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ORIGINAL ARTICLE

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

Türkiye'de Satılan Tıbbi Bitkilerde Ağır Metal ve Okratoksin A Seviyeleri

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ABSTRACT

Objectives: The aim of this study was to determine the levels of Pb, Cd, and OTA in frequently used medicinal plants.

Materials and Methods: Twenty-one samples of linden, chamomile, and sage were obtained during the spring and summer period of 2016 from local markets and traditional bazaars in İstanbul, Turkey. Microwave-assisted digestion was applied for the preparation of the samples and the ICP-OES was used for the determination of Pb and Cd. Determination of OTA was performed using 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, Pb 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 Cd 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.

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 a health risk for consumers according to the exposure assessment. However, it is suggested that other mycotoxins and heavy metal contents should be carefully considered in medicinal plants.

Key words: Lead, cadmium, OTA, linden, chamomile, sage

ÖZ

Amaç: Bu çalışmanın amacı, sıklıkla kullanılan tıbbi bitkilerde Pb, Cd ve OTA seviyelerinin araştırılmasıdır.

Gereç ve Yöntemler: Toplam 21 adet ıhlamur, papatya ve adaçayı örnekleri 2016 yılının bahar ve yaz aylarında İstanbul Türkiye'de geleneksel pazar ve marketlerden satın alındı. Örnekler mikrodalga parçalama yöntemi uygulanarak hazırlandı ve kurşun ve kadmiyum analizi ICP-OES cihazı kullanılarak gerçekleştirildi. OTA tayini immünoafinite kolonu ile temizlemenin ardından HPLC-FLD ile gerçekleştirildi.

Bulgular: OTA sadece 1 adet papatya örneğinde düşük konsantrasyonda (0.034 µg/kg) tayin edildi. ICP-OES analizi sonuçlarına göre; ıhlamur, papatya ve adaçayı örneklerinde sırasıyla kurşun 4.125-6.487 mg/kg, 3.123-5.769 mg/kg ve 3.229-5.985 mg/kg konsantrasyon aralığında; kadmiyum 0.324-0.524 mg/kg, 0.365-0.51 ve mg/kg 0.321-0.474 mg/kg konsantrasyon aralığında bulundu.

Sonuç: Tıbbi bitkilerde Pb ve OTA seviyeleri maksimum izin verilen seviyelerin altında bulunmuş olup, yüksek seviyelerde bulunan Cd seviyelerinin yapılan maruziyet değerlendirmesine göre tüketici sağlığında risk oluşturmayacağı gösterilmektedir. Bununla birlikte, tıbbi bitkilerde diğer mikotoksinler ve ağır metal seviyelerinin dikkatle izlenmesi gerektiği önerilmektedir.

Anahtar kelimeler: Kurşun, kadmiyum, OTA, ıhlamur, papatya, adaçayı

INTRODUCTION

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

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Ochratoxin A (OTA) is a toxic secondary metabolite, mainly produced by several *Aspergillus* and *Penicillium* species under diverse environmental conditions. OTA has received increased attention worldwide due to the deleterious effects to health of humans and animals.[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 biologic roles in living organisms and contaminate environments. Many of these metals have toxic effects and cause 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 quality control and protection of human health. Food safety authorities are responsible for checking product compliance with 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 are needed to assess the contamination of heavy metals and OTA in widely-consumed medicinal plants and to estimate the exposure to the Turkish population. Therefore, the aim of the present study was to investigate the contamination of Pd, Cd, and OTA in selected medicinal plants such as linden, sage, and chamomile, which are widely consumed in Turkey and exported worldwide, and to evaluate their potential risk to humans. The results of this study would contribute to pollution control and the risk management of heavy metals and OTA in medicinal plants.

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

Pd and Cd analysis in medicinal plants

Dried and homogenized medicinal plant materials were weighed as 0.1-0.3 g and placed in Teflon containers (DAP 60, Berghof instruments GmbH, Eningen, Germany). The plant material was wet-digested with 8 mL of 65% nitric acid in a microwave oven (150-190˚C) (Berghof MWS-4 device, Berghof instruments GmbH, Eningen, Germany). After the digestion procedure, the Teflon containers were allowed to cool and the suspensions were diluted with deionized water to 25 mL. The material was passed through syringe-type filters (Chromafil PET-45/25, Macheerey Nagel GmbH, Düren, Germany), and they were then ready for measurement. Samples were run on an inductively-coupled plasma with optical emission spectrometry (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).[12,13] Measurements were performed in triplicate and the mean value was calculated on a dry weight basis (mg/kg, dw). Standard calibration curves were constructed at 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 Trucksess et al.[14] with some modifications. In a 20-mL centrifuge tube, 5 g of sample, 1 g of NaCl, and 25 mL of methanol (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. Five milliliters of the 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 the supernatant (20 mL=1 g sample equivalent) was passed through an OchraTest™ immunoaffinity column at a flow rate of 1 drop/second until air came through the column. The column was washed with 10 mL of PBS buffer containing 1% Tween 20 and 10 mL of purified water, and dried under vacuum. OTA was eluted by passing 1.5 mL of methanol through the column and the eluate was then diluted with 1.5 mL of purified water. One hundred microliters of the aliquot was injected into an HPLC analyzer 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 with an RF-10A XL FLD.[11]

Confirmation of OTA in positive samples was achived 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 the 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 analysis 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 as 0.013 and 0.13 mg/kg and the limit of quantifications (LOQ; signal-to-noise ratio=10) were 0.039 and 0.39 mg/kg of Cd and Pb, respectively for each matrix.

We analyzed 21 medicinal plants including linden (n=7), chamomile (n=7) and sage (n=7) unpacked samples collected from traditional bazaars in Istanbul. As shown in Table 2, the 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) in all medicinal plants; however, Pb levels were lower than the maximum permissible level (10 mg/kg) set by the Food and Agriculture Organization and World Health Organisation for medicinal plants.[16] A number of studies have been conducted to determine the contamination of heavy metals in spices and botanicals. The available data on the contamination of heavy metals in analyzed medicinal plants in Turkey are summarized in Table 3. Our results are consistent with the study from Ozcan and Akbulut[5] who reported that Cd was found in the concentration range of 0.61-1.05 mg/kg, which exceeded the maximum permissible level in medicinal plants,

Table 1. Some characteristics of the 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 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	Başgel and Erdemoğlu[4]
Linden	n.d.	0.26	
Sage	n.d.	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	n.d.	Tercan et al.[9]
Sage	n.d.	n.d.	
Sage (*Salvia officinalis* L.)	0.17	0.039	Ozyigit et al.[10]

n.d.: Not detected, Pb: Lead, Cd: Cadmium, LOD: Limit of detections

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, Pb: Lead, Cd: Cadmium

whereas Pb was found in the concentration range of 0.43-2.73 mg/kg, which is below the maximum permissible level. In contrast, Bilgic Alkaya et al.[8] detected Cd and Pb at low levels (<0.16 mg/kg for Cd and <1.36 mg/kg for Pb) in medicinal plants.

The results of worldwide studies in the analyzed medicinal plants are summarized in Table 4. In the 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]

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

Sample	Cd (mg/kg)	Pb (mg/kg)	Reference
Chamomile (packed sample)	0.094	0.242	Abou-Arab et al.[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*)	n.d. (<0.002)	n.d. (<0.05)	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]

n.d.: Not detected, Pb: Lead, Cd: Cadmium

The European Food Safety Authority (EFSA)[26] established that the tolerable weekly intake for Cd was 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 an OTA calibration curve at the six concentrations (0.03-10 µg/kg) with a correlation coefficient r^2=0.9998. LOD was obtained as 0.01 µg/kg and the LOQ level was 0.03 µg/kg of OTA for each matrix. 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%, and 97.6-92.4% for linden, chamomile and sage, respectively, with a relative standard deviation less than 9.6%.

As shown in Table 6, OTA was determined in only one chamomile sample at a concentration of 0.34 µg/kg. OTA was detected in two chamomile samples and one linden sample under the LOQ level (0.03 µg/kg). We found very low levels 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]

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

	Spiking level (mg/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

SD: Standard deviation, RSDr: Relative standard deviation, OTA: Ochratoxin A

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, LOD: Limit of detections, LOQ: Limit of quantifications, OTA: Ochratoxin A

To our knowledge, there are no studies on 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 mean concentrations of 14 µg/kg, 28.7 µg/kg, and 8.9 µg/kg, respectively, and some 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 in 2010.[31] Omurtag and Yazicioğlu[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.

A few studies have reported the contamination of OTA in linden, chamomile, and sage plants. Halt[33] reported that trace amounts of OTA were detected in medicinal plant materials from *Tilia grandifolia*. Aziz et al.[34] found no OTA in lime tree and chamomile medicinal plants. Santos et al.[35] found that chamomile and sage were contaminated with OTA at concentrations of 0.8-1 µg/kg and 1.1-17.3 µg/kg, respectively.

CONCLUSIONS

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. 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 human health. Occurrence of OTA contamination in medicinal plants from local bazaars in İstanbul 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 in the studied medicinal plants to be very low. However, further studies covering larger numbers of samples that include other types of medicinal plants in open markets are needed to carefully consider the contamination of other mycotoxins and heavy metals.

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