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Quality-Based Microbial Contamination Analysis of Nutraceuticals

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Abstract

The aim of this study was to evaluate the microbial contaminations of medicinal plants used as traditional herbal medicines sold in pharmacies in Cotonou in Benin. Twenty (20) medicinal plants involved in the preparation of improved herbal medicines, eleven (11) improved herbal medicines sold in pharmacies and fourteen (14) improved herbal medicines sold by herbalists were studies. The microbial contaminations (mesophilic bacteria, yeasts and moulds) of the samples were analyzed. Our results revealed that the samples were contaminated with a wide range of pathogenic microorganisms, including Staphylococcus aureus, Pseudomonas aeruginosa and Escherichia coli. Samples sold by herbalists were the most highly contaminated. The herbal medicines sold in pharmacies were also contaminated by Escherichia coli (81.82%), Staphylococcus aureus 27.27% and Pseudomonas aeruginosa (18.18%). These products did not meet the European Pharmacopoeia standards. Our data suggest that nutraceutical processing as well as storage conditions among others must be critically improved in order to meet the standards required for any pharmaceutical drugs.

Keywords: Phytomedicines improved, microbiological quality, specified germs, Benin.

Introduction

Ethnobotany has been the source of several drug discoveries to treat a wide range of human diseases. The development of drug resistance in synthetic anti-malarial drug therapies has led to the use of combined artemisinin-based (CTA) drug therapy. However, there has been lately several accounts of resistance to the ACT therapy. On the other hand, the *Artemisia annua* L (Asteraceae) nutraceutical has been proven to be very effective to treat the disease, without inducing drug type of resistance[1]. Currently in Europe, there is a trend of return to nutraceuticals for human disease treatment. In Africa, the several socio-economical factors including poverty, lack of drug accessibility have forced people to embrace the use of nutraceuticals to treat a wide range of human diseases. The use of nutraceuticals has been further enhanced by the highly diverse and rich African flora and fauna, readily and cheaply available. About 75% of Africans rely on medicinal plants for a variety of ailments[2]. In Benin, more than 85% of the population relies on traditional medicine for health care needs[3]. This explains the rapid expansion of global market of medicinal plant derived nutraceuticals that represents currently over 60 billion U.S. dollars per year[4]. The traditional medicine represents an economically sustainable alternative enabling the developing countries to cope with highly expensive modern medicine therapies. The declaration of Abuja (Nigeria) in April 2001 aimed at making traditional medicine research a priority for Africa. Since then considerable effort has been put on identifying, characterizing and promoting the use of medicinal plants and herbal medicines in Africa. The production of phytochemicals and nutraceuticals has been improved to minimize the cytotoxicity while increasing their efficacy in treating a wide range of ailments. These improved nutraceuticals have been approved to be sold in pharmacies and they have been adopted by the community at large. However, there has been no report on the microbial content analysis of these products, which might occur during packaging, storage and distribution[5]. One of the stringent safety regulations of these products is the quality control before commercialization. Unfortunately, only a quarter of African countries have such safety regulation rules before commercialization of nutraceuticlas[5]**.** The WHA42.43 resolution of World Health Assembly urged the country members to implement the safety control regulation of neutraceuticals before their commercialization[6]. In Benin, there is not safety regulation, and no microbial analytical control is performed before

commercialization of nutraceuticals widely embraced by the population. In this study, we aim to evaluate the microbial content of nutraceuticals as well as pharmaceuticals commercialized in different pharmacies in Cotonou, Benin.

Material and Methods

Materials: The samples used in this study are: medicinal plants purchased at market (table 1), improved and processed medicinal herbals purchased from herbalists, and the nutraceuticals purchased from different pharmacies (table 2). In total we considered 20 medicinal plants were purchased from two highly populated markets of Cotonou (Dantokpa and Gbegamey). In addition we purchased 11 nutraceuticals from pharmacies and 14 other nutraceuticals were purchased at the National Nutraceutical Fair (MTA) of 24 August 2011 at "Stade de l'amitie" in Cotonou.

Survey of registered medicinal plants in form of nutraceuticals: The survey was conducted in ninety-three (93) pharmacies in Cotonou[7] with the guidance of the pharmacists. The survey was performed according to the following criteria:

Inclusion criteria: Available neutraceuticals made through infusion or maceration with no antibacterial and/or antifungal properties, administered orally

Non-inclusion criteria: Unavailable nutraceuticals, with antibacterial and/or antifungal agents, administered by any (s) other (s) path (s) or as a decoction and alcoholic maceration.

Determination of viable aerobic microbial germs: The both agar plate and Sabouraud containing chloramphenicol cell count approach was use to determine the number of bacteria and fungi present in the samples as described in the European Pharmacological guideline**.** A volume of 1 mL of inoculums of SM or its serial dilutions is deposited in the empty Petri dishes and covered by 15 to 20 mL of PCA agar. The SM solutions were seeded in duplicate. Plates were then homogenized and allowed to solidify under sterile hood. The Petri dishes were then incubated at 35 ° C for 48 hours.

About 15 to 20 mL of Sabouraud Chloramphenicol Agar is distributed aseptically in Petri dishes. After cooling, the plates were seeded in duplicate by filing it with 0.1 mL of the SM or its serial dilutions. The microbial drop was on the Petri dish spread and incubated at 25 ° C for 96 hours[8].

Research of specific microbial germs: ***Staphylococcus aureus (SA)****:* the most pathogenic species, SA, responsible for food spoilage and food contamination was assessed on Baird Parker Agar containing potassium telluride.

Pseudomonas aeruginosa: We assessed the highly resistant bacteria that can grow in antiseptic or antibiotic solution on cetrimide agar.

Escherichia coli: To identify *E. coli* bacteria, we traced the marker of fecal contamination, an efficiency indicator from treatment on eosin methylene blue agar. MS previously prepared and used to prepared serial dilutions and kept at room temperature under the sterile hood for six hours (6 pm). 0.1 mL of MS was used to inoculate three selective media respectively. The control containing no bacterial was performed under the same conditions. The duration of incubation was 72 h for all selective media inoculated.

Table-1
The medicinal plants, herbal drugs and the places of purchase

N°	Medicinal plants	Herbal drugs	Purchase locations
01	*Carica papaya*	Leaf	Market Dantokpa
02	*Cassia alata*	Leaf	Market Dantokpa
03	*Citrus aurentifolia*	Leaf	Market Dantokpa
04	*Combretum glutinosum*	Leaf	Market Dantokpa
05	*Combretum micranthum*	Leaf	Market Dantokpa
06	*Crataeva religiosa*	Stem bark	Market Dantokpa
07	*Eucalyptus globulus*	Leaf	Market Dantokpa
08	*Ficus spp*	Leaf	Market Dantokpa
09	*Funtumia africana*	Leaf	Market Dantokpa
10	*Imperata cylindrica*	Leaf	Market Dantokpa
11	*Lippia chevalieri*	Leaf	Market Gbégamey
12	*Lippia rugosa*	Leaf	Market Gbégamey
13	*Lonchocarpus cyanescens*	Leaf	Market Gbégamey
14	*Morinda lucida*	Stem bark	Market Gbégamey
15	*Nauclea latifolia*	Root bark	Market Gbégamey
16	*Newbouldia laevis*	Leaf	Market Gbégamey
17	*Ocimum basilicum*	Leafy stem	Market Gbégamey
18	*Ocimum gratissimum*	Leaf	Market Gbégamey
19	*Panax ginseng*	Root	Market Gbégamey
20	*Phaseolus vulgaris*	Leaf	Market Gbégamey

Table-2
Phytomedicinal forms and places of purchase

N°	Improved phytomedicines (PI)	Forms of administration	Places to buy
01	PI 01	Tea	Pharmacy
02	PI 02	Tea	Pharmacy
03	PI 03	Tea	Pharmacy
04	PI 04	Powder	Pharmacy
05	PI 05	Oral solution	Pharmacy
06	PI 06	Oral solution	Pharmacy
07	PI 07	Aqueous maceration	Pharmacy
08	PI 08	Oral solution	Pharmacy
09	PI 09	Aqueous maceration	pharmacy
10	PI 10	Oral solution	Pharmacy
11	PI 11	Oral solution	Pharmacy
12	PI 12	Oral solution	Stade de l'amitie
13	PI 13	Oral solution	Stade de l'amitie
14	PI 14	Oral suspension	Stade de l'amitie
15	PI 15	Oral suspension	Stade de l'amitie
16	PI 16	Oral suspension	Stade de l'amitie
17	PI 17	Oral solution	Stade de l'amitie
18	PI 18	Infusion bag	Stade de l'amitie
19	PI 19	Infusion bag	Stade de l'amitie
20	PI 20	Infusion bag	Stade de l'amitie
21	PI 21	Tea	Stade de l'amitie
22	PI 22	Aqueous maceration	Stade de l'amitie
23	PI 23	Infusion bag	Stade de l'amitie
24	PI 24	Tea	Stade de l'amitie
25	PI 25	Aqueous maceration	Stade de l'amitie

Results and Discussion

Results of the field survey: Almost all pharmacies in Cotonou, Benin have embraced the selling of nutraceuticals. However, 66.67% of the pharmacies surveyed doubt the quality and/or the efficacy of the medicinal derived nutraceuticals, while 2.22% of pharmacies have not fully embraced the selling of nutraceuticals.

Most of the nutraceuticals found in the pharmacies are not approved and account for 63.64% of the medicines preferred by the folk**.** Marketing approval/license to sell plant based medicines/nutraceuticals is very complicated and generally not enforced. Among the well recognized licensing agencies, the Direction for Food and Applied Nutrition (DANA) is the most structured in the Country. As such, it has issued more than two out of the four marketing licenses of the recognized approved nutraceuticals in the pharmacies. No improved phytomedicine has been approved from the federal recognized agency, the National Program of the Pharmacopoeia and Traditional Medicine (PNPMT).

Herbal medicines are mainly sold as liquid improved infusions (45.46%) either in water or alcoholic drink**.** They are generally packaged in bottles (63.64%) and paper packaging (36.36%) devices**.** For the bottle packaging they are generally in content of 100 mL, 250 mL and/or 1000 mL. In addition, most of these packaging medicines have no expiration date, although relatively very few contain expiration date. Specifically, more than half (54.55%) of herbal medicines sold in improved pharmacies have no expiration date.

Results of counts of detected viable aerobic microorganisms: In this analysis, we only take into account plates containing 30-300 colonies for further characterizations. The results of different microorganisms identified are summarized in tables 3, 4 and 5.

Identification and Characterization of specific microbial germs: The entire medicinal plants (100%) analyzed in this work have been contaminated by the following three microbial organisms: *Staphylococcus aureus*, identified in three samples out of the eleven samples analyzed. i.e. 27.27%; *Pseudomonas aeruginosa*, identified in two out of the eleven samples analyzed, i.e. 18.18% and *Escherichia coli*, identified in all the samples analyzed, i.e. 100%. Additional investigation showed that samples PI 05 and PI 08 were void of *E. coli*, indicating that 81.82% of all analyzed samples were contaminated by *E. coli*. In summary the nutraceuticals sold in pharmacies were most contaminated by *Escherichia coli* followed by *Staphylococcus aureus*. In addition of the microbial contamination data, no nutraceuticals considered in this work meets the European Pharmacopoeia standards.

Among the phytomedicines purchased directly from herbalists *Staphylococcus aureus* was identified in twelve out of fourteen samples analyzed, i.e. 85.71%, *Pseudomonas aeruginosa* was identified in four out of fourteen samples, i.e. 28.57%, while *Escherichia coli* was present in all samples with exception of four samples, i.e. 92.86%. They are also not conforming to the European Pharmacopoeia standards.

In this work, we surveyed eleven herbal medicines from registered pharmacies in the city of Cotonou, Benin (West Africa) and evaluated the microbial contamination profile of the medicines. The size of samples analyzed in this study was higher than that of Coulibaly[9] which was six (06) herbal medicines in Mali. About 88.17% of medicines sold in pharmacies in the city of Cotonou were herbal medicines, indicating that nutraceuticals are the most popular mode of medication in Benin. There are, however, pharmacies that do not sell these nutraceuticals due to their uncertain quality and low hygienic standard of processing. The pharmacies that refused to sell these nutraceuticals generally referred to the lack of marketing authorization and the lack of proper expiration date on the packaging of the medicines. Terminology /indications of improved phytomedicines are very arbitrary in this region of the world. They are not generally related to chemical properties of the plants, and most of the chemicals in the neutraceuticals were not properly studied and were generally unknown. This reflects the need to study for the validation of new indications (*Phaseolus vulgaris*) indicated in the removal of cholesterol, weight loss.

The liquid forms of nutraceuticlas are more available, but also they were the most contaminated by the microorganisms. The form (maceration, infusion or decoction) of processing were not indicated in the packaging device. This is generally important because it can easily affect the storage conditions, the expiration date and the modification of the active ingredients of the nutraceuticals. According to Agassounon et al.[10], at room temperature (26 ° C) macerations are not to be stored as long as decoctions. They generally can be kept more than three days. The shelf life is longer with the preparation that took into account a higher hygienic condition such as sterilization with filter through Millipore membrane 0.4 diameter[10]. The use of 1000 mL bottle packaging, which takes longer to be used are generally the most contaminated because the storage conditions were not generally followed both in the pharmacies and by the patients.

Table-3
Microbial contamination counts in the medicinal plants

S. No.	Medicinal plants	Number of colonies per ml of product (UFC/mL)	
		Bacteria	**Yeasts and molds**
01	*Carica papaya*	32.10^2	3.10^4
02	*Cassia alata*	59.10^2	6.10^4
03	*Citrus aurentifolia*	5.10^2	2.10^4
04	*Combretum glutinosum*	19.10^2	5.10^2
05	*Combretum micranthum*	40.10^2	5.10^4
06	*Crataeva religiosa*	99.10^2	12.10^3
07	*Eucalyptus globulus*	7.10^1	34.10^3
08	*Ficus spp*	4.10^3	52.10^2
09	*Funtumia africana*	27.10^2	8.10^3
10	*Imperata cylindrica*	36.10^2	14.10^4
11	*Lippia chevalieri*	41.10^2	18.10^4
12	*Lippia rugosa*	45.10^2	27.10^3
13	*Lonchocarpus cyanescens*	50.10^2	38.10^3
14	*Morinda lucida*	1.10^2	2.10^4
15	*Nauclea latifolia*	18.10^2	37.10^3
16	*Newbouldia laevis*	5.10^2	3.10^3
17	*Ocimum basilicum*	5.10^3	40.10^3
18	*Ocimum gratissimum*	6.10^3	24.10^3
19	*Panax ginseng*	18.10^1	36.10^3
20	*Phaseolus vulgaris*	25.10^3	25.10^3
Average number		**$4,4.10^3$**	**$3,9.10^4$**

Table-4
Microbial contamination counts in nutraceuticals sold in pharmacies

S. No.	Code of improved phytomedicines	Number of colonies per ml of product (UFC/mL)	
		Bacteria	Yeasts and molds
01	PI 01	000	000
02	PI 02	8.10^2	000
03	PI 03	1.10^4	000
04	PI 04	1.10^2	000
05	PI 05	8.10^3	45.10^2
06	PI 06	16.10^4	000
07	PI 07	15.10^4	5.10^3
08	PI 08	1.10^4	4.10^3
09	PI 09	14.10^4	4.10^3
10	PI 10	3.10^4	5.10^2
11	PI 11	18.10^4	000
Average number		**$6,3.10^4$**	**$1,6.10^3$**

Table-5
The fungal versus bacterial contaminants in herbal medicines purchased from herbalists

S. No.	Code of improved phytomedicines	Number of colonies per ml of product (UFC/mL)	
		Bacteria	yeasts and molds
01	PI 12	000	000
02	PI 13	000	000
03	PI 14	4.10^4	49.10^3
04	PI 15	18.10^5	2.10^7
05	PI 16	000	000
06	PI 17	000	000
07	PI 18	5.10^2	000
08	PI 19	16.10^2	000
09	PI 20	3.10^2	000
10	PI 21	18.10^5	000
11	PI 22	31.10^2	000
12	PI 23	8.10^2	000
13	PI 24	18.10^5	1.10^7
14	PI 25	2.10^6	6.10^5
Average number		**$5,3.10^5$**	**$2,2.10^6$**

The total viable aerobic microorganisms were evaluated A total of twenty (20) medicinal plants were analyzed. Herbal drugs analyzed are mainly leaves (15/20) as they are commonly used in African traditional medicine. Yeasts and molds are the main contaminants; they generally affect the organoleptic characteristics of the herbal products. Places of harvest and drying conditions and storage are generally the main reasons for these contaminations. As above mentioned herbal medicines purchased from pharmacies were mainly in liquid forms (7 out of 11). They are also contaminated with bacteria at the rate of 14.32 times that of medicinal plants. This can be explained by the Proliferation of microbial contaminants of herbal medicine use and/or contamination occurred during the nutraceutical processing. To obtain improved herbal and medicinal plants that meet the approved standards, the plant materials used should always be disinfected. The compliance with good practice (GMP), packaging and appropriate storage conditions are (7 out of 11) not always observed. Compliance with good practice (GMP), appropriate packaging and control of intermediates in the production of herbal medicines need improvement. This study found that herbal medicines purchased from herbalists are mostly in liquid forms (8 of 14). They are slightly contaminated by yeasts and molds. For those contaminated by fungi, the fungal contamination count was very high (10^6 CFU/ml). The result is the same for bacterial contaminants. The most contaminated samples were the oldest products registered in the pharmacies compared to the newer products, which were less contaminated. The nutraceutical manufacturing process also plays a significant in the microbial contamination. The macerations are more easily contaminated than the decoctions.

Medicinal plants purchased from different places (markets and Dantokpa Gbegamey) in Cotonou were all contaminated with *Staphylococcus aureus, Pseudomonas aeruginosa and Escherichia coli*. These results are similar with those obtained by Agassounon et al.[11] who found *E. coli* in all samples of

medicinal plants analyzed. In these markets, the mixture of herbal drugs, the absence of disinfectant during nutraceutical processing and good storage conditions could explain the high level of medicinal contamination. During storage, the conditions for cross-contamination increases the rate of microbial germ counts and complicate the issue of nutraceutical safety. The rate of herbal medicine contaminations reported in this study is similar with that reported by Baba-Moussa et al.[12] (83%) in food sold by hawkers in the city of Cotonou. However, studies conducted by Coulibaly[9] revealed an absence of *Salmonella* and *Shigella* in six (06) improved herbal analyzed.

Herbal medicines purchased from herbalists are heavily contaminated with *Staphylococcus aureus* and *Escherichia coli*. Given the habitat of these two organisms (human digestive tract and genitals), contamination of human origin is suspected in these samples. This reflects a lack of strict hygienic measures.

The strong presence of *Staphylococcus aureus* and *Escherichia coli* in the phytomedicines confirms the involvement of manipulations.

Conclusion

Following this study, eleven (11) improved herbal medicines sold in pharmacies in the city of Cotonou were analyzed. The results show that none of these herbal medicines meets the required European Pharmacopoeia standards. Improved herbal medicines sold in pharmacies and those sold by herbalists are highly contaminated with highly pathogenic microbial germs, including *Staphylococcus aureus, Pseudomonas aeruginosa* and *Escherichia coli.*

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