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Levels of Heavy Metals and Ochratoxin A in Medicinal Plants Commercialized in Turkey

Hakan Özden[1], Sibel Özden[2]

[1]Division of Botany, Department of Biology, Faculty of Science, Istanbul University, 34134, Suleymaniye, Istanbul, Turkey.

[2]Department of Pharmaceutical Toxicology, Faculty of Pharmacy, Istanbul University, 34116 Beyazit, Istanbul, Turkey.

ABSTRACT

INTRODUCTION: The aim of this study as to determine the levels of lead, cadmium and ochratoxin A (OTA) in frequently used medicinal plants.

METHODS: Totally twenty-one samples of linden, chamomile and sage were obtained during the spring and summer period of the year 2016 from local markets and traditional bazaars in Istanbul, Turkey. Microwave-assisted digestion was applied for the preparation of the samples and inductively coupled plasma technique with optical emission spectrometry (ICP-OES) was used for the determination of lead and cadmium. Determination of OTA was carried out using high performance liquid chromatography with fluorescence detector (HPLC-FLD) after immunoaffinity column clean-up.

RESULTS: OTA was detected in only one chamomile sample with a low concentration level of 0.034 µg/kg. According to the results of ICP-OES analysis, lead in the concentration range of 4.125-6.487 mg/kg, 3.123-5.769 mg/kg and 3.229-5.985 mg/kg and cadmium in the concentration range of 0.324-0.524 mg/kg, 0.365-0.51 mg/kg and 0.321-0.474 mg/kg was found in linden, chamomile and sage teas, respectively.

DISCUSSION AND CONCLUSION: We indicated that levels of Pb and OTA were found below the maximum permissible level whereas high levels of Cd were observed in medicinal plants which may not pose health risk for the consumers according to the exposure assessment. However, it is suggested that other mycotoxins and heavy metal content should be carefully considered in medicinal plants.

Keywords: Lead, cadmium, ochratoxin A, linden, chamomile, sage

INTRODUCTION

Herbs play an important role in various traditional medicine and recently they are increasingly used in the primary health care intervention. However, there has been increasing concern on the safety and toxicity of natural herbs. Similar to the agricultural products, herbs may be subjected to natural and chemical contaminations during the one or more stages of the supply chain. Medicinal plants are naturally contaminated with mycotoxins during harvesting, storage and distribution of these products. Besides, herbs may be subjected to chemical residues from heavy metals.

Ochratoxin A (OTA) is a toxic secondary metabolite mainly produced by several of *Aspergillus* and *Penicillium* species under diverse environmental conditions. OTA has received increased attention worldwide due to the deleterious effects to the health on human and animal.[1,2] OTA can be found as a natural contaminant in a variety of foods including cereals, wine, grapes and its products, dried fruits, cacao, coffee and spices.[2]

Some heavy metals, particularly arsenic (As), lead (Pb), cadmium (Cd) and mercury (Hg) have no biological roles in living organisms and contaminate environments. Many of these metals are involved in several toxic effects and developmental disorders. Due to their widespread occurrence, toxicity and persistence in the environment, they should be considered as a potential hazardous threat to human health and crop plants.

Control of chemical hazards in herbs during the food chain is important for the quality control and protection of human health. Food safety authorities are responsible for checking product compliance to the legal limits. So far there has been very little published information on the occurrence of heavy metals and OTA in medicinal plants in Turkey.[3-10] Data is needed to assess the contamination of heavy metals and OTA in widely consumed medicinal plants and to estimate the exposure to Turkish population. Therefore, the aim of the present study was to investigate the contamination of lead, cadmium and OTA in selected medicinal plants such as linden, sage and chamomile widely consumed in Turkey and exported worldwide and to evaluate their potential hazard to human. The results of this study would contribute to pollution control and risk management of heavy metals and OTA in medicinal plants.

MATERIAL AND METHODS

Chemicals

Ultrapure water was used in all experiments obtained using a Milli-Q system (Millipore, Bedford, MA, USA). Analytical grade chemicals were obtained from Merck (Darmstadt, Germany) and Riedel-de Haën (Seelze, Germany).

The metal standard solutions of Cd and Pb for the calibration curves were prepared by diluting a stock solution of 1000 µg/mL which was obtained from VHG labs (Manchester, NH, USA). All plastic and glassware were properly cleaned by soaking them in 2 M nitric acid and rinsed thoroughly with deionized water prior to use.

For OTA analysis, HPLC standard solutions in the final concentrations ranges from 0.03 to 10 µg/kg which are equivalent to 1 g medicinal plant samples were prepared in methanol:water (1:1, v/v) by serial diluting the stock standard solution of OTA (50 ng/µL in benzene:acetic acid; 99:1, v/v) which was obtained from Supelco (Cat. No: 46912). Phosphate-buffered saline (PBS), pH 7.4, for the extraction in the OTA analysis was prepared as previously described.[11]

Sample collection

A total of 21 unpacked samples including 7 linden, 7 chamomile and 7 sage were collected randomly in April and July 2016 year from traditional bazaars in Istanbul, Turkey. Some characteristics of the medicinal plants studied are given in Table 1. The taxonomic identity of each botanical samples was confirmed by the Department of Botany, Science Faculty, Istanbul University. The dried materials were ground by using a Waring Blender (Conair Corp., Stamford, CT, USA) and the ground samples sealed in plastic packages were kept at room temperature until they were analyzed.

Lead and cadmium analysis in medicinal plants

Dried and homogenized medicinal plant materials were weighed to be 0.1-0.3 g and placed in teflon containers (DAP 60, Berghof instruments GmbH, Eningen, Germany). Then, plant material was wet-digested with 8 mL of 65% nitric acid in the microwave oven (150-190ºC) (Berghof MWS-4 device, Berghof instruments GmbH, Eningen, Germany). After the digestion procedure, the teflon containers were allowed to cool and suspensions were diluted with deionized water to 25 mL. The material was passed through the syringe-type filters (Chromafil PET-45/25, Macheerey Nagel GmbH,

Düren, Germany), then ready for the measurement. Samples were run on a inductively coupled plasma technique with optical emission spectrometry (ICP-OES) (Pelkin Elmer Optima 7000 DV, Waltham, MA, USA) for analysis on two different elements of Pb (k: 220,353 nm) and Cd (k: 214,440 nm) wavelength).[12,13] Measurements were done in triplicate and the mean value was calculated on a dry weight basis (mg/kg, dw). Standard calibration curves were performed in the concentrations of 0.25; 0.5; 1; 2; 3; 5 mg/kg for Cd and 0.4; 1; 2; 3; 5; 10 mg/kg for Pb. Blanks were also run with standard solutions and all samples were checked for any loss and cross-contamination.

OTA analysis in medicinal plants

Sample extraction and immunoaffinity clean-up

The immunoaffinity columns OchraTest™ were purchased from VICAM (Watertown, MA, USA). Medicinal plants were extracted according to Truckess et al.[14] with some modifications. In a 20 mL centrifuge tube; 5 g of sample, 1 g of NaCl and 25 mL of metanol-0.5% $NaHCO_3$ (70:30, v/v) solution were added and mixed on a vortex mixer. Then, the mixture was shaken at 400 rpm for 15 min and centrifuged at 3000 rpm for 15 min. A 5 mL of supernatant was diluted with 20 mL PBS buffer containing 1% Tween 20. The diluted extract was centrifuged at 3000 rpm for 15 min. Afterwards, 20 mL of supernatant (20 mL = 1 g sample equivalent) was passed through an OchraTest™ immunoaffinity column at a flow rate of 1 drops/second until air comes through column. The column was washed with 10 mL of PBS buffer containing 1% Tween 20 and 10 mL of purified water, dried under vacuum. OTA was eluted by passing 1.5 mL of methanol through the column and eluate was then diluted with 1.5 mL of purified water. 100 µL of the aliquot was injected into high performance liquid chromatography (HPLC) equipped with fluorescence detection (FLD).

HPLC-FLD analysis and method validation

The chromatographic analysis was performed as previously described using an LC-20A Shimadzu (Kyoto, Japan) liquid chromatographic system coupled to an RF-10A XL fluorescence detector.[11] Confirmation of OTA in positive samples was done following the method of Zimmerli and Dick[15] by formation of the methyl ester derivative.

Validation of the method was performed according to our previous paper[11]. In present study we established the calibration curve with six levels of OTA in the range of 0.03-10 µg/kg. Recovery experiments and precision of the method were performed

on OTA-free blank medicinal plant samples by spiking with the OTA standard solutions in order to obtain final concentrations of 0.5 and 1 µg/kg.

RESULTS AND DISCUSSION

Pb and Cd levels in medicinal plants

Linearity was assessed using Cd (0.25-5 mg/kg) and Pb (0.4-10 mg/kg) calibration curves at the six concentrations with a correlation coefficient r^2=0.9987 and 0.9926, respectively. For the instrumental sensitivity, the limit of detections (LOD; signal-to noise ratio = 3) were obtained to be 0.013 and 0.13 mg/kg and limit of quantifications (LOQ; signal-to-noise ratio = 10) were 0.039 and 0.39 mg/kg of Cd and Pb, respectively for each matrixes.

We analyzed totally 21 medicinal plants including linden (7), chamomile (7) and sage (7) unpacked samples collected from traditional bazaars in Istanbul. As shown in Table 2 medicinal plants contained Cd and Pb in the following concentration range of 0.321-0.524 mg/kg and 3.123-6.487 mg/kg, respectively. Cd levels exceeded the maximum permissible level (0.3 mg/kg for Cd) in all medicinal plants, however Pb levels were lower than the maximum permissible level (10 mg/kg for Pb) set by FAO WHO for medicinal plants.[16] A number of studies have been carried out to determine the contamination of heavy metals in spices and botanicals. Available data on the contamination of heavy metals in analyzed medicinal plants in Turkey were summarized in Table 3. Our results are consistent with the study from Ozcan and Akbulut[5] reported that Cd was found in the concentration range of 0.61-1.05 mg/kg which exceeded maximum permissible level in medicinal plants, while Pb was found in the concentration range of 0.43-2.73 mg/kg, which is below the maximum permissible level. However, Bilgic Alkaya et al.[8], where Cd and Pb were detected in low levels (< 0.16 mg/kg for Cd and < 1.36 mg/kg for Pb) in medicinal plants.

Results of worldwide studies in the analyzed medicinal plants were summarized in Table 4. In United Arab Emirates, Dghaim et al.[24] showed that 55% of chamomile samples contained Cd (0.82 mg/kg) above the maximum level (0.3 mg/kg).[16] Dghaim et al.[24] also reported that 44% of chamomile samples contained Pb (5.37-11.40 mg/kg) and 100% of sage samples contained Pb (12.66-21.76 mg/kg) above the maximum permissible level (10 mg/kg).[16]

European Food Safety Authority (EFSA)[26] established that the tolerable weekly intake for Cd is 2.5 µg/kg bw per week. We calculated the weekly intake of Cd (µg/kg bw per week) considering the mean daily consumption of teas as 2.3 g for Middle Eastern (GEMS/Food Regional Diets)[27] for an adult with a mean body weight of 70 kg and the maximum level of Cd (0.524 mg/kg) found in the linden samples. Accordingly, the highest value for the estimated human weekly Cd intake in this study is 0.121 µg/kg per week, thus representing 4.82% of the tolerable weekly intake as established by EFSA.[26]

OTA in medicinal plants

For the method validation, linearity was assessed using OTA calibration curve at the six concentrations (0.03-10 µg/kg) with a correlation coefficient r^2=0.9998. LOD was obtained to be 0.01 µg/kg and LOQ level was 0.03 µg/kg of OTA for each matrixes. The extraction recoveries are presented in Table 5. The average recoveries at the concentrations of 0.5 and 1 µg/kg were 98.9-87.3%; 99.8-98.9%; 97.6-92.4% for linden, chamomile and sage, respectively with a relative standard deviation (RSD) less than 9.6%.

As shown in Table 6, OTA was determined in only one chamomile sample in the concentration of 0.34 µg/kg. OTA was detected in two of chamomile and one of linden under the LOQ level (0.03 µg/kg). We found very low level of OTA in linden, chamomile and sage plants under the limit of 15 µg/kg for spices regulated by European Commission (EU)[28] and The Turkish Ministry of Agriculture and Rural Affairs (Turkish Food Codex Legislation 2011/28157)[29].

According to our knowledge there has been no study available about the occurrence of OTA in medicinal herbs including linden, chamomile and sage in Turkey. Only one study in Turkey from Tosun and Arslan[30] reported that Aflatoxin B1 was determined in linden (60%), chamomile (100%) and sage (100%) with the mean concentration of 14 µg/kg, 28.7 µg/kg and 8.9 µg/kg, respectively and some of the medicinal plants were found to be contaminated with AFB1 above the EU limits of 5 µg/kg for AFB1 and 10 µg/kg for total aflatoxins in spices established in 2010.[31] Omurtag and Yazıcıoglu[32] presented fumonisin b1 (FB1) and fumonisin b2 (FB2) levels in medicinal plants and they showed no contamination of FB1 and FB2 in linden, chamomile and sage medicinal plants.

There have been few studies that have reported the contamination of OTA in linden, chamomile and sage plants. Halt[33] reported that trace amount of OTA was detected in medicinal plant materials from Tilia grandifolia. Aziz et al.[34] did not find OTA in lime tree and chamomile medicinal plants. Santos et al.[35] found that chamomile and sage were found to be contaminated with OTA at the concentrations of 0.8-1 µg/kg and 1.1-17.3 µg/kg, respectively.

CONCLUSION

In summary, Cd and Pb were detected in the range of 0.321-0.524 mg/kg and 3.123-6.487 mg/kg, respectively. We indicated that Pb levels were found below the maximum permissible level whereas high levels of Cd were observed in medicinal plants. According to the exposure assessment for Cd consumption of the medicinal plants does not represent a threat to the human health. Occurrence of OTA contamination in medicinal plants from local bazaars in Istanbul was observed for the first time and OTA was determined in only one chamomile sample (0.34 µg/kg). Although herbs are open to contamination with variable amounts of mycotoxins, we observed the contamination levels of OTA studied medicinal plants were very low. However, further studies covering larger number of samples which include other types of medicinal plants in open market are needed to carefully consider for the contamination of other mycotoxins and heavy metals.

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Table 1. Some characteristics of studied medicinal plants

Common name	Scientific name	Turkish name	Used part	Origin
Linden	*Tilia argentea*	Ihlamur	Dried inflorescence	Anatolia
Chamomile	*Anthemis cretica subsp anatolica*	Papatya	Leaf and flowers	Anatolia
Sage	*Salvia fructicosa*	Adaçayı	Leaf	Anatolia

Table 2. Contamination of heavy metals in the analyzed medicinal plants

Medicinal plant	**No. of samples**	**No. of samples with heavy level**	**Range of contamination (mg/kg)**		**Mean of contamination (mg/kg) ± SD ***	
			Cd	**Pb**	**Cd**	**Pb**
Linden	7	7	0.324-0.524	4.125-6.487	0,395 ± 0,08	4.357 ± 1.11
Chamomile	7	7	0.365-0.51	3.123-5.769	0,422 ± 0,04	4.374 ± 0.77
Sage	7	7	0.321-0.474	3.229-5.985	0,423 ± 0,05	4.43 ± 0.81

* SD: standard deviation

Table 3. Available data on the contamination of heavy metals in analyzed medicinal plants in Turkey

Sample	Cd (mg/kg)	Pb (mg/kg)	Reference
Sage	-	0.51	Ozcan[3]
Chamomile	0.44	0.72	Basgel and Erdemoglu[4]
Linden	nd	0.26	
Sage	nd	1.14	
Chamomile	1.05	2.73	Ozcan and Akbulut[5]
Lime flower	0.66	0.43	
Sage (*S. aucheri*)	0.79	1.24	
Sage (*S. fructicosa*)	0.61	0.46	
Linden	<LOD (0.025)		Sekeroglu et al.[6]
Chamomile	0.126		
Chamomile (*Matricaria chamomilla*)	0.12	0.12	Leblebici et al.[7]
Linden (*Tilia platyphyllos*)	0.02	0.12	
Sage (*Salvia officinalis*)	0.04	1.36	
Chamomile	0.14	0.07	Bilgic Alkaya et al.[8]
Linden	0.16	0.11	
Sage	0.16	0.16	
Linden	4.35	nd	Tercan et al.[9]
Sage	nd	nd	
Sage (*Salvia officinalis L.*)	0.17	0.039	Ozyigit et al.[10]

Table 4. Available data on the contamination of heavy metals in analyzed medicinal plants in the world

Sample	Cd (mg/kg)	Pb (mg/kg)	Reference
Chamomile (packed sample)	0.094	0.242	Abou-Arab[17]
Chamomile (non-packed sample)	0.211	0.308	
Linden blossom	0.088	0.096	
Linden blossom	0.141	0.121	
Chamomile	1.3	6.19	Abou-Arab and Abou Donia[18]
Sage (*Saliva officinalis*)	0.01	0.8	Chizzola et al.[19]
Matricaria recutita	0.23	0.31	
Chamomile	0.05	1.34	Dogheim et al.[20]
Chamomile (*Matricaria chamomilla*)	0.017	0.128	Alwakeel[21]
Sage	0.017	0.134	
Sage (*Saliva officinalis*)	Nd (<0.002)	Nd (<0.05)	Abu-Darwish[22]
Lime	0.36	2.30	Nordin and Selamat[23]
Musk lime	1.22	2.44	
Chamomile	0.82	5.37-11.40	Dghaim et al.[24]
Sage	0.88	12.66-21.76	
Chamomile blossom	0.22	0.62	Mirosławski and Paukszto[25]

Table 5. Recovery data for OTA in medicinal plants (n = 4)

	Spiking level (μg/kg)	**Recovery (%, Mean ± SD)**	**RSDr (%)**
Linden	0.5	98.9 ± 4.8	4.8
	1	87.3 ± 5.5	6.4
Chamomile	0.5	99.8 ± 2.2	2.2
	1	98.9 ± 7.7	7.7
Sage	0.5	97.6 ± 0.9	0.9
	1	92.4 ± 8.9	9.6

Table 6. Occurrence of OTA in the analyzed medicinal plants

Medicinal plants	**No. of samples**	**No. of samples with OTA level (%) [a]**			**Range of contamination (µg/kg)**	**Mean of contamination [b] (µg/kg)**
		<LOD	LOD-LOQ	>LOQ		
Linden	7	6 (85.7%)	1 (14.3%)	-	-	-
Chamomile	7	4 (57.1%)	2 (28.6%)	1 (14.3%)	0.034	0.034
Sage	7	7 (100%)	-	-	-	-

[a] Percentage of contamination

[b] Mean contamination of positive samples