The Eurasia Proceedings of Health, Environment and Life Sciences (EPHELS)
ISSN: 2791-8033

The Eurasia Proceedings of Health, Environment and Life Sciences (EPHELS), 2022

Volume 7, Pages 8-16

ICMeHeS 2022: International Conference on Medical and Health Sciences

Quality Control of Diet Herbal Teas Commercialized in the Region of Sidi Bel Abbes

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Abstract: Overweight and obesity are defined as abnormal or excessive accumulation of body fat to a level that represents a health risk, it is certainly linked to genetic components but the change in eating habits and the increase in inactivity through urbanization are the main causes of obesity. The most obvious management of obesity consists of a balanced diet and regular practice of physical activity. Traditional herbal remedies exist and can be divided into several groups including laxatives, lipolytic, appetite suppressants, and diuretics. Herbal teas based on these plants must be subject to constant quality control, to ensure patient safety. This study aims to control the quality of five herbal teas; for this, botanical and microbiological controls were done. Falsification detection with mineral substances was carried out using the total ash test. Botanical controls have revealed some elements that do not conform to the standards specified in the monographs, such as the lack of scientific information to guide the patient in his treatment and to identify herbal drugs. Microbiological controls have revealed fungal and bacterial contamination. Mineral falsification was detected in one of the five studied herbal teas.This study has shown the importance of quality control of herbal teas that are widely used by the population, hence the need to adopt stricter policies for their marketing

Keywords: Quality, Control, Herbal, Obesity

Introduction

Overweight and obesity are defined as abnormal or excessive accumulation of body fat to a level that represents a health risk, it is certainly linked to genetic components but the change in eating habits and the increase in inactivity through urbanization are the main causes of obesity. The most obvious management of obesity consists of a balanced diet and regular practice of physical activity. Traditional herbal remedies exist and can be divided into several groups including laxatives, lipolytic, appetite suppressants, and diuretics primaries (Bibalani & Mosazadeh-Sayadmahaleh, 2011). Herbal teas based on these plants must be subject to constant quality control, to ensure patient safety.

However, with their popularity and the global market expansion, herbal product safety has become a major public health concern. Regulatory failures and unsafe distribution channels (including Internet sales) can lead to adverse reactions due to the poor quality of these products. The most common causes are falsification, substitution or misidentification, incorrect dosages, interactions with conventional drugs, use of products contaminated with potentially dangerous substances such as microbial metabolites (e.g. mycotoxins),

- Selection and peer-review under responsibility of the Organizing Committee of the Conference

radioactive particles, heavy metals, and agrochemical residues (Kosalec et al., 2009). In such a marked context by the increasing use of herbal products by the public, it appears imperative to take the necessary measures to evaluate the health claims of these products, and to control and develop quality and manufacturing standards. The main objective of this study was to evaluate the quality of dieting herbal teas available over the counter in Sidi Bel Abbès Community pharmacies.

Materials and Methods

The study focused on five herbal teas: herbal tea Bio 3, herbal tea Fassa, herbal tea Medicaflor herbal tea Wassilat, and herbal tea Santé vie. These products were obtained in a community pharmacy in Sidi bel Abbes. For each herbal tea, a quantity was crushed and stored in a new paper kraft bag in a dry place at room temperature. The remains of unground herbal teas were kept in their packaging.

Control Tests

Control tests were assessed according to the WHO guidelines for assessing quality of herbal medicine (world health organization, 2007). Botanical controls of the five herbal teas were carried out through the setting of a visual control sheet, macroscopic and microscopic observation, total ashes determination, maceration, and observation of the extracts by UV light.

Visual Checklist

Herbal teas were visually inspected in order to discover the signs of a possible counterfeit: defective packaging, bad labeling, and incorrect dosage. A visual checklist form was created for each herbal tea. This form was based on the WHO guidelines for assessing quality of herbal medicine (world health organization, 2007).

Macroscopic Observation

It is an examination, which is made with the naked eye. It allows us to identify the presence of foreign elements (pebbles, snail shells...), but also foreign parts (stems accompanying the leaves for example). A quantity of each tea was put in a beaker. With the help of a pair of tweezers, we looked for foreign elements. Then, we checked the real presence of plants including the composition by organoleptic characteristics like color, smell, and aspect.

Microscopic Observation

Powder small quantity of each herbal tea was spread on a slide with a few drops of Gazet du Chatelier reagent. The slide was covered by a coverslip. For microscopic observation, the focus was made with the l0 x lens before switching to the 40xn lens. The characteristic elements were noted and photographed (Baytop, 1956).

Total Ash

Total ash refers to the total amount of material produced after powder incineration at a temperature of 600 ° C to remove all carbon atoms (organic matter). Two porcelain crucibles were heated for 30 min, cooled in a desiccator, and then weighed. In each crucible, 1.00 g of herbal tea powder was introduced and distributed evenly inside. After drying for 1 h at 100 °C in the oven, the crucibles were incinerated in a muffle furnace at a temperature of 600 °C. After each incineration, the crucibles were placed in a desiccator for cooling and then weighed separately. Incineration was continued until a constant mass was obtained (Leong et al., 2020). The total ash content was calculated according to the following formula:

$$\% \text{ Ash (uncorrected)} = \frac{W3 - W1 \times 100}{W2}$$

Where W1 = mass of crucible
W2 = mass of sample in grams
W3 = mass of ash and crucible in grams

Maceration and Observation under UV Light

For the maceration process, four solvents were used: sulfuric acid, benzene, methanol and chloroform. Sulfuric acid was diluted by half (50% H2SO4. Ten ml of each solvent was placed in volumetric flasks and then 0.5 g of herbal tea was added. The mixture was left to cool for about 24 hours and then the resulting solution was filtered. The supernatant was observed by UV light at two wavelengths: 254 nm and 366 nm. (Liang, 2004).

Microbiological Control

1g of each herbal tea was weighed and introduced into a tube containing 9 ml of sterile physiological water. From the suspension obtained three successive dilutions were carried out: one to the tenth, one to the hundredth and the last one to the thousandth. For each dilution two plating operations were made on petri dishes according to the rake technique, one on Sabouraud medium for the search of fungi and the other on nutrient agar for the search of bacteria. Then incubation was carried out in an oven at 37°C for 48 hours for the medium containing the nutrient agar and at 25°C for five days for the Sabouraud medium. After incubation, the different colonies were counted and the results were expressed in colony forming units per ml. (Adounkpe et al., 2017).

Results and Discussion:

Visual checklist results are summarized in Table 1 and 2.

Table 1. Visual inspection checklist results (part1)

Herbal tea	Sealing and packaging system	Readability information on the packaging	Composition	Leaflet
Bio 3	Hermetically sealed	clear	available	available
Santé vie	Hermetically sealed	clear	available	available
Fass	Hermetically sealed	clear	available	available
Medicaflore	Hermetically sealed	clear	available	Not available
Wassilat	Hermetically sealed	clear	available	Not available

Table 2. Visual inspection checklist results (part1)

Herbal tea	Iindication	Contraindication	Side effects	Mode of use	Shelf life and expiration date
Bio 3	available	available	Not available	available	available
Santé vie	available	Not available	Not available	available	available
Fass	available	available	available	available	Not available
Medicaflore	available	Not available	Not available	available	available
Wassilat	available	Not available	Not available	available	Not available

Table 1 and 2 show that composition, indications and use mode are mentioned on the five herbal teas. The bio 3, Santé Vie and Fassa herbal teas contain a leaflet unlike Medicaflor and Wassilat herbal teas. Contra-indications were mentioned on the bio 3 and Fassa herbal teas but not on Santé Vie, Medicaflor and Wassilat herbal teas. Shelf life and expiration dates are mentioned on the herbal teas, except for Fassa. According to the European pharmacopoeia, herbal teas must be stored in a dry place and protected from light, the shelf life of the mixture is

that of the drug with the shortest shelf life (Edqm, 2012). Therefore, it appears that the Fassa herbal tea does not meet the standards of the pharmacopoeia. The absence of instructions in the Medicaflor and Wassilat herbal teas would be non-compliant because it should serve as a therapeutic guide. The contraindications are a very important information during the treatment. Therefore, their absence in Medicaflor and Wassilat herbal teas is not conform

Macroscopic Study

The macroscopic examination showed foreign mineral elements such as pebbles in the herbal teas Fassa, Medicaflor and Wassilat. Some plant parts included in herbal tea composition was also detected: *Bio 3: leaf and pod of senna (see figure 1) *Healthy Life: fennel, aroma and lemon peel (see figure 2) *Fassa: chrysanthellum and cassia alata leaves (see figure 3) *Medicaflor: globular, rosemary, ash, caraway and mallow (see figure 4). For Wassilat: no plant was identified

Figure 1. Bio 3 herbal tea macroscopic observation

Figure 2. Santé et vie herbal tea macroscopic observation

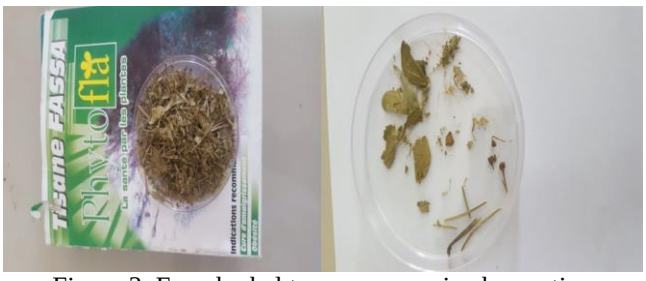

Figure 3. Fass herbal tea macroscopic observation

Figure 4. Medicaflor herbal tea macroscopic observation

Microscopic Study

Microscopic observation has shown the existence of many debris belonging to the plants included in herbal teas composition (see figures 5, 6, 7, 8, 9)

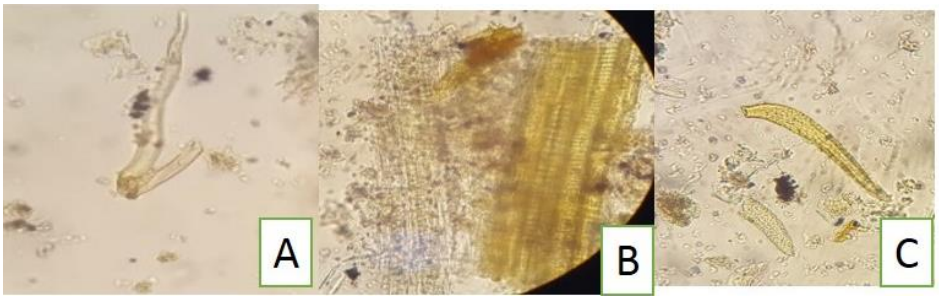

Figure 5. BIO3 herbal tea microscopic observation

A: *Malva sylvestris* trichome, B: *Senna acutifolia* pitted vessels, C: *Senna acutifolia* trichome

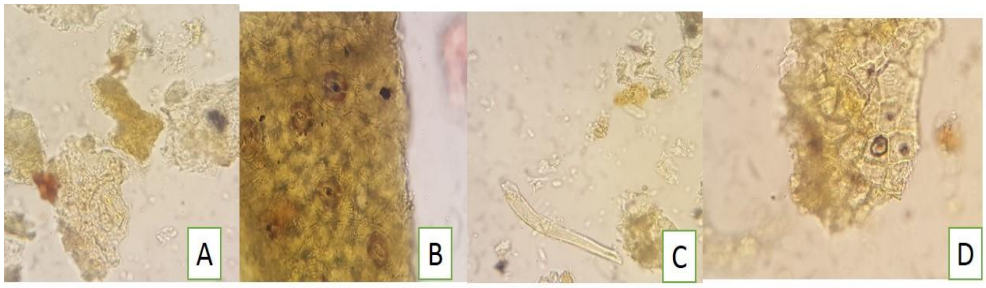

Figure 6. Santé vie herbal tea microscopic observation

A: *Citrus sp* secretory cavities, B: fennel epicarp, C: *Fraxinus sp* trichome, D: *Citrus aurantium* epidermis debris

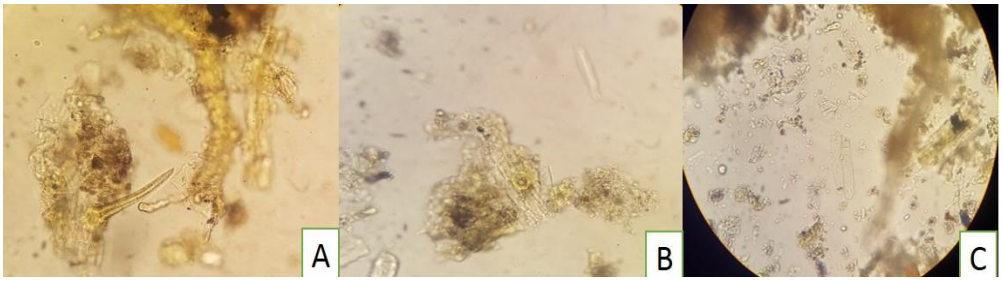

Figure 7. Fass herbal tea microscopic observation

A: *Cassia alata* trichome, B: *Chrysanthellum sp* secretory canal, C: *Chrysanthellum sp* trichome

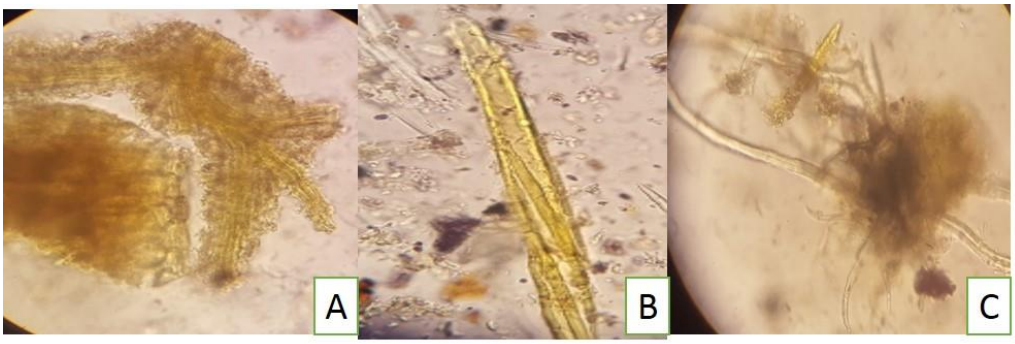

Figure 8. Medicaflor herbal tea microscopic observation

A: *Fraxinus sp* sclereids, B: *Fraxinus sp* gelatinous fibres, C: *Rosmarinus officinalis* trichome

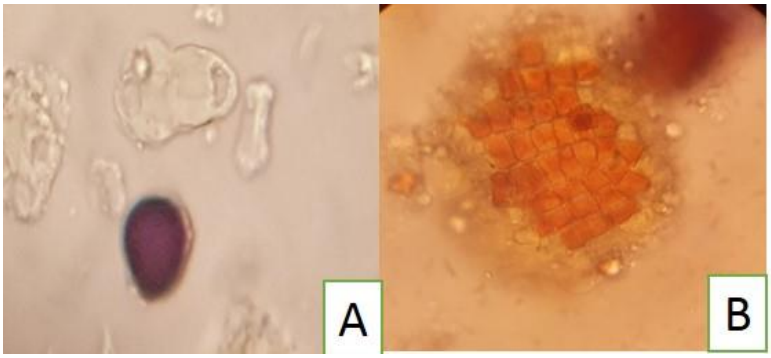

Figure 9. Wassila herbal tea microscopic observation

A: *Artemisia annua* glandular secretory trichomes, B: *Brassica oleracea parenchyma*

According to European pharmacopeia, the identity of each plant present in the herbal tea mixtures must be verified by botanical macroscopic and/or microscopic examination. However, some drugs that appear in the compositions of the herbal teas were not found. According to the same pharmacopeia, herbal tea mixtures should not exceed 10 plants:

- No more than 5 plants considered as active substances, which should represent at least 10% (m/m) of the total mixture
-No more than 3 herbal drugs for flavor improvement with a maximum of 15% (m/m) from the total mixture,
- No more than 2 herbal drugs for appearance improvement with a maximum of 10% (m/m) of the total mixture.
-No information was done about the role of each drug in the five herbal teas. Knowing that, according to the European pharmacopeia, plants used as active substances can be associated between them only if they have identical or complementary medicinal properties and if the herbal teas mode of use (maceration, infusion, decoction) is similar to that of the plant alone, a thing that couldn't be checked especially that there is no information about which drug is used as active substance. It should also be noted that the drug dosage was not indicated on the herbal teas package or leaflet.

Total Ash Values

Total ashes Values are expressed in Table 3

Table 3. Total ashes values

Herbal tea	Total ashes (pourcentage ± SD)
Bio 3	6.865±0.135
Santé vie	6.335±0.395
Fass	7.19±1.81
Medicaflore	9.805±5.045
Wassilat	4.035±0.915

Table 3 analysis shows that the total ash levels and the standard deviation are quite high in Medicaflor herbal tea, a little high for Fassa herbal tea, average for Bio 3 and santé vie herbal teas, and low for Wassilat herbal tea Plant carbonization allows the elimination of all organic matter and the residue weighing, containing only mineral materials, gives an indication of the cleanliness degree of the plant as well as the presence of fertilizing agents (Anton, 2003). Since the herbal tea cleanliness degree is related to the level of total ash, it can be said that Medicaflor herbal tea contains more impurities, followed by Fassa herbal tea, Bio 3 herbal tea, Health Life herbal tea, and Wassilat herbal tea. The high levels of total ash are due to the presence of foreign elements of mineral origin such

Extracts Observation under UV Light

Herbal tea extracts were observed under UV light at two wavelengths: 254 nm and 366 nm. The results are expressed in Tables 4 and 5.

Table 4. Herbal teas benzene and sulfuric acid extract observation

Herbal tea	Benzen			Sulfiric acid		
	daylight	254 nm	366 nm	daylight	254 nm	366 nm
Bio 3	Yellow green	Orange	Orange	Brownish	No fluorescence	No fluorescence
Santé vie	Yellow green	Orange	brick red	Brownish	No fluorescence	No fluorescence
Fassa	Yellow green	Orange	Red	Brownish	No fluorescence	Orange
Medicaflor	Yellow green	Orange	Red	Brownish	No fluorescence	No fluorescence
	colorless	No fluorescence	No fluorescence	purple	No fluorescence	No fluorescence

Table 5. Herbal teas chloroform and methanol extract observation

Herbal tea	Chloroform			Methanol		
	daylight	254 nm	366 nm	daylight	254 nm	366 nm
Bio 3	Yellow green	Yellow	brick red	Yellow green	Yellow	No fluorescence
Santé vie	pale green	Orange	brick red	pale green	Yellow	No fluorescence
Fassa	dark green	Orange	Red	dark green	No fluorescence	No fluorescence
Medicaflor	Yellow green	Orange	Red	Yellow green	Yellow	No fluorescence
Wassilat	colorless	No fluorescence	No fluorescence	colorless	No fluorescence	No fluorescence

There is no fluorescence for sulfuric acid and methanol extract at 254 nm and at 366 nm for all herbal teas. The observation at 254 nm of benzene extract gave an orange color except Wassilat herbal tea where there is no fluorescence. Observation under daylight of sulfuric acid extract gave a brownish color for all the herbal teas except for Wassilat extract which took a purple color. There is no fluorescence for the observation at 366 nm of sulfuric acid for all the herbal teas except for the Fassa tea which took an orange color. There is no fluorescence for all Wassilat herbal tea extracts.

Microbiological Control

After 48 hours and 5 days of incubation respectively for the search of bacterial and fungal contamination, the colony count was summarized in Table 5. As we can see all the studied herbal teas revealed the presence of bacterial and fungal contamination, the results of the count vary from 1.2 x 10 3 CFU /ml to 2.5 x 104 CFU/ml for bacterial contamination and from 6 x 102ufc/ml to 2.9 x 104ufc/ml for fungal contamination. Based on the overall microbial load, it appears that the Bio 3 herbal tea is the most contaminated one; the Wassilat herbal tea is in last position in terms of contamination. The average bacterial and fungal contamination is 9.8 x 103ufc/ml and 1.2 x 104ufc/ml respectively.

Table 5. Microbial load

Herbal tea	Bacterial load (UFC/ml)	Fungal load (UFC/ml)
Bio 3	2.5×10^4	1.63×10^4
Sante Vie	9.1×10^3	2.9×10^4
Fassa	1.3×10^4	1.3×10^4
Medicaflor	9×10^2	2.2×10^3
Wassilat	1.2×10^3	6×10^2

Microbiological examination revealed that all the examined herbal teas were contaminated with fungal and bacterial agents. These results are much higher than those of the study of the same type conducted by Derouiche & Abdennour (2017) on herbal teas marketed in pharmaceutical pharmacies in the region of Constantine. It showed that four out of six teas studied or a percentage of 66.67% were contaminated. The presence of a

significant amount of microorganisms in herbal teas can be explained by contamination by dust from the soil, the natural environment of bacteria and molds, or that from manure (Kandeel et al., 2014). According to the WHO, the main sources of contamination include the soil, the transport and storage process, and post-harvest treatments that plant drugs undergo (WHO, 2007). Cross-contamination from foreign equipment that comes in contact with plant drugs is also a possible source of contamination.

Conclusion

Quality control of the five diet herbal teas that was carried out in this work, showed that these herbal teas are subjected to bad conditions of conservation and storage especially as the microbiological controls revealed fungal and bacterial contaminations. The ash tests showed high levels of total ashes, which would indicate the presence of mineral foreign elements in the herbal teas. botanical controls concluded on the fact that certain vegetable drugs were used in these herbal teas without really caring about the contra-indications and the precautions of use.

Scientific Ethics Declaration

The authors declare that the scientific ethical and legal responsibility of this article published in EPHELS journal belongs to the authors.

Acknowledgements or Notes

* This article was presented as a poster presentation at the International Conference on Medical and Health Sciences (www.icmehes.net) conference held in Antalya/Turkey on November 17-20, 2022.

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To cite this article:

Selka, M.A. Achouri, M.Y. Chenafa, A. & Bellifa, N. (222). Quality control of diet herbal teas commercialized in the region of Sidi Bel Abbes. *The Eurasia Proceedings of Health, Environment and Life Sciences (EPHELS), 7,* 8-16.