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Phytochemical profile and quality control of complex herbal mixtures used to prepare slimming drinks, commercialized in Salvador-Bahia, Brazil

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Traditional herbal products with multiple associated plant species and weight loss claims are widely consumed. The inadequacy of these resources to health standards, the lack of supervision, and the analytical obstacles involved in their investigation are challenges and reasons for this research. In this sense, this study aimed to characterize the profiles: Phytochemical, physical, physicochemical, and microbiological, of eight brands of crude drugs commercialized for the preparation of weight loss teas in the cities of Salvador and Lauro de Freitas in Bahia, Brazil. Phytochemical tests showed the presence of total phenolics and flavonoids using spectrophotometric and spectroscopic techniques. In the spectrophotometric assay of phenolics, two samples, 5A and 6A (same formulation), showed 185 and 140 mg EGA.g^{-1}, respectively. In the content of flavonoids, samples 6A (eight drugs) and 1A (five drugs) obtained 56.2 and 52.8 mg EQ.g^{-1}, respectively, superior results compared to samples with a higher amount of drugs. In microbiological analyses, one sample was outside the bromatological limits for thermotolerant coliforms, and two disagreed with ANVISA legislation for the limits of molds and yeasts. The study points to the need to regulate physical, physicochemical, and microbiological parameters for herbal mixtures, adopt good practices by manufacturers, and expand local health inspection.

Key words: Medicinal teas, traditional products, authenticity, weight loss, phytotherapy.

INTRODUCTION

Interest in herbal medicines has increased significantly in recent years. According to the World Health Organization (WHO), about 80% of the population in developing countries depends on medicinal plants for primary health care (WHO, 2013). In Latin America, for example, more than 400 million people use traditional medicine (Guido et al., 2015). Numerous plants are often associated and used as a single therapeutic resource. The high demand

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is due to easy access, low cost compared to conventional medicine products and the search for natura alternatives l that, according to belief, have fewer side effects (Teschke and Eickhoff, 2015).

Traditional herbal products are commonly used for maintaining/promoting health and treating diseases in different world regions. In some cases, they can be marketed with parts of plants (bark, leaves, flowers, and roots) isolated or intercropped (complex herbal mixtures) (Jamshidi-Kia et al., 2018). Studies reveal that the combination of numerous plant species can give rise to improved and synergistic effects, as investigated for treating some types of cancer and in the fight against obesity (Yang et al., 2014).

According to WHO, worldwide obesity has nearly tripled since 1975. In 2016, over 1.9 billion adults, 18 years or older, were overweight. Of these, more than 650 million were obese. That same year, 340 million children and adolescents aged 5 to 19 years old were overweight or obese (WHO, 2021). Several medicinal plants are used for the treatment of obesity in ANVISA. Green tea is one of the most consumed plant species in the world, whether isolated or intercropped. According to the Food and Agriculture Organization of the United Nations (FAO/UN), world production of green tea will increase by 7.5% per year, reaching 3.6 million tons in 2027. Production could double in China, reaching 3.3 million tons (FAO/UN, 2018). In ethnobotanical research in the country, the most popular species cited for reducing body weight were *carqueja- Baccharis trimera* (Less.) DC; *soursop - Annona muricata* L.; *hibiscus-Hibiscus sabdariffa* L. and *green tea- Camellia sinensis* (L.) Kuntze (Cercato et al., 2015).

The rise of trade in products with complex herbal mixtures raises the attention of public authorities to quality, safety, and efficacy regulation. In terms of quality, the hygienic and sanitary conditions of these products are worrying. As for effectiveness and safety, it is essential to investigate the potential and risks associated with the consumption of these resources (Ndhlala et al., 2011). Another important issue is the terminology, regulatory status, and categorization of products with herbal mixtures in each country, especially those used in traditional medicine (Wardle et al., 2014).

Faced with the need to harmonize regulations on products with complex herbal mixtures, the WHO has shown interest in promoting specific standards for traditional medicine inputs, such as the definition of phytochemical quality markers (WHO, 2000). In 2013, at an event held in China, the agency expressed concern about the trade and consumption of products that needed formal recognition in the countries of origin. On the other hand, eastern nations, such as China and India, stand out for promoting the harmonious integration of traditional medicine with their respective health systems (He et al., 2015).

In Brazil, WHO guidelines were instrumental in building Initiatives aimed at traditional medicine, which was institutionalized as Integrative and Complementary Practices (PICs) (Approves National Policy on Integrative and Complementary Practices “PNPIC” in the Unified Health System “SUS.”, 2006). One of these initiatives, the National Policy on Medicinal Plants and Herbal Medicine, highlights the importance of the State in guaranteeing quality, effectiveness, and safety of herbal medicines in the consumer market (ANVISA, 2012).

Considering the need to reaffirm public policies for traditional medicine in Brazil and the interest of manufacturers and consumers in products with multiple plant species mixed, traditionally used for weight loss, it is necessary to analyze the quality of these resources. In addition to the analytical challenges involved in investigating herbal mixtures, this research has enabled the development of comprehensive approaches to studying the quality of these resources. Thus, this work aimed to characterize the microbiological, physical, physicochemical, and phytochemical profiles of eight brands of herbal mixtures with weight loss functions described on the packaging labels available on the formal market in Bahia.

MATERIALS AND METHODS

Selection of samples, identification, and preparation of hydroethanolic extracts

Visits were made to establishments in the commercial centers of Salvador and Lauro de Freitas in Bahia, Brazil. Market observation forms were filled out to verify which brands of herbal mixtures for slimming teas were most commonly found on the shelves of formal commercial establishments (drugstores and health food stores). The criteria used in choosing the products were: (1) being the most cited in market observation forms; (2) having more than one plant species recognized by the Brazilian population for weight loss in the study locale (Cercato et al., 2015); and (3) having an implicit or explicit term on the labels of the packaging that conveyed some information about weight loss to buyers. Based on the inclusion criteria, eight brands were selected and acquired from the consumer market for the study (1A to 8A) (Table 1).

Herbal mixtures 1A, 2A, 3A, 4A, 7A, and 8A were in powdered form and packaged in primary plastic packaging and secondary kraft paper packaging. These were subjected to pulverization in a TECNAL knife mill (Wiley type). Samples 5A and 6A were in powder form (sachets) and stored in packaging made of polyethylene terephthalate (PET) material, packed in secondary kraft paper packaging and tertiary polypropylene plastic (PP). Before being analyzed, the samples were kept in the original packaging at a temperature of 28 ± 2°C, protected from light and moisture. The analyses were performed in triplicate.

For the phytochemical analyses, 40 g aliquots of each powdered sample were weighed, and then subjected to maceration extraction with 360 mL of 70% ethanolic solution (v/v). The hydroethanolic extracts (EH) were subjected to filtration and subsequent evaporation of the residual solvent under reduced pressure in a rotary evaporator. The crude extracts were placed in a greenhouse with air circulation for 24 h at a temperature of 35°C until complete removal of water, and then the yields were calculated.

Preliminary phytochemical analysis

For the phytochemical characterization of the complex matrices,

Table 1. Name individual drug species and parts used in each sample with herbal mixtures.

Sample	Common name	Part used	Botanical Name *
1A	Porangaba	Leaf	*Cordia ecalyculata* Vell.
	Centella	All the plant	*Centella asiatica* L.
	Horsetail	All the plant	*Equisetum arvense*L.
	Sene	Leaf and seed	*Senna alata* L.
	Chamomile	Flower	*Matricaria chamomilla* L.
2A	Hibiscus	Flower	*Hibiscus sabdariffa* L.
	Green Tea	Leaf	*Camellia sinensis* (L.) Kuntze
	Olive tree	Flower	*Olera europaea* L.
3A	Avocado plant	Leaf and stem	*Persea americana* Mill.
	Green Tea	Leaf	*Camellia sinensis* (L.)
	Carqueja	Leaf	*Baccharis spp.*
	Leather hat	all the plant	*Echinodorus macrophyllus* Kunth.
	Jambolan	Leaf	*Syzygium jambolanum* D.C
	Horsetail	all the plant	*Equisetum arvense*L.
	Melissa	Leaf	*Melissa officinalis* L.
	Douradinha	Leaf	*Waltheria douradinha* St. Hilaire
	Sarsaparrill	Leaf	*Smilax japecanga* Griseb.
	Porangaba Tea	Leaf	*Cordia ecalyculata* Vell.
	Carobinha	Leaf	*Jacaranda caroba* (Vell.) A. DC.
	Sene	Leaf and seed	*Senna alata* L.
	Dandelion	all the plant	*Taraxacum officinale* Web.
	Fennel	Flower	*Foeniculum vulgare* (Mill.) Gaertn.
	Lemon balm	Leaf and flower	*Lippia Alba* (Mill.) N.E.Br
	Seven-Bleeding	all the plant	*Cuphea carthagenesis* (Jacq.) J.F.
	Hibiscus	Flower	*Hibiscus sabdariffa* L.
	Soursop	Leaf	*Anonna muricata* L.
	Fucus	all the plant	*Fucusvesiculosos* L.
	Stonebreaker	Aerial part	*Phyllanthus niruri* L.
	Chile boldo	Leaf	*Peumus boldus* Molina
	Jurubeba	Leaf and fruit	*Solanum paniculatum* L.
	Angelica	Air part	*Angelica archangelica* L.
	Ironwood	Trunk bark	*Caesalpinia leiostachya* (Benth.)
	Cashew tree	Leaf and bark	*Anacardium occidentale* L.
	Spark	All the plant	*Centella asiatica*(L.) Urban
	Artichoke	Fruit and leaf	*Cynara scolymus* L.
	Malva Branca	Leaf	*Sidia cordifolia* L.
	Porangaba	Leaf	*Cordia salicifolia* Cham.
	Velame	Leaf	*Croton campestris* A. St.-Hil.
	juah	Bark	*Ziziphus joazeiro* Mart
	Sucupira	Seed	*Pterodon emarginatus* Vog.
	Ipe Purple	Bark	*Tabebuia heptaphylla* Vellozo
	Jatobá	Bark	*Hymenaea courbaril* L
4A	Avocado tree	Leaf	*Persea americana* Mill.
	Green Tea	Leaf	*Camellia sinensis* (L.) Kuntze
	Carqueja	Leaf	*Baccharis spp.*
	Leather hat	All the plant	*Echinodorus macrophyllus* (Kunth.)
	Jambolan	Leaf	*Syzygium jambolanum* D.C
	Horsey	All the plant	*Equisetum arvense* L.

Table 1. Contd.

	Cane of the brejo	Leaf and stem	*Costus spicatus* SW.
	Douradinha	Leaf	*Waltheria douradinha* St. Hilaire
	Pau de Resposta	Trunk bark	*Anemopaegma arvense* (Vell.)
	Porangaba tea	Leaf	*Cordia salicifolia* Cham.
	Carobinha	Leaf	*Jacaranda caroba* (Vell.) A. DC.
	Sene	Leaf and seed	*Senna alata* L.
	Passion fruit	All the plant	*Passiflora edulis* Sims.
	Mango	Leaf	*Mangifera indica* L.
	Artichoke	Leaf and fruit	*Cynara scolymus* L.
	Malva Branca	Leaf	*Sidia cordifolia* L.
	Porangaba	Leaf	*Cordia salicifolia* Cham.
	Velame	Leaf	*Croton campestris* A. St.-Hil.
	Ipe Purple	Trunk bark	*Tabebuia heptaphylla* Vellozo
	Jatoba	trunk bark	*Hymenaea courbaril* L
	Lemon balm	Leaf and flower	*Lippia Alba* (Mill.) N.E.Br. ex Britt &
	Seven Sangrias	All the plant	*Cuphea carthagenesis* (Jacq.) J.F.
	Mulungu	Bark	*Erythrina verna* L.
	Cinnamon of old man	Bark and leaf	*Miconia albicans* (Sw.) Steud.
	Cashew tree	Bark and leaf	*Anacardium occidentale* L.
	Soursop	Leaf	*Anonna muricata*L.
	Cat nail	Bark	*Uncaria tomentosa* Willd.
	Mangabeira	Leaf and latex	*Hancornia speciosa* Gomes.
	Centelha	All the plant	*Centella asiática* (L.) Urban
	Pau pereira	Bark	*Geissospermum vellosi* Allemao
	Catingueira	Leaf and stem	*Caesalpinia pyramidalis* Tul.
	Marapuama	Trunk bark	*Ptychopetalum olacoides* Benth.
	Green Tea	Leaf	*Camellia sinensis* (L.) Kuntze
	Carqueja	Leaf	*Baccharis genistelloides* Lamarck
	Mate herb	Leaf	*Illex paraguariensis* St. Hill
5A	Mint	Leaf and stem	*Mentha piperita* L.
	Ginger	Root	*Zingiber officinale* Roscoe
	Guarana	Seed	*Paullinia cupana* L.
	Salvia	Leaf	*Salvia officinalis* L.
	Rosemary	Aerial part	*Rosmarinus officinalis* L.
	Green Tea	Leaf	*Camellia sinensis* (L.) Kuntze
	Carqueja	Leaf	*Baccharis genistelloides* Lamarck
	Mate herb	Leaf	*Illex paraguariensis* St. Hill
6A	Mint	leaf and stem	*Mentha piperita* L.
	Ginger	Root	*Zingiber officinale* Roscoe
	Guarana	Seed	*Paullinia cupana* L.
	Salvia	Leaf	*Salvia officinalis* L.
	Rosemary	Aerial part	*Rosmarinus officinalis* L.
	Carqueja	Leaf and stem	*Baccharis gaudichaudiana* DC
	Soursop	Leaf	*Anonna muricata*L.
7A	Hibiscus	Flower	*Hibiscus sabdariffa* L.
	Sene	Leaf	*Senna alata* L.
	Horsetail	Aerial part	*Equisetum arvense*L.
	Mint	Leaf and stem	*Mentha piperita*L.
	Green Tea	Leaf	Camellia sinensis (L.) Kuntze

Table 1. Contd.

	Hibiscus	Flower	*Hibiscus sabdariffa* L.
	Oliveira	Leaf	*Olera europaea* L.
8A	Green Tea	Leaf	*Camellia sinensis* (L.) Kuntze
	Porangaba	Leaf	*Cordia ecalyculata* Vell.
	Carqueja	Leaf	*Baccharis spp.*
	Eggplant	Leaf	*Solanum melongena* L.

*****Information not described on the packaging (except 5A, 6A, 7A and 8A), inserted for didactic purposes. In bold: species described in official Brazilian Health Regulatory Agency (ANVISA) lists in at least one of the categories: Food, Spice, Traditional Herbal Product, and/or Herbal Medicine.

tests were carried out to determine total phenolics (using the spectrophotometric quantification method in microplates with the Folin-Ciocalteau reagent), total flavonoids (using the spectrophotometric method in microplates with aluminum chloride – $AlCl_3$), and spectroscopic analysis through Hydrogen Nuclear Magnetic Resonance (^{1}H NMR). Identification of characteristic phenolic peaks was confirmed by comparison with literature data (Dos Santos et al., 2017; Imai et al., 2020).

The presence of phenolic and flavonoid compounds with bioactive potential, especially antioxidants, has been related to preventing diseases such as cancer. There is no direct relationship between phenolic compounds and weight loss. However, the benefit of eliminating free radicals has been widely disclosed on the labels of herbal mixtures marketed with a slimming objective. In addition, the characterization of phenolic and flavonoid compounds using spectrophotometric and spectroscopic techniques may be useful for assessing the bioactive potential and providing storage conditions.

The results of phenolics and total flavonoids were expressed based on the equation generated after the construction of a standard gallic acid curve (y = 0.0012x – 0.00006; R2 = 0.9982), expressed in terms of mg equivalent of gallic acid/g of extract (mg EGA.g^{-1}) and standard quercetin curve (y = 0.0032x + 0.0012; R2 = 0.9982), expressed in mg of quercetin equivalents/g of extract (mg Q/g^{-1}), respectively.

One-dimensional 1H NMR analysis was performed on a BRUKER Avance III 500 spectrometer (11.75 T) equipped with a Broadband Inverse Detection (BBI) probe with a 5 mm internal diameter and field gradient in the Z direction. The spectra were acquired using a Zgpr pulse sequence, with an acquisition time of 5.24 sec, relaxation time of 8 sec, pulse duration of 9.48 µs, 64K points in the time domain distributed in a spectral window of 25 ppm, with an accumulation of 128 spectra at a temperature of 300 K. The program used in the pre-treatment of the 1H NMR spectra obtained was the ACD/NMR Processor Academic Edition 12.0. Chemical shifts were referenced to the TMS signal at δ 0.00 ppm.

Physical and physicochemical quality control of herbal mixtures

Physical and physicochemical analyses were performed as described in Brazilian Pharmacopoeia (BP) 6th ed by Brazilian Health Regulatory Agency [ANVISA] (2019a). The macroscopic identification of mixtures was based on shape, size, and appearance (aspect). After quartering the sample, the foreign material (ME) content was determined using a magnifying glass. Apparent density was performed in a 100 mL test tube with known weight, using 50 g of sample. The result was obtained through the mass/volume ratio of the samples.

The total ash content was determined in a muffle at 550°C. 3 g of the sample were weighed in previously dried crucibles and incinerated for 6 h. The residues obtained in the total ash test were used to determine the ash insoluble in HCl. The sample was heated with 2 M HCl solution (v v^{-1}), and the acid-insoluble material was collected with filter paper of known ash content. Afterward, the residue's filter paper was transferred to the original crucible, dried in an oven at 40°C for 1 h, and incinerated in a muffle at 550°C for 6 h. The moisture content was determined in a drying oven (model SL 100) at 105°C, using porcelain capsules previously dried. 2 g of powdered samples were weighed and placed in an oven for 6 h. The extractive content was determined by heating the samples in water under agitation, followed by resting and filtering. Afterward, the material was swelled in a volumetric flask, and an aliquot was evaporated in a water bath and dried in an oven at 105ºC for 2 h (ANVISA, 2019b).

Microbiological quality control of complex herbal mixtures

Microbiological analyzes were performed according to the methodology of the American Public Health Association-APHA (APHA, 2001). The search for *Salmonella* spp. was performed using the classic and sensitive cultural method of presence or absence, with a detection limit of 1 CFU/25 g of sample. The procedure followed the steps of pre-enrichment, selective enrichment, differential selective plating, and biochemical confirmations. Thermotolerant coliform analyses were performed using the most probable number (MPN) method. Positive reactions were compared with the NMP Hoskins table (MPN g mL^{-1}) for thermotolerant coliforms. For the count of molds and yeasts (total fungi), the surface sowing technique (spread plate) was used in Petri dishes containing the culture medium Agar Dicloran Rose Bengal Chloramphenicol (DRBC) (Difco, France), and the results determined in Colony Forming Units, per gram of analyzed sample (CFU. G^{-1}).

The results of the microbiological tests were compared to the health parameters established by the National Health Surveillance Agency (Agência Nacional de Vigilância Sanitária) (ANVISA) for dietary and medicinal products. Normative Instruction No. 161, July 1, 2022, establishes microbiological standards for food products (recreational teas). The parameters of the Brazilian Pharmacopoeia 6th ed. (2019) are used by the manufacturer when the products are considered traditional herbal medicines (medicinal teas). In turn, Resolution of the Collegiate Board (RDC) No. 26, of May 13, 2014: “Determines the Publication of Guidance for the Registration of Herbal Medicines and Registration and Notification of Traditional Herbal Medicines” (ANVISA, 2019a, 2022, 2014).

Statistical analysis

The determinations of total phenolics, total flavonoids, and physicochemical analyses were carried out in triplicate, and the

Table 2. Determination of total phenolics and flavonoids in hydroethanolic extracts from commercial samples of complex herbal mixtures.

Sample	Total Phenolics mg EGA.g^{-1}	Total flavonoids mg EQ.g^{1}
1A	88.06 ± 0.03	52.81 ± 0.02
2A	135.28 ± 0.06	24.89 ± 0.05
3A	109.45 ± 0.04	35.10 ± 0.01
4A	108.61 ± 0.01	38.96 ± 0.00
5A	185.28 ± 0.05	42.08 ± 0.01
6A	140.56 ± 0.01	56.25 ± 0.01
7A	100.84 ± 0.03	26.36 ± 0.00
8A	110.28 ± 0.04	29.38 ± 0.00
Standard	411.11 ± 0.02	247.81 ± 0.05

results were expressed as mean ± SEM (standard error of the mean) with the help of the program (Microsoft® Excel® 2019 MSO - Versão 2311).

RESULTS AND DISCUSSION

The results of the phytochemical, physical, physical-chemical, and microbiological analyses are displayed in Tables 2 to 5. Such analyses aimed to know the profiles of the associated drugs and control the quality of these drugs to verify the sanitary suitability of the products on the market.

Phytochemical analysis

The preliminary phytochemical analysis confirmed the presence of total phenolics and flavonoids (Table 2). The results of total phenolics ranged from 88.06 to 185.28 mg EGA g^{-1}, with sample 5A (eight drugs) having the highest value: 185.28 mg EGA g^{-1}, followed by samples 6A (eight drugs) and 2A (three drugs), with 140.56 and 135.28 mg EGA g^{-1}, respectively.

According to data from the literature, in a study carried out with hydroethanolic extracts of Brazilian green tea (*Camellia sinensis* L. var. *assamica*), total phenolics (TP) levels of up to 120 mg were shown. g^{-1} of the sample (Nishiyama et al., 2010). In this study with mixtures, samples 5A and 6A (eight drugs and the same formulation), which allege the presence of green tea (*C. sinensis* L.) in the mix, obtained the highest levels of TP among the studied samples. The study's results with isolated green tea corroborate this research with mixtures since all samples (except 1A) that supposedly contain green tea in the composition showed TP content above 100 mg.EGA.g^{-1} of extract in hydroethanolic solution. In another study that compared the method of infusion and aqueous and alcoholic decoction of calyces of *Hibiscus sabdariffa* L., the TP levels found were higher in alcoholic infusion (up to 71.58 mg. EGA. g^{-1}), demonstrating greater efficiency in the alcoholic extraction of secondary compounds (Sobota et al., 2016). The TP content for the individual aqueous extracts of *H. sabdariffa* L. was below the TP content when this drug was associated with green tea (*C. sinensis* L.) in the hydroethanolic extracts of samples 2A, 7A, and 8A (> 100, 84 mg.EGA.g^{-1}). The results of these three mixtures with *C. sinensis* L. and *H. sabdariffa* L. reaffirm the TP content with the two species present. In another research with three batches of aqueous extracts of nine species belonging to the traditional medicine of the Amazon, commercialized in the market of Belém, in Pará- Brazil, TP levels ranged between: 27.04 and 721.08 mg. EGA.g^{-1} was obtained (Port's, 2011). In this research with Amazonian plants, the soursop extracts (*Annona muricata* L), prepared through aqueous infusion, and showed: 159.22 mg.EGA.g^{-1}, a result above the mixtures containing soursop in this study: 3A, 4A, and 7A (up to 109.44 mg.EGA.g^{-1}). A study carried out with six traditional species from the Eastern Anatolia region of Turkey showed results between 17.4 and 35.3 mg.EGA.g^{-1} for methanolic extracts was obtained for *Malva neglecta* and *Plantago lanceolata*, respectively (Dalar et al., 2012). In a study with different types of extracts (aqueous, methanolic, ethanolic, acetone, and ethyl acetate) of *Cassia angustifolia* (Sene), supposedly present in samples: 1A, 3A, 4A, and 7A, the levels of TP in concentrations of 100 to 250 µg.µL^{-1}, ranged from 0.53 to 2.32 mg. EGA. g^{-1}, with methanolic extract being the most efficient (Ahmed et al., 2016). Most of the results observed in previous studies were inferior to this study with complex matrices. The results of this research with complex herbal matrices showed a relationship between the TP content and the presence of species such as *Baccharis genistelloides* Lamarck *C. sinensis* L. and *H. sabdariffa* L., mainly the samples: 2A, 5A, and 6A. This result can also be verified by analyzing the stacked ^{1}H NMR spectra since all the mixtures that showed a high TP content in the spectrophotometric assays (2A to 8A) showed prominent peaks in the phenolic region (Figure 1).

Baccharis trimera (Less) DC is one of the species on the

Table 3. Sensory and macroscopic analysis of samples with associated drugs, commercialized for preparing weight loss drinks.

Physical analyzes	Samples							
	1A	2A	3A	4A	5A	6A	7A	8A
Organoleptic characterization	Strong aromatic odor, presence in a smaller proportion of leaves and a larger proportion of stems of the erased plants. Whitish to the greenish color	Sweet and fruity aroma, intense purplish color, and presence in a greater proportion of flowers in relation to scratched leaves	Woody and sweet aroma, with brown colored stems in greater proportion to the light green leaves	Presence of a greater proportion of shaved stems and trunks leaves with a greenish-brown appearance, and a sweet aroma	Homogeneous mixture in powder, with a strong refreshing odor characteristic of menthol. Drugs with a fluffy (bulky) appearance.	Homogeneous mixture in powder, with a characteristic odor of menthol, all in the form of fine powder	Compound rich in stems and leaves, with a smaller proportion of flowers. Brownish brown appearance, with purplish traces. Sweet and refreshing aroma similar to menthol	Granular and homogeneous mixture, strong sweet and refreshing aroma. Mix with purple and greenish colors
Strange material * (% w.w $^{-1}$)	0.6	0.0	1.2	0.9	1.3	0.8	1.1	0.0
Apparent density (g.mL^{-1})	0.16	0.16	0.13	0.20	0.35	0.29	0.11	0.25
Amount of drugs in mixtures **	05	03	37	37	08	08	07	06

* Except for parts of vegetables (flowers, leaves, and stems). It should not exceed 2% (w.w^{-1}), according to the Brazilian Pharmacopoeia 6th, ed. (2019). **According to the information described on product labels.

Table 4. Physical-chemical analyzes of the eight commercial samples of complex herbal mixtures.

Assay	Samples							
	1A	2A	3A	4A	5A	6A	7A	8A
Total ash (%) M ± SD*	7.07 ± 0.11	7.20 ± 6.57	7.65 ± 0.51	3.45 ± 0.15	6.45 ± 0.16	7.55 ± 0.22	8.20 ± 0.35	6.67 ± 0.30
Insoluble ash (%) M ± SD	0.70 ± 0.16	0.66 ± 0.32	1.11 ± 1.27	0.46 ± 1.09	0.25 ± 0.20	0.82 ± 0.14	1.62 ± 0.26	0.40 ± 0.14
Moisture content (% w.w $^{-1}$)	16.13	18.15	16.24	18.16	14.49	16.64	17.88	17.75
Water-soluble extractive (% w.v^{-1})	27.97	42.91	22.27	20.13	38.13	30.68	41.27	29.67

official list of 71 Unified Health System (SUS) plants of interest and is widely used in mixtures for weight loss in Brazil (ANVISA, 2014). All samples (except 1A and 2A) that supposedly have species of the genus *Baccharis* in the mixture showed characteristic spectroscopic signals in the three spectral regions, such as pairs of doublets between ^{1}Hδ 5.5 and 11.0 ppm; and singlets between ^{1}Hδ 1.28 to 0.89 ppm and in the region close to: ^{1}Hδ 3.85 and 6.7 ppm (Chaves et al., 2020). Signals characteristic of *Baccharis genistelloides*, supposedly present in samples 5A and 6A, were detected between ^{1}Hδ 7.53 and 6.84 ppm, probably attributed to *trans*-methyloside and Hydroxy-5,4'-dimethoxy-flavone, respectively. Likewise, samples 2A, 5A, 6A, 7A, and 8A, which claim to have green tea (*C. sinensis* L) in the mixtures, showed singlets characteristic of caffeine in the regions between ^{1}Hδ = 3.3-4.0 ppm and another in the phenolic region: ^{1}Hδ = 7.5-7.8 ppm, already evidenced in another study

Table 5. The microbiological analyzes of the commercial mixtures, according to ANVISA's sanitary specifications for dietary teas (mixed tea).

Assay	Specification sanitary*	Sample						
		1A	2A	3A	5A	6A	7A	8A
tal fungus count* (CFU.g^{-1})	N/E	1.4x10^4	<1.0x10^2	1.1x10^4	1.7x10	2.8x10	<1.0x10^2	2.6x10^3
Coliforms to 45ºC (MPN.g^{-1})	1x10^3	>1.1x10^3	< 3.0	9.3x10	4.0	9.0	< 3.0	4.3x10
Salmonella spp.	Absence in 25g	+	-	-	-	-	-	-

*N/E- Not Required by ANVISA in dietary teas, according to ANVISA's NI N°. 161/2022.

(Santos, 2019). Samples 1A, which does not allege the presence of "carqueja" (*Baccharis* sp.) and green tea (*C. sinensis* L.) in the mixture, obtained the lowest TF dosage: 88.06 mg EGA.g^{-1}. All samples claiming to have carqueja (*Baccharis* sp.) and green tea (*C. sinensis* L) species in the mixtures (2A to 8A) obtained characteristic ^{1}H NMR signals in the phenolic region, as well as TF levels between: 100.84 and 185.28 mg. EGA. g^{-1}, which can confirm the presence of these compounds in the studied complex matrices.

The levels of total flavonoids (FL) in the extracts of the mixtures are presented in Table 2. Samples 6A (08 drugs), 1A (05 drugs), and 5A (08 drugs) were the ones that showed the highest levels of FL among the investigated mixtures: 56.25, 52.81, and 42.08 mg.EQ.g^{-1}, respectively. The difference in FL between samples 5A and 6A (with the same composition) is probably due to the difference in drug concentrations in the two mixtures. Among the drugs present in samples 5A and 6A, which demonstrated the presence of flavonoid compounds in other studies, the following stand out green tea (*C. sinensis* L.), "carqueja" (*B. genistelloides* Lamarck) and "yerba mate" (*Illex paraguariensis*). A comparative research related the antioxidant activity and the anticarcinogenic potential in *Illex paraguariensis* and *C. sinensis* L. extracts with the presence of flavonoid compounds (Chandra and De Mejia, 2004). In another study with fourteen samples of green tea (*C. sinensis* L.) purchased from the formal market in Bahia- Brazil, the FL levels found in aqueous extraction, in the form of infusion with mechanical agitation, did not exceed 28.23 mg epicatechin equivalents per gram of sample (Firmino and Miranda, 2015). Samples 5A and 6A, as well as samples 2A, 3A, 4A, 7A, and 8A, which supposedly have *Camellia sinensis* L. in the mixture, showed considerable levels of FL when compared to individual studies of the species (between 24.90 and 56.25 mg.EQ.g^{-1} of the analyzed sample (Da Silva et al., 2013; Do Nascimento and Taveira, 2011).

Researches with the genus *Baccharis* sp. (family *Asteraceae*), supposedly present in samples 3A, 4A, 5A, 6A, and 7A, had already related the presence of polysaccharides and flavonoids, with bioactivity in species of the genus (Chaves et al., 2020; De Oliveira et al., 2014). Likewise, species of the genus *Equisetum* (Equisetaceae family), used as a diuretic, sudoriferous, and astringent in traditional Chinese medicine and, supposedly, present in samples 1A, 3A, and 4A, also demonstrated considerable FL content: between 35.10 and 52.81 mg.EQ.g^{-1} of the extract (Veit et al., 1993). In another study with *C. angustifolia* (Sene) leaf extracts, supposedly present in samples 1A, 3A, and 4A, the flavonoid content present ranged from: 1.29 mg.EQ.g^{-1} in aqueous extracts to 5.00 mg.EQ.g^{-1} in methanolic extracts, lower results of this study with mixtures (Ahmed et al., 2016). Other works with *C. ecalyculata* Vell., Boraginaceae family, supposedly present in samples 1A, 3A, 4A, and 8A, had already confirmed the presence of FL in this species (Assonuma, 2009; Dias, 2004). In another research carried out with nine species belonging to the traditional medicine of the Amazon, sold individually in the market of Belém, in Pará, Brazil, the FL levels varied between: 0.44 to 12.80 mg of epicatechin per gram of extract (Port's, 2011). In most studies with isolated drug extracts, supposedly present in mixtures from 1A to 8A, FL contents were below this study with complex matrices, between: 24.90 and 52, 81 mg.EQ.g^{-1}. This result can be confirmed by analyzing the stacked ^{1}H NMR spectra of the eight complex matrices, as they also showed prominent peaks of flavonoids in the three spectral regions (Figure 01). In the study by Dutra et al. (2020), doublets between ^{1}Hδ 8.0 and 6.52 ppm are attributed to the phenolic hydrogens of kaempferol and 3-O-methyl quercetin, as well as the hydrogen located at C7, attributed to trans-melilotoside. The presence of characteristic signs of the flavonoid 3-O-methyl quercetin, such as pairs of doublets in ^{1}Hδ 7.90 and 7.63; 6.42 and 6.20 ppm, already shown in the literature, were found in all commercial samples claiming to contain species of *Baccharis* sp. in the mixture (except 1A and 2A).

Characteristic singlets were found in samples 5A and 6A, which claim to have *Baccharis genistelloides* in the mix, between ^{1}Hδ 1.28-0.89 ppm and ^{1}Hδ 3.85 and 6.70 ppm (500 MHz, CD3OD). When compared with the literature, they can be typical of sugars, such as 4-O-β-glucopyranosyl-3', 5'-dimethoxybenzyl-tetrahydro- 2H-pyran-2-yl-acetyl (BaIII) (Bonacheva and Botirov, 2014). Characteristic peaks of flavonoids in mixtures 1A, 3A, 4A, and 7A, which claim to have Senna (*C. angustifolia*) in the mix, were also obtained, such as quercemethrin (δ 3.48-3.98), scutellarin (δ 6.22) and

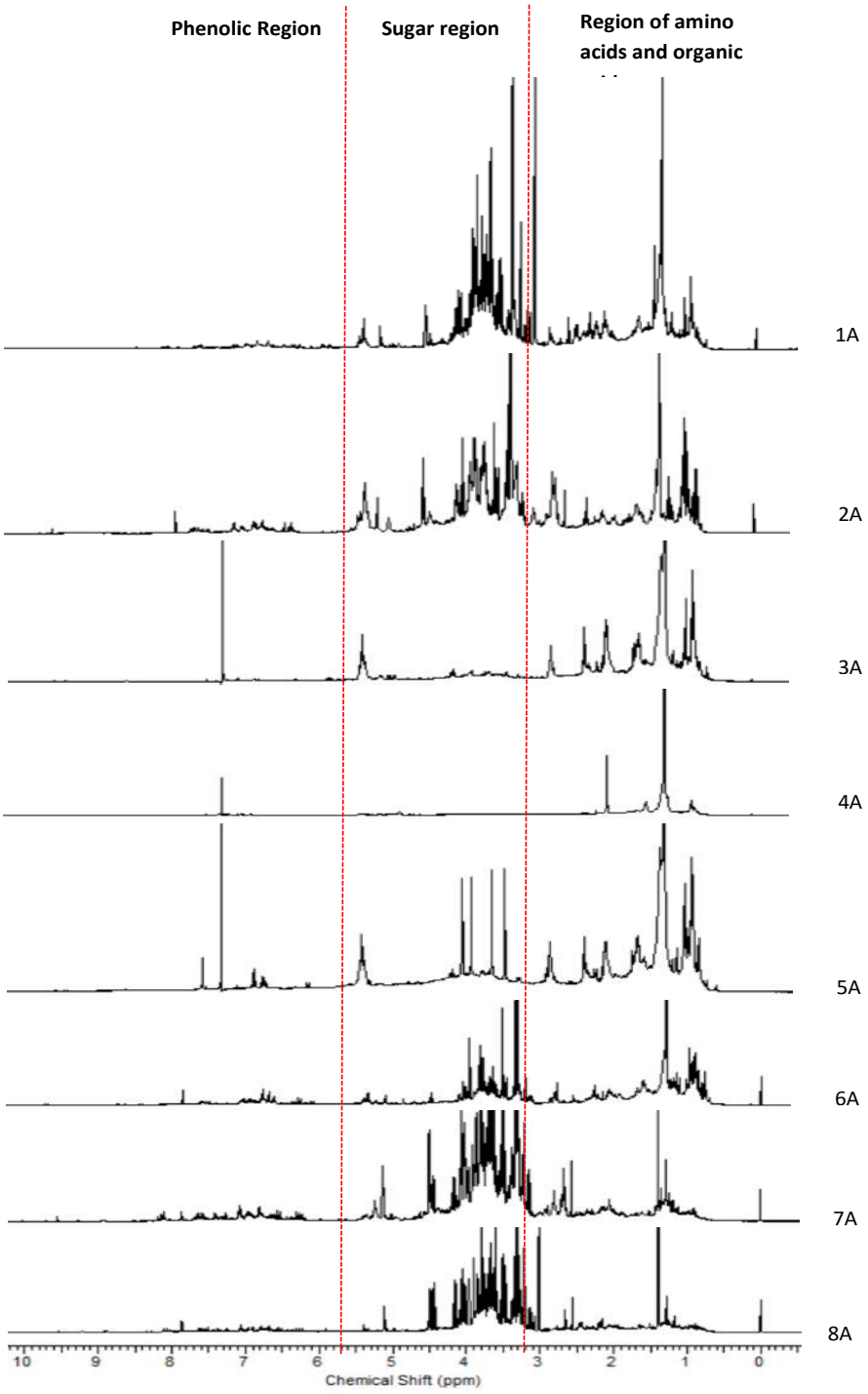

Figure 1. Stacked spectra of the complex matrices (1A to 8A) (^{1}H NMR, 500MHz, CD_3OD).

rutin (δ 1.15) (Ahmed et al., 2016). Two characteristic signals in the region ^{1}H δ = 5.1-5.2 ppm were also evidenced, as well as signals between ^{1}H δ = 1.26-1.30 ppm and 0.89 ppm in the high field, probably belonging to methylenes and methyl-terminal groups of fatty acids, also present in *Cassia* species (Fontella and Gindri, 2013). Species of the genus *Equisetum* (*Equisetaceae*) are used in traditional medicine in China, India, Vietnam, Russia, and North and South America (Bonacheva and Botirov, 2014; Tan et al., 2011). Known in Brazil as

"Cavalinha", the leading representative described in FB 6th ed. (2019), it is *Equisetum arvense* L. even though it is not native (ANVISA, 2019b). Comparing the spectroscopic fingerprints of the literature and the mixtures that claim to have *E. arvense*, it was possible to verify the presence of characteristic signs of flavonoids of the Kaempferol type in the mixtures: 1A, 3A, and 7A (between 26.35 and 52.81 mg.EQ. g^{-1}), as doublets in the region of ^{1}Hδ 8.0 and 6.89 ppm, typical of 3, 5, 7, 4-tetra-substituted flavonol protons. According to the study of *Equisetem silvaticum* L, from the autonomous region of Khanty Mansi, Russia, these signals are typical of flavonoid compounds (Bonacheva and Botirov, 2014). Therefore, the results of this study confirm the presence of flavonoid compounds obtained spectrophotometrically, mainly in the matrices: 1A, 4A, 5A, and 6A (between: 38.96 and 52.81 mg.EQ.g^{-1}). The higher levels of TP in the four samples mentioned above, about the other mixtures, is due to the supposed presence of plant species such as *C. angustiolia*, *E. arvensis*, *C. sinensis* L., and *B. genistelloides* in the combinations. The results of the ^{1}H NMR assays in the four plant matrices with the highest TP contents: 1A, 4A, 5A, and 6A, showed characteristic peaks of flavonoids, mainly in the region of sugars and phenolic acids, also evidenced in this study with mixtures.

Physical and physicochemical quality control of complex herbal mixtures

Several laboratory tests of identity, purity, and technical reports related to the quality control of raw materials and finished products are required by ANVISA to notify medicinal teas as traditional herbal products. However, regulations for comparing teas with associated herbs are still scarce, and studies of this nature are essential to contribute to constructing a database (Pelkonen et al., 2014). Although no standards were established in the FB 6th ed. (2019) for the sensory and macroscopic study of samples with herbal mixtures, only for isolated species, physical characterization was performed as a quality criterion for the first time (ANVISA, 2019b). The samples presented different formats, such as erasures, powder, and granules, whereas those in the form of erasures presented pieces smaller than 1 cm. The physical analyzes are shown in Table 3.

Samples 5A and 6A, with the same species mixed, according to information on the labels, had an odor similar to menthol. Except for samples 5A and 6A, which were presented in powder forms, the other samples were shown as erasures (pieces smaller than 2 cm). Samples 2A, 7A, and 8A showed a purplish color in the mixtures, similar to hibiscus flowers (*Hibiscus sabdarrifa* L.), also described on the product labels.

The maximum percentage of foreign material allowed in FB 6th ed. (2019) in plant drugs, with fungi, insects, and other non-animal contaminants, is 2% (w w^{-1}) (ANVISA, 2019b). The Resolution of the Collegiate Board - RDC N° 623/2022, from ANVISA, used as a parameter in diet teas, establishes the limit of insect fragments indicative of failures in good practices, between 100 and 200 units (associations with mint) every 25 g of analyzed sample; rodent fragments: 1 in 25 g (mixtures with *Peumus boldus* Molina, *Baccharis* species and *Mentha* sp.) and barbule (except pigeon): 50 in 25 g in products with *P. boldus* Molina, according to RDC N° 623/2022: "*Deals with Tolerance Limits for Foreign Matter in Food, the General Principles for Your Establishment and Methods of Analysis for Evaluation Purposes Conformity*" (ANVISA, 2022). No sample showed levels above the Brazilian health recommendations for foreign contaminants in traditional herbal products (medicinal teas) or dietetics products (recreational tea) norms. Other studies consider it stems from other species and the studied drug as foreign material when not specified in an individual monograph. In a survey of 25 samples of five plant species distributed in Health Units in Paraná-PR (Brazil), Alfavaca (*Ocimum basilicum* L.) obtained 36.53% of foreign matter. The result of research with species distributed in health units in Paraná showed an ME content far above the Brazilian health legislation for alfavaca; however, part of this material consists of stems and trunks of the plant itself (Garbin et al., 2013). In another study with green tea [*C. sinensis* (L.) Kuntze] sold in natural products stores, pharmacies, and drugstores in the Federal District- DF, 82% of the samples were rejected, according to parameters of the British Herbal Pharmacopeia (Nascimento and Taveira, 2011). In this investigation with herbal mixtures, it is subjective to distinguish plant contaminants. Good Agricultural Practices (GAP) and Good Manufacturing Practices (GMP) in the production chain of traditional herbal products or food, established by ANVISA, are necessary actions aimed at guaranteeing the quality and safety of these inputs in the market.

The researched samples presented densities between: 0.11 and 0.35 g mL^{-1}. Navarro et al. (2015) and Sobota et al. (2016), when working with drugs isolated from *Hibiscus sabdariffa* L. and *Morus nigra* L., obtained relative densities of 0.15 and 0.36 g mL^{-1}, respectively, similar to that observed in this study with mixed drugs. The apparent density is specific for each formulation and can serve as a parameter of the authenticity of the products on the market. Although this measure is not established by current Brazilian legislation, it is useful in assessing the degree of purity of samples and detecting fraud, such as adding species or substances other than those described in the label information used to add volume to mixtures.

The results of the physical-chemical analyzes are presented in Table 4. Total ash and insoluble in hydrochloric acid can detect organic and inorganic

impurities in vegetable samples, such as non-volatile residues (sand, silica, and metals). Such contaminants negatively influence the quality and may indicate failures in the manufacturing process (ANVISA, 2019b).

Tests to analyze the contents of total ash and acid-insoluble ash aim to determine the presence of organic and inorganic impurities in the samples, such as sand, silica, or glass. The recommended limits for individual species in Brazilian law are 2 to 20% ($w.w^{-1}$) for total ash and 0.7 to 5% ($w.w^{-1}$) for ash insoluble in hydrochloric acid (ANVISA, 2019a). The eight samples analyzed, in triplicate, presented total ash content between 3.45 and 8.20% ($w.w^{-1}$). For the ash content insoluble in hydrochloric acid (2M), the eight samples showed results between 0.25 and 1.62% ($w.w^{-1}$). Brazilian standards do not establish total ash and insoluble ash values for herb mixtures, only for individual herb species. When the results are compared with the averages for individual drugs, the eight samples analyzed demonstrated levels below the limits required by Brazilian legislation, demonstrating that there is no excess of impurities of organic and/or inorganic origin in the herbal mixtures studied. There was no direct relationship between the amount of herbs mixed in the samples and the results of the tests to determine the total and insoluble ash content in this research. Sample 4A (with 37 mixed herbs) and 5A (eight mixed herbs) showed lower levels among the eight samples analyzed. Sample 4A presented a total ash content of 3.45% ($w.w^{-1}$) and an insoluble ash content of 0.46% ($w.w^{-1}$). Sample 5A already showed a value of 6.45% ($w.w^{-1}$) for total ash. As for insoluble ash, sample 5A had a content of 0.25% ($w.w^{-1}$). Sample 7A (with seven mixed herbs) demonstrated the highest content among the eight samples analyzed: 8.20% ($w.w^{-1}$) for total ash and 1.62% ($w.w^{-1}$) for acid-insoluble ash, being lower than the limits established, on average, for individual herbs. The results of the study of a traditional Ayurvedic medicine formula, Hutabhugadi Curṇa (HC), standardized with six crude drugs: *Plumbago zeylanica*, *Apium leptophyllum*, rock salt, *Piper longum*, *Piper nigrum*, and *Terminalia chebula*, demonstrated limits of up to 14.27% ($w.w^{-1}$) for total ash content and 3.12% ($w.w^{-1}$) for acid-insoluble ash content (Pushpendra et al., 2016). Compared to the results of this Brazilian study with eight herbal mixtures, the limits of the Indian formula were higher for total and insoluble ash, which may demonstrate greater flexibility in Indian legislation or lower quality of the raw material used in this country. The definition of physical-chemical limits for herbal mixtures is already being adopted in standardizing traditional medicine formulas in other countries (Dinakaran et al., 2019; Knoess and Wiesner, 2019). In order to define the quality of each plant-based mixture present on the national market, it is necessary to standardize the limits for the content of total ash and acid-insoluble ash by Brazilian health authorities.

The moisture content of the analyzed samples ranged from 14.49 to 18.16%. No limits are established in Brazilian legislation for mixtures of plants; the current recommendation in FB 6th ed. (2019) is for individual drugs (between 8 and 14%) (ANVISA, 2019a). In a study carried out in health units in Paraná, Brazil, with 25 samples of five plant species, humidity between 10.67 and 14.47% was found, where one of the samples, basil or alfavaca was considered unfit for consumption, with moisture content above 14% (Gomes et al., 2008). According to the authors, the high moisture content can demonstrate flaws in these products' processing or inadequate storage.

In an Ayurvedic traditional medicine formula, Hutabhugadi Curṇa (HC), with six drugs mixed, moisture contents of up to 9.22% were adopted as a standardization criterion in local legislation (Pushpendra et al., 2016). The standardization and monitoring of this parameter in mixtures of plants in Brazil are essential since the excess of moisture in plant material (observed in all mixtures) leads to the degradation of constituents and the development of pathogenic microorganisms.

The content of extractives in the water of the analyzed mixtures ranged from 20.13 to 42.91%. The lowest values were observed in samples 3A and 4A (both with 37 drugs mixed), while the highest values were observed in mixtures 2A (three drugs) and 7A (seven drugs), both with the supposed presence of hibiscus (*H. sabdarrifa* L.) and green tea (*C. sinensis* L.) in the mixture. The results show no direct relationship between the number of drugs mixed and the amount of extractives obtained in water. The presence of purplish-colored flowers in samples 2A and 7A, attributed to hibiscus, as well as the presence of green tea, rich in phenolic compounds (tannins) and flavonoids, may explain the of extractives superior when compared to the other mixtures. In a research carried out with hibiscus flowers, purchased in formal trade in Paraná (Brazil), the efficient extraction of these compounds was also evidenced (Sobota et al., 2016). In a formula belonging to Ayurvedic traditional medicine, with six mixed drugs, the content of extractives in water standardized in the local health legislation was up to 42.05% (Pushpendra et al., 2016). Although there are no defined limits in the Brazilian sanitary legislation for water-soluble extractive in complex herbal mixtures, this parameter is important because it indicates the presence of water- soluble compounds in the extraction with this solvent, such as amino acids, sugars, flavonoid heterosides and mucilages (Barni et al., 2009). The results of this research on complex herbal mixtures can contribute to the formation of databases to be helpful in the quality control of these products in the future.

Microbiological analysis of complex herbal mixtures

The results of the microbiological analyzes are shown in Table 5. All samples showed the absence of *Salmonella* sp. in 25 g of the sample. A different result was observed in a study with samples of *C. sinensis* (L.)

Kuntze purchased from Araras, in São Paulo-SP, Brazil. The FB 6th ed. (2019) establishes the absence of *Salmonella* sp. in 10 g of sample analyzed in herbal products, while NI nº 161 (2022), establishes the absence of this microorganism in 25 g of sample in dietary products (ANVISA, 2022).

In analyzing thermotolerant coliforms, such as *E. coli*, sample 1A showed a result above the limit established by NI nº 161 (2022) in dietary products (dietary tea) of up to 10^3 most probable number per gram per sample analyzed (MPN. g^{-1}). Considering the limits of BP 6th ed. (2019) in herbal products (medicinal teas), sample 1A would be suitable for consumption due to the count for Gram-negative bacteria-tolerant bile being below the limit of 10^4 MPN g^{-1} (ANVISA, 2019b). When considering the limits for total fungal counts of the FB 6th ed. (2019) of up to 10^4 CFU g^{-1}, samples 1A and 3A would be unfit for consumption. In turn, the sanitary norm for drinks considered dietetic, the NI nº 161 (2022) does not contemplate the fungus count, despite the predisposition of these microorganisms in this type of sample. These data demonstrate the importance of harmonizing parameters in health regulations for the same type of sample (crude drug); the need to adopt good manufacturing practices by producing establishments, and the expansion of local inspection by competent authorities.

Conclusion

The absence of physical and physicochemical parameters in Brazilian legislation for investigating the authenticity and purity of herbal mixtures, along with the discrepancies found in microbiological boundaries for dietary and traditional herbal products, presented challenges in establishing the global quality of the studied samples. The research underscores the need for adjustments in regulatory aspects, such as standardizing physical and physicochemical boundaries for the analysis of herbal mixtures, which currently exist only for individual species. The study demonstrates the necessity to harmonize microbiological parameters for the same type of sample, as microbiological limits for dietary products are less stringent than those for traditional herbal products. This study also illustrates the bioactive potential in much of the analyzed samples and serves to characterize botanical authenticity using integrated analytical techniques.

Phytochemical analyses through fingerprints obtained by hydrogen magnetic resonance and spectrophotometric analyses in the ultraviolet-visible range successfully confirm the presence of flavonoids and total phenolics in six of the eight commercial matrices analyzed, except for the samples with the highest amount of mixed herbs, which could indicate fraud. Entry into the Brazilian commercial herbal mixing market, particularly for products claiming weight loss functions on packaging labels, should adhere to WHO (World Health Organization) and Anvisa recommendations for traditional herbal products rather than dietary supplements. Moreover, botanical authenticity through vegetable species identification reports before mixing should be adopted by manufacturers and supervised by sanitary authorities as a security prerequisite.

CONFLICT OF INTERESTS

The authors have not declared any conflict of interests.

REFERENCES

Ahmed SI, Hayat MQ, Tahir M, Mansoor Q, Ismail M, Keck K, Bates RB (2016). Pharmacologically active flavonoids from the anticancer, antioxidant and antimicrobial extracts of *Cassia angustifolia* Vahl. BMC Complementary and Alternative Medicine 16(1):1-9. https://doi.org/10.1186/s12906-016-1443-z

ANVISA - Brazilian Health Regulatory Agency (2012). RESOLUTION - RDC NO. 15, OF MARCH 15, 2012: Provides for good practice requirements for the processing of health products and provides other measures. Ministry of Health National Health Surveillance Agency. https://bvsms.saude.gov.br/bvs/saudelegis/anvisa/2012/rdc0015_15_03_2012.html

ANVISA - Brazilian Health Regulatory Agency (2014). Resolution of the Collegiate Board - RDC nº 26 of May 13, 2014. https://antigo.anvisa.gov.br/documents/10181/3078078/%281%29RDC+n%C2%BA+214_2018+-+English.pdf/335b2209-76a6-4813-b4da-3f20d7a5b372

ANVISA - Brazilian Health Regulatory Agency (2019a). Brazilian Pharmacopoeia, 6th edition. Medicinal herbs.

ANVISA - Brazilian Health Regulatory Agency (2019b). Brazilian Pharmacopoeia, 6th edition 1:874. https://doi.org/10.1590/S0102-33062006000100002

ANVISA - Brazilian Health Regulatory Agency (2022). Resolution of the Collegiate Board - RDC nº 623 of March 9, 2022. Deals with tolerance limits for foreign matter in food, the general principles for your establishment and methods of analysis for evaluation purposes conformity. Diário Oficial da União, Brasília, DF, 2022. https://in.gov.br/en/web/dou/-/resolucao-rdc-n-623-de-9-de-marco-de-2022- 386100039

APHA (2001). Compendium of methods for the microbiological of foods: American Public Health Association-APHA 4:676. http://lib.ugent.be/catalog/rug01:000842770

Assonuma MM (2009). Determination of allantoin and pharmacological assessment of *Cordia ecalyculata* Vell. (Bugre tea). In Paulista State University. https://repositorio.unesp.br/handle/11449/97972. Accessed in September 2020.

Barni ST, Cechinel Filho V, Couto AG (2009). Chemical and technological characterization of *Ipomoea pes-caprae* (L.) R. Br., Convolvulaceae, leaves, branches and entire plant as a pharmaceutical raw material. Brazilian Journal of Pharmacognosy 19(4):865-870. https://doi.org/10.1590/S0102- 695X2009000600012

Bonacheva VM, Botirov EK (2014). Kaempferol and its glycosides from *Equisetum silvaticum* L. from the khanty-mansi autonomous area. Russian Journal of Bioorganic Chemistry 40(7):777-780. https://doi.org/10.1134/S1068162014070048

Cercato LM, White PAS, Nampo FK, Santos MRV, Camargo EA (2015). A systematic review of medicinal plants used for weight loss in Brazil: Is there potential for obesity treatment? Journal of Ethnopharmacology 176:286-296. https://doi.org/10.1016/j.jep.2015.10.038

Chandra S, De Mejia EG (2004). Polyphenolic compounds, antioxidant capacity, and quinone reductase activity of an aqueous extract of *Ardisia compressa* in comparison to mate (*Ilex paraguariensis*) and green (*Camellia sinensis*) teas. Journal of Agricultural and Food Chemistry 52(11):3583-3589. https://doi.org/10.1021/jf0352632

Chaves PFP, Adami ER, Acco A, Iacomini M, Cordeiro LMC (2020). Chemical characterization of polysaccharides from *Baccharis trimera* (Less.) DC. Infusion and its hepatoprotective effects. Food Research International 136:109510. https://doi.org/10.1016/j.foodres.2020.109510

Da Silva BC, Da Silva F, Michelin DC (2013). Quality evaluation of *Camellia sinensis* (L.) Kuntze (Theaceae) samples sold in the city of Araras - SP. Journal of Basic and Applied Pharmaceutical Sciences 34(2):245-250.

Dalar A, Turker M, Konczak I (2012). Antioxidant capacity and phenolic constituents of *Malva neglecta* Wallr. and *Plantago lanceolata* L. from Eastern Anatolia Region of Turkey. Journal of Herbal Medicine 2(2):42-51. https://doi.org/10.1016/j.hermed.2012.03.001

De Oliveira SQ, Kappel VD, Pires VS, Lencina CL, Sonnet P, Moreira JCF, Gosmann G. Antioxidant properties of phenolic compounds from Baccharis articulata and B. usterii. Natural Product Communications 9(7):1934578X1400900714.

Dias TG (2004). Pharmacognostic Study of Porangaba (*Cordia Ecalyculata* Vell. - Boraginaceae) and identification of tampering. Master's Dissertation in pharmaceutical Drugs, Faculty of Pharmaceutical Sciences, University of Sao Paulo pp. 1-166.

Dinakaran SK, Chelle S, Avasarala H (2019). Profiling and determination of phenolic compounds in poly herbal formulations and their comparative evaluation. Journal of Traditional and Complementary Medicine 9(4):319-327. https://doi.org/10.1016/j.jtcme.2017.12.001

Do Nascimento FS, Taveira CC (2011). Evaluation of the quality of samples of *Camellia sinensis* (L.) Kuntze (Green tea) commercialized in the Federal District, Brazil. In Anhanguera Educacional Ltda. (Ed.), Student Scientific Initiation Production Yearbook. Faculty of Anhanguera de Brasília - Taguatinga Unit 13(17):63-80.

Dos Santos AE, Barreto Junior BC, Kuster MR (2017). Flavonoids and Antioxidant Potential from the Leaves of *Bauhinia longifolia* (Bong.) Steud. Revista Virtual de Química 9(3):1303-1317. https://doi.org/10.21577/1984-6835.20170076

Dutra LM, Da Conceição SAD, Lourenço AVF, Nagata N, Heiden G, Campos FR, Barison A (2020). 1H HR-MAS NMR and chemometric methods for discrimination and classification of Baccharis (Asteraceae): A proposal for quality control of *Baccharis trimera*. Journal of Pharmaceutical and Biomedical Analysis 184 p. https://doi.org/10.1016/j.jpba.2020.113200

Food and Agriculture Organization of the United Nations (FAO/UN) (2018). Current Market Situation and Medium-Term Outlook. Twenty-Third Session of the Intergovernmental Group on Tea pp. 13-16. http://www.fao.org/3/a-i4480e.pdf. Accessed in September 2020.

Firmino LA, Miranda MPS (2015). Contents of phenolics and flavonoids in green tea (*Camellia sinensis*) from samples of different brands available for sale in Salvador- Ba. Brazilian Journal of Medicinal Plants 17(3):436-443. https://doi.org/10.1590/1983-084X/11_041

Garbin L, Tiuman TS, Kruger RL (2013). Quality Evaluation of Medicinal Plants Distributed by Health Unit to Inner Cities of Paraná. *RECEN* - Revista Ciências Exatas e Naturais 15(1):77-94.

Gomes EC, Negrelle RRB, Elpo ERS (2008). Evaluation of the microbiological and physical-chemical qualities of lemongrass tea (*Cymbopogun citratus D.C*). Acta Scientiarum Health Sciences 30(1):47-54. https://doi.org/10.4025/actascihealthsci.v30i1.4396

Guido PC, Ribas A, Gaioli M, Quattrone F, Macchi A (2015). The state of the integrative medicine in Latin America: The long road to include complementary, natural, and traditional practices in formal health systems. European Journal of Integrative Medicine 7(1):5-12. https://doi.org/10.1016/j.eujim.2014.06.010

He TT, Ung COL, Hu H, Wang YT (2015). Good manufacturing practice (GMP) regulation of herbal medicine in comparative research: China GMP, cGMP, WHO- GMP, PIC/S and EU-GMP. European Journal of Integrative Medicine 7(1):55-66. https://doi.org/10.1016/j.eujim.2014.11.007

Imai A, Lankin DC, Godecke T, Chen SN, Pauli GF (2020). Differentiation of Actaea species by NMR metabolomics analysis. Fitoterapia 146:104686. https://doi.org/10.1016/j.fitote.2020.104686

Jamshidi-Kia F, Lorigooini Z, Amini-Khoei H (2018). Medicinal plants: Past history and future perspective. Journal of HerbMed Pharmacology 7(1):1-7. https://doi.org/10.15171/jhp.2018.01

Knoess W, Wiesner J (2019). The Globalization of Traditional Medicines: Perspectives Related to the European Union Regulatory Environment. Engineering 5(1):22-31. https://doi.org/10.1016/j.eng.2018.11.012

Navarro FF, Guizzo PL, Bredda TCC, Scarpa MVC (2015). Quality control and phytochemical screening of the plant drug in the leaves of *Morus nigra* L. (Moraceae). Journal of Basic and Applied Pharmaceutical Sciences 36(2):259-265. http://seer.fcfar.unesp.br/rcfba/index.php/rcfba/article/viewFile/240/144 Ndhlala AR, Stafford GI. Accessed in January 2021.

Nishiyama MF, Costa MAF, Da Costa AM, De Souza CGM, Boer CG, Bracht CK, Peralta RM (2010). Brazilian green tea (*Camellia sinensis* var assamica): effect of infusion time, mode of packaging and preparation on the extraction efficiency of bioactive compounds and on the stability of the beverage. Ciência e Tecnologia de Alimentos 30(3582):191-196. https://doi.org/10.1590/S0101-20612010000500029

Pelkonen O, Xu Q, Fan TP (2014). Why is research on herbal medicinal products important and how can we improve its quality? Journal of Traditional and Complementary Medicine 4(1):1-7. https://doi.org/10.4103/2225-4110.124323

Port's PS (2011). Phenolic compounds and antioxidant potential of herbs consumed in the Brazilian Amazonian region. Master's Dissertation in Food Science, Faculty of Engineering of Foods, University State of Campinas, pp. 1-82.

Pushpendra P, Sunil Kumar KN, Priyadarshini P, Holla BS, Ravishankar B, Yashovarma B (2016). Quality standards for *Hutabhuga¯di cu¯r*a (Ayurvedic Formulary of India). Journal of Traditional and Complementary Medicine 6(1):78-88. https://doi.org/10.1016/j.jtcme.2014.11.019

Sobota JF, Pinho MG, Oliveira VB (2016). Physical-chemical profile and antioxidant activity of the calyx of the species *Hibiscus sabdariffa* L. from the aqueous and alcoholic extract obtained by infusion and decoction. Fitos Magazine 10(1):33-46. https://doi.org/10.5935/2446-4775.20160004

Tan JM, Qiu YH, Tan XQ, Tan CH, Xiao K (2011). Chemical constituents of equisetum debile. Journal of Asian Natural Products Research 13(9):811-816. https://doi.org/10.1080/10286020.2011.596829

Teschke R, Eickhoff A (2015). Herbal hepatotoxicity in traditional and modern medicine: Actual key issues and new encouraging steps. Frontiers in Pharmacology 6:1-40. https://doi.org/10.3389/fphar.2015.00072

Veit M, Czygan FC, Witte L, Markham KR, Geiger H (1993). New Kaempferol Glycosides from Equisetum Species. *Zeitschrift Fur Naturforschung - Section B* Journal of Chemical Sciences 48(10):1398-1400. https://doi.org/10.1515/znb-1993-1015

Wardle J, Weir M, Marshall B, Archer E (2014). Regulatory and legislative protections for consumers in complementary medicine: Lessons from Australian policy and legal developments. European Journal of Integrative Medicine 6(4):423-433. https://doi.org/10.1016/j.eujim.2014.03.008

World Health Organization (WHO) (2000). General Guidelines for Methodologies on Research and Evaluation of Traditional Medicine pp. 1-80. http://apps.who.int/iris/bitstream/10665/66783/1/WHO_EDM_TRM_2000.1.pdf. Accessed in September 2020.

World Health Organization (WHO) (2013). WHO traditional medicine strategy: 2014- 2023:75. https://doi.org/10.1212/01.wnl.0000282763.29778.59

WHO, World Health Organization (2021). Obesity and overweight. https://www.who.int/news-room/fact-sheets/detail/obesity-and-overweight. Acessed in November 2020.

Yang Y, Zhang Z, Li S, Ye X, Li X, He K (2014). Synergy effects of herb extracts: Pharmacokinetics and pharmacodynamic basis. Fitoterapia 92:133-147. https://doi.org/10.1016/j.fitote.2013.10.010