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Elemental Analysis of *Ginkgo biloba* Leaf Samples Collected during One Vegetation Period

Szilvia Czigle[a]*, Erzsébet Háznagy-Radnai[b], Klára Pintye-Hódi[c], Jaroslav Tóth[a], Daniela Tekeľová[a] and Imre Máthé[b,d]

[a]*Faculty of Pharmacy, Department of Pharmacognosy and Botany, Comenius University, Odbojárov 10, SK-83232, Bratislava, Slovakia*

[b]*Faculty of Pharmacy, Department of Pharmacognosy, University of Szeged, Eötvös 6, H-6720, Szeged, Hungary*

[c]*Faculty of Pharmacy, Institute of Pharmaceutical Technology, University of Szeged, Eötvös 6, H-6720, Szeged, Hungary*

[d]*Centre for Ecological Research, Institute of Ecology and Botany, Hungarian Academy of Sciences, Alkotmány 2, H-2163, Vácrátót, Hungary*

czigle@fpharm.uniba.sk

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The object of our work was the identification and quantification of inorganic elements in *Ginkgo biloba* L. leaves (Ginkgonis folium, Ginkgoaceae) by X-ray fluorescence analysis. The plant material was obtained from a 50-years-old female tree at the Comenius University Botanical Garden (Bratislava, Slovakia). Leaves were collected from early May to late September, with the last sample consisting of fallen leaves. The elements analyzed were: phosphorus, sulfur, potassium, calcium, scandium, iron, zinc, yttrium, molybdenum, tellurium, samarium, gadolinium, dysprosium, iridium, thallium and lead. The amounts of the monitored heavy metals were below the limits specified in Ph. Eur. 7 and PhS 1.

Keywords: *Ginkgo biloba* L., Ginkgonis folium, X-ray-fluorescence analysis, Trace elements.

Phytopharmaceuticals containing standardized *Ginkgo biloba* L. leaf dry extract (EGb 761) are used in central and peripheral circulation disorders (for example, intermittent claudication), dementia syndromes (primary degenerative, vascular or mixed forms), and neurosensory disturbances, such as vertigo and tinnitus [1]. Standardized *G. biloba* leaf dry extract (EGb 761) is produced from the herbal drug Ginkgonis folium (*Ginkgo* leaf, *Ginkgo biloba*, Ginkgoaceae) and contains 22.0 – 27.0% flavonoids expressed as flavone glycosides (M_r 756.7), 5.0 – 7.0% terpene lactones, including 2.8 – 3.4% ginkgolides (A, B and C), and 2.6 – 3.2% bilobalide [2]. The content of inorganic constituents in EGb 761 was reported to be 5% [3].

Ginkgo leaf (dried drug) should not contain less than 0.1% of terpene lactones, calculated as the sum of ginkgolides A, B and, C and bilobalide. It also should not contain less than 0.5% flavonoids, expressed as flavone glycosides (M_r 756.7) [4]. Other compounds, such as alkylphenols (ginkgolic acids, cardanols), organic acids, and sterols are also present in the herbal drug [5]. Besides the major biologically active compounds, the drug also contains inorganic compounds [5]. Total ash should be quantified at maximum 11.0% [4].

Yu *et al.* [6] conducted a study on the content of trace elements in *Ginkgo* leaves (harvested from the Chinese province of Zheijang). They recorded (in ppm) 1.1 – 6.6 of ^{24}Cr, 15 – 73 of ^{25}Mn, 74 – 399 of ^{26}Fe, 2.8 – 6.9 of ^{29}Cu, 6.1 – 17.1 of ^{30}Zn and 0.1 – 2.17 of ^{34}Se [6].

Heavy metals are widespread in industry. When released into the air or rivers they distort the naturally occurring distribution of metals. Plants extract elements from the soil in which they grow and can concentrate these undesirable trace elements up to toxic levels. Heavy metals in this context mean: mercury, cadmium, and lead in the first place. In a broader sense, other toxic elements, such as arsenic (from certain pesticides) and barium are included. Determination of heavy metals is generally performed by either atomic absorption spectrophotometry after acid digestion of the sample or by X-ray fluorescence analysis. The European Pharmacopoeia (Ph. Eur. 7) specifies limits for any identified toxic impurities (Cd, Cu, Fe, Ni, Pb, Zn, As, Hg) [2], and the Pharmacopoeia Slovaca (PhS 1) further specifies limits for the content of trace elements (Pb, As, Ca, Cl^-, F^-, Mg, Fe, PO_4^{3-}, SO_4^{2-}, K, Ni, Al) in analyzed drugs [4].

The aim of this work was to identify and quantify the chemical elements in Ginkgonis folium by X-ray fluorescence analysis. The elements analyzed in the respective samples were phosphorus, sulfur, potassium, calcium, scandium, iron, zinc, yttrium, molybdenum, tellurium, samarium, gadolinium, dysprosium, iridium, thallium, and lead (Table 1).

Table 1: Elements identified in Ginkgonis folium.

Samples	Harvest day	Elements
1.	May 5, 2009	^{15}P, ^{16}S, ^{19}K, ^{20}Ca, ^{42}Mo, ^{52}Te, ^{81}Tl
2.	May 31, 2009	^{15}P, ^{16}S, ^{19}K, ^{20}Ca, ^{21}Sc, ^{30}Zn, ^{42}Mo, ^{52}Te, ^{66}Dy, ^{77}Ir, ^{82}Pb
3.	June 22, 2009	^{15}P, ^{16}S, ^{19}K, ^{20}Ca, ^{42}Mo, ^{64}Gd, ^{66}Dy, ^{77}Ir
4.	July 15, 2009	^{15}P, ^{16}S, ^{19}K, ^{20}Ca, ^{26}Fe, ^{39}Y, ^{42}Mo, ^{77}Ir, ^{82}Pb
5.	August 12, 2009	^{15}P, ^{16}S, ^{19}K, ^{20}Ca, ^{30}Zn, ^{39}Y, ^{42}Mo, ^{77}Ir
6.	September 22, 2009*	^{15}P, ^{16}S, ^{19}K, ^{20}Ca, ^{39}Y, ^{42}Mo, ^{62}Sm

*Note: sample No. 6 – fallen leaves

Table 2: Correlation coefficients of the calibration curves, and salts used.

Elements	Salts	Correlations
^{15}P	Na_2HPO_7	0.9948
^{16}S	Precipitated sulfur	0.9999
^{19}K	KI	0.9893
^{20}Ca	$CaCO_3$	0.9975
^{42}Mo	$(NH_4)_6Mo_7O_{24} \cdot 4\,H_2O$	0.9987

Table 3: Elemental content of *Ginkgo biloba* leaves in the course of a vegetation period.

	Samples					
No.	1.	2.	3.	4.	5.	6.
Harvest day	5. 5. 2009	31. 5. 2009	22. 6. 2009	15. 7. 2009	12. 8. 2009	22. 9. 2009*
Elements	Content [ppm]					
^{15}P	13631.4	6896.0	7278.3	9136.5	9096.0	6032.2
^{16}S	2481.0	1971.8	2778.3	3590.6	8440.8	4993.5
^{19}K	22508.5	13860.4	15048.8	12933.9	16462.76	16546.7
^{20}Ca	5037.8	3672.2	4457.9	5276.7	7208.1	9330.9
^{42}Mo	2484.9	859.6	466.6	788.7	735.6	1335.8

*Note: sample No. 6 – fallen leaves; 1 ppm = 1 mg/kg

The content of elements in the collected samples was calculated from calibration curves (Table 2). These were elaborated for ^{15}P, ^{16}S, ^{19}K, ^{20}Ca, and ^{42}Mo (in concentrations of 0.25 – 5% in methylcellulose). The content of the elements analyzed was different in the respective samples (Table 3). The highest concentrations in all samples were observed for potassium, the maximum value being recorded in early May (22508.5 ppm). Phosphorus concentrations showed a maximum in early May (13631.4 ppm) and later in July (9136.5 ppm). Sulfur content was the highest in August (8440.8 ppm). The amount of calcium at the end of the growing season was 9330.9 ppm. For molybdenum the highest concentration was quantified at the beginning and at the end of the vegetation period. Other identified elements (^{21}Sc, ^{26}Fe, ^{30}Zn, ^{39}Y, ^{52}Te, ^{62}Sm, ^{64}Gd, ^{66}Dy, ^{77}Ir, ^{81}Tl, ^{82}Pb) could not be quantified due to the unavailability of salts suitable for calibration. The amounts of the monitored heavy metals in our samples were below the limits specified in Ph. Eur. 7 and PhS 1, which means that our plant materials can be considered to be from an ecologically clean locality.

Experimental

Plant material: *Ginkgo biloba* L. leaves (Ginkgonis folium, Ginkgoaceae) were obtained from a 50-years-old female tree at the Comenius University Botanical Garden (Bratislava, Slovakia). Leaves were collected in the course of a vegetation period from early May to late September. Herbarium samples have been deposited at the Department of Pharmacognosy and Botany (Comenius University, Bratislava, Slovakia).

Samples (1–6) consisted of powdered leaves mixed with methylcellulose as a vehicle (LACHEMA, Czech Republic) in a 1 : 1 ratio.

Equipment: A Philips Mini-Pal PW 4025 (MiniPal, Philips Analytical, Almelo, Netherlands) energy-dispersive X-ray fluorescence analyzer was used to identify and quantify the samples [7]. Conditions applied: 4 – 8 kV, 200 – 1000 μA and 1 bar helium purge or air. Samples were measured for 30 - 600 s and the measurement was repeated in triplicate for each sample (Table 4).

Table 4: Conditions applied during measurement.

Samples + standards	Voltage [kV]	Electric current [μA]	Time [s]	Carrier gas	Filter
Ginkgonis folium (Samples 1 – 6)	8	200	60	He	(5) none
NaCl (LACHEMA, Czech Republic)	4	1000	600	He	(5) none
Na_2HPO_3 (LACHEMA, Czech Republic)	5	1000	30	He	(5) none
Precipitated sulfur (MICROCHEM, Czech Republic)	12	100	180	air	(0) kapton
KI (LACHEMA, Czech Republic)	8	500	180	air	(1) Al-thin
$CaCO_3$ (LACHEMA, Czech Republic)	8	500	180	He	(1) Al-thin
$(NH_4)_6Mo_7O_{21}$ 4 H_2O (LACHEMA, Czech Republic)	5	900	120	He	(5) none

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