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Microbial quality of five selected commercially herbal formulations sold in Anambra State, Nigeria

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Abstract

The use of Herbal formulation has become a mainstay in developing countries in the last few decades with Nigeria embracing these practices due to the high cost and accessibility of modern medicine.

This study aimed to assess five (5) commercially available herbal formulations sold in Anambra State for their microbial quality using the pour plate method. The isolates were identified based on their morphological, macroscopic, and biochemical characteristics.

A total of five (5) herbal formulations were screened and various degrees of microorganisms were isolated ranging from *Staphylococcus aureus*, *Staphylococcus epidermidis*, and *Proteus* sp. For bacteria. *Aspergillus niger*, *A. flavus*, *Mucor* sp, *Rhizopus* sp, *Candida* sp, and *Trichophyton rubrum* for fungi isolates. The total bacteria count ranged from 1.6×10^3 to 5.0×10^3 CFU/mL and the total fungal count ranging 1.6×10^3 to 2.2×10^4 CFU/mL respectively.

However, it can be confirmed the microbial load is within the World Health Organization safety limit for good manufacturing practices of herbal formulation.

Keywords: World Health Organization; Herbal formulations; Anambra State; Biochemical; Microbial

1. Introduction

The use of herbal medicine has gained a lot of recognition in the developing world including Nigeria partly due to the high cost of orthodox drugs, antimicrobial resistance, and also claims made by the manufacturers that these formulations can be used in the treatment of multiple ailments [1,2,3]. The populace is now turning to these formulations for the treatment of various health ailments in various health settings [3]. Several Asian and African countries, including Nigeria, have begun to promote herbal medicine as an essential component of public health programs [4].

According to the World Health Organization (WHO), four billion people (about 80% of the world population) use herbal formulations for treatment in health care [5,6].

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To meet its millennium goal of providing quality and widely accepted health care to the world's population, the WHO has encouraged countries to use traditional plant-based medicines rationally and has developed safety guidelines for profiling herbal formulations [7].

Herbal formulations/remedies have become increasingly popular in Nigeria over the last two to three decades [8]. With the growing use of herbal medicines and the global expansion of the herbal medicines market, safety has become a major concern for both health officials and the general public [9].

The standard, degree of excellence, and safety of herbal formulations are of major concern because quality is the foundation of reproducible efficacy and safety of herbal drugs, and to ensure the standard of research on herbal medicines, the quality of plant materials or preparations is critical and must therefore be investigated [10].

The World Health Organization (WHO) has advocated for proper identification, prudent exploitation, scientific development, and appropriate utilization of herbal medicines that provide safe and effective remedies in medical care, in recognition of their inherent value to primary health care [11].

Despite the proliferation of herbal products on the market in Nigeria, not much has been done in this field. Some herbal preparation producers in Nigeria lack the necessary expertise to perform quality control on their products. As a result, there is inconsistency in the quality of herbal preparation in the country.

Because of the rapid growth in the use of herbal medicines and the global expansion of the pharmaceutical market, safety has become a major concern for both health authorities and the general public in many countries. This is because many contaminants and residues that may harm consumers have been reported [12].

The quality assessment of herbal preparations is thus critical to validate their acceptance in the modern medical system. Thus, microbiological limit tests of herbal medicinal preparations are required to ensure that the product is risk-free.

Some formulations are not subjected to aseptic conditions during various stages of preparation, packaging, storage, and transportation, as required by NAFDAC (National Agency for Food and Drug Administration and Control) regulatory norms, which aim to achieve high-quality standards for food and drugs, including herbal preparations, in Nigeria.

Plants and plant materials also contain a large number of organisms, the majority of which are soil-borne. Aerobic sporulating bacteria predominate in this, with additional contamination and microbial growth occurring during harvesting, handling, and production [6]. Health professionals and herbalists should be aware of the microbiological safety of these preparations [13].

This study was conducted to assess the microbial quality of five herbal formulations sold in Anambra State, Nigeria if it meets the World Health Organization safety standard.

2. Material and methods

2.1. Collection of herbal samples

Five (5) different brands of liquid herbal formulations were purchased randomly from herbal shops and retail outlets in different parts of Anambra State. All commercial herbal preparation samples purchased were conveyed to the laboratory of the Department of Pharmaceutical Microbiology and Biotechnology, Madonna University, Elele Campus, River State for analysis.

2.2. Preparation of Culture Media

The following agars were used: Nutrient agar (Hi media, India), Nutrient broth (Hi media, India), MacConkey agar (Hi media, India), Eosin Methylene Blue (Hi media, India), Mannitol Salt agar (Hi media, India), Salmonella-Shigella agar, Sabouraud dextrose agar, and Cetrimide agar (Hi media, India). These culture media were made following the manufacturer's instructions.

2.3. Microbial analysis of herbal preparations

The samples were shaken vigorously after which one milliliter (1 mL) of each of the herbal formulations was dispensed into 9 mL of nutrient broth in a test tube. Serial dilutions were made (10^{-1} to 10^{-5}) and viability was assessed using the

pour plate method. The Sabouraud Dextrose Agar supplemented with Chloramphenicol is used to prevent bacterial contamination to determine fungal contamination, while the Nutrient Agar containing Nystatin prevents fungi contamination and yeast growth to determine bacterial contaminants. The methods of Gupta *et al*., (2012)[14] were used in the isolation and identification of microbiological contaminants from herbal medicines, with some modifications. In triplicates, 1 mL of dilution (10^{-3}) of each herbal formulation was added to a petri dish and 15 mL of molten agar (Nutrient agar, Mannitol salt agar, Eosin methylene bleu, MacConkey agar, Cetrimide agar, Salmonella-Shigella agar, and Sabouraud dextrose agar) dispensed into the same petri dish cooled to 45 ^{0}C, swirled and allowed to solidify.

For bacteriological examination, the plates were inverted and incubated at 37 ^{0}C for 24 hours, and for fungal analysis, they were inverted for 5 days under room temperature. The plates (Nutrient agar plate) were placed on a colony counter enumerated for the Total viable count. This experiment was performed in triplicates.

The formed microbial colonies were enumerated and calculated as colony-forming units/ml (CFU/ml) after incubation.

Total colony counts (Cfu/ml = $Number\ of\ colonies\ formed\ \times dilution\ factor \div volume\ plated$.

The discreet colonies were sub-cultured to obtain a pure culture. The pure cultures were tentatively identified based on microscopic, morphological, and biochemical tests using Bergey's Manual for Determinative Bacteriology [15,16,17].

2.4. Identification of bacteria Isolates

Pure isolates were presumptively identified based on cultural and Gram staining characteristics. Bacterial isolates were further identified using biochemical tests such as catalase, oxidase, coagulase, and indole production test [18,8].

3. Results

3.1. Microbial analysis

From the table shown below the Herbal formulations (Ruzu bitters, Yoyo bitters, African iba, Blood Purifier, and Deep root) showed a varying level of total viable bacteria count ranging from 1.6×10^3 ± to 5.0×10^3 CFU/mL (Total bacteria count) with Blood purifier having the highest number of contamination with a value of 5.0×10^3 CFU/mL, Ruzu bitters having the least 1.6×10^3 CFU/mL. The total viable fungi count ranges from 2.4×10^3 to 2.2×10^4 CFU/mL (Table 1). Based on the standard microbial analysis protocols employed in this investigation, the organisms isolated from the herbal formulations were *Staphylococcus aureus*, *Staphylococcus epidermidis*, *Proteus* sp, *Aspergillus flavus*, *Aspergillus niger*, *Rhizopus* sp, *Mucor* sp., *Trichophyton rubrum*, *Penicillium* sp and *Candida* sp (Table 2)

Table 1 Microbial viable count of the five Herbal formulations

Herbal production	Mean Bacteria count (CFU/ml)	Mean Fungi count (cfu/ml)
African iba	2.2×10^3	1.07×10^4
Deep root	4.4×10^4	2.2×10^4
Blood purifier	5.0×10^3	1.6×10^3
Yoyo bitters	4.0×10^3	9.0×10^3
Ruzu bitters	1.6×10^3	2.4×10^3

Key:Cfu: colony forming unit per ml

Table 2 Microbial content of the Herbal formulations

Herbal formulation	Bacteria	Fungi
African iba	*Staphylococcus aureus, Staphylococcus epidermidis, proteus sp*	*Rhizopus sp, Candida sp*
Deep root	*Staphylococcus aureus, Staphylococcus epidermidis, Proteus sp*	*Candida sp., Aspergillus flavus, Trichophyton rubrum.*
Blood purifier	*Staphylococcus aureus, proteus sp*	*Trichophyton rubrum, Aspergillus flavus*
Yoyo bitters	*Proteus sp., Staphylococcus aureus*	*Penicillium sp., Aspergillus niger, Mucor sp.*
Ruzu bitters	*Proteus sp., Staphylococcus aureus*	*Aspergillus niger, Mucor sp, Trichophyton rubrum*

Table 3 Biochemical and Gram staining Table

Isolated Bacteria	Gram stain	Shape	Indole test	Coagulase test	Catalase test	Oxidase test
Staphylococcus epidermidis	+	Cocci	-	-	+	-
Staphylococcus aureus	+	Cocci	-	+	+	-
Proteus spp	_	Rod	+	-	+	-

Keys: (-) Negative; (+) Positive.

4. Discussion

This evaluation found that five (5) herbal formulations were contaminated with various types of microorganisms. *Staphylococcus aureus*, *Staphylococcus epidermidis*, *Proteus* sp, *Aspergillus niger*, *Aspergillus flavus*, *Mucor* sp, *Rhizopus* sp, *Penicillium* sp, *Candida* sp, and *Trichophyton rubrum* are among them. *Staphylococcus aureus* and *Proteus* sp are the most common bacteria, and *Aspergillus* specie is the most common fungi. The majority of the samples were contaminated by more than one organism. It was observed that the organisms isolated from the herbal formulation met the World Health Organization's safety guidelines for evaluating the quality of herbal medicine [19]. Various plant parts have been reported to be a reservoir for a diverse range of bacteria [20].

The presence of *Staphylococcus aureus* in these herbal formulations may not be of public concern to consumers because not all strains of *Staphylococcus aureus* are thought to produce enterotoxins linked to poisoning, and it must grow beyond the safety limit for its toxins to be a major health concern [21,3].

The presence of *Aspergillus niger* and *Aspergillus flavus* is a major health concern because this fungus (*A. flavus*) is known to be spore formers and produces secondary metabolites that could be toxic to consumers and cause degradation of herbal formulation components [3]. The organisms isolated from these herbal formulations are similar to the work done by Ayepola et al (2017) on the Microbial Assessment of Herbal Cleansers (Bitters) sold in Ota, Ogun State, where organisms such as *Staphylococcus aureus*, *Proteus*, *Staphylococcus epidermidis*, Rhizopus sp, *Mucor* sp, *Penicillium* sp, and *Aspergillus niger* were isolated.

Although the microorganisms detected in this study are thought to be within the acceptable microbial safety limit [19], public awareness of good manufacturing practices in HM cannot be overstated to avoid the proliferation of these organisms, which can cause health concerns.

5. Conclusion

It is confirmed that the five (5) herbal formulations complied with the World Health Organization's safety limit (Good manufacturing guidelines and practices). The microorganisms were of acceptable pharmaceutical and microbial quality and were within the WHO limit.

Compliance with ethical standards

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Disclosure of conflict of interest

The authors declare no conflict of interest.

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