ISSN (Print): 2476-8316
ISSN (Online): 2635-3490
Dutse Journal of Pure and Applied Sciences (DUJOPAS), *Vol. 6 No. 2 June 2020*

Microbial Contamination of Liquid Herbal Preparations Marketed in Parts of Abuja Metropolis, Nigeria

***Ya'aba Y[1,2], Izebe KS[1], Mohammed SB[1], Chuku A[2], Abdulmumin AR[1], Abarike M.C[1]**

[1]Department of Microbiology and Biotechnology, National Institute for Pharmaceutical Research and Development (NIPRD) Abuja, Nigeria

[2]Department of Microbiology, Federal University Lafia, Nasarawa State, Nigeria

Email: yakyabnig@yahoo.com

Abstract

There is an increase in the consumption of herbal preparations globally. The quality and safety of these herbal preparations often times are not authenticated and they may be pathogenic microorganisms as contaminants, thereby making, their use a serious Public Health challenge. This study was designed to evaluate the microbial quality of liquid herbal preparation products sold in Abuja markets. Twelve coded samples of liquid herbal preparation products were purchased for certain microbial analyses in accordance to United States Pharmacopoeial methods. Microbes identified were further isolated, and characterized using differential, selective media, and by selected biochemical analyses. The findings from this study showed that nine (75%) coded samples had bacteria contamination with varied counts, while three (25%) coded samples were free from bacterial contamination. Similarly, the fungal contaminations were recorded against eight (67%) coded samples while four (37%) of the coded samples analyzed were free from the any fungal contamination. The total aerobic bacterial mean counts ranged from 20 to 6.7 x10^3 CFU/mL while the total mold count ranged from 10 CFU/mL to 30 CFU/mL. The isolated bacteria in this study included Bacillus sp, Micrococcus sp, Coliforms, Pseudomonas aeruginosa, Salmonella sp while the fungal pathogens isolated were Zygomycetes groups which included Mucor, Rhizopus and Absidia sp indicated that the samples were contaminated. The findings from this study showed poor microbial quality and exhibit contamination by pathogenic microorganisms. Thus, there is need for regular quality evaluation and extended regulatory control by agencies responsible for regulating herbal preparation products to improve microbial quality and safety.

Keywords: Herbal preparation, Contamination, Microbial quality, Pathogens, and Pharmacopoeial

**Author for Correspondence*

INTRODUCTION

The term "herbal preparations" refers to plants and plant parts that have been converted into useful phytopharmaceuticals by means of some simple processes that does not requires sophisticated equipment for its production from harvesting, drying and storage (Abel and Busia, 2005). World Health Organization (WHO) in 2003 stated that herbal preparations (herbs, herbal medicines, herbal materials and finished herbal products) that are contained in plant parts or plant materials in crude or processed form as active constituent and excipients (foreign substances or materials) (WHO, 2003). It is of paramount importance to note that any admixture with chemically defined active substances or isolated ingredients is not considered herbal preparations (Ampofo *et al.,* 2012). It was also defined by the European Medicine Evaluation Agency (EMEA) that herbal preparations are medicinal products containing exclusively herbal drugs or herbal drug preparations of active substances or constituents (Abel and Busia, 2005).

Many scientists had documented that herbal preparations are been used since ancient times in treatment of some disease conditions such as eczema, asthma, migraine, menopausal symptoms, irritable bowel syndrome, premenstrual syndrome, rheumatoid arthritis, chronic fatigue and cancer among others (Kulkarni and Deshpande, 1999; WHO, 2004; Oktem *et al.,* 2007; Khanyile *et al.,* 2009). Although in the past, the medicinal use of herbs and herbal products went into a serious reduction in the Western countries when other predictable synthetic drugs were made commonly available and accessible. However, some developing countries still continue to get gain from the utmost knowledge of medical herbalism. For instance, Kampo medicine in Japan, Unani medicine in the Middle East and South Asia, Ayurvedic medicine in India and traditional Chinese medicine (TCM) are still being used by a large majority of populace (Khanyile *et al*., 2009).

In the developing countries like Nigeria, the World Health Organization (WHO) survey report estimates that approximately four billion people (about 70-80% of the global population) depend on non-conventional medicines that are mainly of herbal origins for their primary health care services (Ampofo *et al.,* 2012). This is a fact that the use of herbal preparations or medicines by traditional practitioners and patients that are sick of one disease or the other are increasing day by day globally due to its readily availability, accessibility and low cost of raw materials compared to the synthetic drugs (Ekor, 2014), thus, there is a need for pharmacists and physicians to have better understanding and knowledge regarding the safety of such preparations (Mosihuzzaman and Choudhury, 2008).

The widespread use of herbal preparations or medicines calls for the assurance of sustainable availability of quality and safe preparations of these herb in order to guaranty its continued access especially for rural or low-income communities, without compromising patient's quality and safety (Kigen *et al.,* 2013). Although, the increase interest in herbal plants preparations as a re-emerging health aids has been fueled by the rising costs of synthetic drugs in the maintenance of personal health related infections and wellbeing. In Nigeria, despite the increase in production of herbal preparations in the market, there is still scarcity of information from researches carried out on its quality because of its public health significant in some geographical locations. Although, most of producers of the herbal preparations in the country (Nigeria) do not have the required expertise to perform quality control and resolving safety issues on the preparations they manufactured. Thus, this may

bring about the challenges of inconsistency on the quality and safety of the herbal preparation in the country.

Microbial contamination is the preclusion of unacceptable substance or impurities (microbiological or chemical or foreign matter) onto raw material to be used, intermediate product or finished herbal preparations during production processes, packaging, storage/perseveration or transport of this preparations or products (WHO, 2007) Generally, the presence of coliforms (*Escherichia coli*) in herbal preparations product implies the possibility of most recent fecal contaminations and inadequate or failure in sanitation measures of the preparation process (Temu-Justin *et al.,* 2011; Onyambu *et al.,* 2013; Khanom *et al.,* 2013). The fecal contamination may be assumed to occur through poor handling by personnel who are infected with pathogenic bacteria during harvesting or collection, post-harvest processing and the finished herbal preparations. The microbial contamination can be avoided by applying best practice guidelines such as Good Manufacturing Practice (GMP) (WHO, 2007).

Furthermore, many microbial contaminants can alter the physicochemical features which can then lead to mischievous changes to the quality of herbal preparations (Onyambu *et al.,* 2013). The rapid expanding markets of herbal preparations use is evidently calling for evaluating issues related to quality and safety of these products for end users (Khanom *et al.,* 2013). It is well documented by some researchers that herbal preparations with microbial contaminations can be a potential source of infections which in turn can result into a variety of challenges from sepsis, gastroenteritis, blindness and even death (Whitcher *et al.,* 2001). In order to prevent and control these undue challenges, it is advised by the World Health Organization (WHO) that all local and international regulatory bodies such as National Agency for Food and Drug Administration and Control (NAFDAC) in Nigeria to enact policies that guarantee the quality and safety herbal preparations for human consumption (Ekor, 2014).

The quality evaluation of herbal preparations is therefore of paramount importance in order to ascertain their acceptance in this era of modern system of technology. It is thus necessary that the microbiological limit determination of herbal preparations be carried out to guaranty that the final product is free from health-related risk. Chitrarekha and co-workers in 2010, reported that plants and plant materials also carry high number of microorganisms that are mainly of soil origin. The aerobic sporulating bacteria frequently predominate as additional contamination in herbal preparations and their growth that occur and persist during harvesting, handling and finished products (Chitrarekha *et al.,* 2010). It is therefore important that traditional practitioners and physicians have the required knowledge about the microbiological safety of the herbal preparations (Mendes *et al.,* 2010). Therefore, this study is aimed at evaluating the level of microbial contaminants of some liquid herbal preparations marketed in Abuja, Nigeria.

MATERIALS AND METHODS

Study Area

This study was carried out in Abuja. Abuja is the developing Federal Capital City of Nigeria lying between latitude 8.25°N and 9.20°E of the equator and longitude 6.45°N and 7.39°E of Greenwich Meridian, with a landmass of approximately 7,315 km^2. It is situated within the

Savannah region with moderate climatic conditions. The territory is located just north of the confluence of the River Niger and Benue River (World Gazetter, 2007).

Sample Collection

A total of twelve (12) different brands of liquid herbal preparations in bottles were purchased at random from different herbal vendors' in parts of Abuja metropolis, Nigeria. All the collected samples were transported to the Microbiology laboratory of the Department of Microbiology and Biotechnology, National Institute for Pharmaceutical Research and Development (NIPRD), Abuja, Nigeria for further analyses between December, 2019 and March, 2020. NIPRD is the apex medical research and referral institution in the country (Nigeria) charged with the responsibility to conduct research into pharmaceutical products and diseases of public health significant.

Microbial load Analyses

The microbial load analyses were carried out based on the techniques described in United States Pharmacopeia (USP). The sample was shaken vigorously after which one millilitre (1.0mL) of each of herbal preparations was dispensed onto 9.0 mL of Tryptic Soya Broth for bacteria and 9.0 ml SDA for fungi. 1.0 mL of these stock solutions was dispensed onto sterile TSA for bacteria and SDA for fungi in triplicates. The inoculated plates were mixed to obtained uniform spread and excess discarded and incubated at 37^{o}C for 24 hrs for bacteria and 25^{o}C for fungi for 7 days. After the incubation period, the colonies on the Tryptic Soya Agar and Sabournd Dextrose Agar were counted in colony coulter. The counted number of colonies were expressed in colony forming units per millilitres (CFU/mL) (USP, 2003).

Isolation for specific pathogens in herbal preparations

One loopful of different stocks of the herbal preparations in the Tryptic Soya Broth and Sabouraand Dextrose Broth was used to streaked on Mannitol Salt Agar, Eosin Methylene Blue Agar, Cetrimide Agar, Salmonella Shigella Agar and Sabourand Dextrose Agar and incubated at 37^{o}C for bacteria and 25^{o}C for fungi for 7 days with daily observation for colonies (Oluwatoyin, and Adelayo, 2016).

Pure bacterial colonies were isolated based on morphologic differences from their axenic cultures. Colony form, elevation, pigmentation and size were the major distinguishing features used in picking the colonies into sterile peptone water for further identification part from Gram's reaction on the colony of interest and biochemical tests (indole test, Voges-Proskeur tests, methyl red test, citrate utilization test, catalase test, urease test, coagulase test, spore stain test, oxidase test, motility test and sugar fermentation test) as described by (Holt *et al.,* 1994).

The fungal pure culture isolates obtained were identified based on their morphology as yeast and molds. The molds were identified using the following test: wet mount by mounting the colony on lactophenol cotton blue solution and was viewed under the microscope.

Statistical Analyses

The quantitative data were analyzed statistically using SPSS (version 20) statistical software. Standard descriptive statistics including means, frequencies and percentages were used to describe some of the findings.

RESULTS

Table 1 depicts the distribution of contaminants in the liquid herbal preparations marketed in parts of Abuja, Nigeria. The microbial contamination in the liquid herbal preparations with coded sample 6 showed highest mean total aerobic bacterial count of $6.7x10^3$ CFU/mL. Coded sample 1 gave $3.4x10^3$ CFU/mL while sample code 2 gave $5.2x10^2$ CFU/mL. The least bacterial count was observed in coded samples 8 and 9 with 10 CFU/mL and 2.0 CFU/mL respectively. The fungal counts also indicated the mean highest total fungal count was 30.0 CFU/mL and least coded samples with no fungal count analysed. The bacteria identified in the liquid herbal preparations in question included *Bacillus sp, Citrobacter sp*, *Coliforms, Enterobacter sp*, *Micrococcus sp*, *Serratia sp, Actinomycete sp, Pseudomonas aeruginosa and Salmonella sp* while the identified fungi included *Mucor sp*, *Rhizopus sp and Absidia sp.*

Table 1: Total Bacterial and Fungal Counts in the Liquid Herbal Preparations

Coded Sample	Total aerobic bacterial count (CFU/ML)	Total mold count (CFU/ML)
01	$3.4x\ 10^3$	ND
02	5.2×10^2	3.0×10^1
03	4.0×10^2	3.0×10^1
04	$2.0\ x10^2$	$1.0\ \ x\ 10^1$
05	$2.0\ x10^1$	$1.0\ \ x\ 10^1$
06	6.7×10^3	ND
07	1.0×10^1	ND
08	1.0×10^1	$2.0\ \ x10^1$
09	2.0×10^1	$1.0\ \ x\ 10^1$
010	Absent	10×10^1
011	Absent	3.0×10^1
012	Absent	ND

Key: ND = Not Done

Table 2: Gram's Staining, Morphological and Biochemical Identification Test Results

Coded sample	Bacterial / fungi isolated	Identification parameters									
		GR	MOR	CAT	COA	LAC	CIT	IND	URA	OXI	LPB
01	*Bacillus sp,*	+	RS	+	ND	+	ND	-	ND	ND	ND
	Citrobacter sp	-	CB	+	ND	+	-	-	-	+	ND
	Coliforms	-	R	+	ND	+	+	+	-	-	ND
	Enterobacter sp	-	R	+	ND	+	+	-	-	-	ND
	Fungal not isolated										
02	*Bacillus sp*	+	RS	+	ND	+	ND	-	ND	ND	ND
	Enterococcus sp	+	CS	-	-	+	ND	ND	ND	ND	ND
	Micrococcus sp	+	C	+	-	-	ND	ND	ND	ND	ND
	Mucor sp										MS
	Rhizopus sp										MRS
	Absidia sp										MSS
03	*Bacillus sp*	+	RS	+	N	+	+	ND	ND	ND	ND
	Serratia sp	-	CB	+	ND	+	+	-	-	-	ND
	Mucor sp										MS
	Rhizopus sp										MRS
04	*Bacillus sp*	+	RS	+	ND	+	+	-	ND	ND	NA
	Actinomycetes sp	+	FR	NA	NA	NA	+	NA	NA	NA	NA
	Mucor sp										MS
	Micrococcus sp										
05	*Bacillus sp*	+	C	+	-	-	ND	ND	ND	ND	NA
	Coliform sp	+	RS	+	ND	+	+	ND	ND	ND	NA
06	*Bacillus sp*	-	R	+	ND	+	+	+	-	-	NA
	Citrobacter sp	+	RS	+	ND	+	+	ND	ND	ND	NA
	P. aeruginosa	-	CB	+	ND	+	+	-	-	+	NA
	Salmonella sp	-	R	+	ND	-	+	-	+	+	NA
	Bacillus sp	-	R	+	ND	-	+	-	-	-	NA
07	*Micrococcus sp*	+	RS	+	ND	+	+	ND	ND	ND	NA
08	*Arthrobacter sp*	+	C	+	-	-	ND	ND	ND	ND	NA
	Absidia sp	-	CB	+	ND	-	+	-	-	-	NA
	Mucor sp										MSS
	Bacillus sp										MS
09	*Coliforms*	+	RS	+	ND	+	+	ND	ND	ND	NA
	Absidia sp	-	R	+	ND	+	+	+	-	ND	NA
	Rhizopus sp										MSS
	Mucor sp										MRS
10	*Mucor sp*										MS
11	*Absidia sp*										MS
	Rhizopus sp										MSS
	Bacteria and fungi not										MRS
12	*detected*										

Keys: GR: Gram Reaction, MM: Microscopic Morphology, CAT: Catalase, COA: Coagulase reaction, LAC: Lactose fermentation, CIT: Citrate utilization, IND: Indole Test, URE: Urea teat, OXI: Oxidase Reaction, LPC: Lactophenol cotton Blue reaction. + Positive, - Negative, RS- Rod/Spore CB-Coccobacillus, FR-Filamentous/Rod, R-Rod, C- Cocci, ND-Not Done, MS-Mycelia/spores, MRS- Mycelium with roots/spores, MSS-Mycelium with attached spores.

DISCUSSION

All of the twelve (12) liquid herbal preparations considered in this present study were orally consumed as drugs and none of them had any form of microbial/faecal based tests carried out by the producers, which may account for the high recovery rates of coliforms. The risk of the presence of microorganisms such as coliform and others in a pharmaceutical-products including herbal products depends on its nature, finality of the use and potential damages that may be caused to the consumers (Mullika *et al.,* 2003; Arani *et al.,* 2014).

As documented in US pharmacopoeia (USP 30), the total aerobic microbial count of herbal preparations in this study was not more than 10^5CFU/mL. Therefore, the microbial load of marketed herbal preparations in parts of Abuja, Nigeria which was analysed in this study were not acceptable based on microbial limit (WHO, 2007). These findings revealed that raw herbal preparation had some initial predispose microbial levels of contaminants of natural origin which may be related to the plant or growing environmental conditions of the herbal plants. This is comparable to the findings that have been also reported in an earlier study on the microbiological quality of some pharmaceutical raw materials (Kosalec *et al.,* 2009).

The observed coliforms which are members of the family *Enterobacteriaceae* in this study, are the most strong and reliable indicators of faecal contamination, therefore, these samples that revealed their presence is an index of the degree of contamination, which may showcase a possible presence of hazard and disease-causing microorganisms (APHA, 1992; Pelczar *et al.,* 1996; Jay, 1997). These coliforms make up an estimated 10% of the intestinal microorganisms of human and other animals. The importance of faecal coliforms is that if these specific bacteria are present, then other harmful microorganisms may also be present, such as *Pseudomonas sp* and *S. aureus* (Forest 2004; Hester 2004).

Other potential pathogenic microorganisms isolated from the coded samples in this study included: *Pseudomomas aeuroginosa*, *Salmonella sp*, *Enterobacter aerogenes*, and *Staphylococcus aureus*. *Staphylococcus aureus* has been associated with a number of adverse challenges especially to immune-compromised persons. It can produce proteins that damage tissues and disable the immune system and may also release exotoxins which cause gastroenteritis (Lowy, 1998).

Some results obtained during this study showed that the mean total aerobic bacterial counts ranged from 20 to 6.7 x10^3 CFU/mL (Table 1) while the mean total mold count ranged from 10 to 30 CFU/mL and varied bacteria isolated included *Bacillus sp*, *Micrococcus sp*, *Coliforms, Pseudomonas aeruginosa, Salmonella sp* while the fungal isolated were *Zygomycetes* groups which included *Mucor, Rhizopus and Absidia sp*. The detection of *E. coli, Salmonella sp* and *Shigella sp* in the liquid herbal preparations render them unacceptable based on WHO specification (WHO, 2007). The presence of these bacteria including *S. aureus* and other coliforms were also recorded in previous related studies (Danladi *et al.,* 2009; Noor *et al.,* 2013; Oluwatoyin and Adelayo, 2016; Archibong *et al.,* 2017). These organisms have been reported as contaminants of causing serious health hazards (Erich *et al.*, 2001; Okunlola *et al.,* 2007). However, no *Salmonella sp* and *Shigella sp* were recorded in Noor *et al.* 2013 thus, conformed with the acceptable standard. The absence of salmonellae, E. coli and Gram-negative bacterial species have been used as indicators of microbiological quality. Gram negative bacteria such as *E. coli, Salmonella sp* and *Shigella sp* should be absent per gram/ml of the herbal medicine.

In this present study, it was revealed that nine coded samples (75%) had bacteria contamination with varied counts, while three samples (25%) were free from bacterial contamination. Similarly, the fungal contaminations were recorded against eight samples (67%), while four of the samples analyzed (37%) were free from the any fungal contamination. There were seven (76%) liquid herbal preparations with the mean total aerobic bacterial count within the acceptable limit of count based on United States Pharmacopeia (USP 30) and WHO, (2015) standards (10^2 CFU/mL)) for herbal medicine, while five of the samples (34%) had higher mean counts of 200 to 6700 CFU/mL. The findings of this study were not comparable to a similar study carried out by Archibong *et al.* (2017) at Awka in Anambra State who reported mean count 1.0 x 10^3-2.1 x 10^6 CFU/mL for total aerobic counts and total coliform count of 1 x10^3 -7.8 x10^4 CFU/mL. However, Abbas *et al.* (2009) in a similar study at Kaduna metropolis also reported mean count of total aerobic count greater than 5 x 10^5 CFU/mL. There were three (32%) out of the seven samples with high total aerobic count that had fecal contamination with *Escherichia coli* (8%), *Pseudomonas aeruginosa* (4%), *Salmonella sp* (8%) and *Coliforms* (4%). The presence of these pathogens in herbal preparations render it not fit for human consumption. This is comparable to the studies carried out by Abbas *et al.* (2009) on herbal medicines contamination that the group isolated *Salmonella* and *Shigella sp* (19.33%), *Escherichia coli* (58.8%), *Staphylococcus aureus* (68.9%). This is also comparable to study done by Archibong *et al.* (2017) in which they reported the presence of contaminants in liquid herbal preparation and isolated *Escherichia coli* (21.6%), *Enterobacter asburiae* (25%) and *Staphylococcus sp* (16.6%) in Awka in Anambra State. Onyemeluwe *et al.* (2019) also reported a similar finding to this present study in which *Staphylococcus aureus* was found (7.3%), *Escherichia coli* (18.2%) and *Pseudomonas aeruginosa* (20%) in their studies on microbial contamination of herbal medicines in Enugu metropolis.

In this study, the major heterophilic bacteria isolated was *Bacillus sp* (36%) in most of the liquid herbal preparations which are contaminated from the raw materials during production (Forest, 2004). This finding in this study was in agreement to that reported by Onyemelukwe *et al.* (2019) at Enugu metropolis in which (38%) of *Bacillus sp* was isolated.

The high levels of microbial contamination (6.7x 10^3 CFU/mL) observed in this study may be attributed to the methods of their preparation and lack of implementation and stringent regulation of herbal preparations in developing countries like Nigeria (Ekor, 2014). The producer could introduce microorganisms to the products during handling of the raw materials and packaging of products. This is in agreement with study reported by some scientists from Kaduna-Nigeria, Nairobi - Kenya and Dhaka – Bangladesh (Okunlola *et al.*, 2007; Khanom *et al.*, 2013; Onyambu *et al.*, 2013) of high contamination rates of traditional herbal preparation products. Thus, Good Manufacturing Practices (GMP) are necessary throughout the process of harvesting, drying, storage, handling and preparation of the herbal preparation products (Abbas *et al.*, 2009).

There were fungi identified in the herbal preparation which accounted for forty per cent (*Mucor* sp), thirty-seven per cent (*Rhizopus*) and twenty-three per cent (*Absidia* sp). The twelve herbal samples were free from pathogenic fungi. Onyemelukwe and his colleagues in 2019 also reported a similar finding of the presence of *Rhizopus sp* (17.8%) and *Mucor sp* (5.3%). The presence of these molds like fungi in this study may be of additional concern, because they are associated with food poisoning and may be likely responsible for infections particularly in immunocompromised individuals (Lin, 2001; Bateman, 2002).

The unacceptably high contamination of liquid herbal preparation in this study were shown to may be because low level of education, lack of formal training, poor handling and packaging as well as unboiling solvent used to mix liquid herbal preparation. The unhygienic practices in the processing of herbal preparation can potentially contaminate these products were also documented in studies carried out in Dar es salaam, Tanzania (Temu-Justin *et al.,* 2011) and South Africa (Govender *et al.,* 2006). As a matter of reality, there is a critical need to have specific policies and regulations addressing liquid herbal preparation safety which are specifically focused on prevention of microbial contamination through direct involvement of manufacturers or vendors of liquid herbal preparations in parts of Abuja, Nigeria.

CONCLUSION

This study showed that liquid herbal preparations marketed in parts of Abuja metropolis, Nigeria are highly contaminated with pathogenic microorganisms. The high bacteriological contamination of the herbal preparation might increase health hazard to consumers rather than curing infections. The merits associated with using herbal preparations to treat infections or diseases cannot be over emphasized and should be complemented with sound bacteriological quality in order to ensure safety. Therefore, these preparations require regular monitoring even after they might have been satisfied by regulators.

RECOMMENDATIONS

It is recommended that sensitization and awareness on microbial contamination and their health related impact on our communities to avoid transmission of communicable diseases in the population and consequently need to extend government's regulations on herbal preparation products in order to ensure that their processing, preparation or manufacturing comply with Good Manufacturing Practices (GLP) and thus lessen risks to consumers.

All control points including the source of the herbal substance, the manufacturing processes and any decontamination procedure used, bacteriological purity of excipients, the protective capacity of the packaging material chosen and preventive measures are to be put in place rather than interventions for decreasing the contamination.

The microbiological quality and safety of herbal preparations be upgraded and sustained by collective effort from the producers, healthcare workers, quality assurance officers, quality control officers and regulatory agencies.

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