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Botanical Adulterants Prevention Program
BOTANICAL ADULTERANTS BULLETIN

Adulteration of *Ginkgo biloba* Leaf Extract

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Goal: The goal of this bulletin is to provide timely information and/or updates on issues of adulteration of ginkgo (*Ginkgo biloba*) leaf and ginkgo leaf extracts to the international herbal industry and extended natural products community in general. It is intended to give a brief overview on the occurrence of adulteration, known adulterants and analytical means to detect them, the market situation, and consequences for the consumer and the industry.

1. General Information

1.1 Common name: Ginkgo[1]

1.2 Other common names:

English: Maidenhair tree[1,2]

French: Ginkgo, arbre aux quarante écus, noyer du Japon[3]

German: Ginkgo, Fächerblattbaum, Mädchenhaarbaum, Elefantenohrbaum, Tempelbaum[3]

Italian: Ginkgo, ginko, ginco, albero di capelvenere

Spanish: Ginkgo, árbol sagrado, árbol de las Pagodes, árbol de los quarantos escudos

Chinese: *yin xing* (银杏)[4]

1.3 Accepted Latin binomial: *Ginkgo biloba* L.[1]

Ginkgo
Ginkgo biloba
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1.4 Synonyms: Existing synonyms, e.g., *Ginkgo macrophylla* K.Koch, *Salisburia biloba* (L.) Hoffmanns., or *Salisburia macrophylla* Reyn.,[5] are no longer used in science or in commerce.

1.5 Botanical family: Ginkgoaceae

1.6 Plant part: Two parts of the ginkgo tree are sold on the market, the leaf and the seed.

This bulletin focuses mainly on the ginkgo leaf extract characterized by 24% flavonol glycosides and 6% triterpenes, which has been subject to repeated and increasing levels of adulteration over the past decades. Products derived from the seeds are not addressed in this document.

1.7 General use(s): Ginkgo leaf extract is used to improve mental performance, increase the pain-free walking distance in peripheral arterial occlusive disease, and for vertigo and tinnitus.[6-8] Other indications include (age-associated) cognitive impairment and quality of life improvement in mild dementia.[6,8,9] Ginkgo leaf extracts may help to prevent symptoms related to altitude sickness.[10,11]

2. Market

2.1 Importance in the trade: In the United States, ginkgo is mainly sold in the Mainstream Multi-Outlet channel (previously referred to as the Food, Drug and Mass Market channel) where it has ranked among the top 25 best-selling dietary supplements from 2009-2014, with sales between US $11.1 and $16.0 million (excluding sales at Walmart). Sales in this channel have slightly increased in 2015 and 2016, but the sales rank has dropped to number 23 at the same time (Table 1). Sales in the Natural channel were between US $3.9 and $4.7 million (excluding sales at Whole Foods) in the same time frame, ranking ginkgo at numbers 15-20 in the years between 2009 and 2016.[12-18] Sales figures from the *Nutrition Business Journal* ranked ginkgo at #13 of herbal dietary supplements in the United States in 2011, with overall sales of US $90 million.[19] Total worldwide sales of ginkgo products in 2012 were US $1.26 billion, with China accounting for 46% (US $578 million) of the sales, Germany for 12% (US $152 million), Australia for 4.8% (US $61 million), France for 4.2% (US $53 million), and Brazil for 3.8% (US $48 million).[20]

2.2 Supply sources: The majority of the ginkgo extracts on the market are made from leaves cultivated on plantations in China (Jiangsu, Shandong, Zhejiang, Hubei, Anhui, and Guangxi provinces), France, and the United States.[6] Other ginkgo plantations are found in Australia, Korea, Japan and New Zealand. (H. Wohlmuth [MediHerb/Integria Healthcare] e-mail communication, September 25, 2015) In China, ginkgo trees are cultivated in small family farms under the supervision of the local Government.[21]

2.3 Raw material and finished product forms: Whole, cut, and powdered dry leaf, dry leaf extracts, and liquid leaf extracts (e.g., tinctures). The extracts are primarily quantified to contain 24% flavonol glycosides and 6% terpene lactones (24/6), based on the widely clinically tested extract EGb 761®, manufactured by Willmar Schwabe GmbH & Co. KG (Karlsruhe, Germany). The Schwabe extract is patented and follows a complex production process: the leaves are harvested from plantations only when chemical tests show the flavonoid content at a maximum. When the leaves have lost 75% of their weight after the drying process, they are subjected to an acetone-water (6:4) extraction. Successive treatments are used to remove other, less desirable compounds (e.g., ginkgolic acids). The extract is eventually concentrated (30–70:1), and quantified to the labeled content.[3] There are many other dry extracts that use a manufacturing process different from the EGb 761 on the market; additionally, a number of liquid extracts are sold, which are usually made using water-alcohol mixtures at various ratios; with some exceptions, these ginkgo preparations are not the subject of published human clinical trials.

2.4 Market dynamics: Statistics on the annual production volume of ginkgo leaves are difficult to find and the available numbers show some discrepancies. According to a review on ginkgo published in 2008, the production of

Table 1: Sales data for ginkgo dietary supplements from 2013–2016

Channel	2013		2014		2015		2016	
	Rank	Sales [US$]	Rank	Sales [US$]	Rank	Sales [US$]	Rank	Sales [US$]
Natural[a]	20	3,988,705	18	4,076,228	17	4,659,417	20	4,598,986
Mainstream Multi-Outlet[b]	19	11,488,186	19	11,147,066	22	12,810,392	23	12,917,783

[a]According to SPINS; This includes data from co-ops, associations, independent retailers, and large regional chains. These data do *not* include sales data from Whole Foods Market, nor does it capture non-electronic tracking systems such as sales through health professionals, private internet distributors, and many health food stores.

[b]According to SPINS/IRI; This channel coverage includes the food, drug, and mass-market sector, military commissaries, and select buyer's clubs and so-called dollar stores. SPINS/IRI data does not include discount department store sales, e.g., possible sales at Walmart and club stores are excluded)

Sources: Smith T, et al.[18] T. Smith (American Botanical Council) e-mail to S. Gafner, September 2, 2015 and September 3, 2015. K. Kawa (SPINS) e-mail to S. Gafner, July 11, 2016.

ginkgo leaves from China, France, and the United States (specifically, South Carolina) was approximately 8,000 metric tons.[22] However, another report in 2008 estimated the yearly production in China alone at 20,000 metric tons.[23] Global demand for dried ginkgo leaves was estimated at 60,000 metric tons in 2014.[24] Between 2009 and 2016, the sales of ginkgo supplements in the United States have been relatively flat, or even declining, at least in the Mainstream Multi-Outlet channel.[12-17] However, global demand in leaf extracts has seen a consistent growth from US $118 million in 2013 to US $162 million in 2016.[25] Given that the supply chain and market for ginkgo seem fairly steady, it is not likely that the factors underlying ginkgo adulteration are due to changing market dynamics.

3. Adulteration

3.1 Known adulterants: The main concern with ginkgo adulteration is the addition of pure flavonols/flavonol glycosides or extracts from other botanicals which are rich in flavonol glycosides:

Pure flavonols:

- rutin
- quercetin
- kaempferol

Flavonol glycoside-rich extracts:

- *Styphnolobium japonicum* (L.) Schott, (syn: *Sophora japonica* L., Fabaceae)
- *Fagopyrum esculentum* Moench, Polygonaceae

A different type of issue was evidenced in regulatory actions by the Chinese government in 2015, which took actions against companies that allegedly adulterated ginkgo extracts by using 3% hydrochloric acid instead of the more expensive organic solvents (ethanol, acetone) in the manufacturing process of ginkgo extracts, providing extracts with higher amounts of free quercetin, kaempferol, and isorhamnetin.[26] The use of unconventional solvents in the manufacturing process also raises the question of just how similar the phytochemical composition of some 24/6 extracts from China are to the clinically tested EGb 761 extract. This is especially relevant considering the active constituents of ginkgo extract are not well defined.

3.2 Sources of information supporting confirmation of adulteration: There is ample evidence of the production and sale of adulterated ginkgo extracts in the international supply chain.

In 2003, an investigation of the quality of nine commercial ginkgo extracts from suppliers in Europe, Asia, and North America found one sample with an unusually high content of rutin, a flavonol glycoside that occurs in many plant species, and one sample with almost no ginkgo terpene lactones and no ginkgo flavonols. The authors suggested that pure rutin was added to one sample to increase the contents of total flavonols.[27]

Similarly, four out of 14 commercial ginkgo products sourced in the Edmonton, Alberta (Canada) area were likely adulterated with pure flavonols (rutin, quercetin, kaempferol, and isorhamnetin),[28] and in a comparison of high-performance liquid chromatography (HPLC) fingerprints of ginkgo extracts from 19 suppliers from the Jiangsu and Zhejiang provinces in China, three products were found to be adulterated with exogenous rutin.[29] Whether the deviations in concentrations of quercetin, kaempferol, and isorhamnetin are indeed due to spiking of ginkgo extracts with synthetic flavonols, as suggested by Liu et al.,[28] is an issue that has been questioned by experts. Higher amounts of these flavonols, which are present in only trace amounts in fresh ginkgo leaves, may be found in an extract due to an unusual manufacturing process or improper storage (H. Wohlmuth e-mail communication, September 27, 2015).

The issue of ginkgo adulteration was again raised in a presentation by Kurth in 2008. In this presentation, rutin (sourced from buckwheat [*F. esculentum*] or Japanese sophora [*S. japonicum*], also known as Japanese pagoda tree), and/or quercetin, and/or kaempferol were found as adulterants of ginkgo extracts.[30]

In 2010, the German Central Laboratory for Pharmacists (Zentrallaboratorium Deutscher Apotheker) investigated 10 ginkgo food supplements purchased in Germany. The researchers calculated the quercetin/(kaempferol + isorhamnetin) ratio after hydrolysis, and found a range of 0.8 – 1.2 for authentic ginkgo leaf material. However, in seven of the ten commercial samples, the ratio was above 1.7 (1.78 – 7.70), suggesting that these products were adulterated.[31] In the same year, analysis of 16 commercial finished products (seven of the 16 samples represented three products sold under three, two, and two different brand names, respectively) from the European market by proton NMR metabolomics showed that one Italian ginkgo product was fortified with extraneous quercetin.[32]

The adulteration of ginkgo extracts with pure flavonoids or flavonoid-rich extracts was also detailed in 2011. In this study, Chandra et al. reported that chromatographic profiles of three out of eight products labeled to contain ginkgo extracts closely resembled those of commercial extracts obtained from Japanese sophora. The HPLC chromatogram of the unhydrolyzed products at 370 nm of the particular commercial sophora fruit extract showed rutin as a single peak, while quercetin was the main peak in the commercial sophora flower extract. Isoflavonoids from sophora, like genistein and its glycosides that are proposed by some experts as markers for adulteration, barely absorb at 370 nm, and may therefore go undetected.[33]

A study in Japan looked at 22 commercial products (16 ginkgo products from Japan and six from Germany and France) by HPLC-UV/MS without prior hydrolysis, finding three products with unusually high amounts of quercetin.[34]

Also in 2012, Harnly et al. analyzed 18 ginkgo dietary supplements purchased in the Beltsville, MD area or from the internet. Comparison of chromatographic and spectral fingerprints with authentic ginkgo extract led to the conclusion that seven products were clearly adulterated with either rutin, quercetin, or an unidentified flavonol glycoside.[35]

Adulteration of commercial ginkgo products with rutin purchased in the Turkish market was reported by Demirezer in 2014.[36]

In 2014, Australian researchers published a relatively simple method to detect adulteration of ginkgo extract in commercial dietary supplement products. The authors discovered admixtures of the free flavonols quercetin and kaempferol without prior hydrolysis in three of the eight commercial samples that were analyzed. The three adulterated samples also contained genistein, an isoflavone that is characteristic of some plants in the pea family (Fabaceae), as noted above. The authors hypothesize that the genistein could have come from extracts of the fruit of Japanese sophora.[37]

Clear evidence of ginkgo adulteration with Japanese sophora extracts was provided in a study by Avula et al., where 11 out of 25 tested supplements, purchased online from suppliers in the United States, showed flavonol-glycosides that are typically found in *S. japonica* fruit, such as genistein-4'-*O*-glucoside and genistein-4'-*O*-neohesperidoside.[38]

The authenticity of bulk standardized ginkgo leaf extracts (n = 15) and commercial finished ginkgo products (n = 14) were also investigated by Canadian researchers Ma et al. in 2016. The powdered extracts were received from various Canadian suppliers, and commercial samples were purchased in local pharmacies in Canada (n = 7) or China (n = 7). Based on comparison of the HPLC-UV fingerprints with those of authentic samples, occurrence of adulteration was confirmed in four of 15 (27%) bulk standardized extracts and 10 of 14 (71%) finished commercial products.[39]

An analysis of 20 batches of ginkgo extract from one manufacturer in Jiangsu province, China was conducted by Chinese researchers in 2016. The samples were analyzed by HPLC-UV with subsequent multivariate statistical evaluation; 17 batches could be separated into two clusters according to their phytochemical composition, with three samples not clustering within either of these two clusters. Of these three batches, one batch was adulterated, based on the presence of genistin and genistein, one contained unusually high amounts of quercetin, kaempferol, and isorhamnetin, and one had lower amounts of many of the ginkgo compounds overall.[40]

Another report on ginkgo quality was published by Booker et al.[41] The 35 food supplements analyzed in this study were obtained from health food stores, supermarkets, and pharmacies in the area of Central London, UK, and from the internet. Samples were analyzed by high-performance thin-layer chromatography (HPTLC), or proton nuclear magnetic resonance (^{1}H NMR) with subsequent principal component analysis (PCA) using soft independent modeling by class analogy (SIMCA) software. Of the 35 samples, 33 (94%) exhibited elevated levels of rutin (n = 8) and/or quercetin (n=26), or low levels of ginkgo metabolites when compared to reference samples. One sample did not have any ginkgo, but contained a compound with structural similarity to 5-hydroxytryptophan.

López-Gutiérrez et al. analyzed 11 commercial finished ginkgo products purchased in local stores in Almeida, Spain, and Krakow, Poland.[42] The authors found the characteristic terpene lactones in all products, and ginkgolic acids in 10 out of 11 ginkgo supplements. However, they also found unusually high concentrations (27.2 – 38.2 mg/g) of rutin in three products, and isoflavonoid (predominantly genistein) concentrations between 0.02 and 2.41 mg/g.[42] Of particular interest, these authors reported the presence of glycitein in addition to genistein in two products. Glycitein is known from a number of plants from the family Fabaceae, including Japanese sophora.

Evaluation of 18 commercial ginkgo supplements from the North American and European markets, obtained between 2015 and 2017, by reverse phase HPTLC and HPLC-UV (detection at 370 and 260 nm) by NSF International showed that only three products contained authentic ginkgo leaf extracts. Adulteration included uncharacteristically high levels of rutin, quercetin, kaempferol, or extracts from Japanese sophora or green tea. (M. Pan [NSF International] email communication, October 25, 2017)

3.3 Genistein controversy: Many researchers have suggested that the isoflavone genistein can be used as a marker to detect adulteration with extracts of Japanese sophora.

However, other authors have reported that genistein is a genuine constituent in ginkgo leaf, albeit at very small amounts.[43-45] Yao et al. (2017) reported genistein concentrations between 5-28 µg/g dry leaf using a validated HPLC-UV method with detection at 350 nm. The HPLC conditions allowed for a clear separation between genistein and apigenin, two compounds that have the same molecular weight and may be confused even if an MS detector is used. The amounts of genistein in the leaves was dependent on the season, and was found to be highest in September and October.[45] Genistein has also been reported from leaf extracts[46-49] but since ginkgo extract adulteration is widespread, questions about the authenticity of the materials used in these studies remain.

While genistein is known to occur in *S. japonica*, the majority of investigations into the chemistry of ginkgo failed to detect this isoflavone.[50] In addition, the enzyme isoflavone synthase, which is needed to produce isoflavones biosynthetically, has not been reported from ginkgo. Another aspect to consider is the absence of isoflavone glycosides in authentic ginkgo leaves. Since flavonoids in ginkgo are predominantly found as glycosides — e.g., rutin is present in much larger concentrations compared to quercetin — one would expect genistein glycosides (such as genistin) to occur in larger amounts than genistein. On the other hand, biflavones reportedly are mainly found as aglycones, so it cannot be excluded that the same could be true for the isoflavones.

A recent investigation into the question of genistein occurrence in six samples of authentic ginkgo whole leaf material by four separate analytical laboratories (two academic laboratories, one contract analytical laboratory, and one in-house research laboratory at a dietary supplement supplier) by HPLC-MS gave contradictory results, with genistein not detected in two laboratories, while the other two either reported it as "detected" or to occur at concentrations between 37 – 217 ng/g dry leaf (Gafner

S, unpublished results). If present in ginkgo leaves, genistein is found at low concentrations. Therefore, establishing a maximal acceptable limit for genistein could be a way to distinguish authentic ginkgo materials from those containing extracts of *S. japonica*. A limit of 1% sophoricoside (genistein-4'-*O*-glucoside) in ginkgo extracts was set in 2015 by the Chinese Food and Drug Administration.[51] Sophoricoside is a marker compound for the presence of Japanese sophora fruit extracts.

3.4 Accidental or intentional adulteration: Industry experts agree that the adulteration of ginkgo extracts is intentional, due to the financial advantage of adding cheaply-sourced flavonols like rutin or quercetin. According to William Obermeyer, PhD, former FDA natural products chemist and former technical director of ConsumerLab.com, the costs for a *Ginkgo biloba* extract in 2009 varied between $35-$90/kg, while the price of rutin isolated from buckwheat is ca. $10/kg, giving an incentive for adulteration to unscrupulous suppliers and manufacturers.[52] Herrmann Kurth, PhD, director of quality control at *Finzelberg* GmbH & Co. KG, places the cost for the EGb 761 extract at >200€/kg (US $220 at the exchange rate from July 8, 2015), making lower-cost sources to boost flavonol glycoside contents attractive to less scrupulous companies.[30] Costs in 2015 for ginkgo extracts from Chinese manufacturers ranged between US $150-240/kg, while Japanese sophora flower extracts sold for US $30/kg (J. Xie, Y-C Ma, J. Zhang [Canadian Phytopharmaceutical Corp.] oral communications, October 7, 2015). Jay Lee, PhD, president of Beijing Ginkgo Group, said in an interview that "The vast majority of the prices we're up against in the U.S. market do not even support the cost of the raw materials used to manufacture a high quality ginkgo extract. Most contract manufacturers tell us they are simply meeting their customer's specifications, so if the customer simply states 24/6 [24% flavonoids/6% terpenes], then the contract manufacturer will seek the lowest cost 24/6."[53] Conversely, manufacturers unaware of the complexity of ginkgo analysis may unknowingly incorporate adulterated ginkgo extracts into their products, depending on either internal or external analysis that confirm the constituent profile desired, namely 24% flavonol glycosides and 6% diterpene lactones. At the same time, knowledge of ginkgo extract adulteration has been reported for two decades. Therefore, if a supplier or contract manufacturer does not take steps to ensure the extract they are selling or buying is made from authentic ginkgo leaves and is free of adulterations as outlined in this bulletin, they are likely entering adulterated products into trade and are subject to the liability that their actions entail.

3.5 Frequency of occurrence: There are no reliable comprehensive data on the number of adulterated ginkgo products on the market. Most of the published studies included only a small number of samples. The largest study on commercial ginkgo supplements evaluated 40 products using DNA mini-barcodes. In this study, DNA was retrieved in 37 products, and 31 of these 37 products (84%) contained ginkgo DNA. However, since the DNA barcoding is unable to detect pure isolates, and the author did not use specific primers to detect DNA from Japanese sophora, or any other potential adulterant, the addition of pure flavonols, or flavonol-rich materials would have gone undetected.[54] The largest studies specifically looking at admixture of flavonols from origins other than ginkgo leaf found adulteration rates of 14% (three of 22 samples), 48% (10 of 21 samples), 44% (11 of 25) and 48% (14 of 29 samples).[34,38,39,55] In the study by Avula et al., eight additional samples (32%) contained variable amounts of genistein, a proposed marker compound for ginkgo adulteration (although the presence of genistein could be also due to soy-based excipients, which would not be considered a case of adulteration), increasing the number of potentially adulterated products to 76%.[38] Finally, an investigation initiated by the British Broadcasting Corporation (BBC), which linked up with a research team of the University College of London's College of Pharmacy, concluded that many ginkgo *food supplements* had little or no ginkgo extract in them.[56] (Food supplements are regulated in the European Union under different quality control requirements than "Traditional Herbal Remedies," which are a type of drug, requiring higher quality assessments for identity and authenticity.) Additional details of this study were published in 2016. Of the 35 food supplements analyzed, 26 (74%) contained elevated levels of rutin and/or quercetin, and one sample did not contain any chemistry consistent with ginkgo at all.[41]

3.6 Possible therapeutic issues: The admixture of pure rutin, quercetin, kaempferol, isorhamnetin, or extracts of flavonol-rich plant materials is not considered a safety concern. However, the adulterated products are not phytoequivalent to the EGb 761 extract, or other clinically

Ginkgo
Ginkgo biloba
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tested ginkgo products. In addition, adulterated low quality ginkgo extracts may contain higher concentrations of less desirable ginkgo leaf constituents, e.g., ginkgolic acids, which are known contact allergens and have shown to cause contact dermatitis.[57] (Ginkgolic acid levels are required to be no more than 5 ppm [µg/g] in the *European Pharmacopoeia* [Ph. Eur] and the *United States Pharmacopeia* [USP]).[58,59]

3.7 Analytical methods to detect adulteration: Most often, extracts of ginkgo in bulk or finished products are analyzed by HPLC using evaporative light scattering (ELSD) or mass spectrometric (MS) detection for the terpene lactones, and ultraviolet detection (UV) at 370 nm for the flavonols. Since ginkgo leaves contain over 20 flavonols, predominantly glycosides of quercetin, kaempferol, and isorhamnetin, the total flavonol content is generally measured after hydrolysis, which cleaves the sugar portion from the flavonol aglycone, greatly simplifying the analysis to the quantification of quercetin, kaempferol, and isorhamnetin. Thus, although the 24/6 specification in pharmacopeias refers to 24% flavonol *glycosides*, the prescribed analytical methods quantify the free aglycones that form as a result of hydrolysis. Accordingly, since glycosides of quercetin, kaempferol, and isorhamnetin are found in many plants, materials that are particularly rich in these flavonols can potentially be added to ginkgo in order to increase the flavonol content.

Once the hydrolysis reaction is performed, signs of adulteration become less obvious. The American Herbal Pharmacopoeia monograph lists the typical ratio of quercetin, kaempferol, and isorhamnetin in ginkgo leaf extracts after hydrolysis as between 6:5:1 and 5:4:1.[6] In order to prevent the adulteration of ginkgo extracts with extraneous flavonols, the USP adopted criteria in 2012 for the kaempferol to quercetin (not less than 0.7) and isorhamnetin to quercetin (not less than 0.1) peak ratios after hydrolysis.[60] Despite this, some manufacturers have found ways to fraudulently spike ginkgo extracts while complying with the USP criteria.[37] Therefore, the USP monograph for *Powdered Ginkgo Extract* now includes an additional test: *Limit of Rutin and Quercetin*, which specifies maxima of 4% for rutin and 0.5% for quercetin in unhydrolyzed extracts.[59,61]

In May 2013 the California company Ethical Naturals, Inc. issued the report "Ginkgo Adulteration & Identification with Fructus sophorae (*Sophora japonica*)", a revised version of its 2006 report on ginkgo adulteration, which suggests the use of HPLC with an UV detection wavelength of 260 nm in addition to 360 nm as a means to detect genistein.[49]

Other HPLC-UV methods propose to measure the entire chemical composition without hydrolysis and compare this HPLC fingerprint to a fingerprint of authentic ginkgo leaves.[33,35,39] Wohlmuth et al. suggest using the HPLC conditions outlined in the USP and analyzing the ginkgo extracts before and after hydrolysis in order to detect admixture of pure flavonol aglycones, and to test for the presence of genistein.[37] Other identity tests use HPTLC for identification,[38,58,59] but the admixture of pure flavonols may be difficult to detect. However, this technique has proven successful in the detection of spiking with fruit or flower extracts from Japanese sophora.[38] An additional derivatization step added to the original HPTLC method for identification was presented in 2017 by Frommenwiler et al. Besides added rutin and/or quercetin, the modified method allows the detection of buckwheat and Japanese sophora fruit or flower extracts.[62]

Finally, a comparison among HPLC-UV, UV spectrophotometry, and near infrared (NIR) spectroscopy with subsequent statistical evaluation by multivariate statistical analysis concluded that UV spectrophotometry was more sensitive to minor variations in the chemical composition than HPLC-UV. The NIR approach was not able to distinguish between authentic and adulterated samples since the excipients, which are part of a solid-state NIR spectrum, have a significant impact on the final result.[35]

3.8 Perspectives: In the report on ginkgo adulteration published by Natural Products Insider, a dietary supplement industry trade publication, William Obermeyer, PhD looked back at ginkgo dietary supplement testing at ConsumerLab. Based on tracking of the kaempferol to quercetin ratios, he suggested that the quality of ginkgo products worsened over the years from 1999 to 2007. In the same article, Jochen Muehlhoff, PhD, former Marketing Information Manager at Willmar Schwabe GmbH & Co. KG, called the adulteration of ginkgo extracts a global problem, though he noted that it is less of a problem in Europe, but definitely a problem in the United States, and even more pronounced in some Asian countries.

4. Conclusions

Economically motivated adulteration of ginkgo extracts with pure flavonol-glycosides, flavonols, or flavonol-rich extracts of other species is an ongoing problem in the dietary supplement industry and elsewhere. While the addition of flavonols is not considered a safety problem, the health benefits of sub-standard ginkgo extracts spiked with pure flavonols or flavonol-rich extracts have not been established. The addition of extraneous flavonols can be detected by HPLC with UV or MS detection. Considering this adulteration has been reported for decades and that methods for the detection of adulteration have been available, there is little excuse for this to continue to occur. It is suggested that contract manufacturers, independent analytical laboratories, and manufacturers with internal analytical capabilities need to take the lead in preventing low-cost adulterated extracts from unscrupulous extract manufacturers, most of which appear to originate from China, from entering the global market.

5. References

1. McGuffin M, Kartesz JT, Leung AY, Tucker AO. *Herbs of Commerce.* 2nd ed. Silver Spring, MD: American Herbal Products Association; 2000.
2. Gruenwald J, Brendler T, Jaenicke C. *PDR for herbal medicines,*

2nd edition. Montvale, NJ: Medical Economics Co; 2000.
3. Wichtl M. Ginkgo folium – Ginkgo leaf. In: Wichtl M, ed. *Herbal Drugs and Phytopharmaceuticals*. 3rd ed. Stuttgart, Germany: Medpharm Scientific Publishers; 2004:251-253.
4. Flora of China. Missouri Botanical Garden & Harvard University Herbaria. http://www.efloras.org/florataxon.aspx?flora_id=2&taxon_id=200028370. Accessed May 25, 2017.
5. The Plant List. Version 1.1 http://www.theplantlist.org. Accessed May 19, 2017.
6. Upton R, Graff A, Swisher D. *American Herbal Pharmacopoeia and Therapeutic Compendium: Ginkgo Leaf, Ginkgo Leaf Dry Extract. Ginkgo biloba L.: Standards of Analysis, Quality Control and Therapeutics.* Scotts Valley, CA: American Herbal Pharmacopoeia; 2003.
7. Folium Ginkgo. *WHO monographs on selected plants.* Vol 1. Geneva, Switzerland: World Health Organization; 1999:154-167.
8. Ginkgo leaf. In: Blumenthal M, Goldberg A, Brinckmann J, eds. *Herbal Medicine: Expanded Commission E Monographs*. Newton, MA: Integrative Medicine Communications; 2000.
9. Community herbal monograph on *Ginkgo biloba* L., folium. London, United Kingdom: European Medicines Agency Committee on Herbal Medicinal Products (HMPC); 2015:1-9.
10. Tissot van Patot MC, Keyes LE, Leadbetter G, 3rd, Hackett PH. *Ginkgo biloba* for prevention of acute mountain sickness: does it work? *High Alt Med Biol.* 2009;10(1):33-43.
11. Murdoch D. Altitude sickness. Systematic review. *BMJ Clinical Evidence.* 2010:1209.
12. Cavaliere C, Rea P, Lynch M, Blumenthal M. Herbal supplement sales experience slight increase in 2008. *HerbalGram.* 2009;82:58-61.
13. Cavaliere C, Rea P, Lynch M, Blumenthal M. Herbal supplement sales rise in all channels; sales rise in 2009. *HerbalGram.* 2010;86:62-65.
14. Blumenthal M, Lindstrom A, Lynch M, Rea P. Herbs sales continue growth – up 3.3% in 2010. *HerbalGram.* 2011;90:64-67.
15. Blumenthal M, Lindstrom A, Ooyen C, Lynch M. Herb supplement sales increase 4.5% in 2011. *HerbalGram.* 2012;95:60-64.
16. Lindstrom A, Ooyen C, Lynch M, Blumenthal M, Kawa K. Sales of herbal dietary supplements increase by 7.9% in 2013, marking a decade of rising sales: turmeric supplements climb to top ranking in natural channel. *HerbalGram.* 2014;103:52-56.
17. Lindstrom A, Ooyen C, Lynch M, Blumenthal M. Herb supplement sales increase 5.5% in 2012: herbal supplement sales rise for 9th consecutive year; turmeric sales jump 40% in natural channel. *HerbalGram.* 2013;99:60-65.
18. Smith T, Kawa K, Eckl V. Herbal supplement sales in US increase 7.7% in 2016. *HerbalGram.* 2017;115:56-65.
19. Ginkgo biloba. *NBJs Supplement Business Report*. New York City, NY: Penton Media; 2012:198.
20. *IMS Health data.* Plymouth Meeting, PA: IMS Health; 2012.
21. Schmid W, Balz JP. Cultivation of *Ginkgo biloba* L. on three continents. *Acta Hort.* 2005;676:177-180.
22. Singh B, Kaur P, Gopichand, Singh RD, Ahuja PS. Biology and chemistry of *Ginkgo biloba*. *Fitoterapia.* 2008;79(6):401-418.
23. Chen XJ, Wang C-Z, Ye Y-Z. Research progress of chemcial constituents of *Ginkgo biloba* Linn. leaves and their applications. *Biomass Chem Eng.* 2008;42(4):57-62.
24. Globalink, Ltd. provides update on current company activities [press release]. MarketWatch: TheNewswire, May 25, 2016 2016.
25. *Ginkgo biloba extract market 2017: Global production, growth, share, demand and applications market research report to 2021.* Eugene, OR: Medgadget, LLC;2017.
26. Blumenthal M. Chinese government takes action against companies producing substandard and adulterated ginkgo extracts: Companies in violation will be dealt with 'severely and strictly' *HerbalGram.* 2015;108:24-27.
27. Sloley BD, Tawfik SR, Scherban KA, Tam YK. Quality control analysis for ginkgo extracts require analysis of intact flavonol glycosides. *J Food Drug Anal.* 2003;11(2):102-107.
28. Liu C, Mandal R, Li X-F. Detection of fortification of ginkgo products using nanoelectrospray ionization mass spectrometry. *Analyst.* 2005;130(3):325-329.
29. Xie P, Chen S, Liang YZ, Wang X, Tian R, Upton R. Chromatographic fingerprint analysis--a rational approach for quality assessment of traditional Chinese herbal medicine. *J Chromatogr A.* 2006;1112(1-2):171-180.
30. Kurth H. Extract adulteration – recognizing the fact and fakes. Agency Meeting of the Martin Bauer Group; 2008; Sinzig, Germany.
31. Tawab M, Krzywon M, Schubert-Zsilavecz M. Dietary supplements with ginkgo under the microscope. *Pharm Ztg.* 2010;20:62-67.
32. Agnolet S, Jaroszewski JW, Verpoorte R, Staerk D. H NMR-based metabolomics combined with HPLC-PDA-MS-SPE-NMR for investigation of standardized *Ginkgo biloba* preparations. *Metabolomics.* 2010;6(2):292-302.
33. Chandra A, Li Y, Rana J, et al. Qualitative categorization of supplement grade *Ginkgo biloba* leaf extracts for authenticity. *J Funct Foods.* 2011;3(2):107-114.
34. Kakigi Y, Hakamatsuka T, Icho T, Goda Y, Mochizuki N. Comprehensive analysis of flavonols in *Ginkgo biloba* products by ultra-high-performance liquid chromatography coupled with ultra-violet detection and time-of-flight mass spectrometry. *Biosci Biotech Biochem.* 2012;76(5):1003-1007.
35. Harnly JM, Luthria D, Chen P. Detection of adulterated *Ginkgo biloba* supplements using chromatographic and spectral fingerprints. *J AOAC Int.* 2012;95(6):1579-1587.
36. Demirezer LÖ, Büyükkaya A, Uçaktürk E, Kuruüzüm-Uz A, Güvenalp Z, Palaska E. Adulteration determining of pharmaceutical forms of *Ginkgo biloba* extracts from different international manufacturers. *Rec Nat Prod.* 2014;8(4):394-400.
37. Wohlmuth H, Savage K, Dowell A, Mouatt P. Adulteration of *Ginkgo biloba* products and a simple method to improve its detection. *Phytomedicine.* 2014;21(6):912-918.
38. Avula B, Sagi S, Gafner S, et al. Identification of *Ginkgo biloba* supplements adulteration using high performance thin layer chromatography and ultra high performance liquid chromatography-diode array detector-quadrupole time of flight-mass spectrometry. *Anal Bioanal Chem.* 2015;407(25):7733-7746.
39. Ma Y-C, Mani A, Cai Y, et al. An effective identification and quantification method for *Ginkgo biloba* flavonol glycosides with targeted evaluation of adulterated products. *Phytomedicine.* 2016;23(4):377-387.
40. Yang J, Wang AQ, Li XJ, Fan X, Yin SS, Lan K. A chemical profiling strategy for semi-quantitative analysis of flavonoids in ginkgo extracts. *J Pharm Biomed Anal.* 2016;123:147-154.
41. Booker A, Frommenwiler D, Reich E, Horsfield S, Heinrich M. Adulteration and poor quality of Ginkgo biloba supplements. *Journal of Herbal Medicine.* 2016;6(2):79-87.
42. Lopez-Gutierrez N, Romero-Gonzalez R, Martinez Vidal JL, Garrido Frenich A. Quality control evaluation of nutraceutical products from Ginkgo biloba using liquid chromatography coupled to high resolution mass spectrometry. *J Pharm Biomed Anal.* 2016;121:151-160.
43. Pandey R, Chandra P, Arya KR, Kumar B. Development and validation of an ultra high performance liquid chromatography electrospray ionization tandem mass spectrometry method for the simultaneous determination of selected flavonoids in *Ginkgo biloba*. *J Sep Sci.* 2014;37(24):3610-3618.
44. Kuchta K. Seasonal variations of the contents of genistein and 6-HKA in *Ginkgo biloba* L. leaves from different regions of China. Ninth Joint Natural Products Conference; 2016; Copenhagen, Denmark.
45. Yao JB, Du X, Jin HH, et al. Seasonal variability of genistein and 6-hydroxykynurenic acid contents in *Ginkgo biloba* leaves from different areas of China. *Nat Prod Commun.* 2017;12(8):1241-1244.
46. Wang F, Jiang K, Li Z. Purification and identification of genistein in *Ginkgo biloba* leaf extract. *Chin J Chromatogr.* 2007;25(4):509-513.
47. Wu J, Xing H, Tang D, et al. Simultaneous determination of nine flavonoids in beagle dog by HPLC with DAD and application of *Ginkgo biloba* extracts on the pharmacokinetic. *Acta*

Chromatogr. 2012;24(4):627-642.
48. Tang D, Yu Y, Zheng X, et al. Comparative investigation of in vitro biotransformation of 14 components in *Ginkgo biloba* extract in normal, diabetes and diabetic nephropathy rat intestinal bacteria matrix. *J Pharm Biomed Anal.* 2014;100:1-10.
49. *PhytoReport #5: Ginkgo adulteration & identification w/fructus sophorae (Sophora japonica).* San Anselmo, CA: Ethical Naturals, Inc.;2013.
50. van Beek TA, Montoro P. Chemical analysis and quality control of *Ginkgo biloba* leaves, extracts, and phytopharmaceuticals. *J Chromatogr A.* 2009;1216(11):2002-2032.
51. Supplementary test method for sophoricoside in ginkgo extract, ginkgo leaves and ginkgo capsules. Beijing, China: China Food and Drug Administration; 2015.
52. Obermeyer W. Economically motivated adulteration in the dietary supplement market place. Public Meeting on Economically Motivated Adulteration; 2009; College Park, MD.
53. Myers S. Adulteration stifles the *Ginkgo biloba* market. *Natural Products Insider*2008: 38-41.
54. Little DP. Authentication of Ginkgo biloba herbal dietary supplements using DNA barcoding. *Genome.* 2014;57(9):513-516.
55. Bewicke C. *Ginkgo biloba Extract: Brand QC Analytical Report.* San Anselmo, CA: Ethical Naturals Inc;2006.
56. Trust me – I'm a doctor. Do herbal supplements contain what they say on the label? BBC Two online services. http://www.bbc.co.uk/programmes/articles/4hX30rMYkMv9YjMTH38MY6/do-herbal-supplements-contain-what-they-say-on-the-label. Accessed October 5, 2015.
57. Lepoittevin J-P, Benezra C, Asakawa Y. Allergic contact dermatitis to *Ginkgo biloba* L.: relationship with urushiol. In: Frosch P, Dooms-Goossens A, Lachapelle JM, Rycroft RJG, Scheper RJ, eds. *Current Topics in Contact Dermatitis*. Berlin, Heidelberg: Springer Berlin Heidelberg; 1989:158-162.
58. Ginkgo folium. *European Pharmacopoeia (EP 8.7)*. Strasbourg, France: European Directorate for the Quality of Medicines and HealthCare; 2015.
59. Powdered ginkgo extract. *USP 40-NF 35.* Rockville, MD: United States Pharmacopeial Convention; 2017:7005-7008.
60. Powdered ginkgo extract. *USP 35-NF 30.* Rockville, MD: United States Pharmacopeial Convention; 2012.
61. Bzhelyansky A, Sarma N, Giancaspro GI. Letter to the Editor in response to "Adulteration of Ginkgo biloba products and a simple method to improve its detection" by Wohlmuth et al. *Phytomedicine.* 2014;21(12):1497.
62. Frommenwiler D, Booker A, Heinrich M, Reich E, Cañigueral S. Quality assessment of *Ginkgo biloba* supplements based on a single HPLTC method. *PMIO.* 2017;4(S 01):We-SL-02.

REVISION SUMMARY

Version # , Author,	Date Revised	Section Revised	List of Changes
Version 1, S. Gafner New	n/a	n/a	none