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Determination of Heavy Metals in Herbal Teas by Inductively Coupled Plasma Mass Spectrometry

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ABSTRACT

People get both beneficial and toxic effects on their health from the consumption of heavy metals in herbal teas. Owing to the importance of heavy metals present in herbal teas, this study was carried out to determine their concentrations in herbal teas. Herbal tea samples purchased in Nakhon Pathom, Thailand were digested with nitric acid and analyzed for 11 heavy metals by inductively coupled plasma mass spectrometry (ICP-MS). The results of analysis showed that herbal tea samples contained high concentrations of Cu, Fe, Pb, Zn, Al, Mn, and Ni and low concentrations of As, Cd, Cr, and Hg. Under the Prevention of Herbal Tea Adulteration Act of Thailand the permissible limits have been fixed only for As, Cd, Cu, Fe, Pb, and Zn. The concentrations of Cu, Fe, Pb, and Zn in almost all samples were higher than permissible levels, whereas those of As and Cd from all 30 samples were lower than the permissible limits. Therefore, the analysis data from this current work should provide guidance for quality control of herbal teas.
Keywords: Heavy Metals, Herbal Teas, ICP-MS

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INTRODUCTION

The consumption of some herbal teas was associated with the reduction of serum cholesterol, prevention of low density lipoprotein oxidation, and reduction in risk of degenerative diseases like cardiovascular disease, diabetes, and cancer [1]. However, some literatures reported that the concentrations of heavy metals in herbal teas were higher than the permissible limits under the Prevention of Food Adulteration Act [2-4]. Furthermore, according to the World Health Organization (WHO), toxic heavy metals have to be controlled in herbal plants in order to assure their safety [5]. Therefore, the consumption of herbal teas might give the positive and negative effects on human health. Generally, the concentrations of heavy metals in herbal teas differed according to the types of herbal teas and geological conditions [6]. People widely consumed herbal teas such as *Camellia sinensis* (green tea), *Morus alba* (mulberry), *Ginkgo biloba* (ginkgo), *Glycyrrhiza glabra* (licorice) and *Cinnamomum verum* (cinnamon). They consumed herbal teas for medical purposes and promoting their health. The concentrations of heavy metals including aluminium (Al), arsenic (As), cadmium (Cd), chromium (Cr), copper (Cu), iron (Fe), mercury (Hg), manganese (Mn), nickel (Ni), lead (Pb), and zinc (Zn) have not been reported in herbal teas available in Nakhon Pathom province, Thailand. Thus, this study was intended to determine the concentrations of 11 heavy metals in herbal teas that purchased from local markets and supermarkets in Nakhon Pathom province using nitric acid digestion followed by inductively coupled plasma mass spectrometry (ICP-MS). The data obtained could be used for the quality control process to ensure the purity of herbal teas in Thailand.

MATERAILS AND METHODS

Materials

The ultrapure water ASTM type I, 18.2 MΩ x cm used for analysis of heavy metals was generated by a TKA GenPure ultra pure water machine (TKA Wasseraufbereitungssysteme GmbH, Germany). Nitric acid for sample digestion was of analytical reagent grade (lot C08033, J.T. baker, USA). A stock solution of a multi-element 10 µg/mL calibration standard-2A (Lot#6-108VY, Agilent, USA) was prepared immediately in 5% v/v nitric acid solution prior to use. The glassware containers used for analysis were soaked overnight in 20% v/v nitric acid solution and rinsed several times with ultrapure water to eliminate metal contamination.

Herbal Tea Samples

Thirty types of herbal tea samples (Table 1) were purchased from local markets and supermarkets in Nakhon Pathom province, Thailand, in December 2010. All herbal tea samples were oven-dried at 60°C to a constant weight. Each sample was powdered with a stainless steel hammer mill with sieve no. 30 mesh and transferred into a plastic bag. All pulverized samples were kept in a desiccators at room temperature until analysis. All herbal teas were treated in an identical manner.

Table 1: Botanical name, Thai name, English name, and part used of herbal tea samples

No	Botanical name	Thai name	English name	Part used
1	*Andrographis paniculata*	Fa-ta-lai-choan	Andrographis	Leaf
2	*Caesalpinia sappan*	Fang	Sappan wood	Heartwood
3	*Camellia sinensis*	Cha-kiew	Green tea	Leaf
4	*Carthamus tinctorius*	Dok-kam-foy	Safflower	Petal
5	*Cassia alata*	Chum-hed	Ringworm bush	Leaf
6	*Centella asiatica*	Bua-bok	Asiatic pennywort	Leaf
7	*Cinnamomum verum*	Ob-choi-ted	Cinnamon	Bark
8	*Clitoria ternatea*	An-chan	Butterfly pea	Flower
9	*Curcuma xanthorrhiza*	Wan-chak-mot-luk	Java turmeric	Rhizome and root
10	*Cymbopogon citrates*	Ta-krai	Lemongrass	Leaf
11	*Derris* scandens	Tao-wan-priang	Jewel vine	Stem, Root
12	*Eupatorium odoratum*	Ya-dok-kao	Christmas bush	Stem, Leaf
13	*Ganoderma Lucidum*	Hed-lin-chue	Reishi	Fruiting body
14	*Ginkgo biloba*	Pae-guay	Ginkgo	Leaf
15	*Glycyrrhiza glabra*	Cha-em-taet	Licorice	Rhizome and root
16	*Hibiscus sabdariffa*	Gra-chieb-daeng	Roselle	Flower
17	*Imperata cylindrical*	Ya-kha	Cogongrass	Root
18	*Jasminum sambac*	Mali	Jasmine	Flower
19	*Lagerstroemia speciosa*	In-ta-nin-nam	Queen’s flower	Leaf
20	*Momordica charantia*	Mara-ki-nok	Bitter gourd	Fruit
21	*Moringa oleifera*	Ma-room	Drumstick tree	Leaf
22	*Morus alba*	Bai-mohn	Mulberry	Leaf
23	*Murdannia loriformis*	Ya-pak-king	Angel grass	Whole plant
24	*Nelumbo nucifera*	Bua-luang	Indian lotus	Pollen
25	*Orthosiphon aristatus*	Ya-nuad-maew	Cat's whisker	Leaf
26	*Piper sarmentosum*	Cha-plu	Wild betel	Leaf
27	*Senna alexandrina*	Ma-kham-khaek	Senna	Leaf
28	*Stevia rebaudiana*	Ya-wahn	Stevia	Aerial part
29	*Thunbergia lauriflolia*	Rang-chued	Babbler's bill	Whole plant
30	*Tiliacora triandra*	Ya-nang	Bamboo grass	Leaf

Sample Preparation

Approximately 1 g of each pulverized sample was accurately weighed and thoroughly mixed with 10 ml of 70% v/v nitric acid solution. The mixture was digested on a hot plate until the solution was clear. After cooling at room temperature, the digested solution was filtered and diluted to 10.0 ml with 5% v/v nitric acid solution. Triplicate digestions were made for each type of herbal tea. Samples prepared by this method were analyzed immediately by ICP-MS. Reagent blanks were also checked in parallel in all steps.

Inductively Coupled Plasma-Mass Spectrometry (ICP-MS)

The concentrations of 11 heavy metals (Al, As, Cd, Cr, Cu, Fe, Hg, Mn, Ni, Pb, and Zn) were determined in the digested solutions using inductively coupled plasma mass spectrometer

(Model 7500 ce, Agilent, Palo Alto, CA). The instrument was operated with flame mode conditions. Operating conditions were: RF power 1,500 w, carrier gas flow rate 0.9 l/min, make up gas flow rate 0.16 l/min, and nebulizer pump 0.1 rps.

Validation Method

The parameters of method validation including trueness by recovery at three levels of concentration, range of linearity, limit of detection (LOD), limit of quantification (LOQ), and repeatability were evaluated. The procedure and calculation were modified according to the European Standard for the analyses of heavy metals [7]. Analysis of reagent blanks showed that there was no contamination or interference from the reagents. The quantitative determinations of heavy metals in all samples were done using calibration curves obtained from diluted stock standard solution 10 µg/mL. All results represented means from triplicate determinations and are quoted on a dry weight basis.

RESULTS AND DISCUSSION

Data Validation: The calibration curves for As, Cd, Cr, and Hg were prepared in the range of 0.01-500 µg/L. For determination of Al, Cu, Fe, Mn, Ni, Pb, and Zn, the calibration solutions were ranged from 0.01 to 5,000 µg/L. All calibration curves showed good linear regression ($r^2 \geq 0.9997$) within the concentration ranges. Linearity was determined from the regression plots by the least squares method and expressed as the correlation coefficient (r^2) in the range of 0.9993-0.9999. The regression equations and correlation coefficients of Al was y = 0.57254X + 1.99788 (r^2 = 0.9998), for As y = 0.02548X + 0.01758 (r^2 = 0.9996), for Cd y = 0.04123X + 0.03412 (r^2 = 0.9996), for Cr y = 0.03618X + 0.04689 (r^2 = 0.9994), for Cu y = 0.40136X + 0.99858 (r^2 = 0.9997), for Fe y = 0.54325X – 0.42369 (r^2 = 0.9997), for Hg y = 0.02369X – 0.01402 (r^2 = 0.9994), for Mn y = 0.45639X + 0.32123 (r^2 = 0.9998), for Ni y = 0.06935X + 0.12596 (r^2 = 0.9998), for Pb y = 0.59632X + 0.56982 (r^2 = 0.9996), and for Zn y = 0.33698X + 0.58956 (r^2 = 0.9995). Accuracy was validated by the percentages of recovery of the standard solutions added to the samples during digestion. The average recovery was 94.58-118.47% in 11 heavy metals. The LOD and LOQ were defined as 3 and 10 times of the standard deviation of ten measurements, respectively. The LOD (µg/L) was 2.54 (Al), 4.35 (As), 1.75 (Cd), 0.99 (Cr), 2.56 (Cu), 4.87 (Fe), 3.25 (Hg), 1.26 (Mn), 1.48 (Ni), 4.36 (Pb), and 2.45 (Zn). The LOQ (µg/L) was 8.75 (Al), 14.20 (As), 5.65 (Cd), 3.25 (Cr), 8.48 (Cu), 16.36 (Fe), 10.23 (Hg), 4.42 (Mn), 4.95 (Ni), 16.23 (Pb), and 7.95 (Zn). The intra-day and inter-day repeatability showed the good precision. The precision expressed as relative standard deviations, was found to be 0.80-1.79% for intra-day analysis (n = 10) and 0.98-2.98% for inter-day analysis (n = 10).

Heavy Metal Concentrations: The botanical name, Thai name, English name, and part used of 30 herbal tea samples were shown in Table 1. The average concentrations of 11 heavy metals (Al, As, Cd, Cr, Cu, Fe, Hg, Mn, Ni, Pb, and Zn) in 30 herbal tea samples and the permissible limits of 6 heavy metals (As, Cd, Cu, Fe, Pb, and Zn) defined by the Prevention of Herbal Tea Adulteration Act [8] are shown in Table 2. Under the Prevention of Herbal Tea Adulteration Act, the permissible limits of As, Cd, Cu, Fe, Pb, and Zn were 0.2, 0.3, 5, 15, 0.5 and 5 mg/Kg, respectively.

Table 2: The concentrations (mg/Kg) of 11 heavy metals in herbal tea samples (n=3).

No	Botanical name	The concentrations (mg/Kg) of 11 heavy metals										
		As	Cd	Pb	Zn	Fe	Cu	Al	Mn	Ni	Cr	Hg
1	*Andrographis paniculata*	ND	0.016	6.027	38.997	35.918	3.993	55.274	24.974	0.570	0.292	ND
2	*Caesalpinia sappan*	ND	ND	ND	5.527	16.694	2.032	22.053	5.487	0.304	ND	ND
3	*Camellia sinensis*	0.013	0.011	5.175	26.303	70.374	9.474	473.171	307.698	8.567	0.063	ND
4	*Carthamus tinctorius*	ND	0.001	5.026	28.980	190.802	7.153	253.188	27.896	0.557	0.107	ND
5	*Cassia alata*	0.001	0.010	0.176	43.305	40.000	5.824	64.646	58.257	0.431	0.062	0.001
6	*Centella asiatica*	0.001	0.082	4.436	44.986	81.109	7.058	121.312	86.924	0.322	0.144	ND
7	*Cinnamomum verum*	0.010	0.074	13.597	39.209	36.163	6.816	315.428	20.544	20.759	0.068	ND
8	*Clitoria ternatea*	0.008	0.053	22.425	47.301	49.160	11.285	47.404	46.058	30.960	0.085	ND
9	*Curcuma xanthorrhiza*	ND	0.003	0.176	0.676	22.597	0.054	36.920	18.015	8.718	0.053	0.001
10	*Cymbopogon citratus*	ND	0.030	6.147	35.528	58.944	5.531	60.757	129.980	0.480	0.141	0.001
11	*Derris* scandens	ND	0.018	6.524	44.734	81.759	5.272	166.350	75.989	16.730	0.135	ND
12	*Eupatorium odoratum*	0.012	0.009	4.304	36.807	43.552	11.256	53.587	86.386	12.816	0.123	0.001
13	*Ganoderma Lucidum*	0.001	0.019	0.168	23.421	43.789	5.704	60.368	19.874	10.019	0.221	0.003
14	*Ginkgo biloba*	0.001	0.086	7.374	32.083	192.427	7.219	265.232	32.427	9.819	0.163	ND
15	*Glycyrrhiza glabra*	ND	ND	ND	6.618	14.340	2.938	18.888	26.144	0.599	1.010	ND
16	*Hibiscus sabdariffa*	0.003	0.030	0.140	33.650	36.219	11.073	77.761	142.485	0.515	0.074	ND
17	*Imperata cylindrica*	0.007	0.003	6.903	18.073	184.464	7.394	324.287	22.979	0.557	0.088	0.001
18	*Jasminum sambac*	0.007	0.018	5.153	34.890	17.923	10.872	28.021	41.031	9.613	0.074	ND
19	*Lagerstroemia speciosa*	0.014	0.006	11.062	41.986	43.914	7.189	72.330	178.073	9.820	0.085	0.002
20	*Momordica charantia*	ND	ND	0.108	14.207	61.734	19.323	34.271	70.309	2.678	0.408	0.013
21	*Moringa oleifera*	ND	ND	ND	1.979	22.328	0.792	35.208	33.249	1.065	0.085	ND
22	*Morus alba*	ND	0.010	4.887	37.582	68.476	5.361	62.192	33.208	12.352	0.141	ND
23	*Murdannia loriformis*	0.003	0.012	4.036	86.454	90.078	7.084	43.261	93.960	23.952	0.137	ND
24	*Nelumbo nucifera*	0.007	0.008	4.103	43.376	0.200	15.464	47.117	96.906	64.058	0.042	ND
25	*Orthosiphon aristatus*	0.002	0.004	0.144	26.145	87.079	5.349	56.227	20.852	0.378	0.073	0.001
26	*Piper sarmentosum*	ND	0.003	1.210	46.061	67.053	10.000	128.638	81.047	21.974	0.089	0.001
27	*Senna alexandrina*	ND	ND	0.117	17.185	21.349	12.654	24.229	92.366	4.929	3.294	0.019
28	*Stevia rebaudiana*	ND	0.033	0.210	22.010	35.471	0.328	115.673	69.981	0.019	0.040	0.002
29	*Thunbergia lauriflolia*	0.002	0.009	4.529	49.063	45.913	13.816	56.783	170.145	0.513	0.065	0.002
30	*Tiliacora triandra*	0.005	0.005	4.171	43.402	68.807	5.289	63.348	74.800	12.404	0.074	ND
	Permissible limits	0.2	0.3	0.5	5	15	5	-	-	-	-	-

ND: not determined, SD: 0.001-0.025

As shown in Table 2, the concentrations of As (≤0.014 mg/Kg) and Cd (0.001-0.086 mg/Kg) in all samples were less than permissible limits (As 0.2 and Cd 0.3 mg/Kg). The concentrations of Cu, Fe, Pb, and Zn were in the range of 0.054-19.323, 0.200-192.427, ≤22.425, and 0.676-86.454 mg/Kg, respectively. Among a total of 30 samples, the concentrations of Cu, Fe, Pb, and Zn in almost all samples (Cu, Fe, Pb, and Zn were 24, 29, 19, and 28 samples, respectively) were higher than permissible limits (Cu 5, Fe 15, Pb 0.5, and Zn 5 mg/Kg). The highest concentrations of Cu, Fe, Pb, and Zn in herbal tea samples were found in

Momordica charantia (Cu 19.323 mg/Kg), *Ginkgo biloba* (Fe 192.427 mg/Kg), Clitoria ternatea (Pb 22.425 mg/kg), and Murdannia loriformis (Zn 86.454 mg/Kg) as shown in Table 2. It was suggested that the almost herbal tea samples might be unsafe for tea drinkers since the concentrations of Cu, Fe, Pb, and Zn were found in excess of permissible limits.

The concentrations of Al, Mn, and Ni were in the range of 18.888-473.171, 5.487-307.698, and 0.019-64.058 mg/Kg, respectively as shown in Table 2. The highest concentrations of Al, Mn, and Ni in herbal tea samples were found in *Camellia sinensis* (Al 473.171 and Mn 307.698 mg/Kg) and Nelumbo nucifera (Ni 64.058 mg/Kg). However, the Act established in 2004 did not mention the permissible limits of these 3 heavy metals. These experimental data suggested that herbal teas were major sources of Al, Mn, and Ni. According to the literature data, trace heavy metals might have adverse effects on human health such as accumulation of Al in tea infusion was associated with Alzheimer's disease [9], and exposure to very high level of Mn in drinking-water was known to cause neurological effects [10]. Furthermore, Ni was one of the most important heavy metals in terms of its potential toxicity to plants and animals [11]. With respect to the acceptable daily intake of Al, Mn, and Ni as toxic heavy metals in tolerable daily dietary and safety standards, these analyzed samples might be dangerous for human consumption.

Cr might cause adverse effects such as lung cancer and liver damage [12]. Chronic exposure to high level of Hg in herbal teas might cause damage to brain, kidney, and lung [13]. However, the concentrations of Cr and Hg were $\leq$3.294 and $\leq$0.019 mg/Kg, respectively (Table 2). Furthermore, the concentrations of Cr and Hg in all analyzed samples were very scanty (Table 2), according to previous report [14]. Therefore, the herbal tea samples might be safe for Cr and Hg.

CONCLUSION

It was concluded that the herbal tea samples collected in Nakhon Pathom province, Thailand contained high concentrations of Cu, Fe, Pb, Zn, Al, Mn, and Ni and low concentrations of As, Cd, Cr, and Hg. Based upon the permissible limits set by the Prevention of Herbal Tea Adulteration Act, all samples of herbal teas were found to be unsafe for human consumption.

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