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Importance of the Quality Control of Herbal Teas: Evaluation of Two Senna Leaf Tisanes Brands Commercialized in Costa Rica through Physicochemical and Microbiological Assays Stipulated in the Central American Technical Regulation 11.03.56.09

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Abstract: The senna leaf contains a powerful natural laxative called anthraquinone. Despite this indication, the quality control of this raw material is not strict, compared to other substances with pharmacological properties, which is unacceptable. Therefore, the present investigation sought to evaluate physicochemical and microbiological quality aspects of the senna leaf tisanes from two brands commercialized in Costa Rica, through various assays established in the current Central American Technical Regulation (RTCA) 11.03.56.09. The aforementioned was made in order to identify and compare whether these aspects are constant or not for the different batches of the same product for each of the brands. For that reason, the following assays were done for three batches of two different brands: Labeling, minimum fill, organoleptic, foreign organic matter, identification, loss on drying, total ash, acid-insoluble ash, microbial enumerations and determination of specific microorganisms. The results show that the batches of both brands are in compliance for all the assays, except for the labeling assay (six of the 23 items required for primary and secondary packaging labeling were not found). For all of the above, the raw material commercialized in Costa Rica meet the quality standards according with the requirements of the RTCA. Also, there is reproducibility for the characteristics of the senna leaf between the different batches in each one of the two commercialized brands. This is an indication that the raw material used maintains the quality characteristics required to favor its efficiency and safety.

Keywords: senna leaf, tisane, Costa Rica, quality control, Central American Technical Regulation.

INTRODUCTION

A medicinal plant is defined as one in which one or more of its organs contain substances that can be used for therapeutic purposes or that are precursors for the synthesis of useful drugs [1]. One of the administration forms in which these natural compounds have been used for the prevention and treatment of diseases is the tisane [2]. It is obtained by using water as a vehicle through which the active principles of the vegetal material used pass [3].

A plant of interest used in tisanes is the senna leaf. Its scientific name is *Cassia angustifolia* Vahl. It belongs to the *Caesalpinaceae* family, *Fabaceae* subfamily. It is native from Saudi Arabia, grows in arid areas of Sudan and Egypt, and is grown commercially in India [4]. The tree is a small shrub type, with irregular and scattered, sparse, extended glass, with thin branches in the shape of an umbrella. Its flowers are very showy, they appear in terminal inflorescences, of great color varying from yellow to red, even with mixed tones [5]. In addition, it is one of the crops in the world presented in different official pharmaceutical books (pharmacopoeias) for its therapeutic properties [5]. Its uses include blood purifier, laxative to relieve constipation and treatment for skin diseases. It contains a powerful natural laxative called anthraquinone and is approved by the World Health Organization (WHO) [6].

The plant´s biological activity is attributed to anthraquinones called sennosides A and B, specifically

to its aglycone portion. The breakdown of anthraquinones can occur in two ways. The flora of the intestine can hydrolyze them in a similar way to free active aglycone. Alternatively, in the presence of bile and sugar, the free aglycone can be absorbed into the bloodstream and subsequently, secreted into the colon. The final result is the stimulation of the Auerbach plexus, which results in an increase of the intestinal muscle contraction. Additionally, its mucilage content decreases the absorption of the organism fluids, causing the final laxative action [7].

Despite these indications, the quality control of these products is not strict compared to other substances with pharmacological properties, which is unacceptable. Little is known about the effects of these extracts on the people health [8]. Therefore, they must be examined to assess their quality, medicinal efficacy and safety [9]. Likewise, the quality of the raw material is dependent on the environmental conditions, since it can be affected by temperature or certain biotic factors changes, as well as the harvesting season or the age of the plant, which can give qualitative and quantitative variations in the different manufactured batches [10].

Therefore, the present investigation sought to evaluate physicochemical and microbiological quality aspects of the senna leaf tisanes of two brands commercialized in Costa Rica, through various assays established in the current Central American Technical Regulation (RTCA) 11.03.56.09 [11]. The aforementioned was made in order to identify and compare whether these aspects are constant or not for the different batches of the same product for each of the brands.

MATERIALS AND METHODS

Senna leaf product sampling

Three batches of brand 1 (1, 2 and 3) and three other batches of brand 2 (4, 5 and 6) of senna leaf tisanes were purchased in different supermarkets and establishments located in the Greater Metropolitan Area of Costa Rica (5 boxes per batch).

Selection of procedures for the physicochemical and microbiological assays carried out

Different assays established in the RTCA 11.03.56.09 were carried out [11]. The requirement indicated in this document for the choice of each one is its presence in an official Costa Rican book for quality control of natural products, such as different countries and organizations pharmacopoeias.

Labeling assay

The labeling assay was carried out following the procedure established by the RTCA 11.04.41:06 [12]. It indicates different items to be reviewed in both primary and secondary packaging. They are presented in Table 1.

Minimum fill assay

Following the procedure of general chapter <755> of the United States Pharmacopeia (USP) 40 [13], a sample of 10 sealed bags of each batch of the selected brand were taken, were cut to open and extract quantitatively the content in a way that there was no loss in the process. Then, the contents of each bag were individually weighed on an ADAM® PW254 analytical balance. The percentage of labeling was determined by dividing the weight of the tisane content by the weight indicated on the secondary packaging of each of the batches and the result was multiplied by 100. The average content of the 10 tisanes should not be less than that declared on the labeling, and the weight of each tisane should not be less than 90 % of what was stated for the product to be in compliance.

Organoleptic assays

Ten tisanes were used to establish the organoleptic characteristics (smell, color, texture) as indicated in the British Pharmacopoeia (BP) for the senna leaf [14]. The inspection was done directly using a lamp for proper lighting. The product was in compliance if it had a slight characteristic odor, a greenish-grayish or greenish-brown color and a smooth texture.

Determination of foreign organic matter

Ten tisanes were taken and their content was spread on a white surface, forming a thin layer and carefully observed with the help of a Konus® loupe and tweezers. Then, the foreign organic matter was organized in foreign organs and foreign elements as stipulated in the British Pharmacopoeia [14]. Each material was weighed separately on an Adam® PW254 analytical balance and the percentage it represented was calculated with respect to the total sample. The product was in compliance with the test if the percentage of foreign organs was not greater than 3 % and that of foreign elements was not greater than 1 % of the total sample.

Identification

The identification was carried out following the procedure of the senna leaf monograph of the USP 40 [13]. To carry out the assay, a KOH solution (100 mg of KOH/ml of 95 % alcohol) and 500 mg of product sample were used. First, 10 ml of KOH solution was added to the sample. Then, it was heated to boiling for 2 minutes, diluted with 10 ml of water and filtered. Thereafter, the filtrate was acidified with concentrated HCl. Finally, it was stirred with ethyl ether, then the ethereal layer was removed and stirred with 5 ml of 6 N ammonium hydroxide. The assay was considered positive if an orange or bluish-red color was observed in the aqueous layer.

Loss on drying assay

The loss on drying assay was done according to the general chapter <731> of the USP 40 [13].

Approximately 1 g of senna leaf powdered was weighed from each of the examined batches on an Adam® PW254 analytical balance using a melting pot, which was carried to constant weight previously. For this, the melting pot with the sample was placed in a Thermo Scientific® Heraterm oven and dried for two hours at 105 °C. Then, it was cooled for a few minutes in a desiccator and the melting pot was weighed with the sample to calculate the loss percentage. This drying procedure was repeated in periods of 30 minutes until reaching a constant weight. According to the acceptance criteria established by the pharmacopoeia, the sample should not lose more than 12,0 % of its weight.

Total ash assay

The assay was made according to the procedure described in the general chapter <561> of the USP 40 [13]. Two tisanes were weighed accurately from each of the batches studied (between 2 to 4 g) on an Adam® PW254 analytical balance using a melting pot previously brought to constant weight, and incinerated in a Thermo Scientific® Thermolyne stove gently at first, gradually increasing the temperature to 675 °C for two hours. Subsequently, the sample was dried at 105 °C in the Thermo Scientific® Heraterm oven in periods of 30 minutes until reaching a constant weight. The total ashes should not have been greater than 12,0 % for the test to be in compliance.

Acid-insoluble ash assay

The assay was carried out according to the procedure described in the general chapter <561> of USP 40 [13]. The ash obtained in the total ash test was heated to boil with 25 ml of HCl 3 N HCl for 5 minutes. Next, the remaining ash was filtered using a Boeco® grade 389 filter paper, washed with hot water until the washings did not present an acid pH and the paper was placed in the melting pot, previously used for the respective batch from which the filtered ash came from. Subsequently, it was incinerated in a Thermo Scientific® Thermolyne stove in a gentle way at the beginning, gradually increasing the temperature to 675 °C for two hours. The sample was then dried at 105 °C in the Thermo Scientific® Heraterm oven for 30 minutes to reach a constant weight. When determining the percentage of acid-insoluble ash, calculated with reference to the sample weight for the total ash assay, this should not be higher than 3,0 % to be in compliance with the acceptance criteria of the assay.

Microbial enumeration assays

The assays were done according to the procedure described in the general chapter <61> of USP 40 [13]. For this, 10 g of each product analyzed batch were taken and suspended in 90 ml of Bacto™ casein-soybean digest broth. Next, two Petri dishes were prepared for each medium (Bacto™ casein-soybean digest agar for mesophilic microorganisms and Liofilchem™ potato dextrose agar for filamentous fungi and yeasts) at an appropriate dilution level, according to the limits established by the RTCA 11.03.56.09 [11]. The plates with casein-soybean digest agar were incubated at 33 °C for 48 hours, while those with Sabouraud dextrose agar at 22,5 °C for five days. After the incubation time, the colony forming units (cfu) present in each plate were counted, the arithmetic mean of the counts per batch was taken for each of the culture media used and the number of cfu was calculated per product gram. According to RTCA 11.03.56.09, the total mesophilic microorganism's enumeration should not exceed 10^7 cfu/g, and the filamentous fungi and yeasts enumeration should be no more than 10^5 cfu/g.

Specific microorganisms assays

The procedures of the general chapter <62> of the USP 40 were made [13]. Initially, 10 g were transferred to a suitable container with 90 ml of Bacto™ casein-soybean digest broth for its subsequent suspension. Then, the sample was incubated at a temperature of 33 °C for 24 hours.

In the case of the *Escherichia coli* absence assay, a 1,0 ml aliquot of the sample was pipetted into a container with 100 ml Difco™ MacConkey broth, mixed and incubated at a temperature of 44 °C for 24 hours. After this period, a 1,0 ml sample was taken to inoculate two BBL™ MacConkey agar Petri dishes. These were incubated at a temperature of 33 °C for 24 hours. Finally, the inoculated dishes were examined.

For the absence of *Salmonella* spp assay, an aliquot of 1,0 ml of the sample was taken and added to 10,0 ml of Difco™ Rappaport Vassiliadis Salmonella enrichment broth. It was mixed and incubated at a temperature of 33 °C for 24 hours. At the end of this period, a sample of 1,0 ml was taken to inoculate two plates of Difco™ xylose lysine deoxycholate agar. These were incubated at a temperature of 33 °C for 24 hours.

Both tests were in agreement if, when examining the plates, there was an absence of both pathogenic microorganisms.

RESULTS AND DISCUSSION

The levels of the different components of the raw materials that make up natural products can vary from one batch to another, due to different environmental factors such as soil, altitude, temperature variation, atmospheric humidity, sunlight time, rain pattern, shadow and dew [16]. These factors have consequences on variations that are difficult to predict, to the extent that plants of the same species that are cultivated in distinct environments can vary significantly in the content of particular secondary metabolites [17]. Therefore, it is essential to determine the quality of the raw material of various batches of the same product, in this case, senna leaf.

For the tisanes batches of the senna leaf brands, the four items of the primary packaging and the 19 items of the secondary packaging required for the products were reviewed. The summary of this review is shown in Table 1. Regarding the primary packaging of both brands, they did not provide information about the expiration date and the batch number. This information is important for tisanes, since many consumers tend to take them out of the secondary packaging, which is not optimal. In the case of the batch number, it must appear in order to give traceability to the product [18] in case it is necessary to remove the product as a consequence of security problems related to the product, which potentially avoids more dangerous situations related with the product [19].

Regarding the secondary packaging, information about the quali-quantitative composition of the active ingredients, the interactions, the adverse effects and the special legends was not observed.

Table-1: Fulfillment of the primary and secondary packaging items of the different evaluated batches of two brands of senna leaf tisanes commercialized in Costa Rica

Item	Fulfillment					
	Brand 1			Brand 2		
	Batch 1	Batch 2	Batch 3	Batch 4	Batch 5	Batch 6
Primary packaging						
Brand name	Yes	Yes	Yes	Yes	Yes	Yes
Batch number	*No*	*No*	*No*	*No*	*No*	*No*
Expiration date	*No*	*No*	*No*	*No*	*No*	*No*
Manufacturer laboratory name or logo	Yes	Yes	Yes	Yes	Yes	Yes
Secondary packaging						
Product name	Yes	Yes	Yes	Yes	Yes	Yes
Pharmaceutical form	Yes	Yes	Yes	Yes	Yes	Yes
Indications	Yes	Yes	Yes	Yes	Yes	Yes
Employment form	Yes	Yes	Yes	Yes	Yes	Yes
Quali-quantitative composition of active ingredients	*No*	*No*	*No*	*No*	*No*	*No*
Registration number	Yes	Yes	Yes	Yes	Yes	Yes
Manufacturer name and country of origin	Yes	Yes	Yes	Yes	Yes	Yes
Net amount of the finished product	Yes	Yes	Yes	Yes	Yes	Yes
Batch number	Yes	Yes	Yes	Yes	Yes	Yes
Storage conditions	Yes	Yes	Yes	Yes	Yes	Yes
Expiration date	Yes	Yes	Yes	Yes	Yes	Yes
Contraindications and warnings	Yes	Yes	Yes	Yes	Yes	Yes
Interactions	*No*	*No*	*No*	*No*	*No*	*No*
Adverse effects	*No*	*No*	*No*	*No*	*No*	*No*
General labeling	Yes	Yes	Yes	Yes	Yes	Yes
Special labeling	*No*	*No*	*No*	*No*	*No*	*No*
Posology	Yes	Yes	Yes	Yes	Yes	Yes
Administration route	Yes	Yes	Yes	Yes	Yes	Yes
Use during pregnancy, breastfeeding, erlderly and children under 2 years	Yes	Yes	Yes	Yes	Yes	Yes

In first place, there was no information on the quali-quantitative composition of the active ingredients of the analyzed products. This information must be provided to consumers, since they must be clear that the properties as a laxative are due to senosides A and B, that the quantities in which they are found, which allows to ensure that the pharmacological properties of the product are assured despite the harvest, packaging and storage processes to which the raw material is subjected [20].

Another aspect that is not present in the labeling are the interactions. It is known that the abuse of the laxative effect for a prolonged period can produce hypokalemia and potentiate the action of cardiac glycosides and interact with antiarrhythmic products that induce the reversion of the sinus rhythm (quinidine) and with products that produce the prolongation of the QT wave. As wise, the concomitant use with other products that induce hypokalemia (diuretics and adrenocorticoids) can increase the electrolyte imbalance [21]. In addition, it can increase the effect of digoxin [22, 23]. Likewise, information related to adverse effects should be considered, since cases have been reported as a consequence of the use of senna leaf, among them, hepatotoxicity, hepatitic, portal venous thrombosis and paralytic ileus [24, 25].

Also, special labeling regarding their use should be present. It is known that there are different conditions in which the consumption of senna leaf is not recommended. Some of them are electrolyte disturbances, potassium deficiency, dehydration, diarrhea, gastrointestinal conditions (intestinal blockage, Crohn's disease, ulcerative colitis, appendicitis, stomach inflammation, prolapse, hemorrhoids) and heart disease [26].

Regarding the results obtained for the minimum fill assay (Table 2), for brand 1, the tisane content was 1,1 g, while for brand 2 the indicated weight was 1,3 g. The labeling average percentage of each of the analyzed batches showed a value above what was declared on the labeling and no tisane showed content lower than 90 % of that labeling. This allows to have a notion of whether the dose administered by each tisane meets the specification (average of 10 tisanes not less than 100 % of the product labeling and no tisane with a minimum fill less than 90 % of that indicated in the product). For the three batches of the two brands it was found that there was a consistent result regarding these criteria. However, in three of the studied batches (two of brand 1 and one of brand 2) labeling average percentage over 110 % were found, with one of brand 2 reaching a value of 120 %. This represents a warning for the manufacturer companies, since high values can cause that the administered dose go from therapeutic to toxic, depending on the appropriate dose (20 to 60 mg of sennosides per day in the case of adults and half of the adult dose for children over six years old [27]), which can not be determined with the information present on the labeling of these products, as the quali-quantitative composition is not taken into account.

Table-2: Labeling percentage of the different evaluated batches of two brands of senna leaf tisanes commercialized in Costa Rica.

Tisane	Labeling percentage (%)					
	Brand 1			Brand 2		
	Batch 1	Batch 2	Batch 3	Batch 4	Batch 5	Batch 6
1	108	120	105	104	109	122
2	108	121	116	114	109	122
3	107	114	115	107	105	117
4	105	114	108	102	109	117
5	103	113	111	109	110	127
6	103	115	112	112	105	121
7	104	117	117	115	103	118
8	106	115	111	110	110	117
9	106	119	115	116	97	117
10	105	115	115	113	102	119
Average	105	116	113	110	106	120

Therefore, a stricter control over the filling is required to obtain a greater safety in terms of reaching the dose range at which the active principle is effective without generating unacceptable adverse effects [28].

Another assay was the organoleptic characteristics, (done through a careful visual observation) to have a notion that the content of the tisane consists of senna leaf and not another vegetable species. The organoleptic characteristics are important, given that these attributes make it possible to distinguish between medicinal and non-medicinal plants [29]. In this case, all the evaluated batches for the two senna leaf brands were in compliance with the color, odor and texture characteristics.

To complement the previous test, the presence of foreign organs (senna parts different from the leave) and foreign elements (material distinct from the senna plant) was determined. In many cases, the adulteration of plant material occurs through substitution with inferior commercial varieties, artificially produced substances, cheaper plants or vegetative parts other than those that have the desired therapeutic effect [30]. In the case of senna, the vegetative part used is the leaf [31]. For the analyzed products, Table 3 shows that all the batches showed a percentage of foreign organs of 1%, with which they were found according to what was established by the BP [14]. Within this same test, the presence of foreign materials was not found in the analyzed samples of the batches of both products, which shows the quality of the raw material used to make the teas.

Another essential test to ensure that the material exerts its desired therapeutic effect is that of identification. Said confirmation was due to the fact that in all cases the formation of a similar orange coloration occurred for the three batches shown of each senna leaf product. Also, it was possible to confirm the presence of sennosides in the batches of tisanes analyzed. Also, it was found that the presence of sennosides was similar for the three batches of each of the brands. This result shows that the raw material has not undergone variations in its composition, due to the environmental conditions to which it was subjected. However, it also

reaffirms that the control of the minimum fill of the herbal teas must be stricter, because for the same batch, a person may be consuming a certain amount of sennosides A and B one day, and the next day, a larger amount. With this, the intended effect will not be maintained over time, but may experience greater or lesser effects depending on the tisane or tisanes that have been used on a certain day to consume the senna leaf product.

With respect to the loss on drying assay (Table 4), all the values were below 12,0 %, for which the different batches studied were in compliance with that established by the USP 40 [13]. Reducing humidity is necessary to prevent the active agents of the plant material from being destroyed and their properties to become unfavorable [32].Therefore, the objective of drying natural products is to extend their useful life and preserve their characteristics. In addition, by reducing the water activity of the products, it is intended to inhibit the growth and development of pathogenic microorganisms or that may be capable of deteriorating the product [33]. In the case of analyzed products, their water content is ideal to ensure the expiration date established for the different batches, and the maintenance of the required characteristics for an adequate pharmacological response by the active components that produce the laxative effect in the senna leaf.

Table-3: Percentage of foreign organs and foreign elements of the different evaluated batches of two brands of senna leaf tisanes commercialized in Costa Rica

Brand 1						
Sample	Batch 1		Batch 2		Batch 3	
	Weight (g)	Percentage within the total (%)	Weight (g)	Percentage within the total (%)	Weight (g)	Percentage within the total (%)
Initial	11,5478	100	12,4434	100	12,1357	100
Foreing organs	0,0669	1	0,1036	1	0,0898	1
Foreign elements	0	0	0	0	0	0
Brand 2						
Sample	Batch 4		Batch 5		Batch 6	
	Weight (g)	Percentage within the total (%)	Weight (g)	Percentage within the total (%)	Weight (g)	Percentage within the total (%)
Initial	14,1955	100	13,4355	100	15,1384	100
Foreign organs	0,1139	1	0,0605	0	0,0788	1
Foreign elements	0	0	0	0	0	0

Table-4: Loss on drying percentage of the different evaluated batches of two brands of senna leaf tisanes commercialized in Costa Rica

Brand	Batch	Initial weight (g)	Final weight (g)	Loss on drying percentage (%)
1	1	1,2148	1,0970	9,7
	2	1,2719	1,1488	9,7
	3	1,2287	1,1076	9,9
2	4	1,5547	1,4011	9,9
	5	1,5195	1,3696	9,9
	6	1,4753	1,3300	9,8

Likewise, in Table 5 it is observed that for the total ash and acid-insoluble ash assays there was a compliance with respect to that specified by this same pharmacopoeia for the products constituted by senna leaf. The total ash assay is developed to measure the amount of inorganic material that remains after the ignition of the vegetal material [34], and includes the plant's own ash, as well as that coming from the sand and the soil adhered to the product [35]. Its determination indicates the care with which the preparation of the vegetable raw material was carried out [36]. In what corresponds to the batches analyzed, it is possible to mention that the raw materials used have been prepared in a careful way to avoid the presence of material other than the required plant material. This is shown in this table, where the percentage of total ash for the batches of brand 1 was between 7,8 and 8,1 %, while for those of the brand 2 was between 7,7 and 8,4 %. These values are well below the 12 % specified by USP [13], demonstrating the quality in the preparation of the material.

Table-5: Total ash and insoluble-acid ash percentage of the different evaluated batches of two brands of senna leaf tisanes commercialized in Costa Rica

Brand	Batch	Initial weight (g)	Total ash weight (g)	Total ash percentage (%)	Insoluble-acid ash weight (g)	Insoluble-acid ash percentage (%)
1	1	2,3029	0,1854	8,1	0,0119	0,5
	2	2,4771	0,1925	7,8	0,0059	0,2
	3	2,5651	0,1998	7,8	0,0104	0,4
2	4	3,0781	0,2455	8,0	0,0102	0,3
	5	2,9014	0,2238	7,7	0,0167	0,6
	6	3,0911	0,2610	8,4	0,0248	0,8

However, because the total ash test is not always reliable, because there is a possibility of the presence of non-physiological substances such as earthy materials [36], including silica [37]. So, it is necessary to carry out the acid-insoluble ash assay. In the case of the samples analyzed, the percentages obtained were less than 1 %. This is another indication of the quality of the raw material used in the preparation of senna leaf tisanes for both brands.

Finally, a series of assays were carried out to know the microbiological quality of the different brands for both products. The concern about this quality in the products is due to the potential contamination, considering their natural origin [38]. It is known that natural products usually contain bacteria and yeasts from the soil and the atmosphere. Moreover, soil, harvest, drying, storage conditions and inadequate handling influence on microbiological quality. This represents a serious problem, since the presence of microbiological contaminants can reduce or even inactivate the therapeutic activity of the products and has the potential to adversely affect the patients who consume them [39, 40]. As regards, the results obtained for the three batches of each one of the products studied were consistent for mesophilic aerobic microorganism enumeration, as well as for filamentous fungi and yeasts enumeration, as seen in Table 6. This shows that the companies of these products in Costa Rica have been concerned with ensuring adequate handling of the plant material at all stages of the production of senna leaf tisanes.

Table-6: Compliance of the different batches evaluated of two brands of senna leaf tisanes commercialized in Costa Rica with respect to the distinct microbiological tests carried out.

Assay	Compliance					
	Brand 1			Brand 2		
	Batch 1	Batch 2	Batch 3	Batch 4	Batch 5	Batch 6
Mesophilic aerobic microorganism enumeration	Yes	Yes	Yes	Yes	Yes	Yes
Filamentous fungi and yeasts enumeration	Yes	Yes	Yes	Yes	Yes	Yes
E. coli absence	Yes	Yes	Yes	Yes	Yes	Yes
Salmonella sp. absence	Yes	Yes	Yes	Yes	Yes	Yes

To complete this microbiological control, the presence or absence of *E. coli* and *Salmonella* sp. was determined in these products. *Salmonella* sp. should be evaluated, because when ingested orally it is capable of generating gastroenteritis, which manifests as nausea, vomiting and diarrhea between 6 and 48 hours after ingestion [41], while *E. coli* is also capable of causing enteritis, enterocolitis and colitis when the person ingests it in sufficient quantity [42]. The regulation of Costa Rica only requires the development of tests for the absence of *E. coli* for products that are consumed using boiling water, but not for *Salmonella* sp, since this microorganism does not resist water at its boiling point [43]. However, because in this country many people consume tea using hot water, *Salmonella* sp is able to withstand these temperatures and become a potential hazard if it is presented. For the batches of the brands of interest, all agreed for the absence tests of these two pathogens, with which there is no danger for the consumer of being exposed to bacteria capable of developing serious problems in the gastrointestinal system.

In future research, we hope to continue obtaining information on the quality of other raw materials commercialized in as tisanes in Costa Rica, which have their respective monographs in pharmacopoeias accepted as officials in this country.

CONCLUSIONS

The senna leaf is a natural raw material widely used for its laxative effects. In the case of the three batches of each brand commercialized in Costa Rica, they were in compliance in regard with minimum fill, organoleptic, foreign organic matter, identification, loss on drying, total ash, acid-insoluble ash, microbial enumerations and specific microorganisms assays. However, both brands do not comply with six of the 23 items required for primary and secondary packaging (batch number, expiration date, quali-quantitative composition of active ingredients, interactions, adverse

effects and special labeling). For all of the above, the raw material commercialized in Costa Rica meet the quality standards according with the requirements of the RTCA. The companies in charge of its production must pay attention in the inclusion of the items missing in the labeling and in the improvement of the filling process controls of the herbal tea, so that its quantity will be closer to that established in the labeling. In this way, a product that provides greater safety for consumers of this herbal product will be obtained. Likewise, it is possible to mention that there is reproducibility for the characteristics of the senna leaf between the different batches in each one of the two commercialized brands. This is an indication that the raw material used maintains the quality characteristics required to favor its efficiency and safety.

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