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Original Research Article

Quality Control of a Boldo Tisanes Brand Commercialized in Costa Rica Following the Central American Technical Regulation for Natural Products

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Abstract

One of the medicinal species used as raw material for the tisanes preparation is the boldo leaf (*Peumus boldus*). This plant is commonly used as treatment for a variety of conditions, such as digestive and hepatobiliary disorders. It is traditionally known for its choleretic, cholagogue, diuretic, and digestive properties, among others. The objectives of this work were to evaluate the quality of four batches of boldo tisanes of a brand commercialized in Costa Rica through various physicochemical and microbiological tests established in the Central American Technical Regulation (RTCA) 11.03.56.09, and to identify the reproducibility of the quality parameters for the four batches employed. For this reason, the following tests were done: labeling, organoleptic characteristics, foreign matter determination, minimum fill, lead limit, arsenic limit, loss on drying, total ash, microbial enumeration, and specific microorganisms (*E. coli* and *Salmonella* sp). The four analyzed batches were in compliance for all assays, except the labeling test, since in all cases only 2 of the 4 items established for the primary packaging (batch number and expiration date) and 3 of the 19 items indicated for the secondary packaging (qualitative-quantitative composition, interactions, and adverse effects) were not found. In addition, the batch 2 had a browner color compared to the others, not complying with the organoleptic test specifications, specifically the color. This is reaffirmed by obtaining a greater percentage of branches in its composition during the foreign matter test. For these reasons, greater controls must be made on the raw material used for the product preparation to achieve reproducibility between the quality characteristics required for the different batches.

Keywords: natural product, tisane, boldo, quality control, Central American Technical Regulation.

INTRODUCTION

The nature is a rich source of physical and chemical varieties [1]. Herbal medicines and their derivatives have been incorporated into traditional medicine since the history beginning [2] and have been used by the world's population majority for thousands of years [3]. However, it is until recent times that the increasingly expanded use of medicinal plants has begun to gain acceptance worldwide [2]. For example, more than 1,300 medicinal plants are used in Europe, of which 90% are harvested from wild resources, and in the United States, about 118 of the 150 prescription drugs are based on natural sources [4]. In addition, more than 80% of people in developing countries are totally dependent on medicinal plants as their primary treatment [2, 4, 5]. Studies in Latin America report that medicinal plants are widely used. For example, in Cordoba, Argentina, 100% of the population knew about the use of medicinal plants, while in Brazil, the use frequency varies between 70 and 98% of the total population, depending on the area. In Panama, 84% of the people who went to a primary care unit employed them [6].

One of the pharmaceutical dosage forms for these products is the tisane. Herbal teas should be called as tisanes or infusions. However, they are usually referred as herbal teas by consumers and researchers [7]. The raw material used may consist of fresh or dried leaves, fruits, flowers, pollen, nuts, barks, seeds, and roots of one or several species [8].

These products are usually consumed for their physical or medicinal effects, especially stimulant, relaxing, or sedative properties [9, 10]. Its popularity is due to the high availability of herbal teas and medicinal plants formulations, the low price, the virtual absence of adverse effects and biological aggressiveness, and recently, the tendency to replace or complement conventional medicines and drugs [11].

One of the medicinal species used as raw material for the tisanes preparation is the boldo leaf (*Peumus boldus*). It belongs to the Monimiaceae family and is native of Chile. It grows in areas that have a microclimate with conditions similar to the Mediterranean sector. It can also be found in areas of Ecuador, Bolivia, Argentina, and Peru, as well as acclimated areas in Morocco and North Africa [12]. It is commonly used as infusions, tinctures, or extract for the treatment of diverse conditions [13]. It is traditionally known for its choleretic, cholagogue, diuretic, and digestive properties, among others [14]. Also, the antioxidant activity of its main active principle, boldine, has been determined [15].

In Costa Rica, tisanes are commercialized as natural products. Yet, as with medicines, and due to their pharmacological properties, it is important to evaluate their quality, safety, and efficacy in order to protect the Costa Rican population that consumes these products [16]. Besides, this control is important, since different factors can affect the qualitative and quantitative chemical profile of natural raw materials, such as the geographical origin (climate, soil, photoperiod), the genotype, the plant part used (leaves, stems, root, bark), the time (year, season, time of day), and the harvest, storage, processing, and extraction conditions. All this confirmed that an adequate control is necessary [17]. In fact, the variable content in different batches of the same product may contribute to its negative effects [18].

Previous works related to these products have been carried out for valerian [19], senna leaf [20], and peppermint [21]. Though, this information can not be extrapolated to others, because each raw material is unique. For this reason, the objectives of this work were to evaluate the quality of four batches of boldo tisanes of a brand commercialized in Costa Rica through various physicochemical and microbiological tests established in the Central American Technical Regulation (RTCA) 11.03.56.09 [22], and to identify the reproducibility of the quality parameters for the four batches used.

MATERIALS AND METHODS

Product Sampling and Procedures Selection

Four batches of a boldo tisanes brand were purchased in different supermarkets in the Greater Metropolitan Area of Costa Rica. The batches were identified as 1, 2, 3, and 4.

For the procedures selection of the physicochemical and microbiological tests done, the official books established by the RTCA 11.03.56.09 [22] were used.

Labeling Test

The 4 items stipulated for the primary packaging and the 19 indicated for the secondary packaging were reviewed, according to the RTCA 11.03.56.09 [22]. It was indicated that the labeling of a natural product must be in compliance with the RTCA 11.04.41:06 [23].

Organoleptic characteristics test

The organoleptic characteristics of odor, color, and texture were analyzed. For this, 10 tisanes were used, as established by the British Pharmacopoeia (BP) 2013 [24]. The inspection was carried out with a Konus® luminous lens. The product was considered in compliance by having a characteristic odor (especially when rubbed), a grayish-green color, and a soft texture.

Foreign matter determination test

The test was done according to the procedure indicated in the BP 2013 [24]. For this, 10 tisanes were opened. Their contents were dispersed on a white surface and observed with the help of a Konus® luminous magnifying glass. The foreign matter found was separated and weighed on an Adam® PW254 analytical balance. The foreign matter percentage was determined with respect to the total sample weight. The product was in compliance if the branches percentage was less than 4% and that of foreign elements did not exceed 2%.

Minimum fill test

It was carried out according to the procedure indicated in the general chapter <755> of the United States Pharmacopeia (USP) 40 [25]. To do this, 10 tisanes were taken from each boldo tisanes batch. Then, they were cut to open and carefully extract the content, so that no sample was lost in the process. In an Adam® PW254 analytical balance, the individual content of each tisane was weighed and the value recorded. The product was considered to be in compliance if the net content of any individual tisane was not less than 90% of the declared amount.

Lead limit test

The evaluation of heavy metals was made for the lead element according to the procedure described for method A of the <2.4.8> chapter of the European Pharmacopoeia 5.0 [26].

For the sample, two tisanes were taken in a 100 ml beaker and 22 ml of distilled water were added.

It was left to rest for 5 minutes. After this, the tisanes were extracted by compressing them, depositing the extracted liquid in the same beaker. Then, an aliquot of 12.0 ml was taken for the sample, 2.0 ml for the standard and 2.0 ml for the blank. Each one was put in a different test tube. 10 ml of the lead standard (1 ppm) and 10 ml of distilled water were added to the standard tube and the blank tube, respectively. Next, 2 ml of acetic acid-sodium acetate buffer at a 3.5 pH, 0.5 ml of diluted 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 standard.

Arsenic Limit Test

The test was carried out according to the procedure described for method A of the <2.4.2> chapter of the European Pharmacopoeia 5.0 [26].

In a 100 ml beaker, one tisane was placed and 11 ml of distilled water were added. It was left to rest for 5 minutes. After this, the tisane was extracted by compressing it and the extracted liquid was deposited in the same beaker. Later, an aliquot of 10.0 ml was taken and deposited in a conical flask. Then, 15 ml of 12 M HCl, 0.1 ml of stannous chloride and 5 ml of 16 %w/v potassium iodide were added. It was left to stand for 15 minutes. After that, 3 g of zinc chips were added. 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 ml of standard arsenic solution (1 ppm). After not less than 2 hours, the stain produced on the mercury bromide paper in the sample should not be more intense than that of the reference standard.

Loss on Drying Test

It was done according to the general chapter <731> description of the USP 40 [25]. First, a crucible was dried for 30 minutes at 105 °C in a Thermo Scientific® HeraTherm oven stove, and the process was repeated until constant weight. Next, the content of a boldo tisane was weighed on an Adam® PW254 analytical balance, placed in the crucible and accurately weighed together with its content. Subsequently, it was placed in the oven and dried for two hours at 105 °C. After cooling in a desiccator, it was weighed again in the analytical balance to calculate the loss percentage. These periods of heating and cooling were continued until reaching a constant weight.

Total ash test

To carry out this test, the procedure described in the general chapter <561> of the USP 40 was made [25]. The content of two boldo tisanes was accurately weighed on an Adam® PW254 analytical balance in a crucible taken previously to constant weight. It was incised gently at the beginning and then the temperature was gradually increasing to 675 °C. The procedure was done for two hours. Finally, the ash obtained was cooled in a desiccator and weighed in the analytical balance to calculate the total ash percentage. These periods of heating and cooling were continued until reaching a constant weight. The compliant sample was considered if the total ash was less than 13.0% with respect to the initial weight.

Microbial Enumeration Tests

The tests were carried out according to the procedure described in the general chapter <61> of the USP 40 [25].

10 g of the product to be analyzed were taken and added in 100 ml of Bacto™ casein-soybean digest broth. Next, two Petri dishes were prepared for each medium at the required dilution level. The plates with Bacto™ casein-soybean digest agar were used for the total aerobic microbial count. They were incubated at 33 °C for 48 hours. In the case of yeasts and molds count, Liofilchem™ potato dextrose agar was employed. Then, the plates were incubated at 22.5 °C for five days. After that, the enumeration arithmetic mean of each medium was taken and the number of colony forming units (cfu) was calculated per g of product. According to the RTCA 11.03.56.09, the total aerobic microbial count should not exceed 10^7 cfu/g, while the total combined yeasts and molds count should not exceed 10^5 cfu/g [22].

Microorganisms' specific tests

The procedures of chapter <2022> of USP 40 were followed [25].

***Escherichia coli* absence test**

10 g of the product to be analyzed were added in 100 ml of Bacto™ casein-soybean digest broth. Next, the sample was incubated at a temperature of 33 °C for 24 hours. Then, a 1.0 ml aliquot of the sample was pipetted into a container with Difco™ MacConkey broth. Later, was mixed and incubated at 44 °C for 48 hours. After this period, two samples, each of 1.00 ml of the broth, were taken to inoculate two BBL™ MacConkey agar plates. Plates were incubated at 33 °C for 24 hours. At the end, the inoculated plates were examined.

***Salmonella* sp absence test**

From the sample prepared for the *E. coli* absence test, a 1.00 ml aliquot was taken and added to 10 ml of Difco™ Rappaport Vassiliadis Salmonella enrichment broth. The solution was mixed and incubated at 33 °C for 24 hours. Afterward, two samples were taken, each of 1.00 ml of the solution, to inoculate two plates of Difco™ xylose lysine deoxycholate agar. These were incubated at 33 °C for 24 hours.

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

RESULTS AND DISCUSSION

The quality control of natural products with pharmacological properties is necessary to ensure that they do not cause adverse effects or even endanger the consumers´ lives, who often consider them to be completely safe products, due to their origin [27]. First, the information required in the primary and secondary product packaging of the product was evaluated, which is necessary to reduce the incorrect consumption errors caused by inappropriate labeling (Table 1) [28].

Table-1: Results of the labeling test of the primary and secondary packaging for four batches of a boldo tisanes brand commercialized in Costa Rica

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

As for the primary packaging, the data referring to the product and the manufacturing company names were present. However, no information was found regarding the batch number. The presence of this is essential to follow a product, especially in a complaint case and if a market withdrawal is necessary [29]. Another detail not shown is the expiration date. It provides information related to the time over which the drug potency and integrity in its sealed container remain intact. Providing this is necessary, because it brings information about the extent to which the manufacturing company would guarantee the product safety, full potency, and stability [30].

Regarding the review of the secondary packaging in which these tisanes are commercialized, there was compliance with 16 of the 19 aspects requested by the RTCA 11.04.41:06 [23]. The items not found were the qualitative and quantitative information, as well as the interactions and the adverse effects of boldo consumption. Data about the formulation components and their respective quantities is required, so that each person is aware of what they are consuming. In the case of boldo leaves, it has been reported to contain 1.2% of tannins and 2 to 3% of essential oils (up to 45% of ascaridole, 30% of cineole, and at least 22 other identified constituents, mainly terpenoids). In addition, dried leaves of *Peumus boldus* have been reported to contain alkaloids in the 0.25 to 0.54% or 0.4 to 0.5% range [13, 31], of which approximately 12 to 19% is boldine [31-33]. This alkaloid effect has been associated with an antagonism on human 5-HT3 receptors [33].

Regarding the boldo interactions identified, the European Medicines Agency (EMA) indicates that the information is limited and reports a case about the tacrolimus levels decrease due to a potential interaction

with a boldo preparation [34]. However, information was found regarding interactions with the following drugs: lithium, hepatotoxic drugs (acetaminophen, amiodarone, carbamazepine, isoniazid, methotrexate, methyldopa, fluconazole, itraconazole, erythromycin, phenytoin, lovastatin, pravastatin, simvastatin), anticoagulants, and antiplatelet agents (aspirin, clopidogrel, diclofenac, ibuprofen, naproxen, dalteparin, enoxaparin, heparin, warfarin) [35, 36].

As a complement, the adverse effects information, also absent, indicates that it can cause severe, whole-body allergic reaction, heart rate disorders, and liver toxicity [37]. But, as with interactions, a greater research is needed [34]. Therefore, in case of not having information from international sanitary authorities in terms of interactions and adverse effects, it should be indicated on the labeling about this situation.

Another test done was related with the organoleptic characteristics. In three of the evaluated batches the presence of a characteristic color was found when the tisanes raw material was rubbed, as well as the observation of a grayish green color in it and a soft texture (which could be appreciated despite being quite crushed), as indicated by the BP [24]. Though, the batch 2 showed a browner color than the others, which can be explained by the foreign matters presence, as will be shown later. This is necessary because raw materials are often adulterated or substituted by other low-quality plant materials before harvesting, and during handling and storage [38]. Still, other tests such as the foreign matter determination are done to corroborate these results.

In Table-2, information regarding the branches and other foreign matter presence not related with the plant leaves is shown.

Table-2: Branches and foreign matter percentages in the analyzed batches of a boldo tisanes brand commercialized in Costa Rica.

Batch	Total sample weight g	Branches weight g	Branches percentaje %	Foreign matter weight g	Foreign matter percentage %
1	10.6041	0.3844	4	0.0046	0
2	12.1958	1.2323	10	0.0545	0
3	14.0338	0.5925	4	0.0791	1
4	11.2267	0.1626	1	0.0123	0

The batch 2 does not comply with the requested specification of the BP for the maximum branches percentage in the sample, being this higher than the 4% established [24]. This information is worrisome, because it indicates that the product is not constituted by the plant part required to exert the pharmacological effect [34]. It is an adulteration example, since there is a partial substitution of the crude drug with inferior commercial varieties which may not have any therapeutic potential as that of the original drug [39]. Therefore, this can cause that the expected therapeutic effect is not achieved. Hence, it is necessary for the company to have a greater control of the raw material prior to the tisanes filling. As opposed, the four batches do meet the foreign matter percentage. These components include soil, stone, sand, dust, glass, metal, plastic, mold, insects, and animal excreta [40].

As for the minimum fill test, this was done to determine if the tisanes content met the limit established by the USP [25]. Table-3 shows all the results obtained.

Table-3: Labeling percentage of the analyzed samples of each one of the batches of a boldo tisanes brand commercialized in Costa Rica

Tisane	Batch							
	1		2		3		4	
	Weight g	Labeling percentage %	Weight g	Labeling percentage %	Weight g	Labeling percentage %	Weight g	Labeling percentage %
1	1.0505	96	1.1102	101	1.2645	115	1.0701	97
2	1.0871	99	1.1147	101	1.2394	113	1.1293	103
3	1.0961	100	1.1405	104	1.2601	115	1.2233	111
4	1.0776	98	1.1433	104	1.2943	118	1.1045	100
5	1.0446	95	1.1136	101	1.3051	119	1.1184	102
6	1.0232	93	1.0823	98	1.3026	118	1.1604	105
7	1.0628	97	1.1047	100	1.2427	113	1.1358	103
8	1.0756	98	1.0957	100	1.3032	118	1.1160	101
9	1.0560	96	1.1172	102	1.2871	117	1.0840	99
10	1.0316	94	1.1294	103	1.2868	117	1.0849	99
Mean value	**1.0605**	**96**	**1.1206**	**102**	**1.2786**	**116**	**1.1227**	**102**

For the minimum fill test, the four analyzed batches were in accordance with the stipulation of USP 40 [25]. Although the RTCA indicates that this test should only be done by sanitary surveillance or by received complaints [22], it is important because a large variation in the filling percentage could be reflected in adverse effects on the population that consumes the product.

Through the research carried out, the variations between the batches of *Peumus boldus* 1, 2, and 4 were within a range between 90 and 110% (except for a tisane of batch 4), indicating an adequate filling control. However, in batch 3 all the tisanes were above 110% (between 113 and 119%), something that must be corrected. The concern about these values is due to the fact that the recommended dose is 1 to 2 g of the herbal product 2 to 3 times a day [34]. Ingesting a greater amount can cause a dose higher than the therapeutic range established for it (dosage range, or blood plasma or serum concentration usually expected to achieve the desired therapeutic effect) [41]. This can cause the side effects appearance [42].

Other assays were lead and arsenic limit tests. There are several case reports and studies that had documented that herbal medication may contain toxic ingredients, including heavy metals such as lead, mercury, and arsenic [43]. Its continuous exposure can cause degenerative diseases in the skeletal and nervous systems. Their alteration can be related to different conditions such as multiple sclerosis, Parkinson's disease, muscular dystrophy, Alzheimer's disease, and different cancer types [44]. The metal residues presence in the herbal plants is prevalent, since they are easily contaminated during growth, development, and processing [45]. In the case of lead, depending on the exposure level, it can originate neurological, hepatic, renal, hematological, circulatory, immunological, reproductive, developmental, auditory, gastrointestinal, and cardiovascular pathologies [46]. For the four studied batches, the results showed that the stain obtained for each of them generated a brown color of less intensity than the standard, as established by the European Pharmacopoeia [26].

In relation to the arsenic limit test, it was also in compliance with the specification indicated by the European Pharmacopoeia [26] for the four batches, as it was observed that each sample exhibited a less stain. This metal also represents a danger to humans. Arsenic found in water is almost entirely in the inorganic form and can be stable as both arsenite (As_2O_3) and arsenate (As_2O_5) [47]. Trivalent species (As^{3+}) are more toxic than pentavalent species (As^{5+}) [48]. Arsenic toxicity inactivates up to 200 enzymes, like those involved in cellular energy pathways, and DNA replication and repair. Unbound arsenic also exerts its toxicity by generating reactive oxygen intermediates during its redox cycling and metabolic activation processes that cause lipid peroxidation and DNA damage. Besides, As^{3+} binds thiol or sulfhydryl groups in tissue proteins of the liver, lungs, kidney, spleen, gastrointestinal mucosa, and keratin-rich tissues (skin, hair, and nails) [49]. The chronic toxicity associated generates skin (pigmentation and keratosis) and systemic manifestations (respiratory, gastrointestinal, liver, cardiovascular, and nervous system diseases, hematological effects, diabetes, and different cancer types) [50].

Another assay was the loss on drying one. The medicinal plant drying has enough potential to reduce the post harvest losses and to prevent product spoilage in storage [51]. This spoilage is caused by contaminating microorganisms and their toxins that may lead to diseases, making them hazardous for comsumption [52]. The information regarding this test is shown in Table 4.

Table-4: Loss on drying percentage of the different evaluated batches of a boldo tisanes brand commercialized in Costa Rica

Batch	Initial weight (g)	Final weight (g)	Loss on drying percentage (%)
1	1.2373	1.1135	10.0
2	1.2294	1.1079	9.9
3	1.1125	0.9972	10.4
4	1.1077	0.9981	9.9

In the case of the evaluated batches, the four have values that range from 9.9 to 10.4%. The official literature consulted does not indicate a specific loss on drying percentage to the product made from boldo leaf. Yet, when analyzing the data, it is possible to observe that there is homogeneity in the drying process selected by the manufacturer. The importance of this characteristic is due to the fact that the drying process decreased the plant moisture content, aimed at preventing enzymatic and microbial activity, and consequently, preserving the product for extended shelf life. Also, an adequate process is needed to provide a rapid reduction in the moisture content without affecting the active ingredients quality [53].

As a complement, the total ash amount was determined for the 4 batches of *Peumus boldus*. This information is summarized in Table-5.

Table-5: Total ash percentages of the different evaluated batches of a boldo tisanes brand commercialized in Costa Rica

Batch	Initial weight (g)	Total ash weight (g)	Total ash percentage (%)
1	2.3319	0.2213	9.5
2	2.5306	0.2535	10.0
3	2.3615	0.2171	9.2
4	2.2914	0.2134	9.3

These batches showed compliance with the specification established for this required quality criteria, which is less than 13.0%, according to USP 40 [25]. The residue remaining after plant material incineration represents both physiological and non-physiological ash. Physiological ash is derived from plant tissues due to biochemical processes, while non-physiological one consists of extraneous matter residue (sand, soil) deliberately or non-deliberately adhering to plant samples itself [54, 55]. Therefore, the total ash test is another way to check the purity and/or the natural products adulteration [56, 57].

Finally, a series of microbiological tests were executed. The microbiological contamination control is important, because microorganisms may contaminate the finished product as well as the manufacturing plant. They can be the origin of certain diseases or may cause products spoilage [58], and can appear because of the cultivation and collection methods used, an inappropriate harvesting and cleaning, an unsuitable transportation, a prolonged drying and storage, a poor producers' hygiene, and the prevailing climate conditions related with the raw materials employed [59]. The microbial counts of both mesophilic microorganisms, and yeasts and molds showed values below those established by the RTCA [22] for the four studied batches.

Likewise, for these batches the presence of *E. coli* was not found. The absence of this pathogen must be assured, because although most *E. coli* strains live harmlessly in the colon and seldom cause disease in healthy individuals, a number of pathogenic strains can cause intestinal and extraintestinal diseases both on healthy and immunocompromised individuals [60]. In addition, its presence is an indicative of fecal contamination [61].

The absence of another pathogenic microorganism such as *Salmonella* sp was determined in the four sampled batches. This is considered the main cause of acute human bacterial gastroenteritis worldwide, being *Salmonella enteritidis* and *Salmonella typhimurium* the most frequently reported serovars [62]. *Salmonella* serotypes are associated with three distinct human disease syndromes, bacteremia, typhoid fever, and enterocolitis [63]. The RTCA indicates that this test should not be done for products that are prepared with boiling water [22]. But, in Costa Rica there is a tendency to use hot water, which is not an appropriate practice, given that some strains of *Salmonella* sp. are more resistant to heat [64].

CONCLUSIONS

The four analyzed batches of boldo tisanes were in compliance with the specifications of official books used for the following tests: minimum fill, lead limit, arsenic limit, loss on drying, total ash, microbial enumeration, and *E. coli* and *Salmonella* sp absence.

However, they did not were in compliance with the labeling test, given that only 2 of the 4 items established for the primary packaging (batch number and expiration date) and 3 of the 19 items indicated for the secondary packaging (qualitative-quantitative composition, interactions, and adverse effects) were not found. Another aspect was that the batch 2 had a browner color compared to the others, not complying with the specifications of the organoleptic test, specifically the color. This is reaffirmed by obtaining a greater percentage of branches in its composition during the foreign matter test. Finally, there is a recommendation regarding the need to improve the filling product process to avoid differences in the tisanes as large as those shown in this research. For these reasons, greater controls must be made on the raw material used for the product preparation to achieve reproducibility between the quality characteristics required for the different batches.

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