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Microbiological quality assessment of aqueous herbal teas sold in Cotonou, Benin

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Abstract
The objective of this study was to assess the microbiological quality of herbal teas sold in Cotonou. Thirteen aqueous herbal teas samples were collected from a range of selected sellers located at well-known places. The counting and identification of bacteria and fungi were carried out using International Organization for Standardization microbiological procedures. Chemical and microbiological parameters values were compared to the special norm and quality of drinking water in Republic of Benin. Acidity, pH, Bacteria and moulds were investigated. pH averages values ranged between 8.13±0.34 and 9.91±0.22 and 92% of samples analyzed values did not comply with the National standard of drinking water. Each herbal teas analyzed contained at least one microorganism with a microbial load higher than the national allowed values. The bacteria counting ranged from $9.15\pm2.32x10^7$ to $3.65\pm0.87x10^9$ cfu/ml. The fungal counting ranged from $1.6\pm0.5x10^4$ to $3.5\pm1.1x10^6$ cfu /ml. Total Coliforms, *Escherichia coli*, Mesophilic Aerobic Germs, *Staphylococcus aureus* and *Salmonella Typhi* load were found in higher values than the acceptable limit, respectively in 54% (n=7), 38% (n=5), 100% (n=13), 46% (n=6) and 15% (n=2) of the tested samples. Among moulds identified, *Aspergillus genus* was found in all herbal teas with a microbial load which varied between $0.45\pm0.87x10^5$cfu/ml and $7.30\pm0.87x10^5$cfu/ml. The study unveiled that herbal teas sold and consumed in Cotonou were contaminated with both bacteria and fungi at various levels inducing a potential risk for the regular consumer arising public health issue. Therefore, proper quality control is needed for herbal teas safety.

Keywords: Herbal teas, medicinal plants, quality issues, safety, microbial contamination

1. Introduction
In Africa, up to 80% of the population uses traditional medicine for primary health care. People in different parts of the world use the same or similar plants for the same purpose [1]. Herbal medicine is the first line of choice for home treatment of children with high fever deriving from malaria in many countries of Africa [2]. The reasons for the high use of herbal medicine are the high cost of very effective orthodox medicines [3]. Researchers also explained the high prevalence of herbal medicine use by massive rural-to-urban migration, influence of cultural and social surroundings and the belief that natural products induce no risk [4-5-6]. Therefore, an increase usage of herbal medicinal products such as herbal teas in the treatment and/or the prevention of diseases is clearly noticeable. The relatively high cost of the conventional pharmaceutical drugs and inaccessibility of the orthodox medical services to most people particularly in suburban and rural areas are contributing factors. With this increased usage, authorities in charge of health and health professionals are worried about the safety, efficacy and quality of herbal tea sold in Cotonou. Product quality is obviously the major criteria that could affect not only the efficacy but also the safety of patients or consumers [7]. Herbal teas could carry a large number of microbes originating from the soil that adhere to leaves, stem, flowers, seeds, berries, barks and roots [8]. Additionally, contaminants may be introduced during handling medicinal plant materials and preparation of herbal tea since no conscious efforts are made to decontaminate the herbs other than quickly washing them [8-9]. Therefore, microbial contents in herbal teas should be evaluated as indicator of quality and safety [10]. According to Beninese standard for drinking water [11], water intended for human consumption should not contain any colony of Total Coliforms, *Escherichia coli*, moulds, *Staphylococcus aureus* and Salmonella. Only Mesophilic Aerobic Germs were allowed in a limit of 50 colonies per milliliter. This research was designed to determine the bacteriological and fungal contamination of herbal teas sold in Cotonou with special reference to National standard for drinking water.

2. Material and Methods

2.1 Study Area

The study was conducted at the National Narcotics and Toxicology Laboratory (LNST), located at Cotonou, Benin.

2.2 Sample collection and preparation

Over a period of three-month, an overall of thirteen (13) aqueous herbal teas were purchased from sellers in well-known places in Cotonou for their microbial quality assessment. In each of the thirteen districts of Cotonou, we haphazardly selected a main track where herbal teas are usually sold. A volume of 500 ml of each herbal tea was purchased in a sterile sachets Stomacher. They were then immediately transported to the laboratory for microbiological analysis. The samples were coded as T1, T2, T3, T4, T5, T6, T7, T8, T9, T10, T11, T12 and T13 in reference to each of the thirteen districts of Cotonou where they were respectively collected. These codes were used in laboratory assays, data analysis and report writing.

2.3 Microbiological analysis methods

2.3.1 Samples dilutions and viability assessment

For each sample, 1ml quantity was dissolved in 9ml of sterile peptone water in test tubes and homogenized using the Vortex mixer for 10 seconds. Tenfold serial dilutions were made and viability assessed using the pour plate and/or spread plate method in triplicates and plates incubated at 30°C for 72 hours in the case of bacteria and at 25°C for 3-5 days for yeasts and moulds. All isolates were maintained on nutrient agar slants and stored at - 4 ºC.

2.3.2 Microbial load count

The enumeration of Mesophilic Aerobic Germs was carried out on agar standard for counting, Plate Count Agar (PCA) (Difco, France) according to ISO 4833-1: 2013 [30] method. The Total Coliforms were isolated and enumerated on Bile Lactose agar at Cristal violet and Neutral Red (VRBL) (Merck, France) according to ISO 4832: 2006 [29] method. *Escherichia coli* were isolated and enumerated on selective medium agar TBX, (Biokar Diagnostics, France) according to ISO 16649-2: 2001 [33] method. *Staphylococcus aureus* were isolated and enumerated on agar Baird Parker (Merck, France) according to ISO 6888-2: 2003 [32] method. The search for Salmonella was made by a pre-enrichment in Buffered Peptone Water (Merck, France), followed by enrichment in the broth Muller-Kauffmann Tetrathionate (Merck, France) and isolation on agar Xylose-Lysine-Deoxycholate (XLD) and agar Hektoen (Biokar Diagnostics, France) according to ISO 6579: 2002 [31] method. Moulds were enumerated on *agar Dichloran Rose-bengale Chloramphénicol and on Aspergillus Flavus Parasiticus Agar (AFPA) (*Merck, France) according to ISO 21527-1-2: 2008 [34-35] method. The seeded media were incubated for 3-5 days at 28±2^{0}C. Then, colonies were counted after incubation and counts were expressed as colony forming units per milliliter (cfu.ml^{-1}).

2.3.3 Microbial identification

The pure isolates were examined by their colonial and cell morphology, Gram coloration and other biochemical tests. Identification of species was carried out by assaying pure cultures on API 20E Analytical Profile Index (Ref. 20 190) galleries (BioMérieux, France). The microbiological quality assessment criterion used is based on the National standard for drinking water presented on Table 1.

Table 1: Beninese standard for drinking water

Parameters	Beninese standards for drinking water.
Chemical	
pH	6,5 – 8,5
Microorganisms	
Total Coliforms	0 cfu/100ml
Escherichia coli	0 cfu/100ml
Mesophilic Aerobic Germs	50 cfu /ml
Yeasts /moulds	0 cfu /ml
Staphylococcus aureus	0 cfu /ml
Salmonella	0 cfu /ml

Source of information: Decree No. 2001-094 of February 20, 2001 setting standard for drinking water quality in Benin Republic.

2.3.4 Statistical analysis

Statistical analyses were performed with SPSS software. The statistical differences between values expressed as mean ± S.E.M. were shown by ANOVA (Analysis of Variance) test. A difference between the values is judged statistically significant for a $p<0.05$ value.

3. Results and Discussion

3.1 Results

Chemical analysis

Herbal teas acidity and pH average values are shown in Table 2. pH values ranged from 8.43±0.34 - 9.63±0.12 when titratable acidity from 105.43±11.38 - 116.41±13.22 °D. These mean pH values were not statistically different ($p>0.05$) as well as the means values of titratable acidity.

Microbial burden of Herbal Teas

The microbial load of herbal teas from Plate Count Agar and Dichloran *Rose-Bengale Chloramphenicol Agar* was shown on Table 3. The highest microbial loads of bacteria and fungi were respectively 3.65 ± 0.87x10^{9}cfu/ml and 3.5 ± 1.1x10^6 cfu/ml. The highest microbial counts greater than 1.0x10^9 cfu/ml were found in six (46%) herbal teas samples T1, T2, T3, T5, T18 and T9. There was no difference statistically significant ($p>0.05$) between herbal teas samples analyzed.

Table 2: Herbal teas acidity and pH means values. Results were expressed as the mean ± standard error on the mean (S.E.M.).

Herbal teas names	Chemical Parameters	
	Titratable Acidity **(°D)**	***pH***
T1	107.65±11.34	9.63±0.12
T2	106.53±13.11	8.85±0.17
T3	109.41±11.72	9.17±0.13
T4	115.49±16.31	8.88±0.14
T5	113.23±17.11	8.82±0.15
T6	111.71±16.13	9.91±0.22
T7	116.41±13.22	8.84±0.32
T8	109.47±10.37	8.76±0.22
T9	106.47±14.12	8.43±0.34
T10	105.43±11.38	8.67±0.11
T11	111.34±17.12	8.93±0.41
T12	109.57±13.25	9.19±0.17
T13	106.41±10.27	8.58±0.07

Table 3: Total microbial population load from Herbal teas. Results were expressed as the mean ± standard error on the mean (S.E.M.).

Herbal teas names	Total Bacterial counts on Plate Count Agar	Total Fungal counts on *Dichloran Rose-Bengale Chloramphénicol Agar*
T1	$3.65 \pm 0.87 \times 10^9$	$2.6 \pm 0.7 \times 10^6$
T2	$2.78 \pm 0.63 \times 10^9$	$1.1 \pm 0.3 \times 10^6$
T3	$1.62 \pm 0.07 \times 10^9$	$1.3 \pm 0.6 \times 10^6$
T4	$5.85 \pm 1.38 \times 10^8$	$3.7 \pm 1.0 \times 10^5$
T5	$2.23 \pm 0.42 \times 10^9$	$2.4 \pm 0.9 \times 10^6$
T6	$7.95 \pm 2.08 \times 10^8$	$5.3 \pm 2.3 \times 10^5$
T7	$9.15 \pm 2.32 \times 10^7$	$1.6 \pm 0.5 \times 10^4$
T8	$1.35 \pm 0.02 \times 10^9$	$3.5 \pm 1.1 \times 10^6$
T9	$1.43 \pm 0.03 \times 10^9$	$2.7 \pm 0.7 \times 10^6$
T10	$8.77 \pm 2.20 \times 10^8$	$5.6 \pm 1.8 \times 10^5$
T11	$5.69 \pm 1.27 \times 10^8$	$3.6 \pm 1.3 \times 10^5$
T12	$9.96 \pm 2.62 \times 10^7$	$5.7 \pm 1.8 \times 10^4$
T13	$9.72 \pm 2.43 \times 10^8$	$4.6 \pm 0.9 \times 10^5$

Microorganisms identified from herbal teas

Table 4 has shown microbial contents of herbal teas. Bacterial isolates were identified as Mesophilic Aerobic Germs, Total Coliforms, *Escherichia coli*, *Staphylococcus aureus* and *Salmonella Typhi*. Fungal were identified as *Aspergillus niger*, *Aspergillus flavus*, *Penicillium expansum* and *Fusarium solanii*.

Table 4: Microbial contents of herbal teas

Herbal tea names	Microbial isolate	
	Bacteria	**Fungi**
T1	Mesophyl Aerobic Germs	*Aspergillus flavus Aspergillus niger*
T2	Mesophyl Aerobic Germs	*Aspergillus flavus Aspergillus niger*
T3	Total Coliforms *Escherichia coli* Mesophyl Aerobic Germs *Salmonella Typhi*	*Aspergillus flavus Aspergillus niger Penicillium expansum Fusarium solanii*
T4	Mesophyl Aerobic Germs	*Aspergillus flavus Aspergillus niger*
T5	Total Coliforms *Escherichia coli* Mesophyl Aerobic Germs *Staphylococcus aureus Salmonella Typhi*	*Aspergillus flavus Aspergillus niger Penicillium expansum Fusarium solanii*
T6	Mesophyl Aerobic Germs	*Aspergillus flavus Aspergillus niger*
T7	Total Coliforms *Escherichia coli* Mesophyl Aerobic Germs	*Aspergillus flavus Aspergillus niger Penicillium expansum Fusarium solanii*
T8	Mesophyl Aerobic Germs	*Aspergillus flavus Aspergillus niger*
T9	Total Coliforms *Escherichia coli* Mesophyl Aerobic Germs *Staphylococcus aureus*	*Aspergillus flavus Aspergillus niger Penicillium expansum Fusarium solanii*
T10	Mesophyl Aerobic Germs	*Aspergillus flavus Aspergillus niger*
T11	Total Coliforms *Escherichia coli* Mesophyl Aerobic Germs *Staphylococcus aureus*	*Aspergillus flavus Aspergillus niger Penicillium expansum Fusarium solanii*
T12	Total Coliforms *Escherichia coli* Mesophyl Aerobic Germs *Staphylococcus aureus*	*Aspergillus flavus Aspergillus niger Penicillium expansum Fusarium solanii*
T13	Total Coliforms Mesophyl Aerobic Germs *Staphylococcus aureus*	*Aspergillus flavus Aspergillus niger Penicillium expansum Fusarium solanii*

Percentage of non-compliance of chemical and microbiological test results of herbal teas

The Percentage of non-compliance of chemical and microbiological test results of herbal teas was presented on Table 5. pH average values in 92% of herbal teas analyzed did not comply with Beninese standard for drinking water. None of the herbal teas comply with Beninese standard for microbial quality of drinking water because they each contain at least one microorganism with a microbial load above the limit allowed in Benin.

Table 5: Percentage of non-compliance of chemical and microbiological tests results of herbal teas.

Indicators	Percentage of non-compliance % ($IC_{95\%}$)
Chemical	
pH	92,3 (*87,9 – 96,2*)
Microbiological	
Total Coliforms	53,8 (*45,1 – 60,5*)
Escherichia coli	38,5 (*29,7 – 46,7*)
Mesophyl Aerobic Germs	100 (–)
Moulds	100 (–)
Staphilococcus aureus	46,2 (*37,7 – 55,2*)
Salmonella Typhi	15,4 (*8,7 – 21,3*)

Microbial load of each microorganism identified in the herbal teas

Microbial load of bacteria and fungi identified was shown on Figure 1, Figure 2 and Table 6. At least one microorganism with a microbial load greater than the limit allowed by the Beninese standard for drinking water was found in each herbal tea. However, Total Coliforms, *Escherichia coli*, *Staphylococcus aureus*, and *Salmonella Typhi* were not identified in herbal teas samples named T1, T2, T4, T6, T8 and T10.

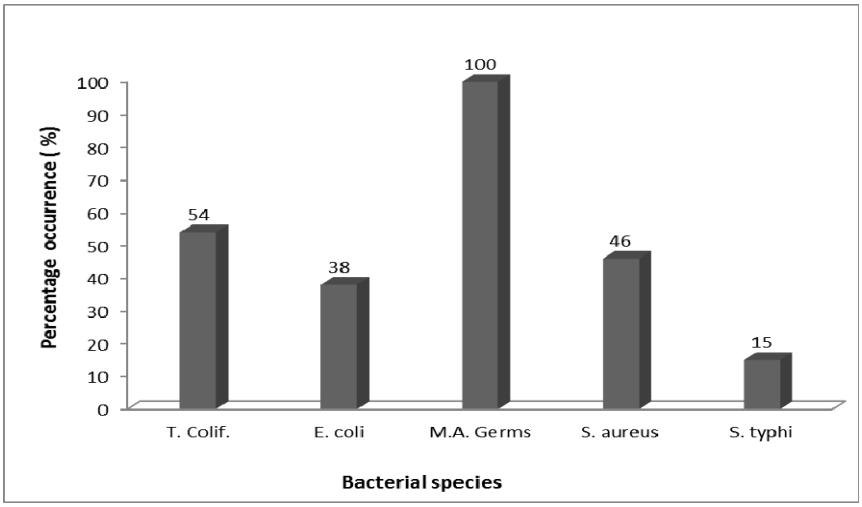

Fig 1: Percentage occurrence of bacteria isolated from herbal teas. T. Colif. = Total Coliforms, E. coli = *Escherichia coli,* M.A. Germs= Mesophyll Aerobic Germs, S. aureus= *Staphylococcus aureus,* S. typhi= *Salmonella typhi*

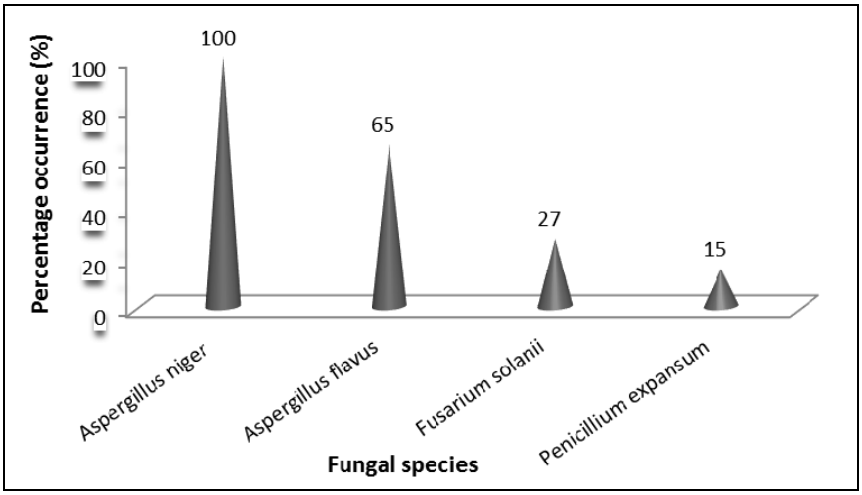

Fig 2: Percentage occurrence of fungi isolated from herbal teas.

Table 6: Mean microbial load of the main microorganisms isolated from herbal teas. Results were expressed as the mean ± standard error on the mean (S.E.M.).

Herbal teas names	Microbial load of the main microorganisms identified from herbal teas					
	TC [a] (10^4 cfu /100ml)	E. coli [b] (10^4 cfu /100ml)	MAG [c] (10^5 cfu /ml)	ASP[d] (10^5 cfu /ml)	SA [e] (10^4 ufc /ml)	ST [f] (10^4 ufc /ml)
T1	-*	-*	2.15± 0.86	0.78± 0.87	-*	-*
T2	-*	-*	1.87±.27	0.85± 0.87	-*	-*
T3	2.15± 0.81	0.32± 0.05	2.80± 0.53	1.01± 0.87	1.05± 0.11	-*
T4	-*	-*	1.60±.27	0.91± 0.87	-*	-*
T5	7.42± 1.87	0.27± 0.04	1.90± 0.17	0.90± 0.87	7.00± 0.97	2.00± 0.21
T6	-*	-*	3.20± 0.35	0.85± 0.87	-*	-*
T7	0.93± 0.15	0.17± 0.03	1.70± 0.32	0.45± 0.87	-*	-*
T8	-*	-*	2.45± 0.37	0.75± 0.87	-*	-*
T9	4.09± 1.03	-*	2.40± 0.11	4.30± 0.87	3.00± 0.63	3.00± 0.87
T10	-*	-*	2.15± 0.01	1.30± 0.87	-*	-*
T11	1.17± 0.37	0.23± 0.05	2.10± 0.08	7.30± 0.87	0.15± 0.01	-*
T12	5.60± 1.13	0.40± 0.08	2.00± 0.03	1.10± 0.87	1.30± 0.15	-*
T13	9.55± 2.04	-*	6.00± 0.71	2.31± 0.87	1.45± 0.13	-*

Key: -* No growth. *TC*= Total Coliforms*; E. coli=Escherichia coli ; MAG*= Mesophilic Aerobic Germs*; SA= Staphylococcus aureus; ST=Salmonella Typhi ; ASP=Aspergillus;* cfu/ml = Colony Forming Units per milliliter *;* ISO Methods References: a= ISO 4832:2006 ; b= ISO 16649-2:2001; c= ISO 4833-1:2013; d= ISO 21527-1-2:2008; e= ISO 6888-2:2003 ; f= ISO 6579:2002

3.2. Discussion

The results depicted considerable variations in different herbal teas samples. Microbial count on aerobic Plate Count Agar and on Dichloran Rose-Bengale Chloramphenicol Agar showed gross contamination of bacteria and fungi in 100% of the herbal teas analyzed (Table 3). Osei-Adjei G *et al.* [12] and Omogbai *et al.* [13] had also found in their studies that herbal teas sold on Ghanaian market and in Nigeria were contaminated with both bacteria and fungi. Some of bacteria and fungi isolated on herbal medicinal products are normal flora from soil, water and vegetation [9]. However, the presence of micro-organisms in herbal teas is common but it is not medically safe for human therapeutic. Okunlola *et al*. [14] and Abba *et al.* [15] had independently reported large scale contamination of herbal remedies sold in Benin City and Kaduna metropolis (Nigeria) which are not different from the present study. Microbial contents of herbal teas could be explained by the fact that they can survive in a dormant state for a long period and could be introduced into herbal teas with the medicinal plants used during the preparations [8]. Besides, a part of the bacterial bio-burden may have originated from environment, air and the personnel handling of the tea materials after processing, especially if strict good manufacturing practices (GMPs) and hygienic conditions were not followed [13]. Four genera of bacteria and three genera of fungi were isolated in this study as shown on Table 4. Some of these microorganisms can be transferred from humans to teas during processing. Total Coliforms were identified in seven: T3, T5, T7, T9, T11, T12, T13 (54%) out of the thirteen herbal teas studied (Fig.2). The coliform bacterium is the primary bacterial indicator for fecal pollution in water [16-17]. *Escherichia coli* were identified in five: T3, T5, T7, T11, T12 (38%) out of the thirteen herbal teas analyzed (Fig.2). *Escherichia coli* are the most accurate indicator for estimating fecal pollution of food. Most of *Escherichia coli* strains are harmless, but some serotypes can cause serious food poisoning in their hosts, and are occasionally responsible for product recalls due to food contamination [18]. Russell JB *et al* [19] have reported that *Escherichia coli* strains can cause gastroenteritis, urinary tract infections, and neonatal meningitis. *Staphylococcus aureus* was identified in six: T3, T5, T9, T11, T12, T13 (46%) out of the thirteen herbal teas analyzed (Fig.2). Aycicek H. *et al.* [20] showed that food handlers and food processing cookware were considered to be the main sources of *Staphylococcus aureus* contamination. *Salmonella Typhi* was identified in two: T5, T9 (15%) out of the thirteen herbal teas studied (Fig.2). *Salmonella Typhi* is the causative agent of typhoid fever, a serious disease, often fatal. Its main source of infection is the ingestion of water or infected food. Jones T. *et al.* [21] and Tirado C. *et al.* [22] have reported that *Salmonella enterica* contamination in various foods is a significant public health concern, domestically and internationally. The fungi isolated from the herbal teas include *Aspergillus niger*, *Aspergillus flavus, Penicillium expansum* and *Fusarium solanii* (Fig.1). *Aspergillus niger* was the most frequently encountered mould found in all of the herbal teas samples tested. Lee and Jo [23] also reported that *Aspergillus niger*, *Aspergillus flavus*, *Rhizopus sp*. and *Alternaria alternata* are common air contaminants probably present in the drying and packing areas. The isolation of *Aspergillus flavus* in 65% of the herbal teas is of the highest concern because it is known to produce mycotoxins including aflatoxin which can prove toxic even at low concentrations [24].

The presence of these bacteria and fungi (Table 6) in herbal teas can be explained by the fact that the medicinal plants used in their preparation are soiled in their natural environment before they are collected. Most of the times, saleswomen did not sufficiently wash medicinal plants well enough or water they use were already contaminated. This presence of microorganisms in herbal teas may be due to contamination when herbal teas have been handled unprotected or when they have been served in already contaminated containers. In addition, some of the saleswomen are used to serve herbal teas accompanied by lemons, roots or juices from cold-extracted plants that are able to contamination. Indeed, medicinal plants are often collected from fields located in suburban areas of Cotonou where people continue to make the stool in the open air. The best way to preserve herbal teas quality is follow strict Good Manufacturing Practices and hygienic conditions during preparation and serving teas [25].

Alteration in water pH is usually accompanied by other physico-chemical parameters; therefore any change in the pH of an ecosystem significantly influences all biochemical activities occurring there [26]. pH average values in 92% of the herbal teas analyzed do not comply with the Benin norm and quality for drinking water. Majority of herbal teas analyzed in Cotonou showed high values of pH. The high value of pH could be attributed to poor hygiene conditions and soil wastes brought by medicinal plants often used in the preparation of herbal teas. High value of pH is the consequence of waste discharge, microbial decomposition of organic material in the water body [27-28].

4. Conclusion

The study demonstrated the presence of microbial contaminants in herbal teas daily sold in Cotonou at levels exceeding the acceptable limits of microbial load count. *Aspergillus* species isolated has the potential to produce aflatoxins; therefore, it is very important that public health departments of the Ministry of Health bring the saleswomen awareness on herbal teas. The risk of microbial contamination can be considerably reduced by following strict GMPs and hygienic conditions when preparing and serving herbal teas.

5. Conflict of Interests

We declare that we have no conflict of interest.

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