture, in particular for Na, P, Cu and K, still the estimation of the concentration of these elements was loaded with a considerable amount of uncertainty. Other elements, which are also important for authentication were also unpredictable with reasonable confidence in raw propolis. Furthermore, those elements present below LOD in the tinctures cannot be estimated in raw propolis. The prediction of the element content in propolis is undoubtedly an option, but generally even an overestimation by two or an equal level of underestimation should be expected. While the geographical authentication by mineral composition is a fragile technique, especially for nearby areas (Soós et al., 2021), this level of uncertainty is unacceptable and

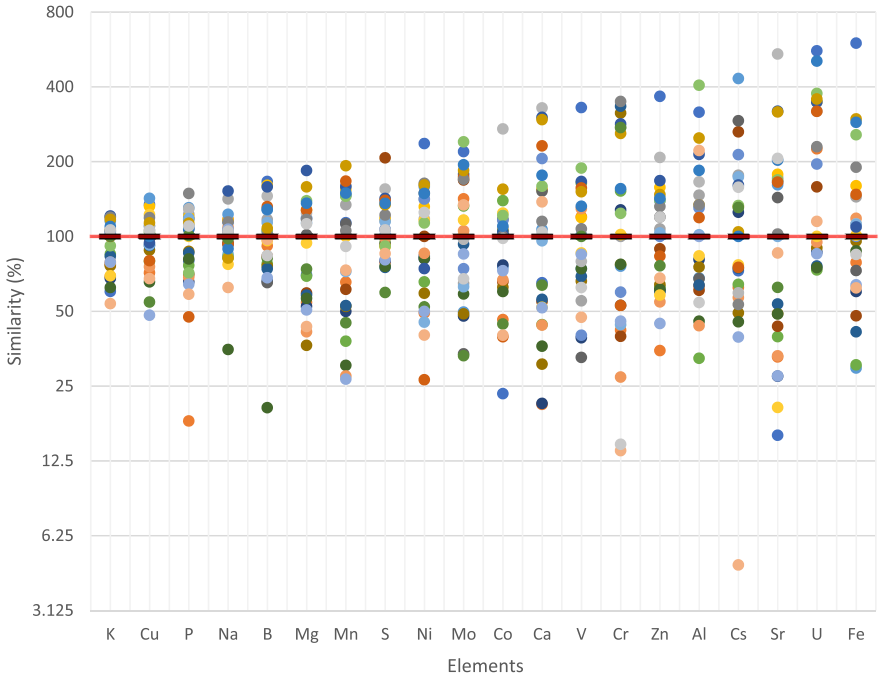

Fig. 4. Estimation of the mineral composition of initial raw propolis based on the element content of its tincture (80% (v/v) ethanol content extractant, 1 week extraction time).

cannot be suggested for that purpose.

Another type of marker is recommended for geographical authentication of tinctures, which is less affected by preparation processes. Polyphenol or flavonoid compositions are often used parameters for geographical authentication of raw propolis (Saftić et al., 2019; Zhou et al., 2008), analyzed from propolis extract made in laboratory conditions. It is proved, that the total flavonoid or total phenolic content is affected by the different extraction parameters applied, that are often used for optimization (Jug, Končić, & Kosalec, 2014; Oldoni et al., 2015), though polyphenol/flavonoid composition or ratio is not studied in relation to the extraction parameters. The effect of the extraction conditions should be tested for the ratio of compounds in the tincture. Furthermore, the isotope ratio analysis is a successfully used technique in wine, which is also a highly processed product (Almeida & Vasconcelos, 2004; Marchionni et al., 2016). These markers should be tested for geographical authentication of propolis tincture, if the mineral content is rejected for that purpose.

4. Conclusion

The element content of tinctures prepared by 80% (v/v) ethanol content extractant was measured parallel to the initial raw propolis. The transfer coefficient varied extremely with regard to the same element. Most of the essential elements (Mo, S, Zn, Mn, Mg, Co, B, P, Na and K) have higher transfer coefficients to the tinctures, than the utmost potentially toxic elements (e.g. Al, Ba, Cd, Cs, Sr and V). The highest TC of potentially toxic elements belong to Ni and Cr, but considering the consumption of tinctures, potentially toxic elements are taken into the body in a nanogram per day magnitude or less. The quantity of the bioactive compounds in the tincture are higher in orders of magnitude, than potentially toxic elements. Nevertheless, raw propolis containing high concentration of potentially toxic element, especially Ni and Cr, is to be avoided. A significant correlation between element content of raw propolis and its tinctures is observed in several cases, but the prediction of the mineral concentration of initial raw propolis is loaded with considerable amount of uncertainty. Generally, even an overestimation by two or an equal level of underestimation should be expected by using the same extraction process, and this level of uncertainty makes the geographical authentication impracticable.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.lwt.2021.112762.

Credit author statement

Áron Soós: Conceptualization; Methodology; Validation; Formal analysis; Investigation Writing-original draft preparation, Writing – original draft; Funding acquisition. **Éva Bódi:** Investigation. **Szilvia Várallyay:** Investigation. **Szabolcs Molnár:** Conceptualization; Resources. **Béla Kovács:** Resources; Funding acquisition.

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