

Determination of Trace Elements in Herbal Tea Products and Their Infusions Consumed in Thailand

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Nineteen elements, Mg, Al, Ca, V, Cr, Mn, Fe, Co, Ni, Cu, Zn, Se, Sr, Sb, Ba, As, Cd, Hg, and Pb, were determined in three types of popular herbal tea products, *Gynostemma pentaphyllum*, *Camellia sinensis*, and *Morus alba*. These herbal tea products, both imported and locally made products, are widely consumed in Thailand and worldwide. Microwave-assisted acid digestion was used for all of the samples, and the element contents were determined by ICP-MS. The concentrations of all elements varied among these herbal teas. Ca and Mg were the most abundant elements in all herbal samples (1384–34070 and 783–7739 mg/kg, respectively). Most elements in these herbal tea powders were also released into the infusions at different percentages depending on types of herbs. *G. pentaphyllum* infusion contained essential elements (Mg, Ca, V, and Fe) at higher levels than *C. sinensis* and *M. alba* infusions. Al and Ni were present at high levels in *C. sinensis* infusion, and Cd level was high in *M. alba* infusion. The daily intake of all elements from these herbal tea infusions (three cups/day) is still within the average daily intake. Therefore, it may not produce any health risks for human consumption, if other sources of toxic metal contaminated food are not taken at the same time.

KEYWORDS: Trace elements; *Gynostemma pentaphyllum*; *Camellia sinensis*; *Morus alba*; herbal tea; infusion

INTRODUCTION

At present, there are many herbal tea products widely consumed in Thailand and worldwide. Among these products, *Gynostemma pentaphyllum* (jiaogulan), *Camellia sinensis* (green tea), and *Morus alba* (mulberry) are the most popular herbal tea products consumed for medical purposes or maintaining good health. *G. pentaphyllum* is a medicinal plant normally grown in southern China, Japan, Korea, and India. It has been used in traditional Chinese and Japanese folk medicines for the treatment of many diseases. The pharmacological activities of this medicinal plant are reduced risk of cardiovascular disease (1), alleviation of asthma and respiratory disorders (2), lowering cholesterol (3), antigastric ulcer effect (4), and anticancer effect (5–7). In Thailand, *G. pentaphyllum* is grown in the northern region.

Green tea, *C. sinensis*, has been used as a beverage in China and Japan for thousands of years. It is also widely consumed in North America and Europe in addition to the equally popular black tea. Tea leaves contain polyphenols such as epigallocatechin 3-gallate (EGCG) that exhibit antioxidant (8), lowering cholesterol (9), hepatoprotective (10), and anticancer activities

(11, 12). The results of an epidemiological study in Saitama Prefecture, Japan, determined that the cancer preventive amount of green tea is 10 Japanese-size cups of green tea (120 mL per cup) daily or ≈ 2.5 g of green tea extract (13).

Another popular herbal tea, *M. alba* (mulberry), has been shown to exhibit antioxidant and hepatoprotective effects (14), anti-inflammatory effect (15), hypoglycemia effect (16), and neuroprotective effect (17).

As these herbal tea products are normally produced in dried-leaf form in tea bags or as an instant tea, they can be easily contaminated with heavy metals and microorganisms from the environment (soil, water, or air) during growth and the manufacturing processes when the ready-made products are produced (18). Additional sources of heavy metal contamination are rainfall, atmospheric dust, plant protective agents, and fertilizers (19–21). Different plants also have different capacities to selectively accumulate some elements, that is, hyperaccumulators.

The human body requires both metallic and nonmetallic elements within certain permissible limits for growth and good health. Therefore, determination of element compositions in foods and related products is essential for understanding their nutritive importance. Several attempts have been made to determine the element contents of herbs, medicinal or aromatic plants, and tea leaves from many parts of the world by using

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various methods such as flame atomic absorption spectrometry (FAAS) (22–26), electrothermal atomic absorption spectrometry (ETAAS) (27, 28), inductively coupled plasma optical emission spectrometry (ICP-OES) (25, 29–31), and inductively coupled plasma mass spectrometry (ICP-MS) (28). ETAAS and ICP-MS are the most effective techniques for ultra-trace element analysis. ETAAS is a single-element technique and very time-consuming if many elements are to be determined in each sample, whereas ICP-MS is a multielement technique with wide linear ranges (32).

The Ministry of Public Health (Thailand) sets the maximum permissible levels of elements in medicinal plant materials and finished herbal products for only As, Pb, and Cd, at 4.0, 10.0, and 0.3 mg/kg, respectively. The contents of trace elements are one of the criteria that make plant raw materials admissible for the production of traditional medicines or tea products. This may be due to the fact that the amount of elements taken into the body increases with repeated doses. The determination of the element contents in medicinal plants should be part of the quality control process to ensure the purity, safety, and efficacy of these herbal products.

G. pentaphyllum (jiaogulan tea), *C. sinensis* (green tea), and *M. alba* (mulberry tea) are widely consumed, especially in Asian countries. However, most studies of these plants were carried out only on the chemistry, pharmacology, and toxicology of the active organic compounds (phytochemicals) and plant extracts. The information on other nutrients or toxic elements is also reported for green tea, wulong tea, and black tea. Essential elements such as K, Ca, Mg, Fe, Cu, and Zn were determined in 46 tea samples including instant tea by using ICP-AES (33). Total Al, Mn, Cr, Cu, Fe, and Ni was determined in tea leaves and their infusions of green tea, wulong tea, black tea, *Hibiscus sabdariffa* (roselle), and *Ilex paraguariensis* (mate) (34, 35). Data on the concentrations and variation of elements in *G. pentaphyllum* and *M. alba* tea products are still limited. Therefore, the objective of the present study is to determine the levels of several trace elements (Mg, Al, Ca, V, Cr, Mn, Fe, Co, Ni, Cu, Zn, Se, Sr, Sb, Ba, As, Cd, Hg, and Pb) in these three herbal tea products and their infusions by a microwave-assisted acid digestion procedure and ICP-MS technique. The data obtained will provide information on whether these herbal teas contain some metal ions in amounts that could be toxic at the normal doses usually consumed as beverages.

MATERIALS AND METHODS

Chemicals. A standard solution of each element was prepared immediately by dilutions of a 1000 mg/L stock solution (Merck, Darmstadt, Germany) prior to use. Milli-Q deionized water (Millipore, Milford, MA) was used throughout this experiment. All solvents and reagents such as HNO₃, HCl, and H₂O₂ were of analytical reagent grade (Merck). All glassware and equipment were soaked with 10% HNO₃ at least overnight and then rinsed with deionized water prior to use. DC73348 bush branches and leaves (approved by China National Analysis Center for Iron and Steel, Beijing, China) and 1573a tobacco leaves (U.S. Department of Commerce National Institute Standards and Technology, Gaithersburg, MD) were used as reference materials.

Apparatus. A Waring Commercial Laboratory Blender (Hartford, CT) was used for grinding the samples. The digestion was carried out in a HP-500 MARS 5 (CEM Corporation, Mathews, NC) poly(tetrafluoroethylene) (PTFE) advanced composite vessel with 100 mL capacity. The measurements for all elements were performed with an inductively coupled plasma mass spectrometer (Agilent Technologies 7500c, Palo Alto, CA).

Plant Materials. Three types of herbal tea products, *G. pentaphyllum*, *C. sinensis*, and *M. alba*, were purchased from different supermarkets in Bangkok, Thailand. They included both imported and locally

made products. The samples, except powdered samples, were powdered with a stainless steel blender. The pulverized and powdered tea samples were transferred into plastic bags and kept at 4 °C until analysis.

Sample Preparation. The digestion was operated using a microwave system. Dried powders of plant samples, 0.25 g, were weighed into PTFE vessels. Two milliliters of H₂O₂ and 6.0 mL of concentrated HNO₃ were added into the vessels. The vessels were closed and placed on the rotating turntable of the microwave oven, and then the digestion process was started. The digestion was allowed to 11.72 bar and 190 °C over 30 min and then maintained at 190 °C for 40 min. After microwave digestion, the digested solutions were filtered through filter paper (Whatman no. 42) and diluted to 50 mL with deionized water.

The tea infusions were prepared following the usual method for tea preparation using deionized water. One bag or 2.0 g of each tea sample was transferred into a glass beaker, 100 mL of boiling deionized water was added, and the sample was left at room temperature for 5 min. The supernatant was then separated by filtering through filter paper (Whatman no. 42). A 5.0 mL volume of this solution was transferred into a PTFE vessel, and 3.0 mL of concentrated HNO₃ was added. The solutions were digested by using the microwave method as previously described. Finally, the digested solution was diluted to 25.0 mL with deionized water. The corrected weight of tea sample in each bag was obtained by subtracting the weight of the tea bag.

Determination of Elements by ICP-MS. Contents of all elements were determined in the clear solutions using ICP-MS. The quantitative determinations of elements in samples were done using calibration curves obtained from diluted stock standard elements 1000 mg/L. Rhodium and germanium were used as internal standards. The excitation power of the plasma was 1500 W; the gas flow rates for plasma gas, carrier gas, and makeup gas were 15.0, 0.9, and 0.3 L/min, respectively. Hydrogen (H₂) and helium (He) gases were used as reaction gases at the flow rates of 4.5 and 5.0 mL/min, respectively.

RESULTS AND DISCUSSION

The accuracy and precision of the method were tested with two standard reference materials (SRMs), SRM1573a (tobacco leaves) and DC73348 (bush branches and leaves). The results indicated that the concentrations of elements determined by the present ICP-MS method are in agreement (within ±10%) with the certified values, except in the cases of As and Hg for SRM1573a and Al and Sb for DC73348, for which the determined values were different from the certified values (Table 1).

In the present work, the concentrations of essential elements (Mg, Ca, V, Cr, Mn, Fe, Co, Ni, Cu, Zn, and Se) and nonessential elements (Al, Sr, Sb, As, Cd, Ba, Hg, and Pb) were determined in three herbal tea products (*G. pentaphyllum*, *C. sinensis*, and *M. alba*). Our results showed that these herbal tea products contained high concentrations of Ca and Mg (Table 2). The concentrations of Ca and Mg were in the range of 1384–34070 and 783–7739 mg/kg, respectively, with the lowest average levels for both elements in *C. sinensis* samples (4473 and 2017 mg/kg, respectively). The concentrations of Ca and Mg were comparable with those previously reported for *C. sinensis* (4252.4 and 1978.2 mg/kg, respectively) (33). The abundance of Ca and Mg in the present study was also in agreement with the previous studies, which indicated that these two elements were the most abundant elements in many medicinal plants and tea leaves (23, 25, 28, 29, 31, 36). The concentrations of Fe varied over wide ranges of 125–2321, 20–318, and 89–408 mg/kg in *G. pentaphyllum*, *C. sinensis*, and *M. alba* samples, respectively. The concentration of Zn varied from 10 to 61 mg/kg, with average values at 46.26, 32.17, and 28.46 mg/kg in *G. pentaphyllum*, *C. sinensis*, and *M. alba* samples, respectively. The concentrations of Fe and Zn were comparable with the levels in tea and herbal samples previously reported by many authors (23, 28, 29, 37). The concentration

Table 1. Assessment of the Accuracy and Precision of the Method by Using Two Standard Reference Materials (SRM1573a, Tobacco Leaves; and DC73348, Bush Branches and Leaves)

element	SRM1573a (mg/kg), <i>n</i> = 15		DC73348 (mg/kg), <i>n</i> = 10	
	certified value	determined value	certified value	determined value
Mg	12000 ^a	10936 ± 508	2870 ± 180	2840 ± 125
Al	598 ± 12	554 ± 34	2140 ± 220	1589 ± 286
Ca	50500 ± 900	50979 ± 3393	22200 ± 1300	20711 ± 1366
V	0.835 ± 0.010	0.769 ± 0.047	2.4 ± 0.3	2.18 ± 0.20
Cr	1.99 ± 0.06	1.78 ± 0.09	2.3 ± 0.3	2.02 ± 0.146
Mn	246 ± 8	238 ± 10.6	58 ± 6	60 ± 1.6
Fe	368 ± 7	344 ± 12.3	1020 ± 67	969 ± 30
Co	0.57 ± 0.02	0.586 ± 0.024	0.39 ± 0.05	0.410 ± 0.020
Ni	1.59 ± 0.07	1.42 ± 0.04	1.7 ± 0.4	1.66 ± 0.03
Cu	4.70 ± 0.14	4.59 ± 1.56	5.2 ± 0.5	4.97 ± 0.22
Zn	30.9 ± 0.7	29.0 ± 5.4	20.6 ± 2.2	21.8 ± 1.05
As	0.112 ± 0.004	0.165 ± 0.022	0.95 ± 0.12	0.94 ± 0.05
Se	0.054 ± 0.003	0.059 ± 0.008	0.184 ± 0.013	0.169 ± 0.027
Sr	<i>b</i>	80.80 ± 6.09	345 ± 11	354 ± 32
Cd	1.52 ± 0.04	1.378 ± 0.045	0.14 ± 0.06	0.18 ± 0.02
Sb	0.063 ± 0.006	0.052 ± 0.006	0.078 ± 0.020	0.056 ± 0.009
Ba	<i>b</i>	59.87 ± 4.43	19 ± 3	17.6 ± 1.4
Hg	0.034 ± 0.004	0.051 ± 0.030	<i>b</i>	43.9 ± 5.9
Pb	<i>b</i>	0.560 ± 0.034	7.1 ± 1.1	7.36 ± 6.06

^a Information value. ^b Not certified.

of Mn ranged from 43 to 1512 mg/kg, with the highest level in *C. sinensis* samples (813.6 mg/kg). The present results showed that the concentration of Mn in *C. sinensis* samples was within the same range as the previous study (824.8 mg/kg) (33) but higher than Mn in other herbal products or medicinal plants (range = 9–188 mg/kg) (23, 25, 28). The concentration of Cu was in the range of 3–22 mg/kg, with the highest level in *C. sinensis* samples (15.20 mg/kg). The results were in the same range as those of other plants from the previous studies (23, 25, 28, 29, 37).

For other microessential elements (V, Cr, Co, Ni, and Se), the concentrations of these elements also varied over a wide range among the three herbal tea products. *G. pentaphyllum* contained V, Cr, and Co at the highest concentrations, whereas *C. sinensis* had the highest level of Ni and *M. alba* the highest level of Se. The present results indicated that these herbal tea products contained large amounts of essential nutrients, and they are especially rich in Ca, Mg, Mn, Fe, and Zn.

Al, Ba, Sr, and Sb, known as nonessential elements, varied between 33 and 6130, between 3.0 and 180, between 1.5 and 212, and between 0 and 0.102 mg/kg, respectively. The highest concentration of Al was found in *G. pentaphyllum* (2014 mg/kg) followed by *C. sinensis* (1179 mg/kg) and *M. alba* (180.3 mg/kg), respectively. Matsushima et al. reported Al contents in green tea and black tea were only 520 and 576 mg/kg, respectively (34). Another study reported Al levels of 919 mg/kg in green tea and 759 mg/kg in black tea (35). Sr and Ba were found at the highest concentrations in *G. pentaphyllum* (96.79 and 81.88 mg/kg) followed by *M. alba* (72.18 and 74.32 mg/kg) and *C. sinensis* (11.91 and 22.74 mg/kg).

It was previously proposed that most investigated foodstuffs (vegetables, meat, and dairy products) contained <5 mg/kg of Al (fresh weight), and high Al concentrations were found in cocoa and cocoa products (33 mg/kg), spices (145 mg/kg), and black tea leaves (899 mg/kg) (38). Levels of Al in 72 samples of 17 different spices and aromatic herbs were determined, and it was found that the concentration of Al ranged from 3.74 to 56.50 mg/kg (27). The average concentration of Al from herbal

teas in the present study was much higher than that reported in previous papers, except for *M. alba* products.

For toxic elements (As, Cd, Hg, and Pb), the concentration of Cd was detectable at high level (>0.3 mg/kg) in 14 of 16 *G. pentaphyllum* products. Three of 16 *G. pentaphyllum* products also contained Pb at >20 mg/kg (the standard limited value is 10 mg/kg). One of 18 *C. sinensis* products contained Pb at 53.89 mg/kg, whereas the others (17 products) contained Pb at <10 mg/kg. It was observed that most *G. pentaphyllum*, both locally and imported, samples were contaminated with Cd at higher levels than the standard maximum value for medicinal plants. *G. pentaphyllum* may accumulate Cd from the growing environment such as soil and water as well as the use of some fertilizers. In addition, cleaning and processing of herbal tea leaves may be other sources for metal contamination in herbal tea products. Some biomaterials such as green tea, aloe, coffee, and Japanese coarse tea were able to adsorb large amounts of Pb and Cd ions after a pretreatment of washing with water followed by drying (39). It was proposed that the processing of tea leaves such as twisting and water-removal stages caused an increase in Pb content in the tea product (40). *C. sinensis* and *M. alba* had Cd concentration of <0.3 mg/kg for all samples. These results suggest that the *G. pentaphyllum* plant can readily absorb Cd from the soil at much higher levels than *C. sinensis* and *M. alba*. Compared to other studies, the average amounts for Pb and Cd in various plants were generally lower than the present results obtained in *G. pentaphyllum* (23, 24, 29, 31, 37, 41). All samples contained As and Hg at levels below those set as the standard maximum values (As and Hg, 4 and 2 mg/kg, respectively). It should be observed that the highest levels of the potentially most toxic elements were detected in *G. pentaphyllum* samples. Therefore, determination of these elements in the raw material before it is used to make the herbal tea product is essential.

The element concentrations in herbal tea infusions were determined to assess the actual amount of exposure to these elements by drinking these beverages. The concentration ranges of 19 elements determined in three types of herbal tea infusions were calculated on the basis of 2 g of herbal tea powders in 100 mL (Table 3). The results also demonstrated the average percentage of each element released into the infusion. The results indicated that there was a large variation of the element concentrations in different herbal tea infusions. The highest average levels of Mg, Ca, V, Cr, Fe, Sr, Cd, Ba, and Pb in infusion were found in *G. pentaphyllum* infusion, whereas the highest average levels of Al, Co, Mn, and Ni were detected in *C. sinensis* infusion. The percent releases of Mg, Ca, Mn, Se, Sr, and Ba were detected at the highest levels in the *G. pentaphyllum* infusion. The highest percent releases of Al, Cr, Co, and Ni were found in the *C. sinensis* infusion. An average percent release of Mg in *G. pentaphyllum* infusion was 75.57%, whereas only 34.26 and 31.85% were found in *C. sinensis* and *M. alba* infusions, respectively.

Mn was released at the highest concentration in the *C. sinensis* infusion (409.7 µg/100 mL), but the highest percent release was found in the *G. pentaphyllum* infusion (48.63%). These results agree with a previous study in which the concentration of Mn in tea infusion was 1.75–6.67 µg/mL in green tea, 0.94–4.04 µg/mL in wulong tea, and 0.78–3.24 µg/mL in black tea (34).

Although a very high Ca concentration was present in all herbal teas (Table 2), the percent of Ca released was very low, especially for *C. sinensis* and *M. alba* infusions, but not for the *G. pentaphyllum* infusion (Table 3). Ca concentration in all herbal tea infusions was in the range of 136.0–40011 µg/100

Table 2. Concentrations of Elements in Three Herbal Tea Products

element	element concentration (mg/kg of DW)					
	<i>G. pentaphyllum</i> ^a (n = 16)		<i>C. sinensis</i> ^b (n = 18)		<i>M. alba</i> ^c (n = 15)	
	range	av	range	av	range	av
Mg	1756–7739	5178	783.6–2549	2017	3078–5188	4243
Al	204.0–6130	2014	564.4–2740	1179	33.00–463.6	180.3
Ca	5583–34070	20460	1384–6550	4473	15286–25182	21048
V	0.207–7.721	2.029	0.081–0.536	0.226	0.060–0.732	0.273
Cr	0.434–12.42	2.892	0.205–10.54	1.476	0.250–1.419	0.790
Mn	43.42–259.4	136.6	229.4–1512	813.6	75.27–352.7	155.6
Fe	125.5–2321	791.4	20.91–318.3	167.1	89.46–408.2	200.0
Co	0.079–1.061	0.392	0.119–0.765	0.294	0.041–0.239	0.095
Ni	0.530–6.600	2.349	2.281–9.194	5.633	0.368–2.171	0.960
Cu	5.141–15.48	9.146	3.075–22.42	15.20	5.724–11.09	8.074
Zn	25.43–61.95	46.26	10.13–55.40	32.17	19.16–34.42	28.46
As	0.070–0.750	0.349	0.010–0.238	0.088	0.041–0.449	0.170
Se	0.00–0.069	0.032	0.014–0.508	0.096	0.037–0.276	0.114
Sr	8.128–212.1	96.79	1.534–24.23	11.91	21.70–138.1	72.18
Cd	0.021–4.772	1.270	0.002–0.100	0.035	0.001–0.022	0.008
Sb	0.008–0.102	0.028	0.002–0.076	0.022	0.00–0.011	0.001
Ba	4.322–168.2	81.88	3.042–50.72	22.74	5.439–180.6	74.32
Hg	0.00–0.041	0.005	nd ^d	nd	0.00–0.032	0.002
Pb	0.361–64.40	9.312	0.060–53.89	3.930	0.118–1.185	0.401

^a Thirteen samples produced in Thailand and 3 samples imported from China. ^b Four samples produced in Thailand and 14 samples imported from China, Japan, Sri Lanka, India, and Indonesia. ^c All 15 samples produced in Thailand. ^d Not detectable.

Table 3. Mean Concentrations of Elements in Herbal Tea Infusions and the Percent of Elements Released into Infusion

element	<i>G. pentaphyllum</i> (n = 8)		<i>C. sinensis</i> (n = 17)		<i>M. alba</i> (n = 14)	
	element contents in infusion ^a (μg/100 mL)	% release ^b	element contents in infusion ^a (μg/100 mL)	% release ^b	element contents in infusion ^a (μg/100 mL)	% release ^b
Mg	2153–9610 (7015)	75.57	764.3–2152 (1414)	34.26	887.6–5218 (2961)	31.85
Al	2.176–95.67 (45.63)	1.46	187.8–1652 (551.5)	21.79	0.00–19.16 (7.085)	3.58
Ca	6272–40011 (26805)	64.22	136.0–751.5 (335.8)	3.66	1576–8506 (4477)	10.23
V	0.00–0.1308 (0.0527)	1.64	0.00–0.0189 (0.0055)	1.35	0.00–0.0392 (0.0123)	2.42
Cr	0.00–1.405 (0.3038)	4.57	0.00–0.6911 (0.2144)	11.45	0.00–0.3968 (0.0596)	2.57
Mn	48.26–276.6 (158.9)	48.63	137.5–741.7 (409.7)	25.52	9.572–94.96 (35.52)	9.42
Fe	5.481–59.55 (28.94)	2.27	4.444–21.62 (7.996)	2.39	0.00–37.61 (13.77)	2.83
Co	0.0444–0.4660 (0.1401)	23.11	0.1092–1.068 (0.3040)	49.27	0.00–0.2138 (0.0531)	20.74
Ni	0.3160–3.370 (1.043)	36.04	3.806–15.54 (8.044)	67.71	0.2932–2.705 (1.108)	49.64
Cu	1.622–7.993 (4.414)	22.96	1.201–8.427 (4.149)	12.96	1.478–7.385 (4.764)	26.74
Zn	7.517–50.19 (25.85)	24.92	5.947–43.32 (21.94)	32.15	2.747–37.60 (20.93)	33.79
As	0.0038–0.2128 (0.0844)	13.76	0.002–0.1533 (0.0441)	23.83	0.0021–0.1863 (0.0949)	30.71
Se	0.0058–0.0352 (0.0183)	24.17	0.00–0.0678 (0.0111)	7.17	0.00–0.0416 (0.0159)	5.85
Sr	7.837–234.1 (124.4)	53.83	0.2264–3.036 (1.523)	6.96	3.793–23.25 (14.68)	9.79
Cd	0.1025–2.307 (0.7120)	18.64	0.004–0.0237 (0.0098)	14.18	0.00–0.0284 (0.0061)	24.03
Sb	0.0009–0.0798 (0.0188)	24.21	0.0005–0.0288 (0.0076)	11.78	0.00–0.1679 (0.0176)	27.93
Ba	0.4001–142.2 (62.95)	33.43	0.00–4.151 (1.396)z	3.93	0.00–13.91 (6.925)	4.44
Hg	nd ^c		nd		nd	
Pb	0.1126–8.017 (2.377)	6.22	0.0004–3.162 (0.4035)	7.11	0.0075–0.1510 (0.0767)	11.61

^a Infusion is prepared from 2.0 g or one bag of tea in 100 mL. The values in parentheses are the mean values (μg/100 mL). ^b Calculated as percentage of the total amount present in tea powder. ^c Not detectable.

mL. For *C. sinensis* and *M. alba* infusions, only 1.79–18.81% of Ca was released, whereas 55.02–74.64% of Ca was leached from the *G. pentaphyllum* infusion. The results from *C. sinensis* and *M. alba* agree with the results of a previous study reporting that only 20% of Ca in Polish herbal products was present in water-soluble form (31). The present results indicated that *G. pentaphyllum* and *C. sinensis* can be other effective sources of Mg, Ca, and Mn for humans.

There were no differences in the percent of infusions of Fe and Zn among these herbal tea infusions. V and Fe had the lowest percent releases in infusions for all herbs. Interestingly, the toxic elements, As, Cd, and Pb, were leached from *M. alba* samples at highest rates, 30.71, 24.03, and 11.61%, respectively. Although the concentrations of As, Cd, and Pb seem to be lower in *C. sinensis* and *M. alba* products than in the *G. pentaphyllum*

product, these toxic elements can be easily released at the higher rate in the infusions. The element concentrations in the infusion are more significant when daily uptake is considered. For example, the average concentrations of As in *G. pentaphyllum* and *M. alba* products were 0.349 and 0.170 mg/kg, respectively, but 0.0844 μg/100 mL (13.76% released) and 0.0949 μg/100 mL (30.71% released) of As were detected in *G. pentaphyllum* and *M. alba* infusions, respectively.

Al concentration in herbs was also high in both *G. pentaphyllum* and *C. sinensis* products, but 21.79% of Al was released from the *C. sinensis* infusion, whereas only 1.46% was released from the *G. pentaphyllum* infusion. The element concentrations in different herbal infusions may be affected by different physiological properties or structures of plants, levels of phytochelating phenolics, other mineral-binding components,

Table 4. Comparison of the Average Daily Dietary Intakes of Each Element

element	estimated dietary intake (mg/day)	calculated intake ^a (mg/day)		
		<i>G. pentaphyllum</i>	<i>C. sinensis</i>	<i>M. alba</i>
Mg	320–420 ^b	6.456–28.83	2.293–6.456	2.663–15.65
Ca	1000–1200 ^b	18.82–120.0	0.4079–2.255	4.727–25.52
Mn	1.6–2.3 ^b	0.1448–0.8298	0.4126–2.2254	0.0287–0.2849
Fe	8–18 ^b	0.0164–0.1786	0.0133–0.0649	0.000–0.1129
Cu	0.700–0.900 ^b	0.0001–0.0014	0.0036–0.0253	0.0045–0.0222
Zn	8–11 ^b	0.0226–0.1505	0.0178–0.1300	0.0082–0.1128
As	0.1050–0.4060 ^c	0.000–0.0006	0.000–0.0005	0.000–0.0006
Cd	0.0084–0.0147 ^c	0.0003–0.0069	0.000–0.0001	0.000–0.0001
Hg	0.0021–0.0091 ^c	0.000–0.000	0.000–0.000	0.000–0.000
Pb	0.0070–0.0126 ^c	0.0003–0.0240	0.000–0.0095	0.000–0.0005

^a Three cups/day (one cup of infusion is prepared from 2 g or one bag of tea in 100 mL of hot deionized water). ^b Recommended dietary allowance (RDAs)/adequate daily dietary intake (www.nap.edu). ^c Estimated dietary intakes based on 70 kg body weight (Committee on Toxicity, 2003).

concentrations of elements in tea powders, the pH of the water used in tea preparation, and, most importantly, the solubility of these elements in hot water.

Because there are no standard limits of trace elements in nonalcoholic beverages, the daily metal intake from the consumption of different herbal teas has been determined to evaluate their potential hazard to health. The total amount of each element in herbal tea infusions was calculated for daily intake. The calculated amounts are based on the concentrations of elements in the infusion and the assumption that the average consumption of herbal teas for a single person is three cups a day (100 mL per cup) with one packet of 2 g each, that is, 6 g, of herbal tea per day. The data in **Table 4** show the ranges of calculated results compared to the average daily dietary intakes (ADDIs) of each element. The *G. pentaphyllum* infusion (5 of 8 samples) contained a higher level of Cd than the limited value recommended for medicinal plants (0.3 mg/kg), whereas *C. sinensis* and *M. alba* infusions contained low amounts of Cd (<0.012 mg/kg).

The concentrations of all elements for daily intake are below the safety levels for human consumption, except for Pb in some *G. pentaphyllum* products. The daily intake of these *G. pentaphyllum* teas may increase Pb intake to over the standard limited value (>0.0126 mg/day). Therefore, long-term consumption of *G. pentaphyllum* product should also be of concern for the accumulation of some toxic elements such as Cd and Pb in target organs, especially the kidney and liver. It is necessary to study in further detail the toxicity of these medicinal plants contaminated with high levels of some toxic metal ions.

In conclusion, three herbal tea products (*C. sinensis*, *G. pentaphyllum*, and *M. alba*), which are widely consumed in Thailand, contained both essential and toxic elements in a wide range. Some of them (*G. pentaphyllum* and *C. sinensis*) can be used as beneficial sources for Ca, Mg, and Mn. At the same time, they may also contain high levels of some toxic elements such as Cd and Pb over the standard limited values for medicinal plants. Although the daily intakes of these elements from herbal tea infusions in the present study are below the standard limits and may not constitute a health risk from toxic elements originating from these herbal teas, it is absolutely essential to have good quality control of plant raw materials and to determine the presence of some contaminants, especially toxic elements, to avoid overconsumption and their cumulative toxicities in long-term use.

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LITERATURE CITED

- Circosta, C.; De Pasquale, R.; Occhiuto, F. Cardiovascular effects of the aqueous extract of *Gynostemma pentaphyllum* Makino. *Phytomedicine* **2005**, *12*, 638–643.
- Circosta, C.; De Pasquale, R.; Palumbo, D. R.; Occhiuto, F. Bronchodilatory effects of the aqueous extract of *Gynostemma pentaphyllum* and gypenosides III and VIII in anaesthetized guinea-pigs. *J. Pharm. Pharmacol.* **2005**, *57*, 1053–1058.
- Megalli, S.; Aktan, F.; Davies, N. M.; Roufogalis, B. D. Phytopreventative anti-hyperlipidemic effects of *Gynostemma pentaphyllum* in rats. *J. Pharm. Pharm. Sci.* **2005**, *8*, 507–515.
- Rujjanawate, C.; Kanjanapothi, D.; Amornlerdpison, D. The anti-gastric ulcer effect of *Gynostemma pentaphyllum* Makino. *Phytomedicine* **2004**, *11*, 431–435.
- Zhou, Z.; Wang, Y.; Zhou, Y.; Zhang, S. Effect of *Gynostemma pentaphyllum* Mak. on carcinomatous conversions of golden hamster cheek pouches induced by dimethylbenzanthracene: a histological study. *Chin. Med. J. (Engl.)* **1998**, *111*, 847–850.
- Chen, J. C.; Chung, J. G.; Chen, L. D. Gypenoside induces apoptosis in human Hep3B and HA22T tumour cells. *Cytobios* **1999**, *100*, 37–48.
- Wang, Q. F.; Chen, J. C.; Hsieh, S. J.; Chen, C. C.; Hsu, S. L. Regulation of Bcl-2 family molecules and activation of caspase cascade involved in gypenosides-induced apoptosis in human hepatoma cells. *Cancer Lett.* **2002**, *183*, 169–178.
- Chandra, S.; De Mejia Gonzalez, E. Polyphenolic compounds, antioxidant capacity, and quinone reductase activity of an aqueous extract of *Ardisia compressa* in comparison to mate (*Ilex paraguariensis*) and green (*Camellia sinensis*) teas. *J. Agric. Food Chem.* **2004**, *52*, 3583–3589.
- Maron, D. J.; Lu, G. P.; Cai, N. S.; Wu, Z. G.; Li, Y. H.; Chen, H.; Zhu, J. Q.; Jin, X. J.; Wouters, B. C.; Zhao, J. Cholesterol-lowering effect of a theaflavin-enriched green tea extract: a randomized controlled trial. *Arch. Intern. Med.* **2003**, *163*, 1448–1453.
- Hasegawa, R.; Chujo, T.; Sai-Kato, K.; Umemura, T.; Tanimura, A.; Kurokawa, Y. Preventive effects of green tea against liver oxidative DNA damage and hepatotoxicity in rats treated with 2-nitropropane. *Food Chem. Toxicol.* **1995**, *33*, 961–970.
- Fujiki, H. Green tea: health benefits as cancer preventive for humans. *Chem. Rec.* **2005**, *5*, 119–132.
- Bettuzzi, S.; Brausi, M.; Rizzi, F.; Castagnetti, G.; Peracchia, G.; Corti, A. Chemoprevention of human prostate cancer by oral administration of green tea catechins in volunteers with high-grade prostate intraepithelial neoplasia: a preliminary report from a one-year proof-of-principle study. *Cancer Res.* **2006**, *66*, 1234–1240.
- Fujiki, H.; Suganuma, M.; Imai, K.; Nakachi, K. Green tea: cancer preventive beverage and/or drug. *Cancer Lett.* **2002**, *188*, 9–13.
- Oh, H.; Ko, E. K.; Jun, J. Y.; Oh, M. H.; Park, S. U.; Kang, K. H.; Lee, H. S.; Kim, Y. C. Hepatoprotective and free radical scavenging activities of prenylflavonoids, coumarin, and stilbene from *Morus alba*. *Planta Med.* **2002**, *68*, 932–934.
- Choi, E. M.; Hwang, J. K. Effects of *Morus alba* leaf extract on the production of nitric oxide, prostaglandin E2 and cytokines in RAW264.7 macrophages. *Fitoterapia* **2005**, *76*, 608–613.
- Singab, A. N.; El-Beshbishy, H. A.; Yonekawa, M.; Nomura, T.; Fukai, T. Hypoglycemic effect of Egyptian *Morus alba* root bark extract: effect on diabetes and lipid peroxidation of streptozotocin-induced diabetic rats. *J. Ethnopharmacol.* **2005**, *100*, 333–338.
- Kang, T. H.; Oh, H. R.; Jung, S. M.; Ryu, J. H.; Park, M. W.; Park, Y. K.; Kim, S. Y. Enhancement of neuroprotection of mulberry leaves (*Morus alba* L.) prepared by the anaerobic treatment against ischemic damage. *Biol. Pharm. Bull.* **2006**, *29*, 270–274.

- (18) Chan, K. Some aspects of toxic contaminants in herbal medicines. *Chemosphere* **2003**, *52*, 1361–1371.
- (19) Harrison, R. M.; Chirgawi, M. B. The assessment of air and soil as contributors of some trace metals to vegetable plants. I. Use of a filtered air growth cabinet. *Sci. Total Environ.* **1989**, *83*, 13–34.
- (20) Harrison, R. M.; Chirgawi, M. B. The assessment of air and soil as contributors of some trace metals to vegetable plants. III. Experiments with field-grown plants. *Sci. Total Environ.* **1989**, *83*, 47–62.
- (21) Djingova, R.; Kovacheva, P.; Wagner, G.; Markert, B. Distribution of platinum group elements and other traffic related elements among different plants along some highways in Germany. *Sci. Total Environ.* **2003**, *308*, 235–246.
- (22) Abou-Arab, A. A. K.; Abou Donia, M. A. Heavy metals in Egyptian spices and medicinal plants and the effect of processing on their levels. *J. Agric. Food Chem.* **2000**, *48*, 2300–2304.
- (23) Ajasa, A. M. O.; Bello, M. O.; Ibrahim, A. O.; Ogunwande, I. A.; Olawore, N. O. Heavy trace metals and macronutrients status in herbal plants of Nigeria. *Food Chem.* **2004**, *85*, 67–71.
- (24) Caldas, E. D.; Machado, L. L. Cadmium, mercury and lead in medicinal herbs in Brazil. *Food Chem. Toxicol.* **2004**, *42*, 599–603.
- (25) Razic, S.; Onjia, A.; Dogo, S.; Slavkovic, L.; Popovic, A. Determination of metal content in some herbal drugs—empirical and chemometric approach. *Talanta* **2005**, *67*, 233–239.
- (26) Maiga, A.; Diallo, D.; Bye, R.; Paulsen, B. S. Determination of some toxic and essential metal ions in medicinal and edible plants from Mali. *J. Agric. Food Chem.* **2005**, *53*, 2316–2321.
- (27) Lopez, F. F.; Cabrera, C.; Lorenzo, M. L.; Lopez, M. C. Aluminium levels in spices and aromatic herbs. *Sci. Total Environ.* **2000**, *257*, 191–197.
- (28) Lozak, A.; Solyk, K.; Ostapczuk, P.; Fijalek, Z. Determination of selected trace elements in herbs and their infusions. *Sci. Total Environ.* **2002**, *289*, 33–40.
- (29) Basgel, S.; Erdemoglu, S. B. Determination of mineral and trace elements in some medicinal herbs and their infusions consumed in Turkey. *Sci. Total Environ.* **2006**, in press.
- (30) Ozcan, M. Determination of mineral contents of Turkish herbal tea (*Salvia aucheri* var. *canescens*) at different infusion periods. *J. Med. Food* **2005**, *8*, 110–112.
- (31) Lesniewicz, A.; Jaworska, K.; Zyrnicki, W. Macro- and micro-nutrients and their bioavailability in polish herbal medicaments. *Food Chem.* **2006**, in press.
- (32) Tyler, G. *ICP-MS, or ICP-AES and AAS? – A Comparison*; Varian Publication ICP-MS-1; Varian Australia: Victoria, Australia, April 1994; pp 1–7.
- (33) Fernandez-Caceres, P. L.; Martin, M. J.; Pablos, F.; Gonzalez, A. G. Differentiation of tea (*Camellia sinensis*) varieties and their geographical origin according to their metal content. *J. Agric. Food Chem.* **2001**, *49*, 4775–4779.
- (34) Matsushima, F.; Meshitsuka, S.; Nose, T. Contents of aluminium and manganese in tea leaves and tea infusions. *Nippon Eiseigaku Zasshi* **1993**, *48*, 864–872.
- (35) Wrobel, K.; Wrobel, K.; Urbina, E. M. Determination of total aluminium, chromium, copper, iron, manganese, and nickel and their fractions leached to the infusions of black tea, green tea, *Hibiscus sabdariffa*, and *Ilex paraguariensis* (mate) by ETA-AAS. *Biol. Trace Elem. Res.* **2000**, *78*, 271–280.
- (36) Jona, S. A.; Williams, I. S. Nutrient elements of commercial tea from Nigeria by an instrumental neutron activation analysis technique. *Sci. Total Environ.* **2000**, *258*, 205–208.
- (37) Al-Oud, S. S. Heavy metal contents in tea and herb leaves. *Pakistan J. Biol. Sci.* **2003**, *6*, 208–212.
- (38) Muller, M.; Anke, M.; Illing-Gunther, H. Aluminium in food-stuffs. *Food Chem.* **1998**, *61*, 419–428.
- (39) Minamisawa, M.; Minamisawa, H.; Yoshida, S.; Takai, N. Adsorption behavior of heavy metals on biomaterials. *J. Agric. Food Chem.* **2004**, *52*, 5606–5611.
- (40) Jin, C. W.; He, Y. F.; Zhang, K.; Zhou, G. D.; Shi, J. L.; Zheng, S. J. Lead contamination in tea leaves and non-edaphic factors affecting it. *Chemosphere* **2005**, *61*, 726–732.
- (41) Abou-Arab, A. A. K.; Kawther, M. S.; El Tantawy, M. E.; Badeaa, R. I.; Khayria, N. Quantity estimation of some contaminants in commonly used medicinal plants in the Egyptian market. *Food Chem.* **1999**, *67*, 357–363.

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