JOANNA KOBUS, EWA FLACZYK, ZBIGNIEW KREJPCIO, HALINA STANIEK

THE EFFECT OF VEGETATION PERIOD ON CONTENTS OF ELEMENTS AFFECTING ANTIOXIDANT CAPACITY OF EXTRACTS FROM GINKGO BILOBA L

S u m m a r y

The aim of the study was to determine contents of macro- and microelements in extracts from yellow and green leaves of *Ginkgo biloba*. The following elements: Mg, Cu, Zn, Cr, Fe and Se were determined by atomic absorption spectrophotometry. The concentrations of these elements varied depending on the vegetation period of leaves and the extraction solvent. The highest concentration of Mg was observed in water extract. The highest levels of iron and the other microelements were observed in water extracts from green and yellow leaves.

Key words: *Ginkgo biloba*, extracts, macro elements, microelements

Introduction

The *Gingko biloba* tree species has a very long and complicated history. It is assumed on the basis of many archeological excavations that ancestors of this tree existed as early as approx. 250 milion years ago. These trees reached their prime in Jurassic Cretaceous periods, i.e. 215 - 280 million years ago, when different varieties of this tree could be found almost everywhere on the northern hemisphere, from North America to Greenland. At present *Ginkgo biloba* is one of the oldest trees found in the world and is considered to be a relic of the Mesozoic era [1, 2, 5, 9, 10].

The tree belonging to family *Ginkgoacae* is a representative of gymnospermous plants with bladed leaves (the other have needles or leaves of other types, e.g. sago palms). Extracts from these leaves have been investigated in many studies in recent years [2, 3, 4, 9]. This plant, traditionally used as a medicinal plant, has an advantageous effect on the vascular system. Extracts are prepared from fruits, but first of all from leaves, and are a component of tablets, capsules, drops or teas. *Gingko biloba*

Mgr inż. J. Kobus, dr hab. E. Flaczyk, dr hab. Z. Krejpcio, dr inż. H. Staniek, Katedra Technologii Żywienia Człowieka, Uniwersytet Przyrodniczy, ul. Wojska Polskiego 31, 60-624 Poznań

extract contains two basic groups of chemical compounds, i.e. flavonoids and terpenes. However, the advantageous action of extracts is connected with the presence of the components exhibiting antioxidant activity, as well as the presence of macro and microelements. Thus the aim of this study was to assess contents of selected macro and microelements in extracts from yellow and green leaves of *Gingko biloba* affecting antioxidant activity.

Material and methods

Experimental material consisted of green and yellow leaves of *Gingko biloba var. Hipocrates* collected in August and October from trees cultivated in Baranowo by the Poznań Univeristy of Life Sciences. Leaves were dried at 40°C and next milled in a laboratory mill. The degree of comminuting of plant material was determined using mesh size of 0.8 mm. Comminuted leaves were extracted with water (15 min, 95ºC), acetone with water (3:2 v/v; 90 min, 40ºC) and ethanol (16 h, 20ºC) to obtain extracts. Samples were incinerated in quartz crucibles in a muffle furnace at 500^0C. Ash was dissolved in 1N HNO_3 (GR, ISO, Merck). Contents of magnesium (Mg), iron (Fe), zinc (Zn), copper (Cu), and chromium (Cr) were determined by flame absorption atom spectrometry using an AAS-3 spectrometer (Zeiss, Jena, Germany) according to Olejnik et al. [7]. Selenium (Se) content was determined by flame absorption atom spectrometry using an AA Varian Spectra 200 Plus spectrometer equipped with a system of a 4-lamp carousel facilitating the use of hollow cathode lamps (HCL) and electrode less discharge lamps (EDL). The accuracy of analytical methods used to determine contents of elements was verified by analyzing certified reference material INCT-TL-1 Tea Leaves. Determined amounts of analyzed elements amounted from 95.2 to 98.6% contents of certified materials. Results of six independent assays were expressed in mg or μg/g d.m. extract. Selenium was expressed in μg/100g d.m. extract. Statistical analysis was performed with the use of Statistica v. 6.0 (Statsoft, Poland). Serial measurements were compared with a two-way analysis of variance (ANOVA).

Results and discussion

Metal contents in tested extracts were analyzed using flame absorption atom spectrometry and the analysis showed a considerable quantitative variation in extracts of both macro- and microelements. Results of analyses in terms of dry matter of extract are presented in Tables 1 and 2.

The content of magnesium as macro element was the highest. Magnesium content in *Ginkgo biloba* extracts differed significantly depending on the applied solvent, while the type of leaves to a lesser extent affected the level of this component. The highest amount of magnesium was determined in water exact from yellow leaves amounting to

16.15 mg/g d.m., while it was lowest in ethanol extract from green leaves - 0.18 mg/g d.m.

Table 1

Content of selenium and calcium of Ginkgo biloba extracts
Zawartość selenu i wapnia w ekstraktach z Ginkgo biloba

Extract		Mg (mg/g d.m.)			Se (µg/100g d.m.)		
Green leaves	Water (GW)	13,97^{c}	±	0,06	0,59^{b}	±	0,03
	Acethone-water (GA)	6,45^{b}	±	0,33	0,49^{a}	±	0,02
	Ethanol (GE)	0,18^{a}	±	0,02	0,74^{d}	±	0,04
Yellow leaves	Water (YW)	16,15^{d}	±	0,18	0,96^{e}	±	0,05
	Acethone-water (YA)	7,79^{b}	±	0,41	0,62^{c}	±	0,03
	Ethanol (YE)	0,34^{a}	±	0,00	1,01^{f}	±	0,05

Results are mean values of three determinations ± standard deviation.
Values sharing the same letter in a column are not significantly different (α= 0.05).

Table 2

Content of microelements of Ginkgo biloba extracts
Zawartość mikroelementów w ekstraktach z Ginkgo biloba

Extract		Fe (µg/g d.m.)			Zn (µg/g d.m.)			Cu (µg/g d.m.)			Cr (µg/g d.m.)		
Green le-	Water	95,86^{e}	±	0,33	39,28^{f}	±	0,66	49,94^{e}	±	0,00	15,28^{f}	±	0,00
	Acethone-water	35,76^{c}	±	0,02	22,76^{e}	±	0,05	5,62^{b}	±	0,00	9,20^{e}	±	0,00
	Ethanol	11,00^{a}	±	0,18	8,98^{a}	±	0,93	13,00^{c}	±	0,00	3,99^{c}	±	0,00
Yel- low	Water	69,66^{d}	±	0,41	18,77^{d}	±	0,65	17,90^{d}	±	0,00	5,30^{d}	±	0,00
	Acethone-water	29,39^{b}	±	0,00	15,75^{c}	±	0,00	6,87^{b}	±	0,00	3,18^{b}	±	0,00
	Ethanol	62,06^{d}	±	0,00	12,05^{b}	±	0,00	0,30^{a}	±	0,00	2,06^{a}	±	0,00

Results are mean values of three determinations ± standard deviation.
Values sharing the same letter in a column are not significantly different (α= 0.05).

Contents of individual microelements varied in analyzed *Ginkgo* extracts. It was found that among analyzed microelements the dominant element was iron, which contents ranged from 11.00 µg/g d.m. in green leaves ethanol extract to 95.86 µg/g d.m. in green leaves water extract, followed by zinc which content also higher in green leave than in yellow one. Water infusion contains the highest level of zinc. Lower contents were determined for copper: from 0.30 µg/g d.m. in ethanol extract of yellow leaves to 49.94 µg/g d.m. in water extract of green leaves and chromium: from 2.06 µg/g d.m. in green leaves ethanol extract to 15.28 d.m. in green leaves water extract. The element which presence was detected in the lowest amounts was selenium. However, its content was higher in ethanol extracts (1.01 µg/100g d.m. in yellow leaves ethanol extract and 0.74 µg/100g d.m. in green leaves ethanol extract) than in water extracts (0.59 µg/100g d.m. in green leves water extract and 0.96 μg/100g d.m. in yellow leaves water extract). The lowest level of selenium was determined in acetone-water extracts. Results of statistical analysis showed that the type of applied solvent had a significant effect on the amount of extracted microelements ($p<0.05$). The total amount of all analyzed microelements extracted with water in case of green leaves was 200.37 μg/g d.m. and it was 2.7 times higher than that obtained after the application of a mixture of acetone and water and 5.4 times higher than that, after the use of ethanol as a solvent. Water extracts from yellow leaves contained two times more microelements than acetone-water extract from yellow leaves (55.20 μg/g d.m.) and 1.5 times more than ethanol extract from yellow leaves (76.47 μg/g d.m.).

Contents of iron and copper in extracts are crucial in the process of fat stabilization, since these microelements may catalyze oxidation processes. Their availability results in the formation of the most dangerous reactive oxygen species, i.e. the hydroxyl radical. In turn, zinc had an antioxidative action, which was shown in many studies [1, 10, 12]. Antioxidant action is found for selenium also, but the level of this element was about 1000 times lower than other elements. According to Szukalska [11], also chromium accelerates the oxidation of fats by catalyzing the dissociation of lipid peroxides into radicals. However, in a study conducted by Ellnain-Wojtaszek et al. [1] it was shown that chromium ions complexes with flavonoids contained in *Ginkgo* extracts increased their antioxidant potential. Moreover, it was found that some complexes of iron and flavonoids may inhibit Fenton reactions, being the primary source of hydroxyl radicals and catalyzed by iron and copper cations [1, 6, 13]. As it results from a study by Ryguła et al. [8], with an increase in the metal-quercetin molar ratio, an increase in the antiradical activity in relation to DPPH radicals was observed, until the maximum value is reached, corresponding approximately to a 1:1 molar ratio. Over this level antioxidant activity of analyzed systems decreased. In turn, at a considerable molar excess of metal in relation to quercetin (the metal-quercetin ratio of over 2.5) a decrees in the antiradical activity of these systems was observed, even below the level of quercetin activity alone, which was particularly manifested for systems containing zinc ions.

Contents of macro and microelements in plant tissues depend first of all on growth conditions, but also plant variety. Differences in contents of elements are also individual traits. In a study by Stefanovits-Banyai et al. [10] significant differences were found both in contents of macro and microelements in *Ginkgo biloba* leaves, depending on the sex of the tree from which leaves originated. Leaves of female trees contained more microelements such as Zn (2.06 times more) and Fe (1.2 times more) than male specimens, whereas male specimens were characterized by higher contents of calcium and magnesium. The authors also investigated the effect of applied extractant, i.e. water and ethanol (80%), on the level of leaching of individual elements. Similarly as in this study, they also found a significant effect of applied solvent on contents of all analyzed elements.

However, in order to estimate the effect of analyzed elements on antioxidant capacity of extracts it would be necessary to perform application analyses in tests estimating antioxidant properties or in accelerated fat stability tests, since - as it was mentioned earlier - antioxidant activity is affected not only by the total content of individual components, but first of all their proportions and the reaction medium.

Conclusion

In the analyses of the composition of elements in Ginkgo extracts it was found that water extracts of both green and yellow leaves contained the highest amount of elements found disadvantageous from the point of view of catalysis of oxidation processes, i.e. iron and copper. Similarly, in those extracts the highest magnesium content was recorded. On the other hand, water extract from green leaves contained the highest amount of zinc, exhibiting antioxidant properties. In ethanol extracts minimum content of magnesium and the highest contents of selenium and zinc were found. Similarly a high zinc content was recorded in acetone-water extracts. Higher selenium contents were detected in extracts prepared from yellow than green leaves.

Literature

[1] Ellnain-Wojtaszek M., Kruczyński Z., Kasprzak J.: Variations in free radical scavenging activity of Ginkgo biloba L. leaves in the period of complete development of green leaves to fall of yellow ones. Food Chem., 2002, 79, 79.

[2] Guoan S., Yongzhen P., Weisheng W., Zhongxiang D., Lingxia Z., Youfang C.,Xiaofen S.: Cloning and characterization of a flavanone 3-hydroxylase gene from *Ginkgo biloba.* Bioscience Reports, 2006, 26 (1), 19.

[3] Jager L. Perfetti G. Diachenko G.: Analysis of ginkgolides and bilobalide in food products using LC–APCI–MS. J. Pharm. Biomed. Analysis 2006, 41, 5 (28), 1552.

[4] Jianping L.: The Use of Ginkgo Biloba Extract in Acute Ischemic Stroke. Explore. J. Sci. Healg. 2006, 2, (3), 262.

[5] Korszun S.: Miłorząb- jeden z najstarszych gatunków drzew nagozalążkowych, Kwiaty 2003, 5-6, 123.

[6] Małolepsza U., Urbanek H.: Flawonoidy roślinne jako związki biochemicznie czynne. Wiad. Bot. 2000, 44, 27.

[7] Olejnik D., Krejpcio Z., Śmigiel-Papińska D., Wójciak W. R, Gawęcki J., Wiśniewska J.: The content of selected minerals (Ca, Mg, Zn, Cu, Fe) in daily food rations of adolescents, comparison of analytical and calculated data. PZH Ann., 1999, 50, 361.

[8] Ryguła K., Szafer K., Szymusiak H., Wybieralska K., Wieczorek D., Zieliński R.: Wpływ wybranych makroelementów na aktywność przeciwrodnikową kwercetyny w modelowych roztworach wodno-metanolowych. Żyw. Czł. i Met., 2005, 32, 841.

[9] Seneta W. Dolatowski J.: Dendrologia, Warszawa, 2004.

[10] Stefanovits-Banyai E., Szentmihalyi K., Hegedus A., Koczka N., Vali L., Taba G., Blazovics A.: Metal ion and antioxidant alterations in leaves between different seeds of Ginkgo biloba L. Life Sci. 2006, 78, 1049.

[11] Szukalska E.: Wybrane zagadnienia utleniania tłuszczów. Tłuszcze Jadalne, 2003, 38, 42.
[12] Zago M. P., Oteiza P. I.: The antioxidant properties of zinc: interactions with iron and antioxidants. Free Radical Biology and Medicine, 2001, 31, 2, 266.
[13] Zając M., Pawełczyk E., Chemia leków. Wyd. AM., Poznań 2000, 123.

WPŁYW OKRESU WEGETACJI NA ZAWARTOŚĆ PIERWIASTKÓW DETERMINUJĄCYCH POJEMNOŚĆ ANTYOKSYDACYJNĄ EKSTRAKTÓW Z LIŚCI *GINKGO BILOBA L.*

Streszczenie

Celem niniejszych badań było określenie zawartości poszczególnych makro i mikroelementów w ekstraktach sporządzonych z suszu żółtych i zielonych liści Gingko biloba. Oznaczono zawartość magnezu, miedzi, żelaza, chromu, cynku oraz selenu za pomocą płomieniowej spektrofotometrii atomowo-absorpcyjnej (AAS). Koncentracja poszczególnych pierwiastków zależała od użytego rozpuszczalnika i okresu wegetacji liści. Najwięcej magnezu zawierały wodne ekstrakty z liści żółtych i zielonych. Spośród mikroelementów najwyższym poziomem żelaza i innych mikroelementów charakteryzowały się wodne ekstrakty z liści zielonych oraz żółtych.

Słowa kluczowe: Ginko biloba, ekstrakty, makroelementy, mikroelementy