Microbial quality and public health risk of selected herbal remedies sold in open markets in Owerri metropolis, South Eastern, Nigeria.

Abstract

Herbal medicines are presently widely used in developed and developing countries for health care due to their affordability, accessibility and availability.

Objective: The microbial quality of selected herbal remedies sold in open markets in Owerri, South Eastern, Nigeria was studied.

Methods: Standard laboratory protocols for microbiological studies and biochemical tests were employed.

Results: The presence ofbacterial species such as *Bacillus*, *Corynebacterium*, *Micrococcus*, *Enterococcus* and *Staphylococcus spp*were recorded. Fungal isolates included *Mucor, Saccharomyces*and *Penicilliumspp*. Bacterial species isolated were all gram positive andbacterial and fungal counts ranged from 1.0 x 10^6 to 7.8 x 10^7cfu/mland 3.0x10^3 to 1.3x10^8cfu/mlrespectively.

Conclusion: The presence of these microorganisms in herbal medicines do not only make them hazardous from the infectious stand point, but might also change the physical, chemical and natural properties of the herbal medicine by altering the contents of active ingredients or convert them to toxic products. The production and consumption of herbal remedies should be properly supervised and monitored to ensure that only good quality products get to the consumers**.**

Keywords:microbial quality, herbal remedies, bacterial counts, fungal counts, hazardous, active ingredients.

1. INTRODUCTION

Use of herbs for medical purposes is the earliest healthcare system known to humanity and has been used in all cultures throughout history. Different herbs have been used since ages to treat various diseases and use of herbs for traditional medicine (TM) has grown and evolved over centuries [1],[2],[3].[4] and [5]in their report stated that about 70 - 85% of the world's population, mainly in developing countries, today still depends on herbs for their primary health care.

Recently in Nigeria, there appears to be an overwhelming increase in the usage of herbal medicinal products for the treatment and/or prevention of diseases. This may not be unconnected to increased public awareness embarked upon by producers and marketers of herbal medicinal products (HMPs), who have probably taken advantage of the relatively high cost of the conventional pharmaceutical products and difficulty in accessing conventional medical services particularly in the rural areas. Most herbal medicine producers and dealers sell their products in local markets, along busy highways, in buses and motor parks where products are displayed openly and the consumers usually consider and believe that the herbal products are safe. However, with this increased usage, the safety, efficacy and quality of these herbal medicines have become an important concern for health authorities and health professionals[6,7]

Medicinal herbs have been reported to be contaminated with microorganisms indigenous to the soil and plants where they are grown. Also, poor conditions during harvesting and post-harvest handling of the herbs and herbal products are predisposing factors to contaminations [8.9]. Studies have reported contamination of herbal products with *Bacillus spp., Enterobacteriaceae, Salmonella spp., Staphylococcus aureus, Penicilliumspp and Aspergillus*spp among others [10,11]

In Owerri, South Eastern Nigeria, there is substantial consumption of herbal medicines due probable to cultural, social, and economic factors as well as the availability of a vast biodiversity of herbs. However, data available is not sufficient enough to address the microbial quality of herbal remedies available to consumers. The present study evaluated the microbial quality of selected herbal remedies sold in open markets in Owerri metropolis, South Eastern, Nigeria

2. MATERIALS AND METHOD

2.1 Study Area and Sample Collection

Thirty (30) samples of each herbal remedy used for this study wererandomly obtained from open markets within Owerri metropolis (Owerri North, Owerri Westand Owerri Municipal).The samples were labelled with their therapeutic claim as presented in Table 1

Table 1: Coded herbal remedies and their therapeutic indications obtained from sale outlets in Owerri

Product code	Therapeutic claim
GL	Malaria

AA	Typhoid
SM	Candidasis
BM	Sex enhancement

2.2 Preparation of media

Nutrient Agar (NA), MacConkey Agar (MA) and Potato Dextrose Agar (PDA) were prepared according to manufacturer's specification (Oxoid. England). NA was used for the isolation of heterotrophic bacteria, MA for coliforms and PDA for heterotrophic fungi.

2.3 Preparation of samples and inoculation

The different types of samples were bulked to obtain a uniform sample for each type of herbal remedy. Each bulked sample of the herbal remedies (GL, AA, SM and BM) was shaken vigorously and 1 ml was aseptically transferred into a test tube containing 9mls of sterile distilled water, followed with a 10-fold serial dilution of each herbal product. From the aliquots, 0.1 ml was transferred into the pre-sterilized and surface dried agar (NA, MA and PDA supplemented with 25ug/ml of chloramphenicol) respectively. The Inoculum was spread evenly to ensure uniform and countable colonies. Plates containing NA, MA,SSA and SSA were incubated at 37^{o}C for 24-48 hours while PDA plates were incubated 28 ± 2 ^{o}C for 72 hrs.

2.4 Determination of Microbial Population

The discrete colonies on the agar plates NA and PDA were selected and counted. Colony counts obtained on the media were expressed as colony forming units per milliliter(cfu/ml) to obtain the total heterotrophic population.

2.5 Characterization and Identification of microbial isolates

Microbial isolates were further characterized based on cultural (colonial), microscopic and biochemical tests with reference to standard manuals.

From the NA and MA plates, colonies were randomly picked and repeatedly sub-cultured on NA for purification. Purified bacterial isolates were stored in slants for further studies. The purified bacterial isolates were characterized by morphology, Gram's reaction and biochemical test using the protocol in Bergey's manual of determinative bacteriology [12,13]. Fungal isolates were

examined macroscopically and microscopically using the wet mount technique and their identification was performed according to the procedure described by[14] and [15]

3.RESULTS

Results revealed that the herbal samples analyzed were contaminated with both bacterial and fungal species.Bacterial species isolated in all the samples included *Bacillus*sp, Corynebacteriumsp, *Micrococcus*sp, *Enterococcus* and *Staphylococcus* sp. on both NA and MA. Fungal isolates included *Mucor, Saccharomyces*and *Penicillium* spp. *Bacillus, Saccharomyces* and *Penicillium*spp were present in all the samples except GL. The highest bacterial count was obtained in sample SM (1.2×10^8 cfu/mL) while the lowest value of 1.0×10^6cfu/mL was obtained in sample GL (Table 2). Similarly, sample BM had the highest fungal count (1.3×10^8 cfu/ml)(Table 4)

Table 2. Total heterotrophic counts of samplesand probablebacterial isolates

Sample code	Total counts (cfu/ml)	Most probable identity
GL	1.0×10^6	*Enterococcus*sp
		Staphylococcus sp
AA	4.0×10^6	*Bacillus* sp
		*Corynebacterium*sp
SM	1.2×10^8	*Micrococcus* sp
		*Enterococcus*sp
		Bacillus sp
		Staphylococcus sp

	BM	7.8 x 10^7	*Bacillus* sp *Corynebacterium*sp *Enterococcus* sp *Micrococcus* sp

Table 3: Occurrence of the bacterial isolates

Bacterial isolates	% Occurrence
*Enterococcu*s sp	25
Bacillus sp	25
*Corynebacterium*sp	16.66
Micrococcus sp	16,66
Staphylococcus sp	16.66

Table 4: Fungal count of samples and colonial characteristics of fungal isolates on PDA

Sample code	Colonial Characteristics	Microscopic characteristics	Fungal count(cfu/ml)	Most probable identity
GL	White cotton wool like hyphae	Hyphae non septate. Spores enclosed in a sporangium	3.0 x 10^3	*Mucor*sp
AA	Large serrated flat cream colonies	Large Gram positive spherical budding cells	1.7 x 10^4	*Saccharomyces*sp
	Green spores with	Septatehyphae. Conidia		*Penicillium*sp

	lemon green reverse	arranged like mop head.		
SM	Large serrated flat cream colonies	Large Gram positive spherical budding cells	5.0×10^7	*Saccharomyces sp*
	Small circular moist and shiny cream colony	Septate hyphae. Conidia arranged like mop head.		*Penicilliumsp*
BM	Large serrated flat cream colonies	Gram positive oval and ellipsoidal budding cells	1.3×10^8	*Saccharomyces sp*
	Small circular moist and shiny cream colony	Septate hyphae. Conidia arranged like mop head		*Penicilliumsp*

Table 5: Occurrence of fungal isolates

fungal isolates	% Occurrence
*Mucor*sp	14.28
Sacchoromycessp	42.85
*Penicillium*sp	42.85

Table.6: Occurrence of bacterial and fungal species in the different samples

Sample	GL	AA	SM	BM
Bacillus	-	+	+	+
Enterococcus	+	-	+	+
Micrococcus	-	-	+	+
Staphylococcus	+	-	+	-
Corynebacterium	-	+	-	+

Mucor	+	-	-	-
Penicillium	-	+	+	+
Saccharomyces	-	+	+	+

- = absent + = present

4. DISCUSSION

In this study, bacterial and fungal species were isolated in the samples. Bacterial species isolated included *Bacillus*sp, *Corynebacterium*sp, *Micrococcus*sp, *Enterococcus* and *Staphylococcus* sp. while fungal isolates recovered from the samples were *Mucor , Saccharomyces* and *Penicillium* species. The microbiological background of herbal medicines depends on several environmental factors such as temperature, humidity and rainfall during pre-harvesting and post-harvesting periods, handling practices and the storage conditions of crude and processed medicinal-plant materials; and by their origin, herbal remedies are prone to contamination by microorganisms from soil, air and water [16]. In order,therefore, to improve on the safety of the products, observation of basic hygiene during preparation, standardization of some physical characteristic such as moisture content, pH and microbiological contamination levels are desirable [17,18,19] Microbial contamination of non-sterile herbal drugs can inactivate or reduce the therapeutic activity of the drugs and may have potential adverse effect to consumers [20].This can be viewed as a serious public health issue that may result to mortality

The totalheterotrophic counts obtained for bacteria ranged from 1.0×10^6 - 1.2×10^8cfu/ml while fungal counts ranged from 3.0×10^3 - 1.3×10^8cfu/ml. According to [16] the contamination acceptable limits are 10^5cfu/g for bacteria and 10^3cfu/g for yeast and molds. The total heterotrophic count of herbal preparations in this study were more than 10^5cfu/mland 10^3cfu/mlfor bacteria and fungi respectively. Therefore, the microbial load of the herbal preparations sold in different parts of Owerri, Nigeria which are not acceptable based on the acceptable microbial limit[1] These findings revealed that the raw herbal preparation had some levels of microbial contaminants; may be of natural origin which may be related to the plant or growing environmental conditions of the herbal plants. This is comparable to the findings of [9] who had reported in an earlier study on the microbiological quality of some pharmaceutical raw materials. As herbal medicinal products are complex mixtures which originate from biological

sources, great efforts should be made to guarantee acceptable quality by ensuring good manufacturing practicesthat will largely determine the microbiological quality of the final products [10].Results from previous studies had confirmed the presence of potential contaminants in herbal preparations [21,16,22,11] thus, manufacturers should ensure the lowest possible level of microorganisms in the raw material, finished product and the packaging materials to maintain appropriate quality, safety and efficacy of the natural products [16].

The presence of some potential pathogenic bacteria detected in the samplesis a major source concern as they constitute health risk. This study detected the presence of *S. aureus. Staphylococcusaureus* has been associated with a number of adverse challenges especially in immune-compromised persons. It can produce proteins that damage tissues and disable the immune system and may also release exotoxins which cause gastroenteritis [23] (Lowy,1998). Other potential pathogenic microorganisms isolated from the samples used in this study included: *Bacillus, Enterococcus* and *Corynebacterium* species.

Staphylococcus aureus and other coliforms have also been recorded in previous related studies [24,20,25]. These organisms have been reported as contaminants capable of causing serious health hazards [26,16]. This renders the products unfit for human consumption. It can be concluded that the herbal medicines that contained these microorganisms were contaminated directly or indirectly by human or animal feces and are therefore unsuitable for consumption

Enterococci have gained significance as the cause of nosocomial infections; and they occur as food contaminants. The two species *Enterococcus faecalis* and *Enterococcus faecium*, have gained significance in recent decades as leading opportunistic pathogens causing nosocomial infections [27,28] and have been associated with various infections, including urinary tract infections, bacteremia, meningitis, wound infections and neonatal infections. Apart from nosocomial infections, E. *faecalis*, has been found in common dental diseases especially periodontitis and dental caries [29,30]where it can also form biofilms [31,32,33,34].

The presence of molds in this study is also of additional concern, because they are associated with food poisoning and may be likely responsible for certain infections particularly in immuno-

compromised individuals [35,36]. They are common natural contaminants of medicinal herbs and as confirmed by the current study *Penicillium* sp., *Mucor* sp. and *Saccharomyces*sp were recovered from the samples. [37] had reported that fungal contamination adversely affects the chemical composition of the raw materials, thereby decreasing the medicinal potency of herbal drugs and possibly synthesize toxically metabolites called mycotoxins.These species may pose a risk when present in products used orally. Many studies have also demonstrated the presence of mycotoxins in preparations derived from medicinal plants and the severity of mycotoxins depends on the toxicity, degree of exposure, age and nutritional status of the individual and the possible synergistic effects of other chemical agents to which they are exposed to.

As reported by [38] product quality is obviously the major criteria that could affect both the efficacy of product and the safety of patients or consumers The presence of thesemicroorganisms in the herbal remedies do not only make them hazardous from the infectious stand point, but may also change the natural properties of the herbal medicine by altering the composition of the active ingredientsor even convert them to toxic products.

The unacceptable contamination of the liquid herbal preparations studiedmay also have resulted from low level of education of the producers, lack of formal training, poor handling and packaging as well as solvent used to mix liquid herbal preparation. These reasonshave also been documented in studies carried out in Dar es salaam, Tanzania [39] and South Africa [40]

5. CONCLUSION

The presence of pathogenic microorganisms in the studied herbal remedy samples make them hazardous and unsafe for consumption. Product quality is obviously a major concern that can affect its acceptability by patients or consumers.Considering the worldwide increase in the use of herbal products as alternative medicines and the risk associated with acquiring natural products contaminated with microbes suspected to be pathogenic, it is necessary to set appropriate standards for herbal medicinal preparations to reduce the risks of infection to consumers in Nigeria. Manufacturers of herbal products should be compelled to adhere to strict quality control measure in order to ascertain the quality, safety and efficacy of their finished products.Good

manufacturing practices in establishments that handle herbal medicines are essential. Beyond compliance, following current national and international legislations, it is also essential to monitor establishments that market herbal medicines by checking that they have a license from appropriate health authority for this trade and also that the products are registered and authorized for consumption.

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