INVESTIGATION ON GINKGOLIDES AND BILOBALIDE CONTENT IN GINKGO BILOBA L. LEAVES

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Abstract

In the present study it was established the concentration of ginkgolides A, B, C and bilobalide in the leaves of 6 Ginkgo biloba trees, located in 6 different settlements in Bulgaria - Plovdiv, Pazardjik, Asenovgrad, Hisar, Karlovo and Kazanluk. Only female trees aged 20-30 years, with very good decorative characteristics, were selected. The material studied was the leaves collected in two weeks, from the appearing of the leaves up to the fall of the leaves. It was found that the concentration of test substances was the lowest in spring and autumn, and the highest in the early summer. The differences between the six studied trees were significant. With the highest concentration of ginkgolides A, B, C and bilobalide were the trees from Plovdiv and Hissar. Of the three types of ginkcolides - A, B and C - the highest content in the leaves is ginkcolid A, followed by ginkcolid B and finally, the lowest content is ginkcolid C.

Key words: *Ginkgo biloba L., ginkolides, bilobalid, content in leaves, seasonal variation*

1. INTRODUCTION

The Ginkgo biloba plant originates in China, with the oldest leaf fossils found to be more than 270 million years old. Because of its exceptional resistance to adverse conditions, Ginkgo is grown in the temperate climate zone around the world for decorative purposes. In China, the cultivation of Ginkgo for the production of nuts and leaves, which are traditionally used in Chinese and Japanese natural medicine, is widespread. In 1974, a Ginkgo leaf extract product codenamed "EGb 761" was first registered in France. Since then, dozens of new pharmaceutical and cosmetic products have been created and hundreds of controlled chemical, biotechnological, pharmacological and clinical studies have been conducted. This gives reason to talk about the development of a new scientific field "ginkgology". In response to the ever-expanding demand for Ginkgo leaf extract, millions of trees are grown in plantations in China, the United States, Japan, South Korea, France and other countries. Ginkgo biloba L. is a species very often used in modern landscaping practice in Bulgaria. Despite the presence of exceptionally suitable natural conditions, Ginkgo is grown as single trees in parks or yards. There are no collections of varieties suitable for planting or leaf production, no traditions and experience in vegetative propagation, in vitro cultivation, and information on the biochemical composition of forms grown in the country. The species was transported to Europe for the first time in 18 century. At first it was used mainly as decorative, but in recent years it has been found that it possesses other medical qualities. From the leaves of the plant have been isolated biologically active substances that have a very good effect on the human organism. Ginkgo preparations are used against various symptoms related to old age and insufficient central or peripheral blood flow. There is a lot of research in this regard. Yongfang, S. (1986) examines the relationship between the leaf age and the concentration of biflavon cynadopitysin, ginketin, isogenetic and albite and finds that the concentration is the highest in the late autumn and the lowest in the summer, and spring of 1.9%, 0.44% and 0.52% respectively. A similar study is conducted by Hasler, A. R. (1990). Despite its relatively low content in May, it found a slight variation in the concentration of common biflavones during the season on the concentration of flavono, a glioside and found the lack of variation from June to November. Little is known about the influence of harvest time on the content of ginkcolides and bilobalides in the leaves. Teng, B.P. (1989) found that the content of ginkcolides was highest during late summer and autumn, and the lowest at the end of autumn. The impact of the season on the contents of bilobalide and gincolides A, B, C studies van Beek, T.A. (1991) and found that the concentration of these substances was highest during the late summer and early autumn. They also find that the concentration of biologically active substances in leaflets of young tree is higher than that

of older ones. In the studies of Panayotov, N. (1996) on some stimulating components applied during vegetation, improvement in development and productivity was observed in a number of crops, as well as a change in the biochemical composition of the product part. There are no studies in this direction in Bulgaria. The purpose of present study is to identify a tree with a high content of biologically active substances in the leaves as well as the season through which these substances have the highest concentration. The detected tree will be used in further research for the vegetative propagation and creation of new clone.

2. MATERIAL AND METHOD

The experiments were carried out during the 2014-2016 period. The plants were marked in the inventory of available genetic resources from Ginkgo biloba L. in Southern Bulgaria and on the Black Sea coast. The criteria for selecting the plants were: vitality, decorativity and sanitary condition.

Vitality - this is the sum of different manifestations of the growth processes in individual specimens: visible slowing and stopping growth at the top of the plant or main branches (without mechanical damage); Yearly growth or length of shoots; Size; Color of the bark of the stem and of the shoots and others. Those with the lowest level of vitality have one or more of these manifestations, to a great extent.

Decorativity - this indicator is the result of the overall appearance, the habitat of the specimen. It includes a height-to-width ratio of the crown; Symmetry of the crown; Obstruction; Shape and color of the leaves; Coloring the leaves during vegetation; Autumn leaf coloring and more.

Sanitary condition - based on the coloring and presence of foliage and crown damage, according to the International Methodology for Forest Systems Monitoring (UN / EIE, 1992). The sanitary condition of each specimen is judged on a five-tier scale: 0 - healthy trees (crown deforestation and damage up to 10%); 1 - trees with a disability and crown defoliation of 11 to 25%; 2 - trees with a disability and defoliation of the crown of 26 to 65%; 3 - trees with a disability and crown defoliation of 66 to 95%; 4 - trees with a disability and defoliation of the crown above 95%; 5 - completely dead trees. Assessments of vitality, decoration, and sanitary status were pooled in one indicator - General Condition.

Six trees were selected in the following settlements: Plovdiv, Pazardjik, Stamboliiski, Asenovgrad, Hissar, Popovitsa. The leaves were harvested in two weeks throughout the growing season - from the appearing of the leaves (mid-April) to the up to the fall of the leaves (mid-October). All procedures for extraction and purification of plant mass and HPL Analysis are described in detail in van Reek, T. A. et al. (1991).

3. RESULTS AND DISCUSSION

Table 1 shows the contents of bilobalide throughout the growing season. The highest concentrations are the leaves during the period 1.08. until 15.08. in the first half of August, or in the middle of the summer.

Table 1. The bilobalide content (mg/g dry leaves) in the leaves of Ginkgo biloba L.

Time Var.	15.04	1.05.	15.05	1.06.	15.06	1.07.	15.07	1.08.	15.08	1.09.	15.09	1.10.	Mean
1.	80.4	94.5	148.7	193.5	247.8	287.5	311.5	387.5	385.3	345.1	278.8	128.1	239.9
2.	69.7	82.3	98.6	135.4	151.8	199.3	217.1	238.1	245.7	223.0	193.4	89.7	162.0
3.	43.5	57.8	68.4	95.4	127.8	138.9	194.5	201.8	211.5	175.4	118.5	73.4	121.9
4.	50.8	71.3	84.7	111.5	125.6	140.1	185.6	193.7	188.7	112.3	97.5	66.8	119.1
5.	95.8	123.4	148.5	201.7	256.1	307.8	356.7	398.3	409.1	387.2	293.5	206.8	255.1
6.	27.8	67.3	95.4	118.3	135.6	171.8	183.4	200.5	211.8	173.4	145.7	95.4	135.5

At the beginning - 15.04. and at the end of vegetation are very low in concentration - from 27.8 mg/g (sample 6) to 95.8 mg/g (baseline 5, from 66.8 mg/g (sample 4) to 206, 8 mg/g (sample 5) for the end of vegetation.

Of all specimens tested with the highest content, a sample of 5 - 398.3 mg / g and sample 1 - 387.5 mg / g. The bilobalid content in the lystadane specimens examined from Ginkgo biloba L. ranged from 119.1 mg / g to 255.1 mg / g averaged over the study period. Higher content is a sample of 1 - 239.9 mg / g and a sample of 5 - 255.1 mg / g, which exceeds the remaining specimens by 40-50%. This difference in the bilobald content is due both to the difference in climatic conditions in which the plants grow and develop, and to the fact that in Bulgaria the majority of the propagating material is produced in the case of seed propagation and the obtained plants differ from one another both by habitus and by chemical composition of fruits and leaves.

A similar trend is observed in the content of ginkcolide A (Table 2) - low in the spring, highest in the summer (15.07 - 15.08.) and again low in autumn. At the highest values are distinguished again 1 and 5 - plants from Plovdiv and Hissar - 93.8 mg / g and 168.3 mg / g, respectively. The very low values of the ginkcolide A content of Stamboliiski plants and the like - 48.6 mg / g and 59.8 mg / g, respectively, were found to be very low, with 67.1 % and 59.4 % lower than the ginkcolid A content of the leaves of the plant of Hissar. In general, the ginkcolid A content is low - averages range from 30.5 mg / g to 100.8 mg / g. It makes the comparatively long period of high levels of ginkcolid A in the leaves of the plants - about four weeks. It is also a positive fact that during periods close to those with high levels of ginkcolid a, its content in the leaves is also very high.

Table 2. The gikgolide A content (mg/g dry leaves) in the leaves of Ginkgo biloba L.

Time Var.	15.04	1.05.	15.05	1.06.	15.06	1.07.	15.07	1.08.	15.08	1.09.	15.09	1.10.	Mean
1.	27.3	48.7	49.5	53.8	58.3	67.9	90.4	93.8	93.5	53.8	32.8	18.7	57.4
2.	16.4	18.5	22.8	34.7	48.6	52.8	59.7	55.6	58.3	42.7	34.5	28.7	39.4
3.	11.3	18.3	20.5	26.8	31.8	40.4	48.6	46.6	49.5	34.8	21.6	16.3	30.5
4.	12.4	17.5	20.4	24.8	38.4	51.7	50.5	55.7	54.8	44.8	37.8	20.4	35.8
5.	48.5	63.8	78.4	95.4	101.8	129.3	147.8	168.3	159.8	100.3	78.5	37.4	100.8
6.	8.7	14.5	22.5	38.1	44.5	56.8	59.8	57.3	60.4	45.3	39.2	22.5	39.1

The content of ginkcolide B (Table 3) is generally much lower than that of ginkcolide A - the highest values are 100.8 mg / g for the Hissar plant and 75.8 mg / g for the plant in Plovdiv. The lowest values for all specimens are observed in the spring, while the maximal values are 1.07. To 15.08. and fall again in autumn. The period in which the content of ginkcolide B has the highest values is quite long - four weeks. This indicator is particularly important in relation to leaf harvesting - the longer the high ginkolide A period, the easier it is to harvest the leaves.

Table 3. The gikgolide B content (mg/g dry leaves) in the leaves of Ginkgo biloba L.

Time Var.	15.04	1.05.	15.05	1.06.	15.06	1.07.	15.07	1.08.	15.08	1.09.	15.09	1.10.	Mean
1.	16.5	23.4	43.7	50.6	64.5	72.8	74.5	73.6	75.8	53.4	34.8	11.7	49.6
2.	12.7	16.3	18.4	28.3	32.5	37.8	30.5	39.7	22.3	19.5	13.4	14.5	23.8
3.	8.4	13.5	19.7	25.3	29.4	35.6	34.8	36.7	37.6	21.5	20.5	17.8	20.1
4.	11.3	18.7	25.6	33.8	41.5	50.4	50.7	57.8	56.8	44.3	37.8	19.3	37.3
5.	25.8	34.8	49.5	57.8	69.7	80.7	89.5	94.5	100.8	80.4	50.7	28.4	63.6
6.	7.5	10.5	12.5	18.6	22.8	37.3	41.8	52.7	50.3	31.4	25.6	18.7	27.5

With the lowest values of the three ginkcolides considered, ginkcolide C (Table 4) values ranged from 3.5 mg / g (sample 3) to 87.5 mg / g (sample 5). The trend observed with other ginkcolides can also be traced here, namely the low concentration of leaflets and leaflets, and the high concentration at the beginning of the summer. The highest values are the plants in Plovdiv and hyssar - 59.6 mg / g for the plant from Plovdiv during the period 15.06. And 87.5 mg / g for the Hissar plant in the period 15.07. Average values between samples are significant - very low are the mean values for the entire study period of the Stamboliiski plant - 12.3 mg / g, which is by 74.1% lower than the average of the plant from Hissar.

Table 4. The gikgolide C content (mg/g dry leaves) in the leaves of Ginkgo biloba L.

Time Var.	15.04	1.05.	15.05	1.06.	15.06	1.07.	15.07	1.08.	15.08	1.09.	15.09	1.10.	Mean
1.	11.8	22.4	38.5	44.6	59.6	58.1	57.3	22.3	11.5	10.8	9.6	7.8	29.5
2.	8.7	11.5	18.3	27.5	33.8	35.9	34.8	21.6	20.5	18.3	17.5	11.8	21.8
3.	3.5	6.7	8.9	10.5	17.3	17.8	19.3	16.7	14.5	12.5	11.8	7.8	12.3
4.	10.5	13.8	17.5	21.5	29.5	30.1	31.5	20.5	16.3	14.8	17.7	12.5	19.7
5.	18.3	28.7	41.5	59.3	60.8	73.1	87.5	67.3	51.9	40.7	29.3	12.8	47.6
6.	4.0	6.6	8.3	10.5	12.5	13.5	14.4	11.7	10.5	11.8	12.5	8.4	10.4

From the obtained results it is possible to conclude that the middle of summer is a very good time to harvest Ginko biloba leaves. Concentrations of ginkgolides and bilobalide are very high but in this time of the yfar leaves can be collected with negative effects to the tree. The results about the time of the maximum concentration for the ginkgolides are not agree with the investigation of van Reek., T.A. et al. (1991). His report of a maximum concentration in the late summer, early autumn cannot be confirmed by results. The possible reason can be the difference in the weather condition in Bulgaria and in Netherland. The study of Teng, B.P. (1989) that a minimum concentration of in late autumn is possible to verity.

It is clear that differences between individual Gingko biloba L. trees are due to weather condition, age, sex of the tree, soil composition and existence of individuals with higher concentration of biological active substances, van Reek, T. A. et al. (1991).

4. CONCLUSIONS

1. The contents of bilobalide and ginkcolides A, B and C were found to be highest in the middle of summer - in the period from 15.06. to 15.08. With the lowest values being the leaves at the beginning and end of the vegetation period - 15.04. and 1.10.

2. The samples of Plovdiv and Hissar - respectively sample 1 and sample 5 - have the highest contents of bilobalide and gincolides A, B and C. They may be used in further studies and propagated vegetatively to obtain a variety suitable for use for medical purposes.
3. Of the three types of ginkcolides - A, B and C - the highest content in the leaves is ginkcolid A, followed by ginkcolid B and finally, the lowest content is ginkcolid C.
4. Differences in the content of bilobalide and ginkcolid A, B and C in the individual specimens are significant and are due to both the different climatic conditions and the seminal method of reproduction.

REFERENCES

Panayotov, N. (1996). The effect of plant growth regulator Atonic on the yield and quality of the reproduce seed of sweet pepper. Acta Horticulturae № 462, Proceeding of the First Balkan Symposium "Vegetable and Potatoes", Beograd, 4- 7 June 1996, v.II, p.757-762

Special issue on Fxtract of Ginkgo biloha EGb 761 (1986) l.a Presse Med. 15. 1438—1604.

Braquet. P. (1987) Drugs Future 12, 643—699.

Schilcher, II. (1988) Z. Phvtother. 9. 119—127.

Schennen. A. (1988) Thesis. Marburg (1988).

Braquet. P.. ed. 11988) Ginkgolides — Chemistry. Biology. Pharmacology and Clinical Perspectives, Vol. 1, 794 pp.. J. H. Prous Science, Barcelona.

Braquet, P.. ed. (1989) Ginkgolides — Chemistry. Biology, Pharmacology and Clinical Perspectives. Vol. 2. 921 pp.. J. R. Prous Science. Barcelona.

Chatterjee. S. S.. Gabard. B. L. Jaggy. 11. F. W. (Dr. V. Schwabe G.m.b.H.). tlilobalide containing pharmaceutical. (',er. Offen. DC 3,338.995 (Cl. A61K31/365), 09 May 1985. AppI. 27 Oct. 1983: 20 pp.

Yongfang, S. (1986) Chem. lod. Forest l'rod. 6, 1 —4.

Hasler, A. H. 1990) Thesis)Diss. FTH Nr. 9353), Zurich.

Hasler, A.. Meier. B.. Sticher. 0. (1990) Schwoiz. Apoth. Ztg. 128,342—347.

'l'eng, B. P. (1989) in: Ginkgolides — Chemistry. Biology. Pharmacology and Clinical Perspectives. (Braquet. P.. ed). Vol. 1. pp. 37—42, J. H. Prous Science, Barcelona.

van Reek, T. A., Scheeren. Ft. A.. Rantio, T.. Molger. W. Ch..Lelyveld, G. P. (1991) J. Chromatogr. 543, 375—387.

Stumpf. H. (1989) Dtsch. Apoth. Ztg. 129. 2794.

Study on supercritical fluid extraction of flavanoides and ginkgolides from leaves of Ginkgo. You Hai, Tao Bingying, Zhang Liqi 200. Journal of Nanchang

Determination of five kinds of ginkolides in Ginko biloba L. leaves extract by reversed – phase high performance liquid chromatography. Zhang Jian, Pan Jian, A. N. Huiming, Jang Zhiyi, Hu Xuediao, Yang Ke Chinese Journal of Analytical Chemistry. 2000, vol. 1.