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Phyto-"similars" May Not be that Similar after All: An Analytical Comparison of Ginkgo Medicinal Products

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Abstract

Ginkgo biloba L. (Ginkgoaceae; G. biloba) leaves extracts are widely recognised for their cognition-enhancing properties in patients with minor and major neurocognitive disorders. Current pharmacopoeias, such as the European Pharmacopoeia, specify a defined content range of Ginkgo terpene lactones and Ginkgo flavonoid glycosides. These constituents are believed to contribute to the pharmacological and clinical effects of *G. biloba* extracts but do not account entirely for their activity. Approximately 70% of the extract composition is not specified and thus can vary between different *G. biloba* preparations. Recently, proanthocyanidins (PACs) have been described as an additional constituent class with both pharmacological activity in preclinical models and quantitatively relevant amounts in Ginkgo extract EGb 761; however, data regarding PAC content of commercial products is still scarce. In the present study, we employed a recently published quantitative HPLC method to analyse the PAC content in registered drug products from the Swiss market containing different *G. biloba* extracts. We found significant differences in PACs, with more than 10-fold differences between products with low versus high PAC content *G. biloba* extracts, ranging from approximately 0.3% to 5%. This variability in PAC content between extracts of different manufacturers may be attributed to different raw materials and manufacturing processes leading to herbal extracts with unique constituent profiles ("product-by-process concept"). The observed quantitative differences in PACs as a potentially pharmacologically relevant constituent group highlight the inherent difficulty in extrapolation and generic use of efficacy and safety data between different Ginkgo products.

Keywords: Proanthocyanidins; Ginkgo biloba; EGb 761

Introduction:

Ginkgo biloba L. (*Ginkgoaceae; G. biloba*) leaves extracts are widely recognised for their cognition-enhancing properties in patients with minor and major neurocognitive disorders and their positive impact on the quality of life in patients with dementia [1–3]. Several *G. biloba* preparations exist in the European market with different manufacturing and quality control processes [4], which can lead to variations in the amount of Ginkgo constituents among *G. biloba* products [5,6]. *G. biloba* special extract, EGb 761®, is a quantified extract with potential therapeutic benefits. Previous clinical trials showed that EGb 761® significantly improved cognitive performance and neuropsychiatric symptoms in patients with mild to moderate dementia [7,8]. Additionally, EGb 761® was found to improve tinnitus volume in patients with tinnitus as either a primary presentation or a concomitant symptom of dementia [9]. Although the molecular mechanisms underlying these effects are not yet fully elucidated, EGb 761® appears to possess neuroprotective properties. Experimental studies demonstrated that EGb 761® improves neuroplasticity [10], reduces oxidative stress in the mitochondria [11], modulates blood viscosity [12], restores cerebral energy metabolism to a near-normal level [13], and suppresses amyloid-β-induced pathological behaviours [14].

Ginkgo biloba extracts are characterised by two main active constituents: ginkgo flavonoids and terpene lactones, which are believed to contribute to the pharmacological and clinical effects [15]. According to the European Pharmacopoeia (Ph. Eur.), Ginkgo biloba extract contains 22-27% ginkgo flavonoids and 5-7% terpene lactones, which include between 2.8% and 3.4% ginkgolides A, B, and C, and between 2.6% and 3.2% bilobalide. Additionally, the product contains less than 5 ppm of ginkgolic acids [16]. However, these requirements lack specifications for the remaining 70% of the extract composition, reflecting a potential variation in the composition of Ginkgo biloba extract.

Proanthocyanidins (PAC) are a class of polyphenolic compounds that are known for their potent antioxidants and anti-inflammatory properties [17]. PACs have recently been described as an additional constituent class with pharmacological activity in preclinical models and quantitatively relevant amounts in EGb 761®. It was found that PACs from EGb 761® reduced reactive oxygen species (ROS) and reversed scopolamine-induced impairment of short-term memory in mice [18]. However, data regarding the PAC content of commercial products is still scarce. In the present study, we employed a recently published quantitative high-performance liquid chromatography (HPLC) method to analyse the PAC content in registered drug products [19] from the Swiss market containing different *G. biloba* extracts.

Materials and Methods:

1. Samples:

Samples included all authorised G. biloba medicinal products in Switzerland with a market share of >1% available between December 2022 and March 2023. Three different batches of the following preparations were acquired in a local pharmacy: Ginkgo-Mepha® (Mepha, Switzerland), Ginkgo Sandoz® (Sandoz Pharmaceuticals Switzerland), Symfona® 240 (OM Pharma Suisse, Switzerland), Rezirkane® 240 (Zeller Medical, Switzerland), and Tebokan® 240 (Schwabe Pharma, Switzerland).

2. Quantification of PACs

2.1 Chemicals and standard solutions

The following solvents and reagents were utilised for the quantification process using HPLC: Methanol, orthophosphoric acid, analytical-grade hydrochloric acid (Roth), and deionised water. PAC standard (procyanidin B2; CAS number 29106–49–8) was purchased from Phytolab for calibration.

2.2 Preparation of standard solutions

The PAC standard solution was prepared by dissolving a precise amount of procyanidin B2 in 1 part methanol + 1 part 1.5 M hydrochloric acid (hydrolysis solution) to yield a concentration of 100 µg/mL, with sonication for 10 minutes, ensuring complete dissolution. Working standard solutions were created from this solution through dilution with hydrolysis solution, resulting in concentrations of 10 µg/mL, intended for the calibration curve generation. All standards were stored and hydrolysed as described in section 2.3. No filtration was performed prior to HPLC analysis.

2.3 Preparation of sample solutions

The average mass of the preparation was determined using a representative sample, which was then ground into a fine powder. A homogeneous quantity of the fine powder was

obtained from each sample, corresponding to approximately 100 mg extract, which was accurately weighed, transferred to 25 mL volumetric flasks and mixed with the hydrolysis solution. The samples were dissolved through ultrasonication (Elmasonic S100) for ten minutes at room temperature, ensuring optimal release of the PACs from the matrix. The solutions were subjected to hydrolysis in 10mL hydrolysis tubes with secure screw caps, ensuring they remained above the water line in a boiling water bath. Through evaluation, 45 minutes was identified as the ideal time for comprehensive hydrolysis of the PACs. Post-

hydrolysis, the sealed tubes were rapidly cooled using an ice bath and then left at room conditions to equilibrate to an ambient temperature of 23°C. No filtration was performed prior to HPLC analysis.

2.4 HPLC

A Chromaster HPLC from Hitachi was employed for the HPLC evaluation. The chromatographic separation was achieved on a reversed-phase C18 column (Waters Symmetry Shield C-18, 5µm, 150 x 4.6 mm). The mobile phase was composed of water, which was adjusted to pH 2.0 using an 85% o-phosphoric acid solution (Eluent A) and methanol (Eluent B). The flow rate was set at 1.0 mL/min. The gradient programme was structured as follows: an isocratic condition for the initial 1.00 min at 60% Eluent A; from 1.00 to 8.00 min, there was a linear decrease from 60% to 54.5% Eluent A; from 8.00 to 9.00 min, the eluent switched to 0% Eluent A for column washing. This was followed by a return to 60% Eluent A between 13.00 to 13.50 min and then a 6.5 min equilibration period, culminating in a total runtime of 20.00 min. Detection was carried out using a DAD detector set to a wavelength of 530 nm. The column temperature was maintained at 25°C, and each run involved an injection volume of 10 µL.

Quantitative analysis was specifically performed for delphinidin and cyanidin peaks. For this purpose, standard solutions of hydrolysed procyanidin B2 were utilised. The sum of both these compound results was used in subsequent calculations. Notably, delphinidin quantification was calculated as cyanidin. Peaks with smaller magnitudes, such as pelargonidin, were excluded from consideration in the sample analysis.

This HPLC method was previously validated as described by Kulić et al. [20].

Results:

Table 1 shows the PAC fraction calculated as procyanidin B2 (% m/m) for the 15 studied batches. The quantification of PACs revealed variability across different G. biloba products and also across batches of the same Ginkgo product. Significantly, Tebokan® 240 (Schwabe Pharma, Switzerland) generally exhibited markedly higher values, with batches 131121, 150122, and 160122 showing 4.669%, 4.945%, and 5.051% PAC content. All other preparations had mean concentrations of PACs between 0.262 – 0.473%, which is one order of magnitude lower.

Table 1. Differences in the PAC content of different ginkgo extracts

Product	Batch	PAC-content % m/m
Ginkgo Mepha 120	145176A	0.43
Ginkgo Mepha 120	144195A	0.521
Ginkgo Mepha 120	143469B	0.324
Ginkgo Sandoz 120	LW8696	0.367
Ginkgo Sandoz 120	MH3894	0.462
Ginkgo Sandoz 120	LS8598	0.277
Rezirkane 240	202223	0.357
Rezirkane 240	202226	0.312
Rezirkane 240	202225	0.425
Symfona 240	2072897	0.262
Symfona 240	2108932	0.473
Symfona 240	2072901	0.332
Tebokan 240	131121	4.669
Tebokan 240	150122	4.945
Tebokan 240	160122	5.051

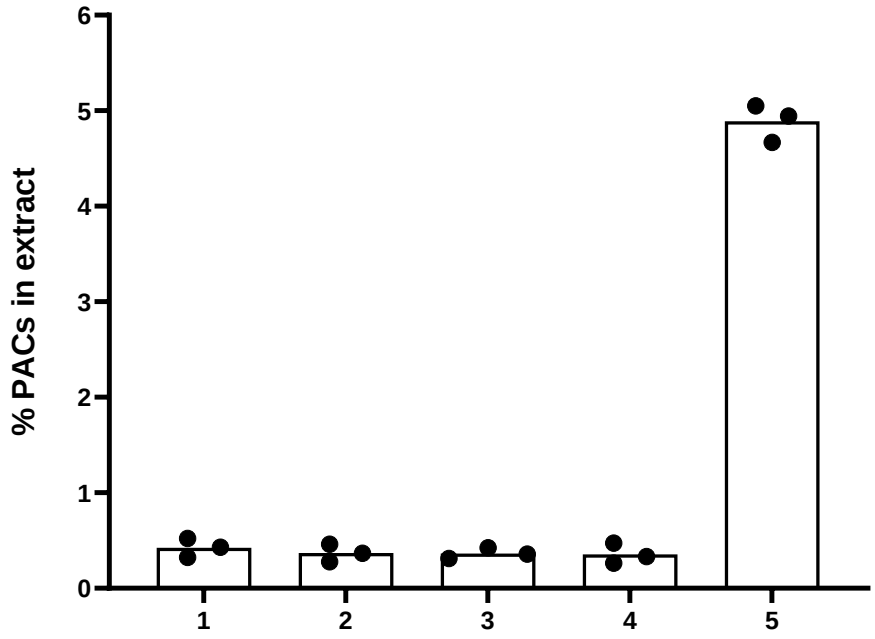

Figure 1: Depiction of measured PAC quantities in the corresponding extracts. 1. Ginkgo Mepha, 2. Ginkgo Sandoz, 3. Rezirkane, 4. Symfona, 5. Tebokan.

Discussion:

The emergence of biosimilars and generics represented a significant shift towards more cost-effective agents with the same therapeutic benefits as their reference products [21]. To gain regulatory approval, biosimilars should demonstrate equivalent pharmacological and functional properties to the reference biologics in several bioequivalence studies [22]. While these bioequivalence validations are pivotal for the marketing approval of biosimilars, the landscape of phytotherapeutic agents is notably different. Phytotherapeutic agents are gaining popularity in medical practice for their potential therapeutic benefits [23]. However, disparities in the phytochemical compositions in extracts derived from the same plant are well-recognised, often arising from a complex interplay between seasonal, environmental and manufacturing factors [20]. Available evidence suggests that extracts from the same plant can differ in their pharmacokinetic and pharmacodynamic properties in men [24]. The extraction and purification process can significantly impact the phytochemical composition of extracts, rendering variable pharmacological profiles [4,24–26].

The present investigation unveiled substantial variability in the PACs content, quantified as procyanidin B2 (% m/m), across different batches of G. biloba medicinal products approved in Switzerland. Comparable differences in PAC contents have also been reported for herbal medicinal products from Germany [19]. Several factors might contribute to the observed discrepancies in PAC content, including the seasonal, geographical and climatic variability of the origin of plant material used for extraction, processing of the plant material and manufacturing conditions [24], all of which are discussed below.

Climatic variations can influence the phytochemical composition of plants, as temperature, precipitation, and seasonal changes can affect plant metabolism and secondary metabolite production [27]. The geographical origin or harvest region and cultivation techniques may also play a role, given that soil composition, altitude, and local microclimates can profoundly affect plant growth and metabolite synthesis [28]. *G. biloba* requires subtropical climates with specific soil conditions and adjusts its leaf histology according to the climate. Optimal planting density and specific cultivation techniques can boost yield and constituent content in the leaves. Additionally, post-harvest treatments, such as exposure to NaCl or UV-B light, can alter flavonoid levels in the leaves [29]. Recent investigations showed substantial variations in the concentrations of flavonol glycosides and terpene trilactones of different *G.biloba* leaves according to their geographical locations and harvesting conditions [30,31]. Therefore, soil quality, climate variations, plant age, and harvest time may have played a role in the observed variability in the PAC content of the *G. biloba* products, as demonstrated by our results.

The extraction practices, including the drying process and the choice of extraction solvent, can also introduce this variability. Every step in the processing chain, from drying patterns to

the specifics of extraction and subsequent procedures, can alter the end product's chemical profile [24]. For instance, different manufacturers utilise varied solvents, solvent mixture ratios, refinement techniques and process sequences. It is worth noting that the current monographs, such as the Ph. Eur. and Herbal Medicinal Products (HMPC) monographs, specify the requirements of only 30% of the constituents of Ginkgo leaf extracts. In comparison, different extraction processes can affect the remaining 70%. As a result, even if products adhere to the same specifications, the residual composition can differ. This was evident in the present study, where the PAC content significantly differed among five approved *G. biloba* herbal medicinal products.

PACs have well-established antioxidants and anti-inflammatory properties [17]. Significant free radical scavenging activity has also been reported for PACs extracted from Ginkgo leaves [32,33]. Preclinical models demonstrate a contribution of PACs to the pharmacological profile of Ginkgo biloba extract preparations: EGb 761® has been observed to enhance endothelial-dependent vasodilation, but the effect was not seen if proanthocyanidins had been removed from the extract [34]. Six individual polyphenols identified in EGb 761®, as well as a fraction of a Ginkgo biloba leave extract containing catechins and procyanidins, exerted potent inhibitory activities towards Aβ42 aggregation and could also destabilise preformed fibrils. The lowest IC50 values were observed for procyanidines [35]. Recently, it was found that PACs from EGb 761® reduced reactive oxygen species (ROS) and reversed scopolamine-induced impairment of short-term memory in mice [18].

An in-vitro modelling study demonstrated that Ginkgo medicinal products from the German market provided by different manufacturers showed heterogeneous protective effects against amyloid beta 42 (Aβ42)-induced alterations in neuronal tissue cultures [17]. The question arises as to what extent such extract diversity can translate into clinically significant variability in the therapeutic safety and efficacy of different phytotherapeutic preparations from the same plant. Few clinical studies directly comparing different Ginkgo preparations are available. Itil and Martorano [25] reported divergent EEG effects of three commercially available Ginkgo preparations in a cross-over trial. Different exposures to Ginkgolides were clearly observed in a bioavailability study comparing two preparations [36].

Conclusions

In the present study, we demonstrated that different G. biloba extract herbal medicinal products available in Switzerland and batches within the products have variable concentrations of PACs. Such phytochemical variability may be attributed to the different raw materials and manufacturing processes. Because PACs likely contribute to the pharmacological profile of G. biloba extracts, these findings highlight the need for more rigorous standardisation to ensure consistency in the efficacy and safety of phytotherapeutic

products. It underscores the importance of standardised starting materials and manufacturing processes leading to herbal extracts with unique constituent profiles ("product-by-process concept").

Author Contributions

WFR participated in the conceptualisation, methodology, formal analysis, investigation, writing of the original draft, and funding acquisition.

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Institutional Review Board Statement

Not applicable.

Informed Consent Statement

Not applicable.

Data Availability Statement

Not applicable.

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Conflicts Of Interest

The author declares no conflict of interest.

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