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RESEARCH ARTICLE

ASSESSMENT OF THE HYGIENIC AND MICROBIOLOGICAL QUALITY OF READY-TO-USE LIQUID TRADITIONAL MEDICINES SOLD IN THE CITY OF OUAGADOUGOU, BURKINA FASO

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ABSTRACT

For the control of multiple new and resurgent diseases associated with microbial resistance to antimicrobials, there is renewed interest in using herbal medicines. However, the efficacy safety issues and good manufacturing practices associated with herbal medicines are major aspects to consider. The presence of pathogenic microorganisms in herbal medicines may adversely affect the therapeutic potential of the product or even make the product harmful to the patient. This study aimed to evaluate the hygienic quality and the microbiological hazard of herbal medicine produced and sold in city of Ouagadougou. Thus, a total of 45 samples of herbal medicines were collected randomly from the production sites of traditional practitioners and the various points of sale in Ouagadougou by herbalists. Research and enumeration of the total aerobic mesophilic flora, total coliforms, thermotolerant coliforms, yeasts, and some specific pathogenic bacteria such as *Salmonella* sp, *Shigella* sp, *Staphylococcus aureus* were performed according to methods based on standard procedures. Good manufacturing practices were also assessed through sample filtration on a 0.45 µm diameter filter and then a cloth sieve to detect physical contaminants. The results were interpreted according to European Pharmacopoeia standards and microbiological criteria for ready-to-eat meals. The results indicate an absence of *Salmonella* and *Shigella*. It was noted the presence of *S. aureus* ($2.91.10^3$ CFU/ml to $6.17.10^3$ CFU/ml). The average loads of the different germs in the traditional medicines of the production and sales sites were respectively $1.26.10^6$ and $4.36.10^6$ CFU/ml for the total aerobic mesophilic flora, $0.88.10^5$ and $1.78.10^5$ CFU/ml for yeast and moulds, $0.34.10^4$ and $1.22.10^4$ CFU/ml for total coliforms, $1.14.10^3$ CFU/ml and $4.15.10^3$ CFU/ml for thermo tolerant coliforms. The search for physical contaminants revealed presence of sand, leaf pieces, and stems in some traditional medicines. In sum, this study demonstrated the presence of some pathogenic bacteria in some ready-to-use oral herbal medicines and lake of good manufacturing practices and hygienic conditions during the production chain. Given the importance of phytomedicines in health systems throughout the world and particularly in developing countries, it is necessary to train and sensitize producers of phytomedicines in good manufacturing practices and hygiene.

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INTRODUCTION

The World Health Organization has defined traditional medicine as the set of knowledge, skills (ability to employ empirical knowledge), and practices based on theories, beliefs, and experiences of different cultures, whether they are explicable or not, and used for the maintenance of health and for the prevention, diagnosis, improvement, or treatment of physical or mental illness (WHO, 2000) Traditional medicine was once relegated to hiding because it was considered witchcraft at a certain time, it is now recognized and unanimously accepted as to the role it plays in health systems and its ability to treat some infectious and metabolic diseases as well as psychological problems. For several reasons, traditional medicine is often the most accessible, affordable, and acceptable to local populations, particularly in their primary health care (Ekor,

2014). In low-income countries of Africa and many over regions of the world, it is estimated that approximately 80% of the population uses traditional herbal medicines as part of their primary health care (Umair *et al*., 2017). In Burkina Faso, traditional medicine and pharmacopeia remain the main source of primary health care for 70% of the population. This high demand for herbal medicine has created an important economic activity. This activity occupied more than 30,000 Burkinabè (Nikiema, 2008). A study revealed that more than one million tons of medicinal plants are sold annually in the cities and countryside of Burkina Faso, and the same quantity is exported. This sale generates a turnover of more than 15219750 US Dollars. The average consumption was estimated at 500 g per year per person, or in value about 3.5 US Dollars. The annual consumption of medicinal plants in the two large cities (Ouagadougou and Bobo Dioulasso) amounted to 7.5 million US dollars per year (Lambert, 2003). Today, herbal medicines have shown their effectiveness against diseases. Indeed, plants contain molecules with anti-inflammatory, ant proliferative, and antioxidant potential (Gayathri *et al.,* 2021). These molecules are supposed to be present in traditional medicines preparation and sold under different galenic forms: powder, herbal teas, capsules, syrups, and ointments to consumers for their care. However, some traditional medicines prepared without good manufacturing practices and unhygienic conditions may present risks to the user. These include contamination by microorganisms, toxins, pesticide residues, and physical contaminants. It is essential to identify the risks associated with the use of herbal medicines, and in this regard, the safety of these products has become an issue of great public health importance (WHO, 2004, 2005). It is therefore necessary to include quality control in the manufacturing process of these traditional medicines. This will contribute to the continuous improvement of traditional medicines. Indeed, the use of traditional medicines can be linked to different risks. The results of this investigation are expected to influence policy decision-makers, health practitioners, traditional herbalists, folk healers, traders, and consumers to develop prevention measures that guarantee the secure and efficient utilization of these medicinal plants.

MATERIAL AND METHODS

Setting, period of study and sampling: This study took place in Ouagadougou in the province of Kadiogo. The different analyses were performed at the Laboratory of Applied Biochemistry and Immunology (LaBIA) at Joseph KI ZERBO University from May 2017 to December 2018. The samples were taken at the production sites and in the markets of the city of Ouagadougou. Fifteen (15) samples of traditional liquid medicines were taken from producers, and thirty (30) others in the same form from vendors in the markets. The samples were obtained by purchase like the other clients. The Traditional liquid medicines were chosen for our study because they are ready to use, easy to use, and more widely used by the Burkinabe population.

Microbiological analysis of traditional medicines

Preparation of the stock solution and its dilutions

The stock solution was obtained by adding 10 ml of aseptically collected traditional drugs to 90 ml of sterile physiological water. Then, 1 ml of the stock solution (10^{-1}) was taken then added to a tube containing 9 ml of sterile physiological water. This gave the 10^{-2} dilution. After homogenization, 1 ml of this solution 10^{-2} was taken and introduced into another tube containing 9 ml of physiological water dilution 10^{-3}. The operation was repeated several times, thus obtaining a range of dilutions from 10^{-2} to 10^{-6}. The stock solution and the decimal dilutions were used for the different microbiological analyses.

Preparation of culture media: The culture media PCA (Plant Count Agar), Sabouraud, EMB (Methylene Blue Eosin), and MSA (Mannitol Salt Agar) were obtained from SIGMA Aldrich and prepared according to the manufacturer's instructions. After reconstitution, the media were sterilized at 121°C for 15 min. On the other hand, the SS agar medium (*Salmonella-Shigella*) was prepared by heating it in a water bath to about 90°C with frequent stirring. After complete dissolution, it was cooled and maintained at 45°C before being poured into sterile Petri dishes.

Enumeration of microorganisms

Enumeration of the total aerobic mesophilic flora: The enumeration was performed according to the ISO standard NF. EN ISO 6887-1:2017 (AFNOR, 2017a). Standard agar or Plate Count Agar (PCA) was used for the enumeration of total aerobic mesophilic flora. The spread plating technique was used. For this purpose, 0.1 ml of the different dilutions were aseptically collected in the middle of the agar and then spread over the entire surface using a sterile Pasteur pipette. All manipulations were performed under sterile conditions next to the Bunsen burner flame. Incubation was done at 30° C for 72 ± 3 hours. After 72 hours of incubation. All colonies were counted on two successive dilution plates. Colonies of various sizes and whitish or yellowish colors were counted.

Research and enumeration of yeasts and moulds: Sabouraud agar supplemented with chloramphenicol was used for yeasts and moulds detection according to ISO 7954 (1988) AFNOR, (2003). A quantity of 0.1 ml of the decimal dilutions was inoculated in sterile Petri dishes. The plates were incubated at 30°C for 48 to 72 hours under aerobic conditions. On the agar, colonies in the form of small circular sizes of milky white color were counted.

Research and enumeration of total and thermotolerant coliforms: Total and thermotolerant coliforms were enumerated on the EMB medium. The plating was done in double layers. The plates were incubated for 24 hours at 37°C for total coliforms and 44°C for thermotolerant coliforms. The coliforms with purplish colonies of 0.5 mm or more in diameter were counted according to ISO 6888-1: (2021) and AFNOR (2021).

Research and enumeration of Staphylococcus aureus: The surface plating technique was used. In each plate, 0.1 ml of the previously prepared decimal dilutions were plated and then spread on the entire surface of Mannitol Salt Agar. Petri dishes were incubated aerobically at 37°C for 24-48 hours according to ISO 6888-1:2021 (AFNOR, 2021). Colonies of *Staphylococcus aureus* appearing yellow and surrounded by a yellow halo were counted.

Research of Salmonella* and *Shigella: The SS medium was used for the research of *Salmonella* and *Shigella*. The search was performed according to ISO 6579-1:2017 (AFNOR, 2017b) in three successive steps pre-enrichment, enrichment, and isolation.

Pre- enrichment: Buffered peptone water (BPW) broth was used for pre-enrichment. 225 ml of sterile BPW broth contained in a flask were added to 25 ml of traditional drugs. Incubation was done at 37°C for 18 to 24 hours under aerobic conditions.

Enrichment: In 09 ml of Rapapport Vassiliadis Soy (RVS) broth contained in sterile glass tubes, 0.1 ml of pre-enriched broth was placed using a sterile pipette. The broths were then incubated in the oven at 37°C for 18-24 hours.

Isolation: SS agar was used for the isolation of *Salmonella* and *Shigella*. 0.1 ml of the suspension was plated with a sterile syringe and streaked with a sterile Pasteur pipette. The seeded Petri dishes were incubated at 37°C for 24 hours of incubation. Suspect colonies will appear bluish with or without a black center.

Colony Counting: The number N of microorganisms was expressed in Colony Forming Units (CFU) according to ISO 7218, (2007) (AFNOR, 2007). Plates with more than 30 colonies and less than 300 colonies were considered. If samples were inoculated in duplicate and if one or two plates inoculated with the same dilution contained colonies. The average number of colonies was calculated and

multiplied by the inverse of the dilution factor to obtain the number of CFU/ml according to the formula:

$$N = \frac{\sum C}{Vi \times d}$$

ΣC = average of the colonies counted on the two plates of the considered dilution
Vi = volume of inoculum in ml
d = considered dilution
If the plates of all dilutions of a sample showed no colonies, the result was reported as "less than 1/*d* UFC per ml" (<1/d UFC/ml).

Interpretation of microbiological results: The results were interpreted according to the European Pharmacopoeia standards (EDQM, 2014) and the microbiological criteria for ready-made meat

Search for physical contaminants in traditional medicines: 100 ml of each traditional medicine sample was filtered through a membrane paddle fitted with a 0.45-micrometer diameter. The deposit was examined by eye.

Data analysis: Data were entered into Microsoft Excel 2016 and analyzed with XL STAT 7.5.2 software to compare the different means of microbiological parameters. The difference between the means was significant when $p < 0.05$.

RESULTS AND DISCUSSION

Physical characteristics of the samples: A total of forty-five (45) samples of traditional liquid medicines were collected (Table 1). Three main modes of preparation were used decoction 75.6% (34/45), maceration 15.6% (7/45) and, infusions 0.8% (4/45). These results corroborate the study of de Souza *et al*. (2011) which had shown that decoction was the most used form at 27.7%. The oral route was the mode of administration in all our samples. The therapeutic indications varied from one drug to another. However, traditional medicines used to treat digestive tract diseases were the most cited and represented 37.8% (17/45). These included hemorrhoids, colopathy, constipation, ulcers, diarrhea, and stomach aches.

Microbiological analyses: Forty-five (45) samples of traditional medicines were analyzed on different culture media according to the germs sought. The microbiological quality of traditional medicines was determined based on the enumeration of the germs of alteration, namely the total aerobic mesophilic flora, the total coliforms, the thermotolerant coliforms, the yeasts, and moulds. In addition, the research on pathogenic germs was also performed through the search for *Staphylococcus aureus*, *Salmonella*, and *Shigella*. The results of the different microbiological analyses expressed in Colony Forming Units per milliliter (CFU/ml) are summarized in Table 2. The total aerobic mesophilic flora gives indications of the preparation conditions and the efficiency of the treatment and preservation processes. According to the European Pharmacopoeia standards (EDQM, 2014), compliant samples had a total aerobic mesophilic flora rate lower than $5{,}10^5$ CFU/ml. Only 8.88% (4/45) of our samples meet this criterion. The remaining 91.11% (41/45) is considered non-compliant for the presence of this germ. Indeed, the results of the analyses show that the total aerobic mesophilic flora is present in all our samples at a level of contamination varying between $1.25.10^5$ to $1.35.10^7$ largely superior to the 5.10^5 CFU/ml maximum admissible number. Our results are superior to other studies that found lower contamination levels. These are Sylla (2000) with 4% and de Souza *et al.* (2011) with 41.11%. This high count of aerobic mesophilic bacteria explains and expresses a general indicator of poor practice in the production of these drugs. Either the extraction temperature is insufficient, the conservation of the drug is prolonged, or the hygiene is deficient (Fernández *et al.*, 1999). Total and thermotolerant coliforms were present in our samples. The contamination rate ranged from $4.02.10^2$ to $4.78.10^4$. The rate of non-conformity in the sample was 95.5% (43/45). Indeed, these samples have a contamination rate higher than 10^3 according to the acceptance criteria of the European Pharmacopoeia standards (EDQM, 2014). However, these rates are below those found by Coulibaly *et al.* (2018) who showed that traditional medicines collected in six communes of Abidjan contained total coliforms in proportions ranging from $1.6.10^4$ to $5.5.10^7$ CFU/ml. Since total coliforms are sensitive to chlorine, their presence in water samples could indicate the existence of a biofilm or a lack of treatment efficiency (Ramani Bai *et al.*, 2022). Their presence in these traditional medicines would explain ineffective heat treatment or subsequent contamination. Their presence could indicate poor cleaning of production equipment (Ramani Bai *et al*., 2022). The high frequency of total coliforms could be explained by the lack of control over hygiene rules and the conservation of finished products. Several studies have shown the presence of total coliforms in traditional medicines at variable rates. Thermotolerant coliforms are present in all samples. The contamination rate varies from 2.10^2 to $2.11.10^4$. The non-compliance rate concerning these germs is 100%. It differs from the work of Gbekley *et al.* (2017) which had not found thermotolerant coliforms in samples used in the control of diabetes in Togo. Indeed, coliforms are indicators mainly related to human or animal fecal contamination and environmental contamination, not controlled by technological treatments. The analysis revealed the presence of yeasts and moulds in all the traditional medicines in proportions ranging from $1.35.10^4$ to $6.6.10^5$. Among the samples, 57.77% (21/45) are non-compliant because they were exceeding the maximum permissible number of 5.10^4 CFU/ml, the acceptance criterion being 10^4 CFU/ml according to the European Pharmacopoeia standards (EDQM, 2014). Our results are superior to the study of Agasounon *et al.* (2001) whose values vary from 3.10^3 to $8.4.10^3$ CFU/ ml. Furthermore, our results are also superior to the study of Coulibaly *et al.* (2018) who reported a total absence of yeasts and moulds in liquid traditional medicines sold in the six communes of Abidjan. The contamination of these samples by fungal germs could be explained by the contamination of the raw material that has been left in the open air for a long time and exposed to humidity or prolonged storage (Tayou, 2007). The high presence of a product leads to the reduction of its quality and represents a risk for the consumer's organism because of mycotoxins which are toxic metabolites that fungi can secrete (*Awuchi et al.*, 2021). Pathogens such as *S. aureus*, *salmonella*, and *shigella* were searched in the traditional drug samples. The analyses showed that all our samples contained *S. aureus* at a level higher than the normal 10^2 CFU/ml according to the microbiological criteria of the ready meals. The non-compliance rate was 100% for this germ. Our results differ from those of Coulibaly *et al.* (2018) who did not identify *S. aureus* in their work. The presence of S. aureus in heated and handled foods after cooking is an indication of human contamination and possibly of poor handling practices and inadequate hygiene of handlers. It may also indicate contamination by raw materials or poor storage conditions. All of these deficiencies may eventually lead to human health risks if corrective actions are not taken. On the other hand, about *Salmonella* and *Shigella*, we did not find evidence of the presence of these germs in our samples, which corroborates the work of Coulibaly *et al.* (2018). However, considering the high frequency of total coliforms in the samples, *Salmonella* may be present because the survival of faecal coliforms in the environment is similar to that of *Salmonella*. In addition, the absence of *Salmonella* could also be explained by the inefficiency of the conventional research methods used. Indeed, Catsaras and Grebot (1984) have shown that the search for Salmonella by the classical method can be negative although the sample contains 10^5 to 10^8 CFU/ml. The overall analysis of the comparison between the microbial loads in the different types of traditional medicines shows that the decocts have overall averages of $3.28.10^6$ CFU/ml and $1.41.10^5$ CFU/ml for total aerobic mesophilic flora and yeasts and moulds respectively. These mean values are lower than the mean values obtained for the macerates, which were $2.22.10^7$ CFU/ml for total aerobic mesophilic flora and $1.38.10^6$CFU/ml for yeasts and moulds. The macerates were more contaminated with total aerobic mesophilic flora and with yeasts and moulds than the decocts. This difference could be explained by the fact that the decocts go through a boiling process that is supposed to

Table 1. Physical characteristics of traditional medicine samples

N°	Mode of preparation	Color	Mode of administration	Therapeutic indications
1	Decoction	Dark brown	Oral	Hemorrhoids
2	Decoction	Black-brown	Oral	Colopathy
3	Maceration	Yellow-orange	Oral	Stomach aches
4	Decoction	Brown	Oral	Heartache
5	Decoction	Brune	Oral	Constipation
6	Decoction	Black-brown	Oral	Rheumatism
7	Maceration	Yellow	Oral	Bloating
8	Decoction	Light Red	Oral	Stomach ulcers
9	Decoction	Black-brown	Oral	Diabetes
10	Decoction	Brown	Oral	Marasmus
11	Decoction	Black-brown	Oral	General fatigue
12	Decoction	Black-brown	Oral	High blood pressure
13	Decoction	Dark brown	Oral	Hemorrhoids
14	Decoction	Black-brown	Oral	Dizziness
15	Decoction	Light Red	Oral	Stomach ulcers
16	Decoction	Black-brown	Oral	High blood pressure
17	Maceration	Yellow-orange	Oral	Malaria
18	Decoction	Brown	Oral	Hepatitis all types
19	Decoction	Brown	Oral	Painful periods
20	Decoction	Dark brown	Oral	Hemorrhoids
21	Maceration	Yellow-orange	Oral	Stomach aches
22	Decoction	Dark brown	Oral	Infection, lungs, kidneys
23	Decoction	Light Red	Oral	Stomach ulcers
24	Decoction	Brown	Oral	Constipation
25	Maceration	Yellow-orange	Oral	Malaria
26	Decoction	Brown	Oral	Diarrhea
27	Decoction	Dark brown	Oral	Hemorrhoids
28	Decoction	Brown	Oral	General fatigue
29	Infusion	Olive green	Oral	Cough
30	Infusion	Olive green (citronnelle)	Oral	Cold
31	Infusion	Olive green	Oral	Cough
32	Decoction	Brown	Oral	Painful periods
33	Decoction	Orange	Oral	Urine retention
34	Decoction	Brown	Oral	Prostatitis
35	Decoction	Dark brown	Oral	Kidney infection
36	Maceration	Yellow-orange	Oral	Malaria
37	Decoction	Brown	Oral	Lung infection
38	Maceration	Yellow-orange	Oral	Stomach aches
39	Decoction	Black-brown	Oral	Diabetes
40	Decoction	Dark brown	Oral	Hemorrhoids
41	Decoction	Black	Oral	Sexual impotence
42	Decoction	Red-dark	Oral	Cirrhosis of the liver
43	Decoction	Brown	Oral	Hepatitis all types
44	Infusion	Olive green	Oral	Cold
45	Decoction	Brown	Oral	Vomiting

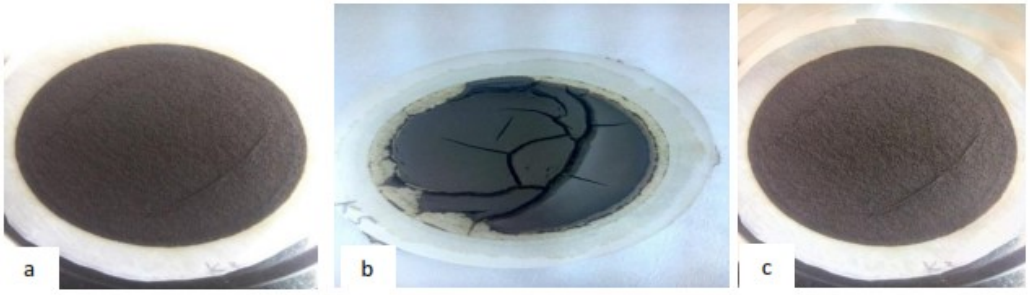

Figure 1. Residues obtained after filtration of traditional medicines on 0,45µm filter

Table 2: Enumeration and research of microorganism in traditional medicines

	Number of germs UFC/ml					
Traditional medicine	TAMF	C t	C th	Y&M	*S. aureus*	SS
Pm001	$1.83.10^6$	$2.10.10^3$	$1.60.10^3$	$3.93.10^5$	$7.50.10^2$	Absence
Pm002	$3.20.\ 10^6$	$1.85.10^3$	$1.15.10^3$	$6.30.10^4$	$2.50.10^2$	Absence
Pm003	$1.49.\ 10^6$	$1.85.10^3$	$1.20.10^3$	$4.00.10^5$	$3.20.10^3$	Absence
Pm004	$1.56.\ 10^6$	$2.75.10^3$	$1.05.10^3$	$2.95.10^4$	$5.40.10^3$	Absence
Pm005	$1.75.\ 10^6$	$3.55.10^3$	$2.10.10^3$	$1.55.10^4$	$1.85.10^4$	Absence
Pm006	$1.59.\ 10^6$	$2.30.10^3$	$7.00.10^2$	$1.65.10^4$	$2.10.10^3$	Absence
Pm007	$1.66.\ 10^6$	$3.90.10^3$	$4.50.\ 10^2$	$1.55.10^4$	$1.25.10^3$	Absence
Pm008	$1.12.\ 10^6$	$2.14.10^4$	$3.80.10^3$	$1.55.10^4$	$3.15.10^3$	Absence
Pm009	$5.50.10^5$	$8.50.\ 10^2$	$2.50.10^2$	$1.50.10^4$	$8.50.10^2$	Absence
Pm010	$1.25.10^5$	$1.45.10^3$	$1.10.10^3$	$7.85.10^4$	$1.05.10^3$	Absence
Pm011	$1.71.10^6$	$1.60.10^3$	$6.50.10^2$	$1.71.10^5$	$1.70.10^3$	Absence
Pm012	$2.75.10^5$	$1.65.10^3$	$6.00.10^2$	$2.95.10^4$	$2.70.10^3$	Absence
Pm013	$1.95.10^5$	$2.60.10^3$	$3.00.10^2$	$3.45.10^4$	$1.20.10^3$	Absence
Pm014	$7.15.10^5$	$1.35.10^3$	$1.25.10^3$	$2.00.10^4$	$4.50.10^2$	Absence
Pm015	$1.10.10^6$	$1.55.10^3$	$3.00.10^2$	$1.55.10^4$	$1.05.10^3$	Absence
Pm016	$5.34.10^6$	$1.49.10^4$	$1.01.10^4$	$1.44.10^5$	$8.80.10^3$	Absence
Pm017	$4.70.10^6$	$3.40.10^4$	$1.50.10^4$	$5.01.10^5$	$4.12.10^4$	Absence
Pm018	$1.35.10^7$	$4.56.10^4$	$2.11.10^4$	$4.36.10^5$	$2.93.10^4$	Absence
Pm019	$3.75.10^6$	$3.85.\ 10^3$	$1.50.\ 10^3$	$2.95.10^4$	$7.00.10^2$	Absence
Pm020	$6.05.10^6$	$3.85.\ 10^3$	$2.25.\ 10^3$	$2.13.10^5$	$5.15.10^3$	Absence
Pm021	$2.42.10^6$	$1.70.\ 10^3$	$8.50.10^2$	$1.08.10^5$	$1.30.10^3$	Absence
Pm022	$7.34.10^6$	$4.40.\ 10^3$	$2.45.\ 10^3$	$1.19.10^5$	$1.50.10^3$	Absence
Pm023	$4.13.10^6$	$1.90.\ 10^3$	$8.00.10^2$	$5.75.10^4$	$4.05.10^3$	Absence
Pm024	$3.47.10^6$	$1.55.\ 10^3$	$3.50.10^2$	$1.01.10^5$	$2.05.10^3$	Absence
Pm025	$6.33.10^6$	$3.10.\ 10^3$	$1.40.\ 10^3$	$2.35.10^5$	$1.01.10^4$	Absence
Pm026	$4.90.10^6$	$2.40.\ 10^3$	$2.00.10^2$	$4.25.10^4$	$1.10.10^3$	Absence
Pm027	$8.73.10^6$	$5.25.\ 10^3$	$1.40.10^3$	$5.80.10^5$	1.65.103	Absence
Pm028	$5.39.10^6$	$3.68.\ 10^3$	$1.25.10^3$	$4.15.10^4$	$8.70.10^3$	Absence
Pm029	$4.13.10^6$	$1.40.\ 10^3$	$1.20.10^3$	$1.65.10^4$	5.50.102	Absence
Pm030	$2.53.10^6$	$1.50.\ 10^3$	$3.00.10^2$	$1.95.10^4$	8.50.102	Absence
Pm031	$3.56.10^6$	$2.35.10^3$	$9.00.10^2$	$2.99.10^5$	$4.60.10^3$	Absence
Pm032	$2.35.10^6$	$2.08.10^4$	$1.13.10^4$	$9.70.10^4$	$1.35.10^3$	Absence
Pm033	$2.65.10^6$	$1.85.10^3$	$3.50.10^2$	$1.96.10^5$	$5.00.10^2$	Absence
Pm034	$2.35.10^6$	$4.67.10^4$	$9.15.10^3$	$2.35.10^5$	$1.30.10^3$	Absence
Pm035	$4.65.10^5$	$4.78.10^4$	$1.10.10^4$	$6.60.10^5$	$1.28.10^4$	Absence
Pm036	$3.35.10^6$	$1.34.10^4$	$4.75.10^3$	$1.10.10^5$	$7.50.10^2$	Absence
Pm037	$2.7.10^6$	$5.85.10^3$	$1.25.10^3$	$2.40.10^4$	$1.85.10^2$	Absence
Pm038	$2.28.10^6$	$1.35.10^3$	$9.50.10^2$	$1.35.10^4$	$1.80.10^3$	Absence
Pm039	$2.75.10^6$	$2.35.10^3$	$1.25.10^3$	$2.05.10^4$	$1.55.10^3$	Absence
Pm040	$5.17.10^6$	$2.89.10^4$	$1.44.10^4$	$3.65.10^5$	$3.40.10^3$	Absence
Pm041	$2.04.10^6$	$1.65.10^3$	$1.05.10^3$	$1.75.10^4$	$1.10.10^3$	Absence
Pm042	$2.59.10^6$	$2.15.10^3$	$8.50.10^2$	$8.85.10^4$	$8.00.10^2$	Absence
Pm043	$4.90.10^6$	$5.95.10^3$	$6.00.10^2$	$2.95.10^5$	$1.45.10^3$	Absence
Pm044	$5.89.10^6$	$1.99.10^4$	$2.75.10^3$	$1.28.10^5$	$9.90.10^3$	Absence
Pm045	$5.17.10^6$	$5.85.10^3$	$2.65.10^3$	$1.46.10^5$	$1.02.10^4$	Absence
Norme	$< 10^5$	$< 10^5$	$< 10^5$	$< 10^4$	Absence (25 ml)	Absence (25 ml)

Legend: Pm: traditional medicines code, TAMF: total aerobic mesophilic flora, Y&M: Yeast and moulds, SS: salmonella-shigella. Ct: Total coliforms; C th: Thermothermotolerant coliforms

Table 3. Average loads of the different germs determined in the traditional medicines

	N	TAMF	Y&M	Total Coliforms	Thermotolerants Coliforms	*S. aureus*	SS
Production site	15	$1.26.10^6$	$0.88.10^5$	$0.34.10^4$	$1.14.10^3$	$2.91.10^3$	Absent
Market	30	$4.36.10^6$	$1.78.10^5$	$1.22.10^4$	$4.15.10^3$	$6.17.10^3$	Absent
Total/average	45	$3.33.10^6$	$1.48.10^5$	$0.93.10^4$	$3.15.10^3$	$5.08.10^3$	Absent
P-value		0,000	0.087	0.035	0.041	0.199	
Significant		S	NS	S	S	NS	

Legend: N : Sample number, TAMF : TAMF : total aerobic mesophilic flora, Y&M : Yeast and moulds, SS : salmonella-shigella ; NS : not significant ; S : significant

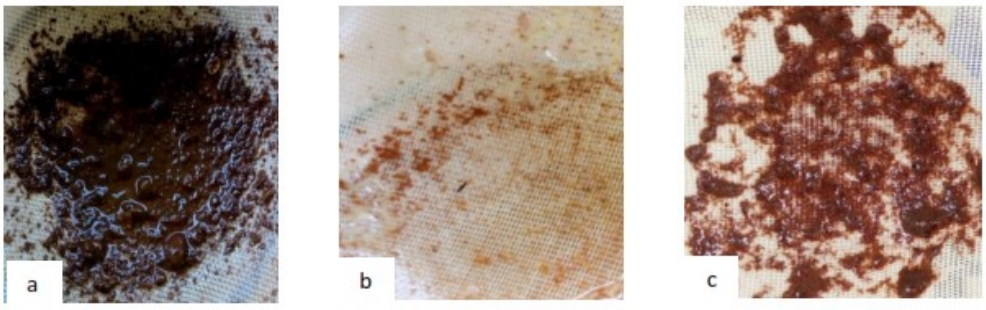

Figure 2. Residues obtained after filtration of traditional medicines on tissu filter

destroy the microorganisms, which is not the case for the macerates. Moreover, this same difference can be observed for the other germs in decocted and macerated products. The decoctates have overall averages for total and thermotolerant coliforms and staphylococci of $8.89.10^3$ CFU/ml, $3.20.10^3$ CFU/ml, and $4.07.10^3 \pm 6.02.10^3$ CFU/ml respectively. These mean values are lower than the mean values obtained in the macerated samples which were $5.58.10^4$ CFU/ml for total coliforms, $1.15.10^4$CFU/ml for thermotolerant coliforms, and $9.42.10^3$ CFU/ml for *Staphylococci*.

Microbiological parameters of traditional medicines: The average loads of the different germs in the traditional medicines according to the sampling site are summarized in Table 3. The microbiological analyses of the different samples of traditional medicines show a total aerobic mesophilic flora ranging from $1.26.10^6$ CFU/ml to $4.36.10^6$ CFU/ml. The microbial load of the samples collected at the market was significantly higher than that of the samples collected at the production site with a p-value of < 0.05. This difference could be explained by the use of recycled materials such as plastic cans and bottles often recovered. Indeed, if this recovered packaging has not been properly cleaned; it can lead to microbial contamination. The lack of good manufacturing practices and good hygiene practices during packaging could also be the cause of microbial contamination. However, the overall average load of $3.33.10^6$ CFU/ml is lower than that obtained by Agassounon *et al.* (2001) in Cotonou which was 6.10^6 CFU/ml. The average yeast and mold load ranged from $0.88.10^5$ CFU/ml to 1.78.105 CFU/ml. The highest value in microbial load of yeasts and moulds is obtained with the samples collected at the market. The lowest value is obtained with the samples collected at the production site. However, the average total load of $1.48.10^5$ CFU/ml is higher than that obtained by Agassounon *et al.* (2001). This high yeast load in our samples reflects the degradation of the sanitary state of the traditional medicine samples. The presence of yeasts and moulds is due either to poor storage conditions or to poor hygiene conditions during packaging (environment, use of soiled packaging). Indeed, these microorganisms and their spores are heat-sensitive. The sources of microbial contaminants in traditional medicines are the packaging and the production environment. Total coliforms (TC) were counted at loads ranging from $0.34.10^4$ CFU/ml to $1.22.10^4$ CFU/ml.

The samples collected from the market were highly contaminated compared to samples collected from the production site. The overall mean value was about $0.93.10^4$ CFU/ml, thus lower than that of Agassounon *et al.* (2001), which ranged from $2.5.10^2$ CFU/ml to $1.1.10^5$ CFU/ml, with a mean of $0.55.10^5$ CFU/ml. The thermotolerant coliforms enumerated in our samples varied on average between $1.14.10^3$ CFU/ml and $4.15.10^3$ CFU/ml with an overall average of 3.15.103 CFU/ml lower than that obtained by Agassounon *et al.* (2001) the values obtained from the samples collected at the market were well above the microbial load of the samples collected at the production site. In sum, the samples from the different sites show a high level of contamination for total coliforms and thermo tolerant coliforms. These levels of contamination are closely related to the general hygiene conditions and the hygiene status of the packaging used. Indeed, the packaging of the product could be a critical step if hygienic measures are not respected, such as the packaging environment of the product and the lack of good hygienic practices during packaging. The Staphylococci counted in the samples collected at the marketplace exceeded those collected at the production site with a variable load ranging from $2.91.10^3$ CFU/ml to $6.17.10^3$ CFU/ml. The overall mean load was $5.08.10^3$ CFU/ml. The contamination of the study samples by staphylococci could be explained by a lack of good manufacturing practices and good hygiene practices on the part of the producers. The presence of these germs constitutes a risk of food poisoning for consumers. *Salmonella* and *Shigella* were absent in all of the traditional medicine samples analyzed. There was no significant difference between the samples from the two collection sites for yeasts and moulds and staphylococci, respectively, with a p-value≥ 0.05. On the other hand, there was a significant difference between the samples from the two collection sites in total aerobic mesophilic flora and coliforms.

The traditional drugs collected at the production site, in contrast to the traditional drugs collected on the market, show the lowest microbial loads in all samples. In general, our microbiological analysis results show a very high bacterial load in our samples which could lead to infections or toxic-infection in the consumer. The lack of hygiene is the main reason that could explain the contamination of our samples of traditional medicines by the different germs we are looking for Traditional medicines, instead of curing a disease, could in these cases cause other diseases if they are not produced under adequate hygienic conditions. Producers of traditional medicines must be sensitized and trained in Good Manufacturing Practices (GMP) and Good Hygiene Practices (GHP). Awareness and training in GMP and GHP would help reduce the contamination found.

Research of physical contaminants in traditional medicines: The forty-five (45) samples of traditional medicines collected were used to search for physical contaminants after the microbiological analyses. The filtration technique was used. The naked eye observation allowed us to detect the presence of plant matter and the touch to detect grains of sand. One or both physical contaminants were found in all filtered samples of traditional medicines. The presence of these physical contaminants indicates a lack of good manufacturing and hygiene practices. Sample b shows more residues after filtration than samples a and c. However, it is more contaminated than sample c. However, it is more loaded with physical contaminants than samples a and c (Figure 1).

Results of the search for physical contaminants in traditional medicines: The presence of plant materials and other impurities such as sand grains were detected in the samples of traditional medicines observed. The presence of these plant materials could be explained by the fact that traditional medicines are produced from plant organs. On the other hand, the presence of sand grains is due to an insufficient level of treatment of the plant organs before preparation or to an external contribution by dust attesting to the lack of good manufacturing and hygiene practices. After filtration with a large mesh cloth, sample a shows more residues after filtration compared to samples b and c and sample c compared to sample b. Physical contaminants are difficult to identify in sample a compared to samples b and c. A piece of tissue was identified in sample b and a stone in sample c (Figure 2). The search for physical contaminants shows a general lack of good manufacturing and hygiene practices during production.

CONCLUSION

This study assessed the microbiological quality of traditional medicines and investigated the physical contaminants contained in traditional medicines sold locally in the city of Ouagadougou. The results of the microbiological analyses showed that these medicines were heavily contaminated with total aerobic mesophilic flora, yeasts and moulds, coliforms, and staphylococci, indicating a lack of respect for good hygiene and production practices. The physical contaminants found in our samples were mainly grains of sand and vegetable matter at a high rate. The grains of sand found in traditional medicines come from the plant organs, especially the roots, if the cleaning is not effective, hence a lack of good manufacturing practices and an exogenous contribution. The plant material found was the remains of plant organs that had escaped the sieve after the traditional medicine had been filtered. In sum, these products represent a sanitary risk as well as a physical danger for consumers and therefore constitute a public health problem. This study shows the need to sensitize and train traditional health practitioners on good practices in the preparation of traditional remedies to preserve consumer health.

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