Contents of ginkgoflavonoglycosides and macronutrients in leaves of maidenhair tree (*Ginkgo biloba* L.)

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Summary

Contents of ginkgoflavonoglycosides and macronutrients were analysed in leaves of maidenhair trees, collected from one- and two-year-old shoots, at four dates in 2002, from a field plantation established from a selected biotype. Ginkgoflavonoglycoside contents were always higher in leaves collected from one-year-old shoots and ranged from 1.28% (30 August) to 1.54% (15 July). The highest contents of nitrogen, calcium and magnesium were recorded in leaves from one-year-old shoots, while that of potassium was higher in leaves collected from one-year-old shoots. Phosphorus content in July and mid-August was similar in one- and two-year-old leaves. In the third decade of August, especially in leaves collected from one-year-old shoots, phosphorus content was the lowest, amounting to 0.20%.

Key words: maidenhair tree, *Ginkgo biloba* L., leaves, ginkgoflavonoglycosides, macronutrients

INTRODUCTION

The maidenhair tree is an ornamental tree of foreign origin. Since ancient times the tree has been planted in China, Japan and Korea in the vicinity of temples and in the gardens. The tree was introduced to Poland at the end of the 18th century. In recent years the species has been planted mainly in urban areas, along streets, avenues and in parks [1]. Because of the content of biologically active chemical
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compounds, mainly ginkgoflavonoglycosides [2], and macronutrients in particular, leaves of maidenhair tree are a valuable raw material for the pharmaceutical industry. Multidisciplinary biological research showed that the therapeutic activity results from complexes of ginkgoflavonoglycosides and terpene derivatives with ginkgolides A, B, C, J and M, as well as sesquiterpenoids, namely bilobalide [3]. The structure of biologically active compounds mentioned above was determined by modern analytical techniques [4]. Both the products and the biological and pharmaceutical preparations are standardised by chemical-analytic methods (HPLC). In intermediate products, the so-called extracts, the content of biologically activated complexes of chemical compounds for ginkgoflavonoglycosides ranges from 24% to 26%, for the terpene fraction the figure is 5–7%, in the case of carboxylic acids 8–10%, for ginkgol acids less than 0.0005%, and for biflavonoids it is less than 0.1% [4]. Medical preparations produced from maidenhair tree leaves are standardized for the content of ginkgoflavonoglycosides. Therefore, it was highly advisable to conduct research as to the point of the vegetation season that leaves should be collected and harvested from shoots on a commercial scale in order to ensure the highest contents of these compounds. It was also investigated whether the content of macronutrients in leaves influences the percentage of biologically active compounds found in these leaves.

MATERIAL AND METHODS

At the Experimental Station of the Department of Dendrology and Nursery at the August Cieszkowski Agricultural University of Poznań there is a plantation of selected biotype of maidenhair tree. Leaves of maidenhair tree were collected there for the purpose of this study at two-week intervals at four different dates, i.e. on 15 July, 30 July, 15 August and 30 August 2002, separately from one- and two-year-old shoots. After desiccation at the temperature of 60°C they become raw material prepared for chemical analyses. At the next stage they were ground and the powdered raw material obtained in this way was liquid-solid extracted with methanol at 60°C in a reflux condenser. Methanol extracts of analysed samples were filtered with a PTFE 0.45 μm filter (Sartorius). After filtration the filtrate solutions were inserted into a LiChrospher RP-18e chromatographic column (250×4.6 mm; 5 μm; Merck). The analysis was conducted in a Merck-Hitachi d-7000 liquid chromatograph, with a 20 μl proportioning loop and a DAD L-7455 spectrophotometric detector. Gradient elution was conducted at room temperature with the use of the mobile phase composed of methanol, water and phosphorous acid. Chromatograms were registered at the detection wavelength of 340 nm. The calculations were performed with HSM (Merck) software using the external standard method, and the result was formulated as the sum of ginkgoflavonoglycosides converted to quercetin (the conversion factor of aglycone to glycoside according to Ph. Eur.). The calibration curve for quercetin (Roth, HPLC purity) was determined
for six different concentrations of the standard substance samples (correlation coefficient \( r^2 = 0.999 \)). The biologically active compounds analysed in the examined material were identified by means of comparing retention times and absorption spectra of standard substances (quercetin, isorhamnetin and kaempferol) at the wavelengths of 200-400 nm.

After mineralization of maidenhair tree leaves by wet combustion the total content of macronutrients was determined, the nitrogen content was assayed according to Kjeldahl, that of phosphorus by colorimetry with ammonium vanadomolybdate, according to Schillak, while those of potassium and calcium by photometry, and that of magnesium by atomic absorption spectroscopy (AAS) [5].

The experiment had two combinations at dates. For each date there were six replications. The results were analysed statistically using a one-or-two-way analysis of variance with STAT software. Means were compared by Duncan's test, at the significance level \( \alpha = 0.05 \) for each date separately.

RESULTS

The leaves collected from one-year-old shoots of maidenhair tree, regardless of the time of harvesting, contained larger amounts of ginkgoflavonoglycosides than those collected from two-year-old shoots (Fig. 1). Depending on the date of harvesting the raw material the content of biologically active substances in leaves collected from younger shoots (one-year old) ranged from 1.28% to 1.54%; however, in the leaves collected from two-year-old shoots at each date the amount was lower by at least 0.28%. The lowest content of ginkgoflavonoglycosides (0.89%) was found in a sample collected on 30 July 2002.

![Graph showing the content of ginkgoflavonoglycosides in leaves of maidenhair tree.](image)

*mean values marked with the same letter do not differ statistically; \( \alpha = 0.05 \).

Figure 1. Contents of ginkgoflavonoglycosides in leaves of maidenhair tree.
Nitrogen content in leaves collected from two-year-old shoots was higher than that assayed in one-year-old shoots, regardless of the date of collection (Fig. 2). In the leaves collected in July the average nitrogen content was determined as 2.76%, while in August the figure was 2.50%. The raw material collected from one-year-old shoots contained from 2.37% to 2.41% of nitrogen.

![Nitrogen content graph](image)

*mean values marked with the same letter do not differ statistically; $\alpha=0.05$

Figure 2. Nitrogen contents in leaves of maidenhair tree.

The phosphorus content in leaves collected from one- and two-year-old shoots on the above-mentioned days was similar, amounting to 0.32% on average, except for the raw material collected from two-year-old shoots on 30 August, where 0.20% content of that macronutrient was found (Fig. 3).

![Phosphorus content graph](image)

*mean values marked with the same letter do not differ statistically; $\alpha=0.05$

Figure 3. Phosphorus contents in leaves of maidenhair tree.
The potassium content in maidenhair tree leaves during the vegetation season varied. The later the samples were collected for the analysis, the higher was the potassium content. Regardless of the date of collecting samples, slightly higher potassium levels were found in leaves collected from younger shoots. In the raw material collected on 30 August, irrespective of the age of shoots, the highest potassium content was assayed in leaves (2.64% in leaves collected from one-year-old shoots and 2.55% in the case of two-year-old ones: Fig. 4).

![Bar chart showing potassium content over different dates and shoot ages.]

*mean values marked with the same letter do not differ statistically; α=0.05.

Figure 4. Potassium contents in leaves of maidenhair tree.

The calcium content in the raw material collected from one-year-old shoots in July was only half of that in the raw material collected from two-year-old shoots, whereas in August it increased, reaching a value three times higher. The calcium content in maidenhair tree leaves was rising from 15 August and at that time reached 1.14% in leaves collected from one-year-old shoots and 3.01% in leaves from two-year-old shoots. In the samples collected on 30 August lower levels of that macronutrient were recorded (Fig. 5).

![Bar chart showing calcium content over different dates and shoot ages.]

*mean values marked with the same letter do not differ statistically; α=0.05.

Figure 5. Calcium contents in leaves of maidenhair tree.
The magnesium content in maidenhair tree leaves harvested between 15 July and 30 August remained similar, amounting on average to 0.3% in the leaves collected from one-year-old shoots and 0.7% in two-year-old ones. At individual dates of raw material collection in leaves harvested from two-year-old shoots a two or three times higher content of magnesium was recorded than in the raw material collected from one-year-old shoots (Fig. 6).

![Graph showing magnesium content in maidenhair tree leaves.]

*mean values marked with the same letter do not differ statistically; α=0.05

Figure 6. Magnesium contents in leaves of maidenhair tree.

**DISCUSSION**

Maidenhair tree starts vegetation in the second decade of May. Fresh and dry weight of leaves increase up to the 95th day of vegetation [1]. For this reason leaves were collected from plants in July and August. Korszun et al. stated that the contents of biologically active chemical compounds in leaves are affected by nitrogen and potassium fertilisation [6]. On the basis of the conducted research it was observed that the content of ginkgoflavonoglycosides decreases with an increase in the potassium content in leaves. The highest contents of these compounds were recorded in leaves collected from one-year-old shoots in July. The maidenhair trees grown on plantations should be properly maintained and trimmed to keep them in a form of shrubs in order to obtain the highest number of one-year-old shoots.

**CONCLUSIONS**

1. The content of ginkgoflavonoglycosides determined in leaves collected from one-year-old shoots of maidenhair tree grown in an open field plantation was highest in July and reached 1.5%.
2. In the leaves harvested from two-year-old shoots the content of ginkgoflavonoglycosides was always lower, amounting to 1.03%.
3. The contents of nitrogen, calcium and magnesium were the highest in the leaves collected from two-year-old shoots.

4. The potassium content was insignificantly higher in the leaves taken from one-year-old shoots, while the phosphorus content remained constant.

REFERENCES


ZAWARTOŚĆ GINKGOFLAWONOGLOKZOYDÓW I MAKROSKŁADNIKÓW W LIŚCIACH MIŁORŻĘBU DWUKLAPOWEGO (GINKGO BILoba L.)

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Streszczenie

Badano zawartość ginkgoflawonoglikozydów i makroskładników w liściach miłorzębu dwuklapowego, pobranych z pędów jednorocznych i dwuletnich, w czterech terminach w 2002 roku, z plantacji połowej założonej z wyselekcjonowanego biotypu. Zawartość ginkgoflawonoglikozydów była zawsze większa w liściach pobranych z pędów jednorocznych i wynosiła od 1,28% (30 sierpnia) do 1,54% (15 lipca). Największą zawartość azotu, wapnia i magnezu miały liście pobrane z pędów dwuletnich, natomiast potasu — liście pobrane z pędów jednorocznych. Zawartość fosforu w lipcu i w połowie sierpnia zarówno w pędach jedno-, jak i dwuletnich utrzymywała się na podobnym poziomie. W trzeciej dekadzie sierpnia, szczególnie w wypadku liści zebranych z pędów dwuletnich, zawartość fosforu była najniższa (0,20%).

Słowa kluczowe: miłorząb dwuklapowy, Ginkgo biloba L., liście, ginkgoflawonoglikozydy, makroskładniki