Trace Elements in Propolis and in its Ethanolic Extract (EEP) as Determined by Neutron Activation Analysis

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Propolis is a natural composite balsam, manufactured by honey bees, and active biochemically. Some of its biological effects are attributed to its ability to stimulate enzymatic systems, which in many instances involve trace elements. In the present study we assayed propolis and its ethanolic extract (EEP) for trace elements, and quantified them with the aid of neutron activation analysis. Four elements that have been detected in propolis and EEP for the first time are Na, Cr, Co (ppm) and Ag (ppb). The wide concentration range of the inorganic components in various batches of propolis and EEP is due to the difference in their extraction capacities, and to the variability in the environmental pollutants and the flora at the beehive sites.

Introduction

Propolis is a natural resin, produced by honey bees and used by them for waxing and strengthening their nests. Propolis and its ethanolic extract (EEP) are rich in chemical constituents including a variety of flavonoids and free amino acids [1], esters, sugars, alcohols, etc. [2], and are claimed to exert antibacterial and antimycotic properties [3, 4]. A study in patients to whom EEP pills were administered orally, demonstrated an increase in the enzymatic activity of their granulocytes, T-lymphocytes and immunoglobulin production system [5]. As many enzymes contain trace elements [6], we were prompted to analyze crude propolis and EEP for their content of trace elements. Over a decade ago we published that propolis contained 1.5–2.0% of trace elements [4], and confirmed the inclusion of the following 14 elements in it: K, Na, Ca, Si, P, Al, Fe, Mg [7], V, Sr, Mn [8], Co and Zn [9] and Cu [10]. In addition we have detected in the spectral analysis of EEP the following 8 elements: Cd, Pb, Sn, Ni, Ag, Ti, Cr and Ba [4]. The purpose of the present study was to complete the qualitative analysis of trace elements in crude propolis and EEP, to quantify these elements by neutron activation analysis (NAA), which is considered the most sensitive assay for inorganic substances, and to compare our results to propolis and EEP analyses by X-ray proton activation which were published in 1980 [11].

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Materials and Methods

Four samples of crude propolis were simultaneously analyzed in this study. Three of the samples were from beehives in southern Poland, and the fourth one was taken from a commercial pharmaceutical preparation – a soft capsule (Aagaard Kapseln), manufactured by Hermann F. Borner and Comp., in Berlin, West Germany. In addition, ethanolic extracts from these four propolis samples were prepared, by a method which had been described earlier [12], and were used for analyses as freeze-dried material, immediately after being dried under vacuum for 72 h.

For neutron activation analyses, the eight samples were irradiated for 96 h in the "EWA" reactor at Swierk, Poland, at a thermal neutron flux of 2.10^{12} $n \cdot cm^{-2} \cdot s^{-1}$. The samples were then allowed to cool for about four weeks, and were transferred to polyethylene bags. They were then counted for 5000 seconds, using a high resolution Ge(Li) detector connected to multi-channel pulse-height analyzer (MTA, Hungary) [13]. The results were expressed as parts-per-million (ppm) or parts-per-billion (ppb) of the respective material tested.

Results and Discussion

Fourteen trace elements in Polish and German propolis batches and their EEP extracts were detected and quantified in the present study. Nine of them (Se, Hg, Cr, Co, Fe, Zn, Rb, Na & Br) were in the ppm range, and one of those (Hg) was not reported before in propolis or EEP. In addition to these fourteen elements – Cs, Ag, Au, Sb & La are also reported for the first time in propolis and EEP, but were detected in the ppb range [4]. Obviously, at

Table I. Trace elements determined, and partially quantified, in propolis and EEP, during the past 25 years. All values are in ppm, except for Ag and Cs, which are in ppb.

Element	Proton Activation Poland [11]		Neutron Activation Analysis Poland (ranges)		West Germany	
	Propolis	EEP	Propolis	EEP	Propolis	EEP
Cl		2344				
K		34				
Na**			110–141	42–82	284	155
Ca**		8.3				
Si**						
Al**						
Fe**	225	1.6	316–391	35–39	1056	155
Mg**						
Sr*						
Mn*	6					
Cu**	2.5	7.9				
Zn*	17	7.7	91–490	12–30	527	147
Cd*						
Pb*	4.2	0.6				
Sn*						
Ni*	0.6					
Ag*			29–113	137–284	286	105
Ti*						
Cr*			2.1–17.6	0.3–0.7	2.6	0.6
Ba*						
Rb	1.5		3.1– 5.1	0.7–1.5	2.0	0.8
Br	0.3		1.0– 1.3	0.7–1.5	3.5	3.8
Zr	0.5					
Se	0.6	0.4	0.1– 0.6	0.1–0.6	0.2	0.2
Hg			0.2– 0.5	0.2–1.1	0.8	0.6
Co			0.2– 0.4	0.0–0.1	0.7	0.3
Cs			9–102	24–101	35	46
Au***						
Sb***						
La***						

* = <0.01%; ** = >0.01% as determined after gel filtration [4]; *** = Detected by NAA in the present study, in both crude propolis and EEP, in 10–20 ppb range.

that range they could not be detected by any other method. The minerals of the highest concentrations in propolis and EEP are iron and zinc, as shown for both analytical methods, in Table I. The correlation between the results obtained by both methods is poor, mostly due to the difference in the environmental pollutants of the various regions where the crude propolis was collected, and the difference in extraction capacities for the various minerals, that were utilized in both studies. This may also explain the fact that some elements were not detected in the proton activation study, and others were not detected in the neutron activation study (Table I).

Naturally, differences between batches of propolis and EEP are mostly attributed to the location where the propolis was collected. In a study published recently by Greenaway *et al.* [2], the authors assayed the major organic components in four samples of crude propolis which had been collected in the Oxford area, England. In spite of the proximity of the four collection sites – differences in some components were as high as 30-fold from one location to the other. The homogenicity in the various elements between the three Polish locations was much higher, and the resemblance between the Polish and German samples were remarkably good (Table I).

Another reason for the non-detection of minerals in the crude propolis or its extract, in neutron activation analysis, is due to the fact that after four weeks of decay (between the end of bombardment and

isotope counting) – the count of some radionuclides is very low, especially of those with low cross sections, and considering the low flux that they were irradiated in. Still, the major reason for "loosing" elements in either analysis is the variability in extraction capacities for the various elements: most organic solvents will retain the minerals, but some may extract some of them, or even remove them together with the extracted waxes. Neither reason blurs the fact that propolis is a product rich in inorganic elements, and as such is an excellent natural source for trace elements for some of our vital enzyme systems.

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