

## CLINICAL INVESTIGATION

# AN ANALYSIS OF GINKGO BILOBA: DO YOU GET WHAT YOU PAY FOR?

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**Background:** The composition of commercially available preparations of *Ginkgo biloba* was determined.

**Methods:** 26 different brands of herbal medicines containing *Ginkgo biloba* as their principle ingredient were obtained from vendors who could be identified from the London, Ontario telephone directory and the Internet. These products included: 1) tablets and capsules (n=14); 2) ginkgo extracts and beverages (n=7); and 3) ginkgo teas (n=5). As the glycoside and terpene fractions of *Ginkgo biloba* are thought to be responsible for the central nervous system effects of *Ginkgo biloba*, the glycoside and terpene content of equivalent weights of all samples and *Ginkgo biloba* Egb 761 standard extract were determined.

**Results:** Capsules and tablets ranged from 4.1% to 26.2% glycoside content and 3.3 to 8.2% terpene content, as compared to 24% glycoside content and 6% terpene content for standardized *Ginkgo biloba* Egb 761 extract. The glycoside content of extracts and beverages ranged from 0.0 to 1.3 mg/ml, while the terpene content of extracts and beverages ranged from 0.0 to 0.7 mg/ml. The glycoside content of brewed teas ranged from 2.3 to 6.8 mg/ml; as terpenes are not water-soluble, they were not found in brewed ginkgo tea.

**Conclusion:** Commercially available *Ginkgo biloba* preparations were shown to have considerable variability in glycoside and terpene content. Variation between capsules and tablets from different manufacturers greatly exceeds the allowable variation for pharmaceutical manufacturers.

**Key words:** Ginkgo biloba, herbal medicine, analysis of products, high pressure liquid chromatography, dementia

## INTRODUCTION

*Ginkgo biloba* is a Chinese tree with an extremely long life. For this reason, Ginkgo may have come to be associated with longevity. Although part of Chinese pharmacopeia for more than 5,000 years, ginkgo was first introduced to the Western pharmaceutical industry in 1965 by Dr. Willmar Schwabe as a defined extract of *Ginkgo biloba* leaves for use in cerebral and peripheral circulatory disturbances. In its present form, Egb 761 ginkgo extract has been available in Europe since the 1970s.<sup>1</sup>

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The natural health products trade in Canada is a multi-billion dollar industry and is growing at a rate of 10-15% per year. More than 50% of Canadians consumed a natural health product in 1997, 20% of which were herbal products.<sup>2</sup> Figures from the United States showed an increase in *Ginkgo biloba* sales from \$160 million dollars (US) in 1995 to \$240 million dollars (US) in 1997.<sup>3</sup>

Under current Canadian law, herbal products are unregulated. Unlike products marketed with medicinal claims, herbal remedies do not require a drug identification number (DIN).<sup>4</sup> Compliance with set standards has been voluntary on the part of manufacturers. Standardized preparations of *Ginkgo biloba* Egb 761 contain 24% ginkgo-flavoneglycosides (hence referred to as glycosides) and 6% terpenelactones (a mix of ginkgolides and bilobalides, hence referred to as terpenes); the recommended daily dose is 120 mg.<sup>5</sup> Independent examination of manufacturers' claims of product content have produced results which both refute and support some manufacturers' data.<sup>3,6</sup> To date, there has been no independent examination of ginkgo-containing products in Canada. The aim of this study was to analyze by high pressure liquid chromatography (HPLC) the glycoside and terpene fractions of commonly available ginkgo-containing products.

## METHODS

**Samples:** A convenience sample of 26 different brands of herbal medicines containing *Ginkgo biloba* as their principle ingredient were obtained from vendors. These products included tablets, capsules, teas, extracts and beverages.

**Vendors:** Herbal vendors in London, Ontario, were identified from advertisements in the telephone directory using the search terms "herbalist", "health food" and "naturopath". Twenty-two vendors were identified, and purchases were made from a conve-

nience sample of eight vendors; three different brands of ginkgo-containing products were obtained from each vendor. A search of the Internet was made for sites advertising *Ginkgo biloba* preparations. Using the search terms “herbs”, “naturopath(y)”, “homeopath(y)”, “ginkgo”, “memory”, “herbalist”, “(w)holistic”, and “Alzheimer”, only one Canadian site was identified, from which samples were ordered; one further sample was purchased from a U.S.-based company advertising on the Internet.

The methodology for HPLC extraction and analysis of the glycoside and terpene fractions of the samples tested was adapted from the literature on natural products chemistry.<sup>7-11</sup> All analyses were blinded, were performed by highly experienced laboratory personnel, and were replicated on two occasions with <10% variation in results. The standard used for all HPLC analyses was an Egb 761 standardized extract 40 mg tablet.<sup>11</sup>

## RESULTS

Table 1 summarizes the content analysis of the 14 samples that were either tablets or capsules. The glycoside content is reported as a percentage of the extract, as per the Egb 761 reference standard. Although the glycoside content claimed in each product was 24% (as per the Egb 761 standard), actual glycoside content varied between 0.4% and 26.2%. Likewise, the terpene content, which com-

poses 6% of the Egb 761 standard, varied between 0.6% and 8.2% of the tested tablets and capsules. Based on the recommended daily dose of 120 mg, the retail cost per 120 mg for each product was calculated, independent of the actual glycoside or terpene content of each product. There was significant cost variability between products, from as little as one cent to as much as \$1.36 (Canadian) for a recommended daily dose of 120 mg.

Table 2 summarizes the measured content of the *Ginkgo biloba* extracts and beverage. Since in most cases, the quantity of standard *Ginkgo biloba* extract in each product was not stated, the amounts were quantified per millilitre of liquid. The glycoside content measured between 0.1% to 1.3% for the six different extracts, while the terpene content of extracts ranged from 0.1% to 0.7%. Extract #7, which is described as a ginkgo-containing beverage, did not contain detectable amounts of either glycosides or terpenes.

Table 3 summarizes the content analysis of the ginkgo-containing teas. There was no stated amount of ginkgo in ginkgo-containing teas. Accordingly, the fractional content of glycosides and terpenes was calculated based on 2 g of sample, which is the approximate content of a standard tea bag. The glycoside content of brewed teas ranged from 2.3 to 6.8 mg/ml, while the terpene content of teas ranged from 1.1 to 3.1 mg/2 g of dried tea leaves. Terpenes are not water-soluble, and were hence not found at all in brewed ginkgo teas.

**Table 1.** Analysis of Glycoside and Terpene content of capsules and tablets

Product Name*	% Glycosides	Terpenes	Retail Cost Per 120 mg (\$)
Ginkgo Biloba Egb761 standardized extract	24.0	6.0	-
Capsule/tablet # 1	12.5	3.3	0.96
Capsule/tablet # 2	23.9	4.5	0.76
Capsule/tablet # 3	23.6	7.0	0.63
Capsule/tablet # 4	18.8	3.9	0.75
Capsule/tablet # 5	4.1	3.6	0.26
Capsule/tablet # 6	21.5	6.2	0.37
Capsule/tablet # 7	23.0	7.8	1.36
Capsule/tablet # 8	0.4	0.6	0.01
Capsule/tablet # 9	18.8	5.2	0.67
Capsule/tablet # 10	22.4	4.6	0.50
Capsule/tablet # 11	26.2	4.8	0.59
Capsule/tablet # 12	24.1	8.2	1.14
Capsule/tablet # 13	12.7	3.9	0.28
Capsule/tablet # 14	21.5	6.5	0.83

\*Product names available upon request from the authors

## DISCUSSION

Since the registration of the first standardized extract of *Ginkgo biloba* in 1965, the majority of the literature on the therapeutic use of *Ginkgo biloba* in disorders of cerebral atherosclerosis and blood-flow has come from Europe. Many studies

**Table 2.** Analysis of Glycoside and Terpene Content of Alcohol-Based and Glycerine-Based Extracts

Product Name	Glycoside content (mg/ml)	Terpene content (mg/ml)
Extract #1	1.3	0.7
Extract #2	0.6	0.1
Extract #3	0.8	0.8
Extract #4	0.2	0.6
Extract #5	0.1	0.1
Extract #6	1.0	0.7
Extract #7	ND	ND

ND = none detected

**Table 3.** Analysis of Glycoside and Terpene Content of Teas

Product Name	Glycosides (mg/ml of brewed tea)	Terpenes (mg/2 g of dried tea leaves)
Tea #1	2.3	2.0
Tea #2	2.7	3.1
Tea #3	2.3	1.1
Tea #4	6.8	1.8
Tea #5	3.6	2.6

have been done demonstrating the effect of *Ginkgo biloba* on cortisol levels, neurotransmitters such as acetylcholine and serotonin, and blood-flow (5,12-16). A recent meta-analysis of *Ginkgo biloba* for cognitive function in Alzheimer's disease, however, found only four studies totaling 424 patients that met their inclusion criteria; the meta-analysis suggests that there is a small (3%, or approximately 1 point, difference in the Alzheimer Disease Assessment Scale – cognitive subtest) but clinically significant effect on cognition with 3- to 6-months treatment at 120 to 240 mg/day.<sup>17</sup>

Only one North American randomized controlled trial of *Ginkgo biloba* in dementia has been carried out.<sup>18,19</sup> This double-blinded study compared 120 mg of standardized Egb 761 extract to placebo, and followed subjects for 52 weeks. Response was assessed using three standardized cognitive assessment tools. Three-hundred and nine subjects over age 45 with mild to severe Alzheimer's or multi-infarct dementia were enrolled, of whom only 137 completed the study. The authors observed statistically significant improvements in two cognitive outcome measures at 52 weeks (a 1.4 point change in the Alzheimer's Disease Assessment Scale – cognitive subtest, and the Geriatric Evaluation by Relative's Rating Instrument). Given the high drop-out rate, however, the clinical significance of these improvements remains unclear.

Overall, there is not yet sufficiently rigorous data to clearly demonstrate clinical benefit from *Ginkgo biloba* standardized EGb 761 extract in persons with dementia. Issues around dosage, timing of intervention, drug tolerance, and the progressive nature of the disease remain to be addressed. Several adverse effects and drug interactions have been observed. These include gastrointestinal upset, headache, and case reports of spontaneous hemorrhages.<sup>19,20</sup> As the ginkgolides (part of the terpene content) are potent inhibitors of platelet activating factor, their use may pose a bleeding risk in patients with clotting disorders, or in patients on anticoagu-

lant therapy.<sup>21</sup>

In our study, high pressure liquid chromatography was used to analyse the chemical content of commonly available ginkgo-containing herbal products currently being marketed to determine product quality. The advantage of HPLC over other methods of chemical analysis is the ability to precisely quantify chemical substances. While detection is accurate, the process is hampered at two points. First, natural products are "dirty" substances, containing a surfeit of chemical substances of varying composition and unclear bioactivity. This can make extraction and purification of the desired chemical fraction difficult. Second, precision varies with the specificity of spectral absorbance. Glycosides have a specific absorbance at 370 nanometres, while the absorbance at 220 nanometres attributed to the terpene fraction can occur with all substances with double bonds. However, the terpene content of the ginkgo-containing products tested here rarely exceeded that found in standardized Egb 761 extract.

Clear differences in measured glycoside and terpene contents were observed between different products. For capsules and tablets, the glycoside content ranged from 1% to 100% of the content claimed by the manufacturer, while the terpene content ranged from 10% to 136% of the content claimed by the manufacturer. Other than true discrepancies from the content claimed by manufacturers, possible causes for these differences may be divided into composition and storage. Tablets and some capsules contain binders and stabilizers which prevent the breakdown of the more volatile ginkgo components, such as bilobalides. Extracts and ginkgo-containing beverages do not have these binders and stabilizers and are more susceptible to oxidation. Temperature is also a factor in the breakdown of natural products. All samples were originally found on shelves at room temperature, which could hasten chemical breakdown. Expiration and manufacture dates were not part of product labels. Because this study focused on obtaining many different products, the effect of a bad lot or expired product on the final analysis would also be magnified.

## CONCLUSION

This study examined manufacturers' claims of standardization of *Ginkgo biloba* products. Our obser-

vations suggest that very large and easily measurable differences exist between standardized ginkgo-containing products. Variation between capsules and tablets from different manufacturers greatly exceeds the allowable variation for pharmaceutical manufacturers.

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