WORLD JOURNAL OF PHARMACEUTICAL RESEARCH
SJIF Impact Factor 7.523
Volume 6, Issue 10, 44-55. Research Article ISSN 2277– 7105

EVALUATION OF THE QUALITY OF NATURAL PRODUCTS FOR THE PREPARATION OF CHAMOMILE AND MINT TEA IN COSTA RICA

Jeimy Blanco Barrantes*[1], Eduviges Bustos Araya[2], Fabio Granados-Chinchilla[3], Arlene Loría Gutiérrez[4]

[1,2,4]Instituto de Investigaciones Farmacéuticas, Facultad de Farmacia, Universidad de Costa Rica, 11501-2060 Ciudad Universitaria Rodrigo Facio, San José, Costa Rica; Tel: +506 2511 8338.

[3]Centro de Investigación en Nutrición Animal (CINA), Universidad de Costa Rica, 11501-2060 Ciudad Universitaria Rodrigo Facio, San José, Costa Rica; Tel: +506 2511 2028 Fax: +506 2234 2415.

Article Received on
12 July 2017,

Revised on 04 August 2017,
Accepted on 25 August 2017

DOI: 10.20959/wjpr201710-9270

***Corresponding Author**
Jeimy Blanco Barrantes
Instituto de Investigaciones Farmacéuticas, Facultad de Farmacia, Universidad de Costa Rica, 11501-2060 Ciudad Universitaria Rodrigo Facio, San José, Costa Rica; Tel: +506 2511 8338.

ABSTRACT

In this study, the quality and safety of chamomile and mint tea marketed in Costa Rica were evaluated. Physical and chemical analyses, the presence of toxic substances and pathogenic microorganisms were carried out, to determine whether they comply with local and international legislation. The sample taken was 1360 envelopes of chamomile and 400 sachets of mint, of 22 products available in the market. A 70 % compliance was found concerning the regulation of labeling of natural products applied in Costa Rica. About the total ash test, a compliance rate of 82 % was observed. Regarding the absence of foreign matter, at least 66 % of the samples showed some of the following structures: insect parts, small stones, plastic fragments. About the levels of cadmium and mercury were found two chamomile products that exceed the maximum limit suggested for medicinal herbs by the WHO. Regarding the evaluation of the microbiological quality of the samples as a criterion of safety, one product was found that exceeds the limit of aerobic bacteria and a 42 % that exceeds the limit of enterobacteria. In the rest of the tests, the products met the specifications. These results highlight the need for quality control of these natural products since not all meet the criteria of quality and safety.

KEYWORDS: Quality, safety, *Matricaria chamomilla*, *Menta piperita*, tea.

INTRODUCTION

In recent decades, consumption of herbal teas has increased and become a regular and scattered product of consumption; of all the preparations with medicinal plants the herbal teas, infusions or fodder, are the most used. That is why with its growing popularity and the expansion of the global market, the safety of these products has become a public health concern.

The World Health Organization (WHO) and official compendia of monographs such as United States Pharmacopoeia, British Pharmacopoeia, International Pharmacopoeia, as well as national and regional regulations establish tests and acceptance criteria for the quality control of some natural products.[1,2]

As for Costa Rica, in 2012, the Central American Technical Regulation came into effect for the verification of the Quality of Natural Products for human use.[3] The legislation aims to harmonize the analytical tests that must be performed to verify the quality of natural products for human use and assure the consumers that the natural products in their final stage of distribution, keep their original characteristics unchanged according to their formulation. The document establishes the quality tests to be performed on powders and crushed some of them include: organoleptic, macroscopic, and microscopic features of the plant material, average mass, incineration residue, determination of the foreign matter, determination of heavy metals, identification general or specific and microbial thresholds.

Quality assurance of herbal teas should include aspects of microbiological and mycotoxicological safety, especially those parts of higher risk of contamination or herbs from hot or humid climates such as our country. It has been determined that the greatest load of fungi spores are found in the leaves, followed by flowers, rhizomes, roots, bark, and seeds.[4] Cross-contamination is also possible by extraneous materials such as plastics, glass, paper and other materials that are used during the production of herbal teas.[5,6]

Metals are among the oldest contaminants known to humans, ingestion, and accumulation of toxic metals in the body can cause chronic toxicity, leading to serious injuries such as kidney and liver failure. Heavy metals are widely scattered in nature and can be freely found in soil

or water. It is important to reduce exposure to these elements by minimizing contamination of herbal teas.[6]

In the Laboratory of Analysis and Pharmaceutical Research (LAYAFA) of the Institute of Pharmaceutical Research (INIFAR), quality control tests of medicines and natural products are carried out routinely, using the techniques and equipment applied for this project. On the other hand, the Animal Nutrition Research Centre (CINA), regularly performs determinations in Costa Rican forages, and silages, including the analysis and development of methodologies for the detection and estimation of trace elements, mycotoxins, and microbiological analyses.
In Costa Rica, the most consumed medicinal plant is *Matricaria chamomilla* L. (chamomile), followed by *Mentha piperita* L. (mint), *Aloe vera* (L.) Burm. F., *Rosmarinus officinalis* L., *Quassia amara* L., as well as black tea and green tea and other plants in the form of herbal teas. According to the National Health Survey for Costa Rica 2006, Profile and habits of consumption of natural products, made by the Central American Center of Population of the University of Costa Rica.[7]According to the Costa Rican Ministry of Health database,[8] there are 26 brands of tea products containing chamomile, 17 of which have chamomile as a single ingredient, and there are 17 registered tea brands in which mint as an ingredient, 5 of which contain mint as the only component.

Although there are both national and international regulations and acceptance criteria for all these determinations, in Costa Rica, there is no routine control for these type of natural products, including all the determinations that were analyzed in this project which allows asserting that medicinal plants would be safe and efficient.

MATERIALS AND METHODS

Sampling

The samples were taken at four different supermarkets in the province of San José. The sample taken was n = 1 360 envelopes of chamomile and n = 400 sachets of mint, of 22 products available in the market. On the other hand, 17 products were produced in Costa Rica and 5 imported from abroad.

Review of Labeling

Compliance with the labeling requirements was verified according to the Central American Technical Regulation (RTCA) "Natural Medicinal Products for Human Use, Labeling Requirements".[3] Which defines the information that must carry the label of the primary

packaging of the product (written in the Spanish language) which must comprehend the product name, pharmaceutical form, indications, method of use, qualitative and quantitative composition of the active ingredients (including scientific name), dosage form, registration number, manufacturer name and country of origin, net quantity or volume of the finished product, batch number, storage conditions, expiration date, posology, and route of administration.

3. Description of the organoleptic characteristics and determination of absence or presence of strange substance in the samples

We used the macroscopic and microscopic identification method of natural products, from the British Pharmacopoeia 2015 (BP), Appendix XI D of Foreign Matter. [10] A description of the morphology of the plant material in the sample was made.

The identification of foreign elements and organs that are not part of the plant was performed by direct stereoscopic observation with 6X magnification, to a minimum of 10 grams of the sample for each product. The foreign matter found was separated and weighed on an analytical balance and the percentage present in the sample was calculated.

Acceptance Criteria: foreign matter should not be more than 2 g/100 g and should be absent from insects, animal excreta, mold.

4. Determination of the average mass of tea bags

The average mass determination was performed according to BP Monographs of Herbal Drugs and Herbal Medicinal Products. The identification of the average mass was determined by weighing the added contents of 20 sachets.

Criteria for acceptance are set depending on the sample mass. For example, when $x < 1,5$, $1,5 < x > 2,0$ and $x > 2,0$ gno, more than 2 of 20 bags, should deviate more than 15, 10 and 7,5 % of the average mass, respectively.

5. Determination of residue of incineration

According to USP 38 <733> "Residue of Incineration".[11] This test was carried out in a crucible incinerated at 600°C and cold in a desiccator, accurately weighed. 1 to 2 grams of sample was weighed, moistened with not more than 1 mL of sulfuric acid and gently heated until the substance was fully carbonized, cooled and the process repeated, then cremated at 650°C until the residue was completely incinerated. It was cooled in a desiccator and

weighed accurately. The residue is calculated from the loss in mass.Acceptance criteria: residue of incineration cannot exceed more than 12g/100g.

6. Quantification of heavy metals (zinc, copper, lead, arsenic, cadmium, and mercury) in tea samples

This determination was performed according to the AOAC® Official Methods℠,[12] and as described elsewhere.[13] Briefly, no more than 0,5 g of sample was weighed into a TFMTM-PTFE vessel (DAP-60+, Berghof, Harretstrasse, Eningen, Germany). A mixture of concentrated nitric acid (6,0mL, ≈ 70 mL/100 mL) and hydrogen peroxide (2,0 mL, ≈ 30mL/100 mL) was added.

The complete digestion of the sample was performed using a Berghof Speedwave Four microwave digestor using the following a temperature program 170, 190, 210 and 50 ºC at 80 bar, 90 percent power and 10 minutes each; designed specifically for the digestion of herbs. Afterwards, Zn and Cu were analyzed using flame atomic absorption spectrophotometry (FAAS, acetylene-air flame) technique (Perkin-Elmer AAnalyst 800 atomic absorption spectrometer, Perkin-Elmer Corp., Norwalk, CT, USA). On the other hand, Pb, As, and Cd analyses were carried out either by graphite furnace atomic absorption spectrophotometry (GFAAS) technique. Finally, Hg was determined using flow injection metal hydride atomic absorption spectrophotometry (FI-MH-AAS) accomplished *in situ* using a 30 g/L $NaBH_4$ in 10 g/L NaOH solution. The radiation sources used included Hollow Cathode and Electrodeless Discharge Lamps for each metal used at wavelengths and spectral slit widths recommended by the manufacturer. For each metal and measurement performed standard calibration curves were prepared. Green Tea (*Camellia sinensis*) Leaves, NIST SRM 3254, were used as a quality control.

7. Determination of the microbial limit and absence of pathogenic micro-organisms

The determination of microbial boundary and absence of pathogenic microorganisms was performed according to USP 38 <2021> and <2022> "Nutritional and dietary supplements."

Determination of Microbial Limit: 10,0 grams of product was weighed into 90 mL of Tryptic Soy Culture Broth. Dilutions of 1 in 100 and 1 in 10 000 were then made. These dilutions were assembled duplicated, 1.0 mL in Petri dishes, with Potato Dextrose Agar and Tryptic Soy Agar and incubated at (22,5 ± 2,5) and (32,5 °C ± 2,5) °C for 5 and 2 days respectively. Subsequently, the presence of colonies in the plates was quantified. The amount found by the dilution factor was multiplied.

Acceptance criteria: There should be no more than 100 000 CFU of bacteria per gram or 1 000 CFU of fungi and yeasts per gram of product.

8. Absence of pathogenic microorganisms

***Escherichia coli*:** 10,0 grams of product were weighed into 90 mL of Tryptic Soy Culture Broth, incubated at (32,5 ± 2,5) °C for 2 days. Subsequently, 1 mL was transferred to 10 mL of MacConkey Broth and incubated at (43 ± 1) °C for two days, then streaked on MacConkey Agar to find suspicious colonies.

Acceptance criteria: There should be no *Escherichia coli* in 10 grams of product.

***Salmonella spp.*:** 10,0 grams of product were weighed into 90 mL of Tryptic Soy Culture Broth, incubated at (32,5 ± 2,5) °C for 2 days. Subsequently, 1 mL was transferred to 10 mL of Broth. Rappaport was incubated at (32,5 ± 2,5) °C for 48 hours, then streaked on Xylose Lysine Desoxycholate Agar to find suspect colonies.

Acceptance criteria: There should be no *Salmonella spp.* in 10 grams of product.

9. Determination of toxygenic fungi

The analysis of toxigenic strains of *Aspergillus flavus* and *Aspergillus parasiticus* was performed in parallel with the analysis of fungal and yeast counts, these species are slanted in plates with *Aspergillus Flavus* and *Parasiticus* Agar (AFPA base with 100 mg chloramphenicol supplementation, CM0731, Oxoid, Hampshire, England), as described previously. [14] Aflatoxigenic strains are identified by a yellow-orange color of the agar on the back of the plate. *A. falvus* var. *flavus* and *A. parasiticus* toxigenic strains were purchased from CBS-KNAW Fungal Biodiversity Centre (Utrecht, Netherlands) and used as quality control strains during assays.

10. Quantification of aflatoxins and ochratoxin a (OTA)

Aflatoxin analysis was performed using a modified ISO/IEC 17025 accredited version of the AOAC method 2003.02, as described in detail previously.[15] Several modifications were included to span the analysis for herbs. For the case of OTA, a representative (25.0 ± 0.1) g sample was used for extraction, 100 mL of an aqueous acetonitrile solution (60 mL/100 mL ACN) was added to the sample. The mixture was forced into contact and homogenized using a digital Ultra-turrax® at 18 000 rpm (T25, IKA® Werke GmbH & Co. KG, Staufen im Breisgau, Germany) during 3 min. The supernatant was removed and filtered by gravity

through a Whatman® 541 ashless filters (GE Health Life Sciences Little Chalfont, Buckinghamshire, United Kingdom). A representative aliquot of 3 mL was diluted to a total volume of 50 mL with phosphate saline buffer. The whole volume was passed through an immunoaffinity column (IAC) (OCHRAPREP®, R-biopharm, Darmstadt, Germany) using a SPE 12 port vacuum manifold (Supelco, Visiprep™, Bellefonte, PA, USA) operating at 15 mm Hg (ca. 0.55 mL per minute). Finally, 3 mL of methanol were used to elute analytes. The total volume recovered was concentrated tenfold under vacuum at 60 °C (Centrivap, LABCONCO, Kansas City, MO, USA) before injection into the chromatograph. An isocratic high-performance liquid chromatography method based on AOAC method 991.44 was used to assess OTA. Equipment consisted of an Agilent 1260 Infinity series HPLC with a quaternary pump (G1311B), a column compartment (G1316A) kept at 25 °C, a fluorescence detector (G1321B) and an autosampler system (G1329A) set to inject 20 mL (Agilent Technologies, Santa Clara, CA). Peak separation was accomplished using a 5 mm Agilent Zorbax Eclipse C18 column (3,0 mm × 150 mm). The mobile phase was set at a flow rate of 0,7 mL min^{-1} and consisted of acidified water (Type I, TOC 2 mg L^{-1}, 0,055 mS cm^{-1}, 1 µL formic acid per mL solution, pH = 2,1), and ACN 50:50. Native fluorescence for OTA was monitored at 247 and 480 nm (emission and excitation wavelengths, respectively).

RESULTS AND DISCUSSION

Review of Labeling

The study was set to verify compliance with the labeling requirements. We found that the product name and the legible label are the requirements that were met in all cases. The data that are mostly omitted in the labeling are administration route, dose, composition, and mode of use. The exclusion of these data represents a health risk, since the quality specifications, especially the microbiological specifications, are defined accordingly. The fact of not declaring doses or contraindications and warnings can contribute to reinforcing the population's belief that the natural implies innocuousness,[16] so it is a factor that should be included. The mode of use is another critical information that presents a low percentage of compliance since the microbiological legal stipulations are particular for products to which boiling water is added before consuming.

In general, mint herbal teas have a higher percentage of compliance with labeling requirements compared to chamomile tea. However, the number of teas of chamomile (*n* = 1

360) sampled was greater than mint teas (*n* = 400), so the results cannot be directly compared.

When comparing the percentage of compliance of the national products with the imported ones, it was observed that the domestic products presented greater labeling requirements compliance, 78 % about those introduced as result of trade in (i.e., 42 %).

Description of the organoleptic characteristics and determination of absence or presence of strange substance in the samples

The general organoleptic characteristics for 100 % of the herbal teas were reviewed, and the following description was found: "Filter paper envelope containing organic material with a dry appearance of different size and color." Since it was a grounded natural product, it was not possible to perform the macroscopic identification of the structures of the organic material contained in the products. Regarding the absence of foreign matter, at least 66 % of the samples showed some of the following structures: insect parts, small stones, plastic fragments. This type of contamination may be due to the environmental factors associated with the site of growth of the plants; these places may also have nearby, cattle or wild birds that lead to the presence of insects.[17] On the other hand, the lack of implementation of good manufacturing practices can favor contamination of the distinctive order along the production chain.[18]

Determination of the average mass of tea bags

From the total of *n* = 22 products analyzed, using British Pharmacopea criteria, *n* = 21 were evaluated under criterion A, since they weighed less than 1,5 g and only *n* = 1 used criterion B since its labeling was 2 g. Only one product did not meet the specification because 14 bags of this product, presented variations of up to 50 % concerning the average weight of the bags, evidencing with this, that the filling process is not under control.

About this test, it is important to mention that the criterion of acceptance is related only to the uniformity in the filling of the bags and does not take into account the amount of product declared per bag. If the criterion takes on account the product reported in the label, the percentage of non-fulfillment would be higher. Bearing in mind the quantity tagged, values between 73 % and 130 % of the labeling were observed, the average content of seven products is below 100 % of the labeling; Two products were below 90 % and five above this value but less than 100 %. This parameter is significant when considering natural products

with medicinal properties since the amount declared is directly related to the dose of the product.

Determination of residue of incineration

Compliance with the limit allowed for total ash was 50 % for mint tea and 69 % for chamomile tea. In both cases, a greater conformity of this test was observed when it was carried out during the dry season, as compared to the ones made during the rainy season.

The method allows quantifying the percentage of mineral components present in the plant. These minerals may be from the plant or come from foreign material that adheres to it by its contact with the soil and can be sand as well, so an ash content higher than allowed is indicative of an inadequate collection and storage procedure in most cases. These results are consistent with the assay of determination of absence or presence of strange substances.

Quantification of heavy metals zinc, copper, lead, arsenic, cadmium, and mercury in tea samples

Regarding the determination of the heavy metals, none of the samples of the analyzed products showed zinc [38,4-14,6] mg kg^{-1}, copper [12,1-5,5] mg kg^{-1}, lead and arsenic [31,2-4,5] µg kg^{-1} concentrations higher than the limit value suggested for medicinal herbs by the World Health Organization.[19,20] Nevertheless, Cadmium values were obtained in the different analyzed products that vary in the range of [585,2–7,25] µg kg^{-1}. In the case of Cadmium, the regulations have been established between (0,3-0,5) mg kg^{-1}; *n* = 2samples exceed these values. The case of mercury is similar concentrations have been obtained for samples tested between (164,97–64,8) µg kg^{-1} of which *n* = 2 exceed the maximum limits of 0,1 mg $kg^{-1}$for this type of product. The legislation for mercury is relatively lax and allows concentrations in the mg/kg range. From the limits that are available internationally, our results seem to indicate the presence of arsenic in concentrations of [27,8 – 4,5] µg kg^{-1} which are relatively small and far from what is established by regulation. A low prevalence of arsenic in tea has been described in other studies.[21] Considering the low solubility of these contaminants in water and the presence of these in the dried tea herbs, a reduction of the concentrations of theses contaminants in the infusion. It has been shown that the contaminant fraction transferred to the liquid, will depend on the infusion preparation time, also the authors suggest establishing the legislation for herbal infusions.[21]

Determination of the microbial limit and absence of pathogenic microorganisms

A 100% compliance of the products was evidenced in the lack of *E coli* and *Salmonella spp.*, as well as with the specification for fungi and yeasts. Few products (n = 2, [$3{,}0\times10^{2}$ to $3{,}6\times10^{6}$ CFU g^{-1}]) of the n = 22 analyzed exceeded the limit of aerobic bacteria (< 10^{5} CFU g^{-1}); This concerning the specifications established in the Central American Technical Regulation Pharmaceutical Products, Natural Medicinal Products for Human Use, Quality Verification.

Noteworthy, the products that did not meet the acceptance criterion for screening aerobic bacteria and limit of enterobacteria are of national production. However, if the USP specifications for these tests were applied, 95 % of the samples do not meet the specifications for fungi and yeast count and, 38 % exceeded the limit of enterobacteria (i.e., < 100 g^{-1}). The RTCA does not contemplate the limit of enteric bacteria, which is an important parameter of quality that is not being evaluated in Central America; as well as the determination of *Salmonella spp*.

Determination of toxygenic fungi quantification of aflatoxins and ochratoxin A

In none of the n = 22 samples analyzed was the presence of *A. flavus* or *A. parasiticus* which are mainly responsible for aflatoxin contamination.[22] Furthermore, for all four aflatoxin fractions (i.e., B_1, B_2, G_1, and G_2) samples showed values below their limit of detection (i.e., 0,005, 0,078, 0,099, and 0,067, respectively).Regarding the presence of OTA, in the herbal teas,, mint teas exhibited significantly higher concentrations of OTA, i.e., [0,53-0,32] µg kg^{-1}, when compared to those obtained from chamomile tea; i.e., [0,41-0,14] µg kg^{-1} (p < 0,05, student t, independent samples). No association was found between OTA total fungi counts (r = 0,085) and n = 4 samples were found below analytical limit of detection (i.e., < 0,004 µg kg^{-1}). On the other hand, the presence of OTA justifies the detection of the potentially toxicogenic *Fusarium* and *Penicillium* fungi.[23]

CONCLUSION

Products were found, both domestic and imported; which do not comply with the RTCA quality specifications, and monographs of pharmacopeias, it is evident the need to continue to carry out such monitoring by the National Regulatory Authority, to avoid the risk of the population consuming products that can endanger their health.

When comparing the quality specifications in different official references, there were differences concerning limits for contaminants such as heavy metals and microbial counts

and determination of pathogens. Overall, mint tea showed significantly lower concentrations of contaminants including fungi.

It is important for the natural products industry to implement WHO Good Manufacturing Practices for herbal medicines, WHO guidelines on assessing quality of herbal medicines with reference to contaminants and residues, as well as, proper storage and distribution practices to ensure the quality and safety of their products with a view to meeting the objective of the WHO strategy about traditional medicine 2014-2023.

ACKNOWLEDGEMENTS

The Office of the Vice Provost for Research of the University of Costa Rica supported this project financially, grant B3228.

REFERENCES

1. World Health Organization. Monographs on selected medicinal plants. (1) Geneva, 1999; 86-92.
2. WHO Department of Essential Medicines and Health Products. The International Pharmacopoeia. Sixth edition, 2016.
3. Decreto Ejecutivo N° 37079-COMEX-MEIC-S. Reglamento Técnico Centroamericano: Productos Farmacéuticos, Productos Naturales Medicinales para Uso Humano. Verificación de la Calidad. RTCA 11.03.56:09. Vigente desde el 9 de mayo de 2012.
4. Kabelitz L, Sievers H. Contaminants of medicinal and food herbs with a view to EU regulations. Innovations Food Technol, 2004: 25-7.
5. World Health Organization (WHO). Guidelines for assessing quality of herbal medicines with reference to contaminants and residues, 2007: 11.
6. Halt, M, Klapec, T. Microbial populations in medicinal and aromatic plants and herbal teas from Croatia. Ital. J. Food Sci, 2005; 17: 349–354
7. Centro Centroamericano de Población de la Universidad de Costa Rica. Perfil y hábitos de consumo de productos naturales. Encuesta Nacional de Salud para Costa Rica, 2006.
8. Disponible en: http://www.ministeriodesalud.go.cr/index.php/empresas-servicios-productos-registrados-ms, accesado el 2 de abril de 2013.
9. Decreto Ejecutivo N° 37079-COMEX-MEIC-S. Reglamento Técnico Centroamericano: Productos Farmacéuticos, Productos Naturales Medicinales para Uso Humano. Requisitos de Etiquetado. RTCA 11.04.41:06. Vigente desde el 9 de mayo de 2012.
10. British Pharmacopoeia 2015 BP.

11. United States Pharmacopeial Convention USP XXXVIII: United States Pharmacopoeia. 38 ed. Estados Unidos de Norte América: United Book Press, 2015.
12. Official Methods of Analysis^{SM} of AOAC$^{®}$ International, 20th edition, 2015.
13. Granados-Chinchilla F, Prado Mena, S, Mata Arias L. Inorganic contaminants and composition analysis of commercial feed grade mineral compounds available in Costa Rica. Int J Food Contam, 2015; 2: 8 DOI 10.1186/s40550-015-0015-1.
14. Pitt JI. *Penicillium vindicatum*, *Penicillium verrucosum* and production of ochratoxin A. Appl Environ Microbiol, 1987; 53: 266-9.
15. Granados-Chinchilla F, Molina A, Chavarría G, Alfaro-Cascante M, Bogantes-Ledezma D, Murillo-Williams A. Aflatoxins occurrence through the food chain in Costa Rica: Applying the One Health approach to mycotoxin surveillance. Food Control, 2017; 82: 217-226.
16. Manteiga R, Park DL, Ali SS. Risks associated with consumption of herbal teas. Rev Environ Contam Toxicol, 1997; 150: 1-30.
17. Zweifel C, Stephan R. Spices and herbs as source of Salmonella-related foodborne diseases. Food Res Int, 2012; 45: 765-769.
18. Chan K. Some aspects of toxic contaminants in herbal medicines. Chemosphere, 2003; 52: 1361-71.
19. World Health Organization (WHO). Guidelines for assessing quality of herbal medicines with reference to contaminants and residues, 2007. http://apps.who.int/medicinedocs/documents/s14878e/s14878e.pdf.
20. Kosalec I, Cvek J, Tomić S. Contaminants of medicinal herbs and herbal products. Arh Hig Rada Toksikol, 2009; 60: 485-501.
21. Shekoohiyan S, Ghoochani M, Mohagheghian A, Mahvi AH, Yunesian M, Nazmara S. Determination of lead, cadmium and arsenic in infusion tea cultivated in north of Iran. Iranian J Environ Health Sci Eng., 2012; 9: 37. doi:10.1186/1735-2746-9-37.
22. Dutta BK, Dutta S, Nath PK. Mycotoxin production potential of mycoflora in tea. In: Jain, N.K., Rahman, F., Baker, P. (Eds.), Economic Crisis in Tea Industry, Studium Press LLC, 2008; 221–232.
23. Cabañes FJ, Bragulat MR, Castellá G. Ochratoxin A Producing Species in the Genus *Penicillium*. Toxins, 2010; 2: 1111-1120.