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Research Article

Physicochemical and Microbiological Assays for Quality Evaluation of a Brand of *Mentha piperita* Tisanes in Costa Rica Market: Employment of the Central American Technical Regulation

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ABSTRACT

Mentha piperita is a natural hybrid of water mint (*Mentha aquatica*) and spearmint (*Mentha spicata* L.), that are consumed as tisanes. For that reason, the present work evaluated physicochemical and microbiological aspects for the quality control of a commercial brand of *Mentha piperita* herbal tea distributed in Costa Rica, using assays established by the Central American Technical Regulation (RTCA) 11.03.56.09 (Pharmaceutical products. Natural medicinal products for human use. Quality verification), and determined if they have uniformity for different batches of this brand. To verify the quality of three batches of this product, the following assays were done: Labeling requirements, organoleptic, minimum fill, foreign organic matter, loss on drying, total ash, acid-insoluble ash, lead and arsenic limits, microbial enumerations and specific microorganisms (*E. coli* and *Salmonella* sp.) assays. As conclusion, the batches were in compliance for all the assays, except for labeling test, since information corresponding to the primary (batch number and expiration date) and secondary packaging (qualitative-quantitative composition of active ingredients, interactions and adverse effects) were not presented. Furthermore, one of the batches did not have information about the employment, contraindications and warnings. Also, the manufacturing company maintained a good reproducibility between one batch and another, and the improvements that can be made are in the product labeling and better controls in its filling.

Keywords: Natural product, *Mentha piperita*, quality control, tisane, pharmacopoeia, Central American Technical Regulation.

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INTRODUCTION

Since the beginning of humanity, individuals have been given the task of exploiting the environment. Plants have become an important component, especially those for medicinal use¹. Through various studies, the benefits of

natural products with pharmacological properties have been proven in the well-being and health conservation².

One of these products is peppermint, whose scientific name is *Mentha piperita*. This plant belongs to the Lamiaceae family³, it grows in Europe, North America and North Africa temperate climates⁴, and is a natural

hybrid of water mint (*Mentha aquatica*) and spearmint (*Mentha spicata* L.)^{5, 6}. It has been reported to possess many properties, including antimicrobial, anti-inflammatory, medium anesthetic, antispasmodic, antiulcer, cytoprotective and hepatoprotective⁷, antioxidant, antidiabetic and antitumor⁸. In addition, the aqueous extract of its leaves has demonstrated significant antiviral activity against viruses: Influenza A, Newcastle, Herpes Simplex, *Vaccinia* and West Nile in egg and cell cultures⁹. These qualities, together with its aromaticity and therapeutic effects, have opened the way for its use in the pharmaceutical industry for the development of cosmetics, medicines and personal care products¹⁰. However, its presentation and preparation influence the amount of active ingredient or natural product available at the time of consumption, affecting its therapeutic effect¹¹.

One of the consumption forms of peppermint is through the tisanes use. The popularity of these herbal beverages is mainly due to the high availability of herbal teas and medicinal plant formulations, their low price, and the virtual absence of side effects and biological aggressiveness, and a recent trend observed regarding with their use as replacement or complement for medicines and traditional drugs¹². Precisely because of these advantages, there is usually no control over the ingested quantity, as well as the populations that could present a greater susceptibility to a certain active principle and, therefore, have an undesired effect. In addition, their compounds can vary their content qualitatively and quantitatively in the different batches sold, since the raw material can be found in different environmental conditions¹³. Other important factors that may compromise the herbal teas quality is the inadvertent contamination by microbial or chemical agents during any of the production stages and the substitution of the authentic plant (substitution by a different vegetable species or an incorrect part of the plant)¹⁴. Therefore, quality control of these products is necessary. Thus, its use and its commercialization in any market will comply with the quality, safety and efficacy standards stipulated in the national regulations.

In this way, the present work evaluated physicochemical and biological aspects for the quality control of a commercial brand of *Mentha piperita* herbal tea distributed in Costa Rica, using assays established by the Central American Technical Regulation (RTCA) 11.03.56.09 (Pharmaceutical products. Natural medicinal products for human use. Quality verification)¹⁵, and determined if they have uniformity for different batches of this brand.

MATERIALS AND METHODS

Sampling and selection of quality control assays

Three batches of *Mentha piperita* tisanes were sampled (five boxes of each one), whose brand is commercialized in Costa Rica. These batches were obtained in different national supermarkets located in the Great Metropolitan Area. The assays performed are established by RTCA 11.03.56.09¹⁵. In this regulation, it is established that the requirement to use a procedure of a certain requested

assay is that it is in an official book for Costa Rican regulations.

Labeling requirements

The four items of the primary packaging and the 19 items of the secondary packaging indicated in the RTCA 11.04.41.06 (Natural medicinal products for human use. Labeling requirements)¹⁶ were evaluated. They are shown in **Table 1**.

Organoleptic assays

The odor, color, and flavor characteristics were determined from ten tisanes of the three available batches, as indicated in the British Pharmacopoeia 2016¹⁷. The content of the tisanes had to have a characteristic and Penetrating smell, as well as a moss green color and an aromatic flavor.

Minimum fill assay

The procedure of the general chapter <755> of the United States Pharmacopeia (USP) 40¹⁸ was carried out. A sample of 10 tisanes was selected and each of the envelope content was individually weighed on an Adam® PW 254 analytical balance. The net content of the 10 tisanes should not be less than the declared amount, and the net content of any individual envelope should not be less than 90 % of the declared amount.

Determination of foreign organic matter

The foreign matter test was carried out according to the British Pharmacopoeia 2016¹⁷. The inspection of foreign material was carried out with the help of a Magnifier Lamp® 8611A-D and the exact weight was determined using an ADAM® PW 254 analytical balance. The criteria established that a maximum of 5 % of stems and a maximum of 2 % of foreign matter should be obtained.

Loss on drying assay

The procedure of the general chapter MGA-FH 0080 of the Herbalist Pharmacopoeia of the Mexican United States was done¹⁹. Around 3 g of each of the batches was accurately weighed using the ADAM® PW 254 analytical balance, using a melting pot that was previously brought to constant weight. Then, the melting pot with the sample was placed in a Thermo Scientific® Heratherm OG5 100 oven at a temperature of 105 °C for two hours. Subsequently, it was cooled in a desiccator and then the melting pot was weighed again with the dried sample to calculate the lost percentage. The sample should be dried until two consecutive weights did not differ by more than 0,0005 g. The lost percentage should not be greater than 15 %.

Total ash assay

The procedure of the general chapter MGA-FH 0060 of the Herbalist Pharmacopoeia of the Mexican United States was followed¹⁹. Around 2 g of the tisane sample of each batch was accurately weighed on an ADAM® PW 254 analytical balance, in a melting pot previously brought to constant weight. Then, the material was incinerated by gradually increasing the temperature to

600 °C in a Thermo Scientific® F47915 muffle furnace, for two hours. Subsequently, it was cooled in a desiccator for 30 minutes and the weight of the ashes was determined using the analytical balance. After that, the samples were continued drying until the difference of two weights was less than 0,0005 g. The acceptance criteria implied that the percentage of total ash should not be greater than 15 %.

Acid-insoluble ash assay

The procedure of the general chapter MGA-FH 0060 of the Herbalist Pharmacopoeia of the Mexican United States was carried out¹⁹. To each of the total ash samples obtained from the previous test, 25 ml of 3 N hydrochloric acid was added. Then, they were covered with a watch glass and heated to boiling for 5 minutes. Next, the insoluble material was collected on a Boeco® grade 389 filter paper, which was then washed with hot water. Subsequently, the filter paper containing the insoluble material was transferred to the melting pot, which was previously brought to constant weight. Then, it was incinerated for two hours at a temperature of 600 °C in a Thermo Scientific® F47915 muffle furnace. Subsequently, it was cooled in a desiccator for 30 minutes and the weight of the acid-insoluble ashes was determined using an ADAM® PW 254 analytical balance. After that, the samples were continued drying until the difference of two weights was less than 0,0005 g. The acceptance criteria implied that the insoluble ash percentage should not be more than 1,5 %.

Heavy metal limit assay (lead)

The evaluation of heavy metals was made according to the procedure described for method A in the general chapter 2.4.8 of the European Pharmacopoeia 5.0²⁰. For each batch sample, two tisanes were taken and 22,00 ml of distilled water were added. It was left to rest for 5 minutes and after this time, the tisanes liquid was extracted by compressing them the maximum, pouring it in the same beaker. After this, an aliquot of 12,00 ml was taken for the sample, of 2,00 ml for the standard and of 2,00 ml for the blank, which were poured in test tubes. 10,00 ml of lead standard (1 ppm) was added to the tube used for the standard and 10,00 ml of distilled water for the tube used for the blank, respectively. Next, 2 ml of acetic acid-sodium acetate buffer (pH 3,5), 0,5 ml of dilute acetic acid and 1,2 ml of thioacetamide solution (previously heated in a steam bath) were added to each tube. The result was consistent if both the blank and the sample had a brown color of less intensity than the pattern.

Arsenic determination limit assay

The test was carried out according to the procedure described for method A in the general chapter 2.4.2 of the European Pharmacopoeia 5.0²⁰. In a beaker the tisane was placed and 11,00 ml of distilled water was added. It was left to rest for 5 minutes and after this time the tisane was extracted, compressing as much as possible to pour the liquid in the same beaker. After this, an aliquot of 10,00 ml was taken and placed in a conical flask. Then, 15 ml of 12 M HCl, 0,1 ml of stannous chloride and 5 ml of potassium iodide 16 %w/v were

added. It was allowed to stand for 15 minutes and then 3 g of zinc were added to heavy chips on a Sartorius® M-prove scale balance. Next, the two parts of the apparatus were assembled and the flask was placed in a water bath with a temperature range between 90 and 100 °C. The standard was prepared in the same way, using 1,00 ml of arsenic standard solution (1 ppm). After two hours, the stain produced on the mercury bromide paper in the sample should not be more intense than that of the reference standard.

Microbial enumeration assay

The procedure of general chapter <201> of USP 40 was performed employing the plate count method¹⁸. For the total aerobic microorganism enumeration, a sample of 10 g of each of the batches was taken and dissolved in 90 ml of Bacto™ casein-soybean digest broth. Then, two Petri dishes were prepared for each culture medium (Bacto™ casein-soybean digest agar for mesophilic microorganisms and Liofilchem™ potato dextrose agar for filamentous fungi and yeasts), according to an appropriate dilution for the subsequent counting. The Petri dishes with casein-soybean digest agar were incubated at a temperature of 33 °C for 48 hours, while those of potato dextrose agar were incubated at 22,5 °C for 5 days. After the incubation, the colony forming units present in each of the dishes were counted, the arithmetic mean of the counts for each of the culture media was calculated and the number of colony forming units (CFU) per gram of product was determined. According to RTCA 11.03.56:09¹⁵, the product is in compliance if the total aerobic microorganism enumeration does not exceed 10⁷ CFU/gram of product and the filamentous fungi and yeast enumeration does not exceed 10⁵ CFU/gram of product.

Specific microorganisms assays

The procedure of general chapter <202> of USP 38 was carried out for the determination of the presence of *Salmonella* sp. and *E. coli*¹⁸.

E. coli absence assay

Around 10 g sample of each batch were transferred to a flask with 90 ml of Bacto™ casein-soybean digest broth. Subsequently, the sample was incubated at a temperature of 33 °C for 24 hours. Next, a 1,00 ml sample aliquot was taken to a flask with 100 ml MacConkey broth, mixed and incubated at 44 °C for 24 hours. After this period, a 1,00 ml sample was taken to inoculate two MacConkey agar Petri dishes. The dishes were incubated at 33 °C for 24 hours and after that, the dishes were examined. According to RTCA 11.03.56:09¹⁵, the product is in compliance with the *E. coli* absence.

Salmonella sp. absence assay

From the sample prepared for the absence of *E. coli* assay, an 1,00 ml aliquot of each batch sample was taken and added to 10,00 ml of Difco™ Rappaport Vassiliadis *Salmonella* enrichment broth. It was mixed and incubated at a temperature of 33 °C for 24 hours. Then, a 1,00 ml sample was taken to inoculate two Petri dishes of Difco™ xylose lysine deoxycholate agar. These were incubated at a temperature of 33 °C for 24 hours and the

inoculated plates were examined. According to the RTCA 11.03.56:09¹⁵, the product is in compliance in case of *Salmonella* sp absence.

RESULTS AND DISCUSSION

To begin the evaluation of the quality of the three batches of a peppermint tisanes brand commercialized in Costa Rica, a checklist was developed. In this way, it is possible to check the presence of the labeling information in the primary and secondary packages, as shown in **Table 1**. The information present on the labeling allows the consumer to make conscious and informed use of the product. Therefore, it must be easy to read and understand, and thus, allow the transmission of information to be effective²¹. Incorrect labeling with unclear or absent information can lead to dosage confusions, treatment discontinuations, incorrect administration, and many other inconveniences and errors that can generate a risk to the people's health²².

As of the review carried out, the three batches lack important information about the batch number and expiration date for the primary package. The expiration date marks the point at which the active ingredient has not been tested in a longer time in terms of safety and efficacy²³. Its absence may endanger the consumer safety, given that if the product is consumed subsequent to it, it is not possible to ensure its microbiological and physical stability, as well as its declared nutritional content²⁴. In addition, by not presenting the batch number it would be impossible to determine the tisane consumed tisane origin and in case of any inconvenience, it would not be possible to remove the rest of the product, since the batch to which it belongs is not known²⁵.

In the case of secondary packaging, no information was found regarding the quali-quantitative composition of the active ingredients (including scientific name), the interactions and the adverse effects associated with the use of the product (**Table 1**). In addition, the labeling of batch 3 did not have information about the use mode and the contraindications. In the case of the active ingredients, the information is essential, given that this

medicinal plant has different active ingredients that generate its pharmacological effect, and that can be found in distinct concentrations. The essential oils are menthol (33-60 %), menthone (15-32 %), isomenthone (2-8 %), 1,8-cineole or eucalyptol (5-13 %), menthyl acetate (2-11 %), pulegone (0,5-1,6 %) and carvone (1 %). It is important to mention that these values vary according to environmental factors such as plant maturity, variety, geographical region and processing conditions²⁶.

Regarding the interactions, it is known that peppermint oil can orally increase blood levels of felodipine and simvastatin, as well as cyclosporine. It can also interfere with the processing of certain drugs that use the cytochrome P450^{27, 28}. Other possible interactions are with antacids, calcium channel blockers and drugs that lower blood pressure²⁷, as well as with warfarin²⁹.

Regarding the adverse effects, it is indicated that they are claimed to be usually mild, such as gastro esophageal reflux, heartburns, nausea, vomiting, allergic reactions and diarrhea³⁰. In other words, it has a relatively low profile of adverse effects, which makes it a favorable treatment^{31, 32}.

As for the organoleptic characteristics, it was found that the three batches were in compliance with the specifications established in the British Pharmacopoeia¹⁷. These characteristics are determined according to the similarities and differences of the organoleptic properties described objectively for a plant³³. These characteristics allow determining the degree of adulteration of the raw material, which can cause health and safety concerns and have implications for public health³⁴. With the assays, it was obtained that the three samples of the analyzed batches showed moss green color and a penetrating smell, as well as the aromatic flavor. This is consistent with that established in the literature used. In addition, in the ten tisanes evaluated for each batch, other characteristics of the leaves could not be observed, such as the presence of purple veins³⁵, due to the degree of crushing of the raw material.

Table 1: Results of the labeling assay of the primary and secondary packaging items for three batches of a brand of *Mentha piperita* tisanes commercialized in Costa Rica.

Required information	Fulfillment		
	Batch 1	Batch 2	Batch 3
Primary packaging			
Brand name	Yes	Yes	Yes
Batch number	No	No	No
Expiration date	No	No	No
Manufacturer laboratory name or logo	Yes	Yes	Yes
Secondary packaging			
Product name	Yes	Yes	Yes
Pharmaceutical form	Yes	Yes	Yes
Indications	Yes	Yes	Yes
Employment form	Yes	Yes	No
Quali-quantitative composition of active ingredients	No	No	No
Registration number	Yes	Yes	Yes

Manufacturer name and country of origin	Yes	Yes	Yes
Net amount of the finished product	Yes	Yes	Yes
Batch number	Yes	Yes	Yes
Storage conditions	Yes	Yes	Yes
Expiration date	Yes	Yes	Yes
Contraindications and warnings	Yes	Yes	No
Interactions	No	No	No
Adverse effects	No	No	No
General labeling	Yes	Yes	Yes
Special labeling	Yes	Yes	Yes
Posology	Yes	Yes	Yes
Administration route	Yes	Yes	Yes
Use during pregnancy, breastfeeding, elderly and children under 2 years	Yes	Yes	Yes

On the other hand, the results for the minimum fill assay are shown in **Table 2**. They denote that the net content of the 10 tisanes of each of the used batches was not less than the declared amount. Also, the net content of each individual tisane was not less than 90 % of the declared amount, so the three batches are in accordance with the specifications of USP 40¹⁸. However, it is not specified which is the maximum that the samples can contain with respect to the declared labeling. For batches 1 and 2, there was not a very distant average with respect to the labeling. But, in the case of batch 3, the average of the ten tisanes was 1,4884 g, that is 13 % of excess with respect to that indicated on the product secondary packaging.

In the absence of a maximum limit, it can not be determined if this batch would be rejected in a quality control. This absence is worrisome, because as with

drugs in general, there is a therapeutic range, which is a ratio that compares the blood concentration at which a drug causes a therapeutic effect with respect to the amount that causes death (in studies animals) or toxicity (in human studies)³⁶. According to the literature, the usual dose for peppermint corresponds to 1,5 to 3 g three times a day as an infusion (adults), 3 to 5 g daily (children from 4 to 10 years) and 3 to 6 g (children of 10 to 16 years)³⁷. Along with this, it is known that high doses of *Mentha piperita* can be hepatotoxic and nephrotoxic, and even cause interstitial nephritis and acute renal failure³⁰. Therefore, it is advisable to evaluate the effect of the excess of tisane in the consuming population and to evaluate whether by using more stringent statistical controls in the filling process of the herbal teas, it would be possible to decrease this variation³⁸.

Table 2: Results of the minimum fill assay for different batches of a *Mentha piperita* tisanes brand commercialized in Costa Rica.

Tisane	Batch 1		Batch 2		Batch 3	
	Weight (g)	Labeling percentage (%)	Weight (g)	Labeling percentage (%)	Weight (g)	Labeling percentage (%)
1	1,3453	103	1,3607	105	1,4640	113
2	1,3627	105	1,3870	107	1,4966	115
3	1,3194	101	1,3640	105	1,4521	112
4	1,2794	98	1,3774	106	1,4408	111
5	1,3519	104	1,3444	103	1,5398	118
6	1,3410	103	1,3567	104	1,5202	117
7	1,3237	102	1,3648	105	1,5053	116
8	1,3253	102	1,3817	106	1,4811	114
9	1,2803	98	1,4083	108	1,5028	116
10	1,3312	102	1,2509	96	1,4808	114
Mean value	1,3260	102	1,3596	105	1,4884	114

With reference to the determination of foreign organs and foreign matter, both components were separated and weighed. The data obtained is shown in **Table 3**. The percentage of foreign organs was between 0,1 and 0,5 % for the three analyzed batches, while for foreign matters it was found between 0,2 and 0,3 %. The final result of this assay was in compliance of the three batches. It

should be mentioned that in batch 1 small stones, red seeds and a small arthropod were found, while for the other two, only small stones were seen. As for foreign organs, it was possible to appreciate woody tissues and stems in the three batches. This determination is relevant, because it ensures that the stated herbal drugs were made from the specific part of the plant and are

devoid of other parts of the same plant or other plants. If values higher than those established for the pharmacopoeia are found, this may mean the natural product adulteration. From the point of view of natural products, adulterations may involve both the presence of

a part of the plant other than the recommended or the presence of dust, mold, whole insects, fragments thereof or other plants³⁹. Regardless of the adulteration found, the final result is obtaining a product with a quality, safety and efficacy lower than required.

Table 3: Foreign organs and foreign matter in samples of 10 tisanes for three different batches of a *Mentha piperita* tisanes brand commercialized in Costa Rica.

Batch	Initial sample weight (g)	Foreign organs weight (g)	Foreign organs percentage (%)	Foreign matter weight (g)	Foreign matter percentage (%)
1	13,6242	0,0742	0,5	0,0306	0,2
2	13,5419	0,0548	0,4	0,0463	0,3
3	14,6422	0,0214	0,1	0,0281	0,2

For the loss on drying assay, values of 9,6, 9,8 and 10,1 % were obtained (**Table 4**), which are below the maximum value established by the Herbalist Pharmacopoeia of the Mexican United States. Therefore, the three batches of *Mentha piperita* tisanes commercialized in Costa Rica were found to be in compliance for this assay. This has been cataloged as an older, critical and fundamental unit operation technique for the postharvest conservation of medicinal plants⁴⁰.

The importance of this amount of water is due that the reduction of the product moisture content below a certain value reduces the physicochemical and microbiological deterioration of the product and allows its storage in a stable condition, increasing the useful life of the final product^{41,42}. Therefore, it represents one of the best alternatives to increase its stability. These values reaffirm the results that were obtained in the microbiological tests, as will be discussed later.

Table 4: Loss on drying percentage of the different evaluated batches of a brand of *Mentha piperita* tisanes commercialized in Costa Rica.

Batch	Initial weight (g)	Final weight (g)	Loss on drying percentage (%)
1	2,8136	2,5305	10,1
2	2,6930	2,4282	9,8
3	2,6384	2,3838	9,6

In relation with the results obtained for the total ash and insoluble-acid ash assays, both showed lower values for all the analyzed batches than established by this same pharmacopoeia, being between 8,1 and 9,6 %, and between 0,5 and 0,6 % for total ash and insoluble-acid ash, respectively. In this way, there was compliance for both assays in each of the analyzed batches. The information is summarized in **Table 5**. The determination of total ash reveals the content of mineral salts or inorganic matter present in the tisane, which could be physiological (coming from the mineral components of the plant itself) or derived from foreign matter that is attached to the surface of the product⁴³. On the other hand, the procedure to determine acid insoluble

ashes from the incineration of the peppermint tisane under study measures the amount of silica present in the sample, especially in the form of sand and siliceous earth⁴⁴. Therefore, both methods are simple and important to evaluate the quality and purity of herbal medicine⁴⁵. In summary, no significant contamination was obtained from inorganic matter and mineral salts in the *Mentha piperita* tisanes. This shows that the necessary care during the manufacturing process did exist. It is essential to mention that the presence of more of these ashes with respect to the established also means adulteration, contamination, substitution or neglect in the preparation of the product⁴⁶.

Table 5: Total ash and insoluble-acid ash percentages of the different evaluated batches of a brand of *Mentha piperita* tisanes commercialized in Costa Rica.

Batch	Initial weight (g)	Total ash weight (g)	Total ash percentage (%)	Insoluble-acid ash weight (g)	Insoluble-acid ash percentage (%)
1	2,7186	0,2209	8,1	0,0154	0,6
2	2,2058	0,2117	9,6	0,0116	0,5
3	2,7389	0,2242	8,2	0,0159	0,6

Other quality control assays carried out were those corresponding to the limit assays for the lead and arsenic determinations. Various items including foods have been

found to contain toxic compounds, including these mentioned⁴⁷. Both metals contained in this type of herbal products enter the human body and can cause negative

effects over the normal functions of different organs and systems, such as central nervous system, liver, lungs, heart, kidney and brain, leading to hypertension, abdominal pain, skin eruptions, intestinal ulcer and different types of cancer⁴⁸. In the case of lead, is a heavy metal that is stored in bone, blood and soft tissues (brain, spleen, kidneys, liver, and lungs). Its presence leads to the production of free radicals that causes oxidative damage of cellular components, and interferes DNA transcription, vitamin D synthesis, and enzymes function that maintains the cell membranes integrity⁴⁹. Other symptoms related to the lead poisoning are abdominal pain, arthralgia, muscle stiffness, anorexia, weight loss, tiredness, lethargy, insomnia, headache, neuritis, convulsions, delirium tremens, coma, renal failure, hypertension, encephalopathy, psychological and behavioral changes⁵⁰. In the case of the conducted assay for this heavy metal, the three studied batches were in compliance according to the European Pharmacopeia, because the lead standard exhibited a less intense color precipitate in comparison with the samples of these batches.

Furthermore, for the arsenic test, the three batches of peppermint tisanes were also found according to what was indicated in the European Pharmacopeia, that is, it was lower than the allowed limit. This was due to the fact that none showed a more intense color stain with respect to the arsenic pattern. The assay is made because arsenic can be found in the groundwater, frequently used for human consumption, as well as for crops irrigation⁵¹. Therefore, the arsenic contamination in groundwater is a serious public health threat worldwide⁵². In terrestrial environment, the inorganic forms of As (trivalent arsenite (As^{3+}) and pentavalent arsenate (As^{5+})) are more prevalent and toxic than the organic forms in general. Its ingestion causes effects on general protein metabolism by reacting with sulfhydryl groups existing in cysteine residues⁵³. The ingested arsenic can cause lung, bladder and skin cancer in humans⁵⁴.

Finally, the microbial enumerations of mesophilic aerobic microorganisms, as well as of filamentous fungi and yeasts were made. These assays are developed since soil contamination can cause microbial contamination of herbal products. Likewise, after harvesting, microorganisms can grow and cause spoilage or even produce toxins during transport and storage if the conditions, mainly temperature and water content, are favorable⁵⁵. Microbiological assessment of non-sterile products can reduce or even eliminate the therapeutic effect of drugs or cause drug-induced infections, because they can change the chemical, physical and organoleptic properties of the drugs or change the contents of active ingredients. Furthermore, microorganisms can convert drugs to toxic products⁵⁶. In the case of samples from the three batches evaluated, a smaller number of CFU per gram of product was found with respect to the limit established by RTCA 11.03.56:09¹⁵. Therefore, all of them were satisfied for the presence of these microorganisms. The presence of acceptable quantities of bacteria, fungi and yeasts in the product is indicative of Good Manufacturing Practices (GMP) in conjunction

with the use of resources in good state and in the adequate conditions.

Regarding the specific microorganisms, in all the three samples of the analyzed batches of *Mentha piperita* there was an absence of *E. coli* and *Salmonella* sp. The *E. coli* is an important cause of infectious diarrhea⁵⁷. This poisoning is caused by the Shiga toxin, which generates in patients a picture of abdominal cramps and diarrhea that can progress to bloody diarrhea⁵⁸.

In the case of *Salmonella* sp., its infection may be manifested as gastroenteritis, septicemia, or enteric fever. Enteric fevers are caused by the human-specific pathogens *S. enterica* serovars *typhi* and *paratyphi*. Infection severity may vary by the resistance of each individual and the immune system as well as the virulence of *Salmonella* isolate^{59,60}. In the case of this last bacterium, the RTCA does not establish an assay for its absence for products that are consumed after the addition of boiled water (because the bacteria are not able to resist boiling water). However, because people in Costa Rica use hot water instead of boiling water, it is better to determine the presence or absence of this pathogen in order to provide a greater security to the consumers of peppermint tisanes.

CONCLUSIONS

The three batches of the *M. piperita* tisanes used to perform the quality control assays were in compliance with the organoleptic, minimum fill, loss on drying, total ash, insoluble-acid ash, foreign organic matter, and arsenic and lead limits assays, according to the different official books used. In addition, the limits of total enumerations of mesophilic aerobic microorganisms, filamentous fungus, and yeasts were fulfillment. Finally, there was an absence of *Escherichia coli* and *Salmonella* sp.

Nonetheless, nonconformities were found for the criteria established by the RTCA for the labeling test, since information corresponding to the primary and secondary packaging was missing. In the case of primary packaging, there was absence of the batch number and the expiration date, while the secondary packaging did not present fundamental aspects such as the qualitative-quantitative composition of active ingredients (including scientific name), interactions and adverse effects. The third batch did not have information about the employment form, contraindications and warnings.

The importance of this information is that, at least in the case of this brand of peppermint tisanes, the manufacturing company maintained a good reproducibility between one batch and another. The improvements that can be made are in the product labeling and better controls in its filling.

Finally, it is expected to continue this research with other raw materials used in Costa Rica in this pharmaceutical form, that have information in different pharmacopoeias and official books. This is because the compliance of a product cannot ensure that for others the results will be the same.

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