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Microbial Assessment of Herbal Cleansers (Bitters) Sold in Ota, Ogun State, Nigeria

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Abstract: The use of herbal medicines is on an increasing trend globally. Herbal medicines may be beneficial but are not completely harmless due to deficient quality control in certain cases. This study evaluated the microbial content of some herbal cleansers (bitters). Eight herbal cleansers obtained from pharmacies in Ota were evaluated for their microbial content. Bacterial and fungal isolates identified from the samples include *Bacillus subtilis, Staphylococcus aureus, Staphylococcus epidermidis, Lactobacillus spp., Proteus spp., Pseudomonas spp., Shigella spp., Aspergillus niger, Rhizopus spp., Geotrichum spp., Fusarium spp., Alternaria spp., Penicillium spp., Mucor spp., Diplosporium spp. and Trichothecium spp*. The mean total aerobic plate count values of the herbal cleansers were in the range of $1.0x10^4$ and $3.0x10^5$ cfu/ml. The results of this study revealed the presence of certain pathogenic organisms in these formulations and this emphasizes the importance of having stringent quality control measures in the manufacture of herbal preparations.

Keywords: Herbal cleansers; microbial assessment; quality control

Introduction

The use of herbal medicines and phytonutrients or nutraceuticals continues to expand rapidly across the world with many people now resorting to these products for treatment of various health challenges in different healthcare settings [1]. It is estimated that up to four billion people (representing 80% of the world's population) living in the developing world rely on herbal medicinal products as a primary source of healthcare and traditional medical practice [2]. There

are varieties of herbal preparations in Nigeria today. However, some may not have been subjected to aseptic conditions during preparation, storage and transportation as required for pharmaceutical preparations [3]. Herbal preparations assumed to be safe could be contaminated with microbial and foreign materials such as heavy metals, pesticide residues or even aflatoxins due to the unhygienic way some of them are produced. The presence of any of the possible contaminants is a potential health risk to a vast population that depends on herbal medicine for their health care need [4]. The incidence of microflora in non-sterile preparations generally is influenced by the nature of the ingredients (whether natural or synthetic), the quality of the vehicle and the care and attitude of personnel involved in their handling [5, 6]. Several cases of infections associated with the use of contaminated non-sterile pharmaceuticals have been reported [7]. It is also common knowledge that the safety of most herbal products is further compromised by lack of suitable quality controls, inadequate labelling, and the absence of appropriate patient information [8]. Common hazardous microorganisms in the pharmaceutical products and premises may include *Escherichia coli*, *Salmonella* spp., *Pseudomonas aeruginosa*, *Staphylococcus aureus*, *Burkholderia* spp., *Alcaligenes* spp., *Flavobacterium* spp., *Chromobacter* spp., *Serratia* spp., *Bacillus subtilis*, *Bacillus megaterium*, *Enterobacter aerogenes* and *Enterobacter cloacae*, *Proteus* spp., *Streptococcus faecalis*, *Clostridium* spp. and the opportunistic bacterial pathogens which are capable of multiplying within both bulk and finished products [9]. Despite the widespread use of herbal medicines globally and their reported benefits, they are not completely harmless. Therefore the inappropriate and non-regulated use of several herbal medicines may put the health of their users at risk of toxicity and infection [10]. This study evaluated the microbial quality of some herbal cleansers sold in Ota, Ogun State, Nigeria.

Materials and Methods

Eight different herbal bitters were purchased from pharmacies in Ota, Ogun state. The sealed bottles of herbal preparations were cleaned with 70% ethanol before opening to prevent contamination. Samples selected were analyzed between January and March 2014. Serial dilutions of each of the herbal bitters (10^{-1}-10^{-5}) were made and 0.1ml of the dilutions was plated in replicates on nutrient agar (Nutrient agar, MacConkey agar and Potato dextrose agar plates). Nutrient agar and MacConkey agar plates were incubated aerobically at 37^{o}C for 18 h for bacterial isolation and viable count estimation. Potato dextrose agar plates were incubated plates 25°C for 5-7 days. Bacterial colonies were counted by using a Colony Counter. Fungal growths were examined both macroscopically and microscopically.

Results and Discussion

The results obtained from the microbial assessment of the herbal cleansers revealed the presence of bacterial isolates such as *Staphylococcus aureus, Bacillus subtilis, Lactobacillus spp., Staphylococcus epidermidis Proteus spp. Pseudomonas aeruginosa and Shigella spp.* The fungal isolates identified were *Aspergillus spp., Fusarium spp., Mucor spp., Geotrichum spp., Rhizopus spp., Alternaria spp., Trichothecium spp., Diplosporium spp.*

and *Penicillium spp.* (Figure 1). *Bacillus subtilis* was the most common isolated bacterium (Figure 1) and *Aspergillus niger* had the highest occurrence among fungal isolates (Figure 2). The mean total aerobic plate count values of the herbal cleansers were in the range of $1.0x10^4$ and $3.0x10^5$ Cfu/ml (Figure 3).

This study has shown the presence of pathogenic and non-pathogenic organisms in the herbal cleansers. Contamination by microorganisms is influenced by the environment, improper handling and storage of medicinal plants [11-12]. The presence of microbial contaminants in non-sterile pharmaceutical products can reduce or even inactivate the therapeutic activity of the products and has the potential to adversely affect patients taking the medicines [13]. Herbal medicinal products usually contain bacteria and moulds from soil and atmosphere. The limits of microbial contamination are: total aerobic bacteria 10^5cfu/g, yeasts and moulds 10^3cfu/g, enterobacteria and other Gram negative organisms 10^3cfu/g and *E. coli* and *Salmonella* should be absent [14].

The results of this study indicated the presence of *Staphylococcus aureus, Bacillus subtilis, Lactobacillus spp., Staphylococcus epidermidis, Proteus spp. Pseudomonas aeruginosa and Shigella spp.* The presence of *Staphylococcus spp, E. coli, Bacillus spp., Streptococcus spp, Pseudomonas, Proteus, Salmonella, Yersinia* and *Corynebacterium diphtheria* in some herbal products has been reported [15]. The most common bacterial contaminant in the present study was *Bacillus subtilis*. *Bacillus* and allied species are potential contaminants of non-sterile pharmaceuticals. It is probable that the ubiquity of endospore-forming species in the environment (soil, dust, air and water) and the resistance of their endospores to physical and chemical treatments are the main reasons for their dominance in pharmaceuticals [16]. The presence of *B. subtilis* may indicate failure to control the moisture levels of medicinal plants during transportation and storage, as well as failure to control the temperatures of liquid forms and finished herbal products. The presence of *Staphylococcus spp.* is indicative of possible human contamination as they are normal flora with the ability to produce an enterotoxin which can cause serious gastroenteritis [17]. However the presence of *Staphylococcus aureus* in oral preparations may not necessarily constitute a potential hazard to users since not all strains of *S. aureus* produce the enterotoxin that causes poisoning and the organism would have to grow to a density of several million cells/g for its toxin to constitute a problem [5]. The presence of Gram negative bacterial contaminants such as *Proteus spp, P. aeruginosa and Shigella spp* is however noteworthy. The presence of *Proteus spp* and *Shigella spp* may indicate faecal contamination of the formulations. *Pseudomonas aeruginosa* is primarily a soil bacterium and is the cause of various infections of burns as well as the urinary and respiratory tracts [13].

The most common fungal isolate in this study was *Aspergillus spp*. Fungi are spore formers, thus ubiquitous and can be found in air, packaging materials and thereby contaminate these herbal cleansers [18]. The presence of moulds such as *Aspergillus flavus* is harmful because of the production of metabolites that may be toxic to consumers and also cause the biodegradation of the different components of the formulation [5].

Microbial infections may not only occur due to physical occurrence of pathogens, but also due to their metabolites/toxins which are very harmful even if they exist in very small quantities. The contamination of the herbal bitters is of public health concern. It is important that manufacturers adhere to standard regulations in all stages of manufacturing, packaging and distribution of the products.

Table 1: Microorganisms isolated from the herbal cleansers

Samples code	Identity of bacteria isolated	Identity of fungi isolated
A	*a)Staphylococcus aureus*	*Aspergillus spp.*
	b)Bacillus subtilis	*Mucor spp.*
		Penicillium spp.
B	*a)Lactobacillus spp.*	*Aspergillus spp.*
	b)Staphylococcus aureus	*Mucor spp.*
		Fusarium spp.
C	*a)Staphylococcus*	*Aspergillus spp.*
	b)Bacillus subtilis	*Geotrichum spp.*
	a)Staphylococcus epidermidis	
D	*a)Bacillus subtilis*	*Mucor spp.*
	b)Proteus spp.	*Alternaria spp.*
E	*a)Bacillus subtilis*	*Apergillus spp.*
	b)Pseudomonas spp.	*Mucor spp.*
	c)Shigella spp.	*Fusarium spp.*
F	*a)Bacillus spp.*	*Aspergillus spp.*
	b)Staphylococcus aureus	*Diplosporium spp.*
		Rhizopus spp.
G	*a)Staphylococcus epidermidis*	*Aspergillus spp.*
H	*b)Lactobacillus spp.*	*Rhizopus spp.*
		Mucor spp.

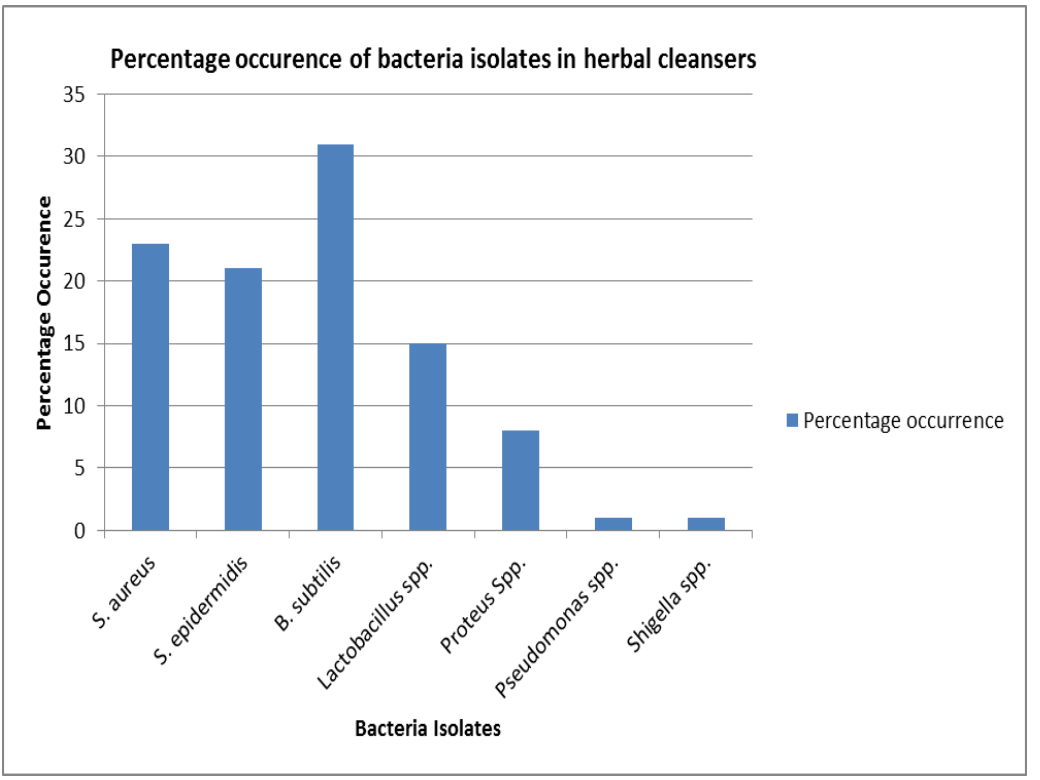

Figure 1: Percentage occurrence of bacteria isolated from herbal cleansers

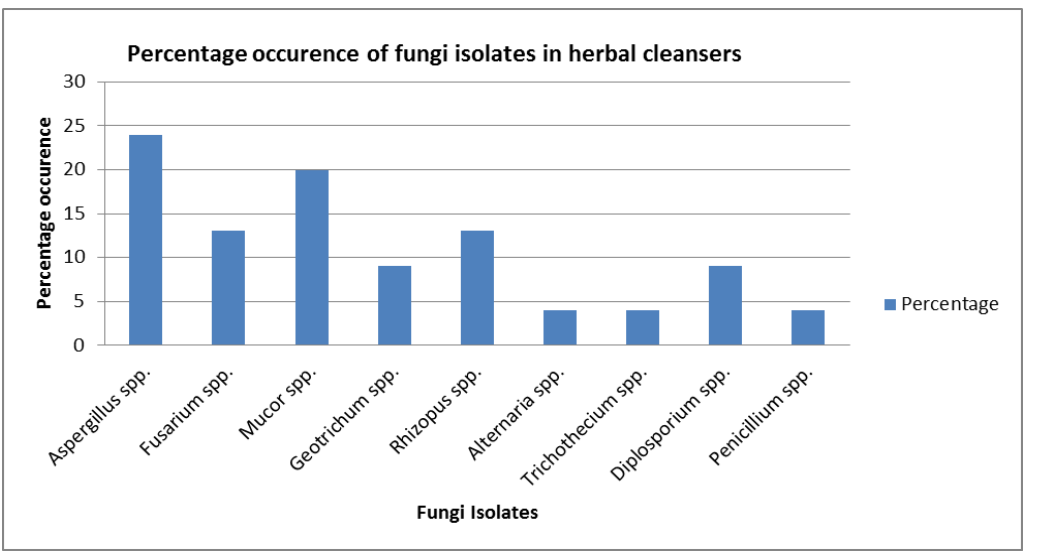

Figure 2: Percentage occurrence of fungi isolated from herbal cleanser

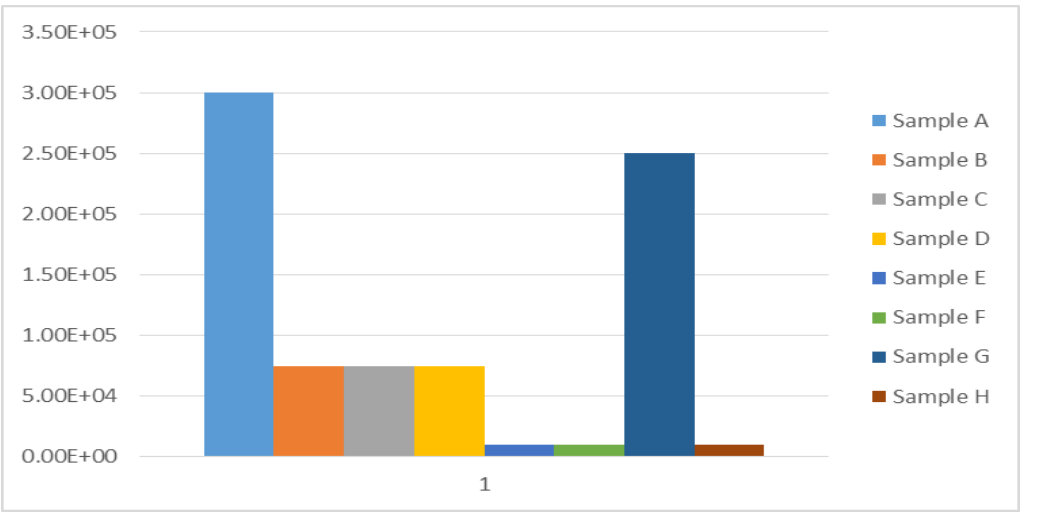

Figure 3: Mean total aerobic plate count of herbal cleansers

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