Original Article

Natural Product Communications
June 2019: 1–8

DOI: 10.1177/1934578X19857900
journals.sagepub.com/home/npx

SAGE

Analysis of Volatile Constituents of *Ginkgo* Leaf

Szilvia Czigle[1], Éva Héthelyi B[2], Erzsébet Háznagy-Radnai[3], Imre Máthé[3,4], and Jaroslav Tóth[1]

Abstract

Ginkgo biloba L. (Ginkgoaceae) is one of the oldest trees on earth. The medicinal use of its seeds and leaves has been a tradition for thousands of years. The standardized extract (known as EGb 761) contains several biologically active components, among them are terpenes and flavonoid glycosides that are responsible for the pharmacological activities of Ginkgonis folium. According to European Union herbal monographs (EUHM), the leaves of *Ginkgo* are recommended for the treatment of dementia, cerebral vascular insufficiency, and disorders of the peripheral circulation. The aim of our work was to analyze volatile constituents of *Ginkgo* leaf. Leaves of 3 *Ginkgo* trees were analyzed; 2 of which grow in the Medicinal Plants Garden (young trees A and B) and 1 at the Botanical Garden (old tree C) in Bratislava. The leaves were collected in 2014. The essential oil was isolated and quantified using hydrodistillation according to European Pharmacopoeia (Ph. Eur.) The volatile constituents of Ginkgonis folium were evaluated qualitatively and quantitatively using gas chromatography-mass spectrometry (GC-MS) and gas chromatography with flame ionization detection (GC-FID). We identified 16 constituents of the leaves of tree A, 18 in tree B, and 14 in tree C. The volatiles of the 3 trees differ in the respective amounts of monoterpenoids, hydrocarbons, fatty acids, and their methyl esters. The following constituents were identified in all of the 3 trees in largest percentage: hexahydrofarnesyl acetone (23.6%, 16.0%, and 27.7%), α-linolenic acid methyl ester (14.8%, 20.7%, and 15.1%), and pentacosane (22.2%, 22.4%, and 21.9%). Other identified compounds include the monoterpenes (*E*)-α-ionone and (*E*)-β-ionone.

Keywords

Ginkgo biloba, hydrodistillation, GC-MS, volatile compounds

Received: February 11th, 2019; Accepted: March 9th, 2019.

Ginkgo biloba L. (Ginkgoaceae) is a unique plant. The *Ginkgo* tree was able to survive millions of years as the only member of a whole class of plants and its leaf extracts are commonly used as phytomedicines.[1] Herbal medicinal products (EGb 761) were used for the improvement of (age-associated) cognitive impairment and of quality of life in mild dementia. Traditional herbal medicinal products (Ginkgonis folium) were used for the relief of heaviness of legs and the sensation of cold hands and feet associated with minor circulatory disorders, after serious conditions have been excluded by a medical doctor.[2-5] Terpenes, flavonoids, organic acids, polyacetate derived compounds, hydrocarbons, and miscellaneous organic and inorganic compounds were found in *Ginkgo* leaf. Most of the isolated compounds are found ubiquitously in the leaves of higher plants with the exception of certain unique terpene trilactones (ginkgolides and bilobalide) and flavonoids.[6-9]

The wood of *G. biloba* contains small amounts of essential oil, consisting of mono- (C10), sesquiterpenes (C15), and phenylpropanoids.[10] Hirao and Shogaki analyzed the essential oil of *Ginkgo* leaves and found polycyclic aromatic hydrocarbons such as acenaphthene and 2,5,8-trimethyldihydronaphthalene. Furthermore, monoterpenes (C10) *p*-cymene and 1,4-dimethyl-2,5-diisopropylbenzene were found. Oxygenated compounds were acyclic alcohols as

[1] Faculty of Pharmacy, Department of Pharmacognosy and Botany, Comenius University in Bratislava, Slovakia
[2] Hungarian Chemical Society, Budapest, Hungary
[3] Faculty of Pharmacy, Department of Pharmacognosy, University of Szeged, Hungary
[4] Centre for Ecological Research, Institute of Ecology and Botany, Hungarian Academy of Sciences, Vácrátót, Hungary

Corresponding Author:
Szilvia Czigle, Faculty of Pharmacy, Department of Pharmacognosy and Botany, Comenius University in Bratislava, Odbojárov 10, SK-83232, Bratislava, Slovakia.
Email: czigle@fpharm.uniba.sk

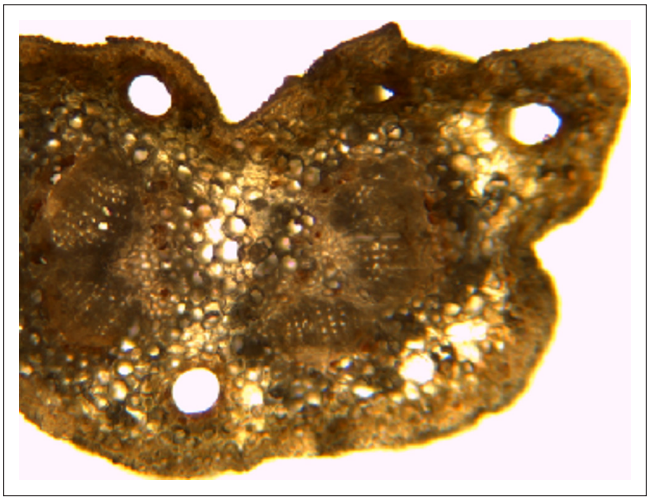

Figure 1. Microscopic Features of the Transverse Section of *Ginkgo* Leaf.

cis-3-hexenol, *cis*- and *trans*-4-hexenol, heptadeca-3,6,9-trien-1-ol, oxygenated monoterpenes (C10), 2-isopropylphenol, thymol, α- and β-ionone, and *trans*-linalool oxide. One phenylpropanoid, *p*-tolylpropylene, was identified.[11] Irie et al isolated further sesquiterpenes (C15) called dihydroatlantones.[12] Bilobanone, a sesquiterpene, bears an isobutyl substitution on the ring framework.[13,14] Zhao et al detected 26 constituents in the ether extract of Cretaceous *Ginkgo coriacea* leaves using GC-MS, including 7 fatty acids (C8, C9, C10, C12, C14, C16, and C18), 14 *n*-alkanes (C16-C29), 4 phtalates - contaminants, and 2,5-bis(1,1-dimethylethyl)-phenol. Similarly, 21 constituents were identified in *G. biloba* leaves preserved for 150 years and 13 constituents were found in extant *G. biloba*. In the preserved leaves, the constituents include 5 fatty acids (C14, C15, C16, C18, and C18:2), 10 *n*-alkanes (C14-C21, C26, and C27), phtalates - contaminants, and 3 phenolic compounds (3-undecylphenol, 3-tridecylphenol, and 3-pentadecylphenol). In extant *G. biloba* leaves, the constituents include 5 *n*-alkanes (C15-C18, C27), 4 fatty acids (C14, C16, C18, and C18:2), 2 phtalates - contaminants, and 2 phenolic compounds (3-tridecylphenol and 3-pentadecylphenol).[15]

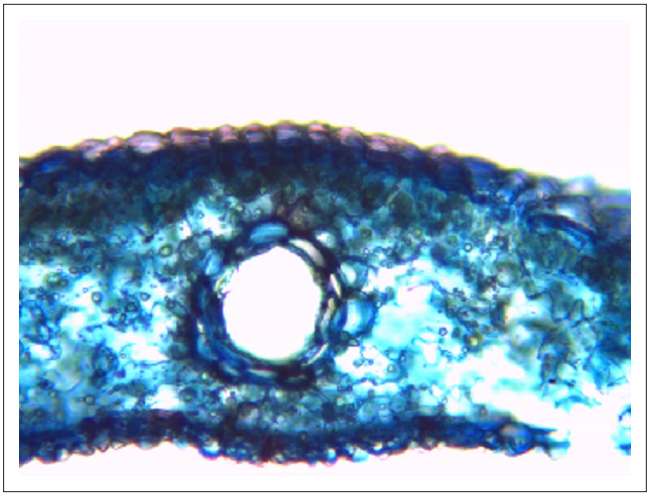

Figure 2. Microscopic Features of the Transverse Section of *Ginkgo* Leaf (Schizogenic Canals).

The aim of our work was to analyze volatile constituents of *Ginkgo* leaf with focus on seasonal/vegetation variability and the influence of plant gender of 3 *Ginkgo* trees were analyzed; 2 of which grow in the Medicinal Plants Garden (young female and male trees A and B) and 1 at the Botanical Garden (old female tree C) in Bratislava. The leaves were collected in 2014. Leaves were collected in the course of a vegetation period from early May to late November. The volatile oil (yellowish color, woody odor) was isolated and quantified using hydrodistillation according to Ph. Eur.[16]

The content of essential oil in our samples was at most 0.2 mL/kg (tree A), 0.3 mL/kg (tree B), and 0.7 mL/kg (tree C) (midsummer samples). The authors[11] reported the content of essential oil 0.9 mL/kg in a female tree and 0.8 mL/kg in a male tree. The oils were distilled into *n*-hexane as an absorbing medium and dried over anhydrous sodium sulfate. The respective volatile compounds were analyzed by thin layer chromatography (TLC) to give violet-purple spots (toluol:diethylether (97:3), detection: vanillin in H_2SO_4).[17]

Ginkgo leaf shows the following diagnostic microscopical characteristics: the upper epidermis consisting of elongated cells with irregular sinuous walls, the lower epidermal cells smaller, with a finely striated cuticle and each cell shortly papillose; stomata about 60 µm wide, deeply sunken with 6-8 subsidiary cells, are more numerous in the lower epidermis; mesophyll with clusters of calcium oxalate of various sizes (1-100 µm), sometimes showing prism of calcium oxalate; vascular bundle (in groups, with lignified walls); mesophyll cells smaller than palisade cells. Schizogenic canals can be observed in the peduncle and in the mesophyll of the blade of *Ginkgo* leaf, possibly containing volatile secrets, similar to other gymnosperms (Figures 1 and 2).

The volatile constituents of Ginkgonis folium were qualitatively and quantitatively evaluated using GC-MS and GC-FID. The essential oil components were identified using a library of spectra, Kovats indices, and partly authentic samples.[18-20] Percentage data were calculated by the area normalization method without applying FID response factor correction and each oil composition was determined 3 times. The relative standard deviation was below 5% for each compound. We have identified 16 constituents of the leaves of tree A, 18 in tree B, and 14 in tree C. The compounds identified in the volatile oils of *Ginkgo* leaf are listed in Tables 1-3.

We identified alkanes: octadecane (C18), nonadecane (C19), eicosane (C20), heneicosane (C21), docosane (C22), tricosane (C23), tetracosane (C24), pentacosane (C25),

Table 1. Percentage Compositions of Oil Components of *Ginkgo* Leaf (Tree A).

				Tree A						
				2014-05-19	2014-06-17	2014-07-17	2014-08-19	2014-09-24	2014-10-20	2014-10-20 (fallen-off leafage)
RT (min)	Essential oil compounds	RI	RI[19]	(%)	(%)	(%)	(%)	(%)	(%)	[%]
4.16	2-Hexenal[a]	n.d.	n.d.	29.7 ± 0.4	-	19.5 ± 0.1	25.8 ± 0.3	-	6.4 ± 0.0	24.5 ± 0.6
11.97	M^{+b}	1290	-	2.6 ± 0.0	-	5.6 ± 0.1	3.0 ± 0.1	-	3.0 ± 0.1	3.1 ± 0.1
20.20	Hexahydroxyfarnesyl acetone	1842	1843-1855[c]	1.0 ± 0.0	23.6 ± 0.4	11.5 ± 0.1	11.2 ± 0.2	10.7 ± 0.4	14.8 ± 0.2	7.1 ± 0.1
20.92	C16:3 Fatty acid methyl ester	1899	-	8.2 ± 0.2	-	1.4 ± 0.0	-	-	-	-
20.93	Nonadecane (C19)	1900	-	-	-	-	-	-	1.1 ± 0.0	-
21.25	Palmitic acid methyl ester	1926	std	1.5 ± 0.0	-	-	-	-	1.2 ± 0.0	1.2 ± 0.0
21.69	Palmitic acid (C16)	1964	std	0.2 ± 0.0	-	-	-	-	0.8 ± 0.1	-
22.17	Eicosane (C20)	2000	std	-	-	-	-	-	0.9 ± 0.0	-
23.37	α-Linolenic acid methyl ester	2102	std	14.8 ± 0.4	13.4 ± 0.3	4.4 ± 0.0	2.3 ± 0.2	-	1.6 ± 0.0	-
24.35	α-Linolenic acid (C18:3)	2144	2098-2139[c]	1.0 ± 0.0	-	-	-	-	-	-
24.47	Docosane (C22)	2200	std	-	-	-	-	-	1.3 ± 0.2	0.8 ± 0.1
25.55	Tricosane (C23)	2300	-	-	-	2.1 ± 0.0	2.8 ± 0.0	14.8 ± 0.1	3.1 ± 0.1	2.1 ± 0.0
26.59	Tetracosane (C24)	2400	std	-	-	2.0 ± 0.0	2.5 ± 0.0	-	2.9 ± 0.2	1.8 ± 0.0
27.60	Pentacosane (C25)[d]	2500	-	-	22.2 ± 2.0	11.5 ± 0.4	14.9 ± 0.4	-	12.3 ± 0.1	9.9 ± 0.4
28.72	Hexacosane (C26)	2600	std	-	-	1.1 ± 0.0	-	-	-	1.0 ± 0.1
30.90	Heptacosane (C27)	2700	-	-	-	2.5 ± 0.0	-	-	2.9 ± 0.1	1.9 ± 0.1

n.d., non detected; RI, (Kovats) retention index; RT, retention time; std, authentic standard.
[a]Correct isomer not identified.
[b]Unknown chemical formula (M_r 192).
[c]NIST 08 Mass Spectral Library.
[d]Coelution with unknown compounds (C25).
[e]Adams' Database.[19]

Table 2. Percentage Compositions of Oil Components of *Ginkgo* Leaf (Tree B).

				Tree B						
				2014-05-19	2014-06-17	2014-07-17	2014-08-19	2014-09-24	2014-10-20	2014-10-20 (fallen-off leafage)
RT (min)	Essential oil compounds	RI	RI[19]	(%)	(%)	(%)	(%)	(%)	(%)	(%)
4.16	2-Hexenal[a]	n.d.	n.d.	14.9 ± 0.3	14.6 ± 0.4	17.5 ± 0.1	13.1 ± 0.2	11.3 ± 0.3	-	24.5 ± 0.6
11.97	M^+192[b]	1290	-	2.5 ± 0.0	2.9 ± 0.0	5.6 ± 0.1	1.3 ± 0.0	2.2 ± 0.1	-	3.1 ± 0.1
14.27	(*E*)-α-Ionone	1430	1426-1429[c]	1.2 ± 0.0	-	10.5 ± 0.1	-	-	-	-
15.17	(*E*)-β-Ionone	1489	1442-1497[c]	1.7 ± 0.0	-	1.6 ± 0.0	-	-	-	-
19.64	Octadecane (C18)	1800	std	-	0.5 ± 0.0	-	-	-	0.6 ± 0.0	-
20.20	Hexahydroxyfarnesyl acetone	1842	1843-1855[c]	2.5 ± 0.1	7.0 ± 0.2	-	16.0 ± 0.2	7.7 ± 0.3	2.1 ± 0.1	7.1 ± 0.1
20.92	C16:3 Fatty acid methyl ester	1899	-	13.0 ± 0.2	3.4 ± 0.1	-	-	2.3 ± 0.1	-	-
20.93	Nonadecane (C19)	1900	-	-		-	-	-	0.7 ± 0.0	-
21.25	Palmitic acid methyl ester	1926	std	2.0 ± 0.0	0.8 ± 0.0	2.4 ± 0.0	-	-	-	1.2 ± 0.0
22.17	Eicosane (C20)	2000	std	-	0.7 ± 0.0	-	-	-	0.7 ± 0.0	-
23.37	α-Linolenic acid methyl ester	2102	std	-	-	-	-	-	0.8 ± 0.0	-
24.35	α-Linolenic acid (C18:3)	2144	2098-2139[c]	20.7 ± 0.3	7.1 ± 0.2	1.1 ± 0.0	4.9 ± 0.2	4.2 ± 0.1	-	-
24.47	Docosane (C22)	2200	std	-	1.3 ± 0.1	2.0 ± 0.0	2.0 ± 0.0	0.6 ± 0.1	1.0 ± 0.0	0.8 ± 0.1
25.55	Tricosane (C23)	2300	-	1.4 ± 0.0	3.5 ± 0.1	11.5 ± 0.4	5.2 ± 0.2	2.4 ± 0.1	1.4 ± 0.1	2.1 ± 0.0
26.59	Tetracosane (C24)	2400	std	1.0 ± 0.0	-	1.1 ± 0.0	5.4 ± 0.1	2.6 ± 0.1	1.6 ± 0.1	1.8 ± 0.0
27.60	Pentacosane (C25)[d]	2500	-	6.0 ± 0.4	7.5 ± 0.9	2.5 ± 0.0	15.4 ± 0.4	22.4 ± 1.2	3.3 ± 0.7	9.9 ± 0.4
28.72	Hexacosane (C26)	2600	std	-	-	-	3.7 ± 0.0	1.9 ± 0.1	1.7 ± 0.1	1.0 ± 0.1
30.90	Heptacosane (C27)	2700	-	-	-	-	5.4 ± 0.1	4.2 ± 0.0	1.6 ± 0.1	1.9 ± 0.1

n.d., non detected; RI, (Kovats) retention index; RT, retention time; std, authentic standard.
[a]Correct isomer not identified.
[b]Unknown chemical formula (M_r 192).
[c]NIST 08 Mass Spectral Library.
[d]Coelution with unknown compounds (C25).
[e]Adams' Database.[19]

Table 3. Percentage Compositions of Oil Components of *Ginkgo* Leaf (Tree C).

				Tree C						
				2014-05-19	2014-06-17	2014-07-17	2014-08-19	2014-09-24	2014-10-20	2014-10-20 (fallen-off leafage)
RT (min)	Essential oil compounds	RI	RI[19]	(%)	(%)	(%)	(%)	(%)	(%)	(%)
4.16	2-Hexenal[a]	n.d.	n.d.		8.9 ± 0.4			18.1 ± 0.6	13.4 ± 0.0	15.1 ± 0.2
11.31	Limonene	1033	std		32.0 ± 1.6					
11.97	M^+192[b]	1290	-		4.4 ± 0.2	6.4 ± 0.2	2.3 ± 0.0	10.5 ± 0.3	12.4 ± 0.0	16.0 ± 0.0
20.20	Hexahydroxyfarnesyl acetone	1842	1843-1855[c]	24.4 ± 0.2	6.0 ± 0.3	24.3 ± 0.7	27.7 ± 0.1	15.4 ± 0.2	19.4 ± 0.0	15.3 ± 0.2
20.92	C16:3 Fatty acid methyl ester	1899	-		5.5 ± 0.3	4.6 ± 0.1	1.9 ± 0.0		1.1 ± 0.0	
21.25	Palmitic acid methyl ester	1926	std		2.2 ± 0.1		6.7 ± 0.0		0.5 ± 0.0	
21.69	Palmitic acid (C16)	1964	std				1.1 ± 0.0			
23.37	α-Linolenic acid methyl ester	2102	std		15.1 ± 0.6	11.9 ± 0.4	6.7 ± 0.0		3.4 ± 0.0	
24.47	Docosane (C22)	2200	std				1.1 ± 0.1		0.6 ± 0.0	0.9 ± 0.0
25.55	Tricosane (C23)	2300	-	7.5 ± 0.0	1.5 ± 0.1	4.7 ± 0.1	5.5 ± 0.0	3.4 ± 0.1	2.1 ± 0.0	3.5 ± 0.1
26.59	Tetracosane (C24)	2400	std	6.9 ± 0.0	1.1 ± 0.1	3.4 ± 0.0	5.4 ± 0.0	3.4 ± 0.1	2.0 ± 0.0	2.9 ± 0.0
27.60	Pentacosane (C25)[d]	2500	-	21.9 ± 0.2	4.2 ± 1.8	12.5 ± 0.0	13.5 ± 0.1	9.7 ± 2.5	5.9 ± 0.3	6.9 ± 0.2
28.72	Hexacosane (C26)	2600	std				3.2 ± 0.1		1.1 ± 0.0	1.5 ± 0.0
30.90	Heptacosane (C27)	2700	-	10.6 ± 0.1	1.5 ± 0.1	5.2 ± 0.0	7.6 ± 0.0	4.3 ± 0.1	2.2 ± 0.0	2.7 ± 0.0

n.d., non detected; RI, (Kovats) retention index; RT, retention time; std, authentic standard.
[a]Correct isomer not identified.
[b]Unknown chemical formula (M_r 192).
[c]NIST 08 Mass Spectral Library.
[d]Coelution with unknown compounds (C25).
[e]Adams' Database.[19]

hexacosane (C26), and heptacosane (C27). Some alkanes, such as octadecane (C18), nonadecane (C19), and eicosane (C20), were identified only in *Ginkgo* leaves collected from young trees A and B. Zhao et al[15] identified in *G. biloba* leaves preserved for 150 years and in the ether extract of Cretaceous fossils (*Ginkgo coriacea* leaves) these *n*-alkanes: tetradecane (C14), nonadecane (C19), eicosane (C20), heneicosane (C21), docosane (C22), hexacosane (C26), and heptacosane (C27) using GC-MS. Pentadecane (C15), hexadecane (C16), heptadecane (C17), oktadecane (C18), and heptacosane (C27) were described in fresh *Ginkgo* leaves. Hirao and Shogaki[11] analyzed the essential oil of *Ginkgo* leaves and found polycyclic aromatic hydrocarbons such as acenaphthene and 2,5,8-trimethyldihydronaphthalene.

In all trees (young trees A and B, old tree C), fatty acids were identified [palmitic (C16), *α*-linolenic (C18:3) together with its methyl ester]. Free linolenic acid (C18:3) was present in tree A only. Palmitic acid (C16) was identified in young tree A and in tree C (oldest). Ross[9] described the following fatty acids in *Ginkgo biloba* (leaf, seed): myristic (C14), *α*-hydroxypalmitic (C16), stearic (C18), oleic (C18:1), linoleic (C18:2), α-linolenic acid (C18:3), arachidonic (C20:4), and behenic (C22). We identified *α*-linolenic acid (C18:3) in addition to his findings in our samples.

Hirao and Shogaki[11] identified these monoterpenes (C10): *p*-cymene, 1,4-dimethyl-2,5-diisopropylbenzene, *cis*-3-hexenol, *cis*- and *trans*-4-hexenol, heptadec-3,6,9-trien-1-ol, 2-isopropyl-phenol, thymol, *trans*-linalool oxide, and *α*- and *β*-ionone. We identified limonene in *Ginkgo* leaves collected from the oldest tree C, and *α*- and *β*-ionone in leaves from the youngest tree B. An aldehyde, 2-hexenal (degradation product of fatty acids), hexahydrofarnesyl acetone (phytone), and an unknown compound (M_r 192) were found in trees A, B, and C.

The major volatile compounds in leaves of the 3 trees A, B (young), and C (old female) are hexahydrofarnesyl acetone (23.6%, 16.0%, and 27.7%), *α*-linolenic acid methyl ester (14.8%, 20.7%, and 15.1%), and *n*-pentacosane (22.2%, 22.4%, and 21.9%), respectively.

Ginkgonis folium extracts contain several biologically active components, among them are terpenes (ginkgolides and bilobalide) and flavonoid glycosides that are responsible for the pharmacological activities. Leaves were collected in the course of a vegetation period from early May to late November. The content of terpene lactones (ginkgolides and bilobalide) varied during this period, with lower levels in spring and autumn, and a maximum in midsummer.[6-9] The content of flavonoid metabolites in Ginkgo leaf varies typically during a vegetation period as well, with the highest percentage in autumn and in fallen leaves.[21-23]

We tried to confront our results with hydrometeorology data. The major volatile compound is hexahydrofarnesyl acetone in August samples, when sunshine duration and air temperature were very high. Hexahydrofarnesyl acetone and *n*-pentacosane (C25) reached maximum content in summer (trees A and C) or in autumn (tree B). *α*-Linolenic acid methyl ester maximum levels were observed in spring (trees A and B) and summer (tree C). The content of *n*-pentacosane (C25) was maximal in fallen-off leafage samples (trees A and B).

Hexahydrofarnesyl acetone (phytone) [CAS 502-69-2] has a long-lasting fresh jasmine, celery, mild waxy, and fresh oily odor. It is often used in jasmine-type compositions as a flavor and fragrance agent. It is assumed that this compound is perceived as the major odor component of Ginkgo leaf volatiles.[24]

Experimental

Plant Material

Ginkgo biloba L. leaves (Ginkgonis folium) were obtained from a 15- and a 14-year-old tree (trees A and B) at the Medicinal Plants Garden, and from a 50-year-old female tree (tree C) at the Comenius University Botanical Garden (Bratislava, Slovakia). Leaves were collected in the course of a vegetation period from early May to late November. Herbarium samples have been deposited at the Department of Pharmacognosy and Botany (Comenius University in Bratislava, Slovakia).

Plant Samples Preparation

Volatile oils were isolated (from frozen leaves) by hydrodistillation for 4 hours in a Clevenger apparatus, the oils being distilled into *n*-hexane (CENTRALCHEM, Slovakia) as an absorbing medium and dried over anhydrous sodium sulfate (CENTRALCHEM, Slovakia). The oils were stored in glass bottles at 4°C prior to analysis.

Microscopic Analysis

Optical microscope: LEICA DME, trinocular, planachromatic objective lens, zoom objective 20×, tube ½; digital camera: LEICA EC 3 Mpix; software: LEICA application suite 2.4.0 R1, LAS EZ ver. 1.3.0.

GC-FID Conditions

The composition of volatile oil was analyzed on an AGILENT 6890/5973N GC/FID (Santa Clara, CA, United States) and CTC Combi PAL sampler (CTC Analytics AG, Zwingen, Switzerland). The operating conditions were as follows: I. GC parameters: (a) capillary column: DB-5MS (Sigma-Aldrich, Saint Louis, MO, United States), 25 m × 200 µm ID, 0.33 µm film thickness, stationary phase: 5% diphenyl-/95% dimethylpolysiloxane fused; (b) the oven temperature was then programmed at 8°C/min from 60°C to 260°C (1 minute isothermal); (c) the injector temperature was 240°C and the injector vents closed for 10 s after which the

split ratio was 1:30; (d) samples: 1.0 µL; (e) high quality helium was used as carrier gas (flow rate 1.2 mL/min, 37 cm/s); II. FID parameters: (a) the temperature was 260°C; (b) frequency: 50 Hz; (c) data were evaluated by the use of MSD ChemStation D.02.00.275 (Agilent) software; and (d) percentage data were calculated by the area normalization method without applying FID response factor correction and each oil composition was determined 3 times. The relative standard deviation was below 5% for each compound.

GC-MS Conditions

The composition of volatile oil was analyzed on an AGILENT 6890/5973N GC/MS (Santa Clara, CA, United States) and CTC Combi PAL sampler (CTC Analytics AG, Zwingen, Switzerland). The operating conditions were as follows: I. GC parameters: (a) capillary column: SLB-5MS (Sigma-Aldrich, Saint Louis, MO, United States), 30 m × 250 µm ID, 0.25 µm film thickness, stationary phase: 5% diphenyl-/95% dimethylpolysiloxane fused; (b) the oven temperature was then programmed at 8°C/min from 60°C to 260°C (4 minutes isothermal); (c) the injector temperature was 240°C and the injector vents closed for 10 s after which the split ratio was 1:30; (d) sample: 1.0 µL; and (e) high quality helium was used as carrier gas (flow rate 1.0 mL/min, 37 cm/s); II. MS parameters: (a) the instrument was operated at 70 eV in electron impact (EI^+) mode, quadrupole analyzer; (b) full-scan analyses were performed in the mass range 40-500 m/z, 3.15 scan.s-1; (c) data were evaluated by the use of MSD ChemStation D.02.00.275 (Agilent) software; (d) the identification of the compounds was done by comparing the retention times and the recorded spectra with spectra from the literature, Kovats indices,[18-20] and spectral data in our own library based on authentic standards.

Acknowledgment

This paper is dedicated to Prof László Tóth (University of Szeged) on the occasion of his birthday.

Declaration of Conflicting Interests

The author(s) declared no potential conflicts of interest with respect to the research, authorship, and/or publication of this article.

Funding

The author(s) disclosed receipt of the following financial support for the research, authorship, and/or publication of this article: This work was supported by the Slovak Grant Agency Projects VEGA/2/0044/15 and APVV-15-0308.

References

1. Czigle S, Tóth J, Jedlinszki N, Háznagy-Radnai E, Csupor D, Tekeľová D. *Ginkgo biloba* food supplements on the European market - Adulteration patterns revealed by quality control of selected samples. *Planta Med*. 2018;84(6-07):475-482.
2. *European Union herbal monograph on Ginkgo biloba L., folium EMA/HMPC/321097/2012*. London: European Medicinal Agency; 2015:1-8.
3. *ESCOP Monographs*. 2nd edition. Stuttgart: Thieme Verlag; 2003:178-210.
4. *WHO Monographs on Selected Medicinal Plants*. Geneva: WHO; 1999:154-167.
5. *The complete German Commission E monographs*. Austin: American Botanical Council; 1998:160-170.
6. van Beek TA. *Ginkgo Biloba*. 1st edition. Amsterdam: Harwood Academic Berlin, Publishers; 2000:1-532.
7. DeFeudis FV. *Ginkgo biloba Extract (EGb 761)*. Paris: Elsevier; 1991:1-187.
8. van Beek TA. Chemical analysis of *Ginkgo biloba* leaves and extracts. *J Chromatogr A*. 2002;967(1):21-55.
9. Ross IA. *Medicinal Plants of the World*. Totowa: Humana Press; 2001:157-190.
10. Hölzl J. Inhaltsstoffe von *Ginkgo biloba*. *Pharm Unserer Zeit*. 1992;21(5):215-223.
11. Hirao N, Shogaki T. The essential oil of *Ginkgo biloba* L. *Kinki Daigaku Rikogakubu Kenkyu Hokoku*. 1981;16:47-50.
12. Irie J, Murata M, Homma S. Glycerol-3-phosphate dehydrogenase inhibitors, anacardic acids, from *Ginkgo biloba*. *Biosci Biotechnol Biochem*. 1996;60(2):240-243.
13. Kimura H. Studies on the components of *Ginkgo biloba* L. IV. Structure and reduction of bilobanone by lithium in ethylenediamine. *Yakugaku Zasshi*. 1962;82(2):214-218.
14. Kimura H, Irie H, Ueda K, Ueo S. The constituents of the heartwood of *Ginkgo biloba* L. V. The structure and absolute configuration of bilobanone. *Yakugaku Zasshi*. 1968;88(5):562-572.
15. Zhao Y-X, Li C-S, Luo X-D, Wang Y-F, Zhou J. Palaeophytochemical constituents of cretaceous *Ginkgo coriacea* florin leaves. *J Integr Plant Biol*. 2006;48(8):983-990.
16. *European Pharmacopoeia (Ph. Eur. 9)*. 9th edition. Strasbourg: Council of Europe; 2019: 01/2011:.1828.
17. *Anfärbereagenzien für Dünnschicht und Papier-Chromatographie*. Darmstadt: E. Merck AG; 1966:1-75.
18. *NIST 08, Mass Spectral Library (NIST/EPA/NIH)*. Gaithersburg, MD: National Institute of Standards and Technology; 2008.
19. Adams RP. *Identification of Oil Components by Gas Chromatograhy/Quadrupole Mass Spectroscopy*. Carol Stream, IL: Allured Publ. Corp.; 2007:1-804.
20. Kováts E. Gas-chromatographische Charakterisierung organischer Verbindungen. Teil 1: Retentionsindices aliphatischer Halogenide, Alkohole, aldehyde und ketone. *Helv Chim Acta*. 1958;41(7):1915-1932.
21. Hasler A, Gross GA, Meier B, Sticher O. Complex flavonol glycosides from the leaves of *Ginkgo biloba*. *Phytochemistry*. 1992;31(4):1391-1394.
22. Hasler A, Meier B, Sticher O. Quantitative HPLC analysis of flavonoid aglycones in different medicinal plants. *Planta Med*. 1990;56(6):575-576.

23. Czigle S, Tekeľová D, Tóth J, Mrlianová M. Variability of flavonoid content in leaves of young trees of *Ginkgo biloba* L. during a vegetation period. *Farm Obzor*. 2011;80(6):169-175.

24. Müller PM, Lamparsky D. *Perfumes: Art, Science and Technology*. London: Springer-Science + Business Media, B. V; 2012:1-665.