

## TOTAL PHENOLIC CONTENT AND ANTIOXIDANT CAPACITY OF GINKGO TEAS

N. KOCZKA<sup>a\*</sup>, A. OMBÓDI<sup>a</sup>, Z. MÓCZÁR<sup>a</sup> and E. STEFANOVITS-BÁNYAI<sup>b</sup>

<sup>a</sup>Institute of Horticulture, Faculty of Agricultural and Environmental Science, Szent István University, H-2100 Gödöllő, Páter K. utca 1. Hungary

<sup>b</sup>Department of Applied Chemistry, Faculty of Food Science, Corvinus University of Budapest, H-1118 Budapest. Villányi út 29–43. Hungary

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Total phenolic content and antioxidant capacity (FRAP method) of *Ginkgo biloba* L. leaves and of different commercial ginkgo teas were determined and compared. Different water extracts (infusions and decoctions) were prepared varying the time of infusing, boiling, and steeping, and also aqueous ethanolic (water/ethanol 80/20 v/v) extract was made.

Total phenolic contents and FRAP values of collected ginkgo leaves were similar to those of commercial ginkgo mono teas, while these parameters were significantly higher for ginkgo teas containing ginseng or green tea. Decoction was more effective than infusion for extracting antioxidative compounds, in contradiction to suggested preparation methods by the producers. Aqueous ethanolic extracts had significantly higher total phenolic content and antioxidant capacity than water extracts. The correlation between phenolic content and FRAP values was very strong and positive for water extracts of collected leaves, while it was weak and negative for the tea products.

**Keywords:** *Ginkgo biloba* L., ginkgo extracts, phenolics, FRAP, boiling time, steeping time

*Ginkgo biloba* L. is an imposing dioecious tree, the only remaining species of the order *Ginkgoales*. Its high adaptability to diverse ecological conditions and therapeutical importance have attracted the attention of scientists of different disciplines. Leaves of *Ginkgo biloba* L. contain several active compounds, have platelet-activating factor antagonism, antioxidant and free radical scavenging properties (SASAKI et al., 2003). Ginkgo preparations are mainly used for the treatment of peripheral vascular disease or cerebral insufficiency (VAN BEEK & MONTORO, 2009). Ginkgo leaves are rich in phenolic acids and flavonoids which possess antioxidant activity (ELLNAIN-WOJTASZEK et al., 2003; SINGH et al., 2008). Recently, besides standardized ginkgo extracts, also ginkgo teas are being consumed in high amount. Phenolic content and antioxidant activity of ginkgo leaves and standardized ginkgo extracts were investigated recently (SASAKI et al., 2003; AOSHIMA et al., 2007; MASTEIKOVA et al., 2007; KOBUS et al., 2009; MILOŠEVIĆ et al., 2011). However, the antioxidant properties of commercial ginkgo teas and the effect of preparation method on those were not investigated in details.

The aim of our investigations was to determine and compare total phenolic content and antioxidant capacity of *Ginkgo biloba* L. leaves and commercial ginkgo tea products in water extracts prepared by applying different infusing, boiling, and steeping times and in aqueous ethanolic extract.

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\* To whom correspondence should be addressed.

Phone: +36-28-522071; e-mail: koczka.noemi@mkk.szie.hu

## 1. Materials and methods

### 1.1. Materials

Green ginkgo leaves were collected from male trees in the Botanical Garden of the Eötvös Loránd University of Budapest. Leaves were dried at 30 °C. GOH and co-workers (2003) documented that the particle size of ground ginkgo leaves has a determining effect on the antioxidant capacity of their extracts. In our investigation, collected ginkgo leaves were pulverized after drying to have to the same appearance as the commercial teas.

Five commercial teas containing ginkgo were analysed: Tea1, Tea2, Tea3 – ginkgo mono teas of different companies, Tea4 – ginkgo tea containing ginseng roots (50%), and Tea5 – ginkgo tea containing green tea (70%).

### 1.2. Methods

*1.2.1. Sample preparation.* Water extracts were prepared by addition of 100 ml water to 1 g of dried leaves or teas. Infusions and decoctions were made in different ways, which were the followings:

*Infusions:* material was infused with boiling water and steeped for 3, 5, 10 min, or 24 h; *decoctions:* material was boiled for 3, 5, 10, or 20 min.

Aqueous ethanolic extracts were made from 1 g of dried leaves or tea drugs infused with 100 ml aqueous ethanol (20 °C; water/ethanol 80/20 v/v) and stored at room temperature for 24 h.

All extraction methods were replicated three times. After steeping for the given time water and aqueous ethanolic extracts were filtered and centrifuged (13 000 r.p.m., 10 min). The supernatants were analysed.

*1.2.2. Total phenolic content.* Total phenolic content was measured using the method of SINGLETON and ROSSI (1965) and was expressed as milligram gallic acid equivalent (GAE) per gram dry matter (DM).

*1.2.3. Antioxidant capacity.* For determining the antioxidant capacity the FRAP assay was used (BENZIE & STRAIN, 1996). Standard curve was prepared using different concentrations of ascorbic acid. Data are given in millimoles ascorbic acid equivalent (AAE) per gram DM.

*1.2.4. Statistical analysis.* All analytical determinations were performed in two parallels. Means of three replications were presented with plus/minus standard deviation. Two-way analysis of variance was performed on the data with extraction method and sample as factors. Means for extraction methods and means for samples were separated by Fisher's protected least significant difference test. Correlation between phenolic content and antioxidant capacity was investigated by performing linear regression analysis on the data. All statistical analyses were realized using Microsoft Excel 2007 software (Microsoft Inc., Redmond, WA).

## 2. Results and discussion

### 2.1. Total phenolic content

Significant differences were found among the phenolic contents of the different extraction methods in average of all samples (Table 1). Total phenolic values for collected ginkgo leaves were similar to those found in the commercial mono teas (Tea1, -2, -3), however, there were

Table 1. Effect of different extraction methods on total phenolic content (mg g<sup>-1</sup> GAE) of collected ginkgo leaves and commercial ginkgo teas

Extraction methods	Ginkgo leaves <sup>a</sup>					Commercial teas <sup>a</sup>					Mean for extraction methods <sup>b</sup>	
	Tea1	Tea2	Tea3	Tea4	Tea5	Tea1	Tea2	Tea3	Tea4	Tea5		
Infusion												
3 min	21.11±0.61	21.25±0.90	22.36±0.55	25.01±0.92	28.83±0.32	20.93±0.39	21.25±0.90	22.36±0.55	25.01±0.92	28.83±0.32	23.25±0.72 E	
5 min	24.53±0.44	20.29±0.12	24.42±0.96	31.20±0.91	29.86±0.60	20.59±0.03	20.29±0.12	24.42±0.96	31.20±0.91	29.86±0.60	25.41±0.99 CD	
10 min	26.67±0.12	23.41±0.23	25.83±1.44	23.17±1.15	26.89±1.09	23.30±0.46	23.41±0.23	25.83±1.44	23.17±1.15	26.89±1.09	24.88±0.57 D	
24 h	33.42±0.40	20.51±0.40	21.22±0.38	31.07±0.73	32.33±0.44	18.94±0.64	20.51±0.40	21.22±0.38	31.07±0.73	32.33±0.44	26.25±1.50 B	
Decoction												
3 min	29.03±0.91	22.98±0.64	24.76±0.88	29.88±1.12	39.97±1.15	26.21±0.37	22.98±0.64	24.76±0.88	29.88±1.12	39.97±1.15	28.80±1.43 B	
5 min	24.72±0.16	23.69±0.92	24.58±0.32	25.01±0.60	45.15±0.77	25.52±0.69	23.69±0.92	24.58±0.32	25.01±0.60	45.15±0.77	28.11±1.87 B	
10 min	30.04±0.09	22.46±0.48	27.31±1.05	33.44±0.70	28.00±1.11	17.02±2.23	22.46±0.48	27.31±1.05	33.44±0.70	28.00±1.11	26.38±1.34 C	
20 min	34.22±0.80	20.37±1.05	20.76±0.73	29.62±0.47	24.78±1.57 D	15.84±0.57	20.37±1.05	20.76±0.73	29.62±0.47	27.88±0.42	24.78±1.57 D	
20% aqueous ethanolic												
24 h	75.74±0.96	85.51±1.63	98.28±0.29	113.23±1.07	147.14±1.20	96.30±0.92	85.51±1.63	98.28±0.29	113.23±1.07	147.14±1.20	102.70±5.58 A	
Mean for samples <sup>b</sup>	33.27±3.05 C	29.11±3.92 E	32.17±4.62 D	37.96±5.27 B	45.12±7.18A	29.41±4.70 E	29.11±3.92 E	32.17±4.62 D	37.96±5.27 B	45.12±7.18A		

<sup>a</sup>Ginkgo leaves collected in Budapest; Tea1, Tea2, Tea3: ginkgo mono teas of different companies; Tea4: ginkgo tea containing ginseng roots; Tea5: ginkgo tea containing green tea

<sup>b</sup>Means indicated with different letters in the column or in the row, significantly differ from each other at  $P \leq 0.05$  according to Fisher's least significant difference test

differences among the mono teas of different companies. Tea4 and Tea5 (containing ginseng and green tea, respectively) had significantly higher phenolic content in average of the nine extraction methods than the collected leaf sample and ginkgo mono teas. Significantly the highest phenolic concentration was found in Tea5. This was an expected result, because of the well-known high phenolic content of green tea (HARA, 2001).

For infusions, increasing steeping time generally resulted in significantly higher phenolic content. This trend was more explicit for collected leaves than for commercial tea products. For commercial mono teas quantity of extracted phenolic compounds increased up to 10 min steeping, but then decreased by steeping for 24 h.

Except for the 20 min steeping time, decoction extracted significantly more phenolic compounds than infusion. For decoctions, the boiling time did not have any effect on the phenolic content in average of the samples. However, there was a considerable difference between the trends for collected leaves and tea products. For collected leaf sample longer boiling times significantly raised the phenolic content of the extracts, while for tea products longer boiling times significantly reduced the phenolic content of the extracts. Based on these results, we suppose that phenolic compounds of recently collected samples were more heat stable, and needed more time to be extracted than phenolic compounds of commercial teas.

Total phenolic content of ginkgo leaf extracts exhibited significant differences depending on extraction solvents: aqueous ethanolic extraction resulted in three times higher phenolic contents than water extraction. Similar result was found by KOBUS and co-workers (2009), who studied phenolics in water, acetone-water, and ethanolic extracts of ginkgo leaves. Although extraction with aqueous ethanol was much more effective than water extraction, ginkgo teas are used as herbal tea, which corresponds to a water extract.

In our investigations, phenolic concentrations of water extracts ranged from 21.11 to 34.22 mg g<sup>-1</sup> GAE for leaf samples, and from 15.84 to 45.15 mg g<sup>-1</sup> GAE for commercial teas (Table 1). Phenolic content of aqueous ethanolic extracts was 75.74 mg g<sup>-1</sup> GAE for the collected ginkgo leaf sample, and it ranged from 85.51 to 147.14 mg g<sup>-1</sup> GAE for the commercial tea drugs. AOSHIMA and co-workers (2007) measured 0.53 mmol ml<sup>-1</sup> GAE in water extracts, while KOBUS and co-workers (2009) reported total phenolic content of water and ethanolic extracts of ginkgo leaves as 56.9±2 and 204.4±1 mg g<sup>-1</sup> quercetin equivalents, respectively. Total phenol content in ethanolic extracts of ginkgo leaves was reported being 34.0±0.29 mg ml<sup>-1</sup> and 66.86 mg g<sup>-1</sup> GAE by MASTEJKOVA and co-workers (2007) and MILOŠEVIĆ and co-workers (2011), respectively.

## 2.2. Antioxidant capacity

Measured antioxidant capacity values (using FRAP assay) of extracts of the investigated samples are presented in Table 2. There were significant differences among the FRAP values of the samples. In average of the nine extraction methods the leaf sample had higher antioxidant capacity than the commercial mono teas (Tea1, -2, -3). Similarly to findings for phenolic concentrations, Tea5 had significantly the highest mean antioxidant capacity, due to the green tea content. GOH (2004) detected for green tea leaves 7-to 9-fold higher FRAP values than for ginkgo leaves. JAIN and co-workers (2011) reported that the combination of ginkgo extracts shows a synergistic effect with green tea, producing higher antioxidant activity than individual extracts.

Table 2. Effect of different extraction methods on antioxidant capacity (mmol g<sup>-1</sup> AAE) of collected ginkgo leaves and commercial ginkgo teas

Extraction methods	Ginkgo leaves <sup>a</sup>					Commercial teas <sup>a</sup>					Mean for extraction methods <sup>b</sup>
	Tea1	Tea2	Tea3	Tea4	Tea5	Tea3	Tea4	Tea5	Tea5		
<b>Infusion</b>											
3 min	16.68±0.29	14.10±0.38	14.87±0.08	16.23±0.35	18.00±0.77	23.08±1.04	17.16±0.74 H				
5 min	18.27±0.24	14.27±0.05	15.39±0.47	17.61±0.44	23.81±0.98	25.39±1.07	19.12±1.02 G				
10 min	21.36±0.43	18.24±0.29	18.96±0.69	19.55±1.13	24.35±0.64	34.32±0.17	22.78±1.38 F				
24 h	26.42±0.83	22.18±0.85	23.84±0.73	24.93±0.80	24.46±2.26	32.60±0.62	25.74±0.80 CD				
<b>Decoction</b>											
3 min	22.20±0.55	23.58±1.13	24.08±0.88	19.47±1.04	17.64±0.39	37.44±0.52	24.15±1.56 E				
5 min	24.95±0.50	26.48±0.16	24.16±0.40	19.68±0.55	19.19±0.21	35.10±0.74	24.93±1.29 DE				
10 min	28.54±0.63	28.76±0.56	27.29±0.19	24.16±0.72	20.44±0.87	26.58±2.03	25.96±0.78 C				
20 min	31.91±0.45	30.14±0.38	31.34±0.77	30.78±1.56	25.21±1.38	26.77±0.65	29.36±0.65 B				
<b>20% aqueous ethanolic</b>											
24 h	69.12±0.52	62.76±1.54	64.28±0.83	69.99±0.87	87.69±2.24	130.29±1.01	80.69±5.74 A				
Mean for samples <sup>b</sup>	28.83±2.94 B	26.72±2.73 C	27.18±2.76 C	26.93±3.10 C	28.98±4.12 B	41.28±6.25 A					

<sup>a</sup>Ginkgo leaves collected in Budapest; Tea1, Tea2, Tea3: ginkgo mono teas of different companies; Tea4: ginkgo tea containing ginseng roots; Tea5: ginkgo tea containing green tea

<sup>b</sup>Means indicated with different letters in the column or in the row, significantly differ from each other at  $P \leq 0.05$  according to Fisher's least significant difference test

Statistically significant differences were found among the antioxidant capacity of the different water extracts. Both for infusions and decoctions the longer extraction time was the higher the FRAP value became. Similar result was obtained by GOH and co-workers (2003), who investigated infusions with 3, 5, 10, 15, and 20 min steeping. This trend was well detectable for all investigated samples, except for Tea5, for which longer boiling times (10 and 20 min) significantly reduced the antioxidant capacity of the extracts compared to shorter boiling times (3 and 5 min). From the view point of antioxidant capacity, we have found more effective extraction methods (infusing for 24 h or decoction) for preparation of the commercial teas than the ones recommended by the producers on the packaging (steeping for 10 min). Hence, disregarding of a very long steeping, it is expedient to make a decoction from ginkgo leaves and their tea products.

The antioxidant capacity of the aqueous ethanolic extracts was about three times higher than that of the water extracts. FRAP values of the water extracts ranged from 16.68 to 31.91 mmol g<sup>-1</sup> AAE for the collected leaf sample, and from 14.10 to 37.44 mmol g<sup>-1</sup> AAE for the commercial teas. GOH (2004) measured FRAP values in water extracts of ginkgo leaves between 63.29–68.25 mg g<sup>-1</sup> AAE. The antioxidant capacity of the aqueous ethanolic extracts was 69.12 mmol g<sup>-1</sup> AAE for the collected leaf sample, and its values ranged from 62.76 to 130.29 mmol g<sup>-1</sup> AAE for commercial tea drugs.

### *2.3. Correlation between total phenolic content and antioxidant capacity*

Positive correlation was shown between total phenolic content and antioxidant capacity for plants with a high phenolic content, including ginkgo (STEFANOVITS-BÁNYAI et al., 2006; HEGEDŰS et al., 2008; KOREKAR et al., 2011; VELJKOVIĆ et al., 2013). On the contrary, in a recent study, RONOWICZ and co-workers (2013) documented a significant negative correlation ( $R=-0.7668$ ,  $P\leq 0.01$ ) between antioxidant activity (using DPPH method) and total phenolic content of ginkgo preparations.

Based on all of our data, a strong positive correlation ( $R=0.9273$ ,  $N=162$ ,  $P<0.001$ ) was found between total phenolic content and antioxidant capacity of the investigated ginkgo extracts. However, the high correlation value evolved primarily because of the distinguished phenolic and FRAP values of the aqueous ethanolic extracts. Investigating this correlation by omitting the values of the aqueous ethanolic extracts, we have found a tendentious difference between the collected leaf sample and the mono tea products. The correlation was very strong and positive for the collected leaves ( $R=0.8433$ ,  $N=24$ ,  $P<0.001$ ), while it was much weaker and negative for the mono tea products ( $R=-0.2480$ ,  $N=72$ ,  $P<0.05$ ) (Figs 1 and 2).

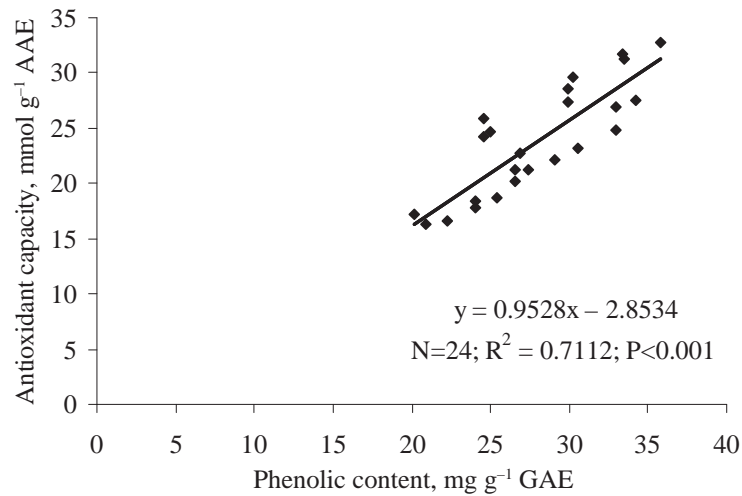


Fig. 1. Correlation between total phenolic content and antioxidant capacity in water extracts of ginkgo leaves

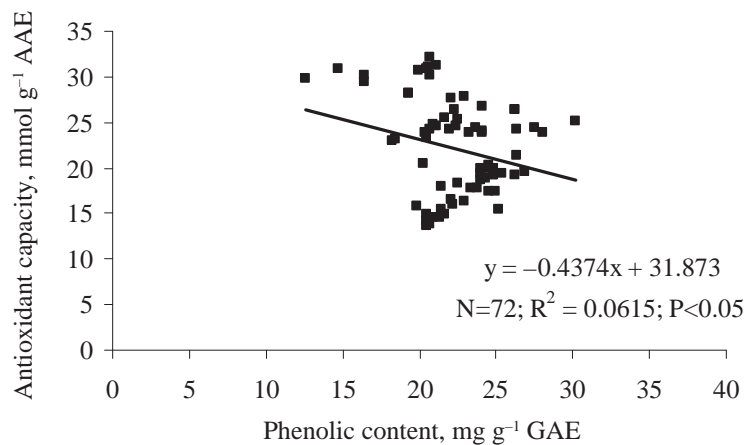


Fig. 2. Correlation between total phenolic content and antioxidant capacity in water extracts of ginkgo mono teas

### 3. Conclusions

Aqueous ethanolic extraction proved to be the most effective method for extracting phenolic materials and other antioxidative compounds either from collected leaf samples and commercial tea products of ginkgo. However, from the viewpoint of human consumption of the extract, the water extraction practice is much more common.

Decoction was more effective than infusion for extracting phenolic compounds. Applying longer boiling time was more effective for the collected leaves, while commercial tea drugs should not be boiled longer than five minutes, based on our results. For realizing higher antioxidant capacity, decoctions prepared with boiling for 20 min was the most

effective method for almost every investigated material. In contradiction to the preparation methods suggested by the producers on the packaging (infusing for 10 min), decoction prepared with at least 10 to 20 min boiling should be recommended. It is regrettable that, based on our data, there are marked differences among the quality of commercial ginkgo tea products.

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