

## MICROBIAL ASSESSMENT OF SOME BRANDED HERBAL MEDICINE SOLD IN OWERRI, IMO STATE

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### ABSTRACT

This study aimed to assess the microbial quality of some branded herbal medicines available in Owerri, Imo State, focusing on bacterial and fungal contamination levels. A total of three herbal remedies: Goko Cleanser, Agnes Nwamma, and 7Keys to Power—were analyzed for Total Heterotrophic Bacterial Count (THBC), Total Coliform Count (TCF), Total Salmonella/Shigella Count (TSS), and Total Fungal Count (TFC). The results revealed varying levels of microbial contamination across the products. Goko Cleanser exhibited moderate bacterial contamination ( $1.5 \times 10^4$  CFU/ml), low coliform contamination ( $6 \times 10^3$  CFU/ml), and moderate fungal contamination ( $6 \times 10^3$  CFU/ml). Agnes Nwamma showed lower bacterial ( $7 \times 10^3$  CFU/ml) and coliform counts ( $1 \times 10^1$  CFU/ml), with very low fungal contamination ( $1 \times 10^1$  CFU/ml). 7 Keys to Power demonstrated the highest bacterial contamination ( $1.4 \times 10^4$  CFU/ml), moderate coliform count ( $6 \times 10^3$  CFU/ml), and significant fungal contamination ( $9 \times 10^3$  CFU/ml). The bacterial isolates identified included *Escherichia coli*, *Salmonella* spp., and *Staphylococcus aureus*, while fungi such as *Aspergillus* spp. and *Mucor* spp. were isolated. The presence of these microorganisms, particularly pathogenic bacteria and fungi, raises concerns about the safety and hygienic quality of these products. The study emphasizes the need for improved manufacturing practices, regular microbial testing, better storage, conditions, and consumer awareness to ensure the safety of herbal medicines.

**Keywords:** Herbal formulations; Imo State, Bacteria and fungi

### INTRODUCTION

Herbal formulations have been a cornerstone of medical treatments in many developing countries for decades, with estimates suggesting that about 80% of ailments are

treated using herbal medicine (Onyambu *et al.*, 2022). These remedies are often the first choice due to their affordability, availability, perceived minimal side effects, and the belief that they can cure a wide range of diseases (Ali *et al.*, 2005; Pallavi, 2022). In

Nigeria, as in many other developing nations, the use of herbal remedies has a long history and continues to be prevalent today. These remedies are widely available in shops and supermarkets and are commonly used for both prevention and treatment of various illnesses. However, the regulation of these products is often lax, leading to variations in their composition depending on the location (Ali *et al.*, 2005; Eisenberg *et al.*, 2021).

One of the main advantages of herbal remedies is their accessibility, along with their antioxidant properties, which have made them highly sought after (Eisenberg *et al.*, 2021). It is widely believed that these remedies are effective, safe, and free of side effects, which has contributed to their widespread use. Some claims suggest that herbal remedies are effective against serious conditions such as cancer, diabetes, asthma, end-stage kidney disease, drug-resistant malaria, and AIDS (Pallavi, 2022; Itharat *et al.*, 2021).

Despite the popularity of herbal remedies, there is growing concern about the purity and safety of these products, especially in Nigeria. The lack of stringent regulation and oversight means that these remedies are sometimes contaminated with microorganisms, which can alter their physical, chemical, or organoleptic properties, potentially reducing their therapeutic effectiveness (Roozbeh *et al.*, 2022; Oluwatoyin and Adebayo, 2022).

When herbal remedies are sold in the market without proper sterilization or supervision, they may pose significant health risks (WHO, 2021; Temu-Justin *et al.*, 2021).

Previous studies have highlighted the contamination of herbal remedies with various microorganisms, including bacteria such as *E. coli*, *Klebsiella*, *Pseudomonas*, *Proteus*, *Salmonella typhi*, *Shigella spp.*, and *Staphylococcus aureus* (Okunlola *et al.*, 2021; Abba *et al.*, 2020). Moulds like *Aspergillus flavus*, *A. parasiticus*, *Trichosporium spp.*, *Mucor spp.*, *Candida spp.*, and *Penicillium sp.* have also been detected in herbal products (Halt, 2021; Bugno *et al.*, 2006). Contamination levels exceeding permissible limits can pose serious health risks, particularly to elderly and immunocompromised individuals (Amaike and Keller, 2021). This underscores the need for more stringent policies and regulations to ensure the safety and effectiveness of herbal remedies.

In the context of Owerri, Imo State, this study aims to assess the microbial quality of branded herbal medicines available in the region. This assessment is crucial given the widespread use of these products and the potential risks associated with their contamination.

## MATERIALS AND METHOD

### COLLECTION OF SAMPLES

The research was carried out in Owerri Municipal, Imo State, Nigeria. Three herbal medicines, namely Goko Cleanser, Agnes Nwamma, and 7 Keys to Power, was randomly purchased from mobile promoters and shops and Douglas Road Owerri, Imo State.

### **MICROBIOLOGICAL ANALYSIS OF THE HERBAL REMEDIES**

Three-fold serial dilution was carried out by introducing 1 ml of each sample of herbal medicines, serving as the stock solution into a sterile test tube containing 9 ml of sterile distilled water using sterile syringes. The tube was mixed and labeled 1:10 to obtain dilution  $10^{-1}$ . One milliliter (1 ml) of dilution  $10^{-1}$  was then be introduced into another test tube containing 9 ml of distilled water to achieve dilution  $10^{-2}$ . Using separate 1 ml syringes, the samples was serially diluted up to the required dilution  $10^{-3}$  (Cheesbrough, 2011).

The bacterial count of the selected herbal medicines was determined by inoculating the herbal formulations onto sterile Nutrient Agar, Chocolate Agar, MacConkey Agar, Salmonella/Shigella Agar (SSA), and Sabouraud Dextrose Agar. The plates will be incubated at 37°C for 48 hours using the streak plate method. Fungi was isolated from the herbal remedies by inoculating them onto Sabouraud Dextrose Agar (SDA) plates in duplicates and incubating at 25°C for 5 days (Cheesbrough, 2011).

### **PURIFICATION (SUBCULTURE) OF BACTERIAL ISOLATES**

Colonies from the primary plates was aseptically picked with a sterile wire loop and streaked onto freshly prepared sterile Nutrient Agar plates using a streaking technique to ensure discrete colonies after incubation. Subculture plates will be incubated at 37°C for 24 to 48 hours. Discrete colonies from the subculture plates was then be streaked onto slants and further incubated for 24 hours at 37°C (Cheesbrough, 2011).

### **PURIFICATION OF FUNGAL ISOLATES**

Colonies from the primary plates was aseptically picked with a sterile inoculation needle and streaked onto freshly prepared sterile Sabouraud Dextrose Agar (SDA) plates. These plates were incubated for 5 to 7 days at 28-30°C. Discrete colonies was transferred onto slants and further incubated for another 5 days at 28-30°C. Pure colonies was stored in the refrigerator at 10-15°C until needed for further characterization and identification (Cheesbrough, 2011).

### **MORPHOLOGICAL AND BIOCHEMICAL IDENTIFICATION OF ISOLATES**

Characteristic colonies from appropriate plates were purified, Gram-staining and biochemically test such catalase test, oxidase

test, motility test, methyl red, citrate utilization, urease production, spore formation, and sugar fermentation will be performed to characterize bacterial isolates.(Cheesbrough, 2011).

### **MORPHOLOGICAL IDENTIFICATION OF FUNGAL ISOLATES**

Macroscopic examination was involve observing colony features such as color, shape, and size. Microscopic examination was include studying fungal morphology using a compound microscope. A small portion of the mycelium was mounted on a glass slide, stained with lactophenol cotton blue, and observed. This process wasallowed for the identification of fungal structures, including hyphae and sporangia.

**TABLE 1. TOTAL HETEROTROPHIC BACTERIAL COUNT (THBC)**

<b>Herbal Remedy</b>	<b>THBC (CFU/ml)</b>
Goko Cleanser	$1.5 \times 10^4$
Agnes Nwamma	$7 \times 10^3$
7 Keys to Power	$1.4 \times 10^4$

### **RESULTS**

Table 1: The Total Heterotrophic Bacterial Count (THBC) for Goko Cleanser was found to be  $1.5 \times 10^4$  CFU/ml, indicating moderate bacterial contamination. This suggests that while the product may have been exposed to environmental bacteria, the level is not excessively high. In contrast, Agnes Nwamma showed a much lower count of  $7 \times 10^3$  CFU/ml, representing a minimal bacterial presence, which suggests relatively good hygiene or preservation practices. However, 7 Keys to Power exhibited  $1.4 \times 10^4$  CFU/ml, which shows significant bacterial growth and could be an indicator of improper handling, storage, or contamination during production, suggesting the product may be more prone to bacterial spoilage.

Table 2: The **Total Coliform Count (TCF)** serves as an indicator of fecal contamination. **Goko Cleanser** had a coliform count of  $6 \times 10^3$  CFU/ml, reflecting a low level of coliform contamination. Although low, this still indicates the possibility of fecal matter or unsanitary handling during

preparation. **Agnes Nwamma** showed  $1 \times 10^1$  CFU/ml, which is very low and suggests that the product is relatively safe in terms of coliform contamination. However, **7 Keys to Power** demonstrated  $6 \times 10^3$  CFU/ml, signifying moderate coliform contamination, which raises concern for its cleanliness, as coliform bacteria are typically found in fecal matter and can be harmful if ingested.

**TABLE 2. TOTAL COLIFORM COUNT (TCF)**

Herbal Remedy	TCF (CFU/ml)
Goko Cleanser	$6 \times 10^3$
Agnes Nwamma	$1 \times 10^1$
7 Keys to Power	$6 \times 10^3$

Table 3: The **Total Salmonella/Shigella Count (TSS)** is crucial for assessing the risk of gastrointestinal illness caused by pathogenic bacteria. **Goko Cleanser** tested **0** CFU/ml for Salmonella/Shigella, indicating no detectable contamination from these pathogens, which is a favorable result. On the other hand, **Agnes Nwamma** showed a trace presence of  $1 \times 10^1$  CFU/ml, which could be due to limited contamination, but still warrants caution as Salmonella and Shigella are associated with severe foodborne illnesses. **7 Keys to Power** had  $4 \times 10^3$  CFU/ml, a moderate presence of Salmonella/Shigella, suggesting that this product may pose a higher health risk, especially if consumed without proper preparation or storage, as these pathogens are known to cause serious gastrointestinal diseases like dysentery and food poisoning.

**Table 3. Total Salmonella/Shigella Count (TSS)**

Herbal Remedy	TSS (CFU/ml)
Goko Cleanser	0
Agnes Nwamma	$1 \times 10^1$



7 Keys to Power

$4 \times 10^3$

The **Total Fungal Count (TFC)** provides insights into the level of fungal contamination in the herbal remedies. **Goko Cleanser** showed  $6 \times 10^3$  CFU/ml, indicating moderate fungal contamination. This could be attributed to inadequate storage conditions, such as exposure to moisture or improper sealing. **Agnes Nwamma** exhibited  $1 \times 10^1$  CFU/ml, which is considered low and suggests better preservation methods or less exposure to fungal spores. In contrast, **7 Keys to Power** showed a higher fungal count of  $9 \times 10^3$  CFU/ml, indicating significant fungal contamination. This is concerning, as excessive fungal growth can lead to the production of mycotoxins, which are harmful if ingested, especially in immunocompromised individuals.

**Table 4. Total Fungal Count (TFC)**

Herbal Remedy	TFC (CFU/ml)
Goko Cleanser	$6 \times 10^3$
Agnes Nwamma	$1 \times 10^1$
7 Keys to Power	$9 \times 10^3$

**TABLE 5. MORPHOLOGICAL AND BIOCHEMICAL IDENTIFICATION OF SUSPECTED BACTERIA**

Presumed Bacterial Isolate	Morphological Characteristics	Biochemical Test Results
<i>Escherichia coli</i>	Gram-negative, rod-shaped, facultative anaerobe	Catalase positive, oxidase negative, lactose fermentation positive
<i>Salmonella sp</i>	Gram-negative, rod-shaped, motile	Catalase positive, oxidase negative, H <sub>2</sub> S production, non-lactose fermenter

<i>Staphylococcus aureus</i>	Gram-positive, cocci in clusters	Catalase positive, coagulase positive, mannitol fermentation positive
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The bacterial isolates identified in the herbal remedies suggest common contaminants found in herbal products. *Escherichia coli*, a Gram-negative, rod-shaped bacterium, was identified in the samples, which is often a marker for fecal contamination. The presence of *E. coli* in any product is a concern, as it indicates unsanitary conditions during processing or storage. Additionally, *Salmonella* was identified in some samples, particularly **7 Keys to Power**, where it was present in moderate amounts. *Salmonella* is a significant pathogen associated with foodborne illnesses, and its presence raises serious health concerns. *Staphylococcus aureus*, another pathogen found in the herbal remedies, is known for its ability to produce toxins and cause food poisoning, especially if the product is not handled properly.

**TABLE 6. IDENTIFICATION OF FUNGI**

Assumed Fungi Species	Microscopic Appearance	Hyphal Structure	Conidia	Colony Appearance (on agar)	Sporulation	Color of Colony (initial)
<b>Aspergillus spp.</b>	Conidiophores are branched with a swollen vesicle at the tip, producing conidia.	Septate hyphae (with internal divisions between cells).	Conidia are small, round, and arranged in chains.	Green, yellow, or blackish, velvety or powdery appearance.	Produces conidia from a vesicle in radial pattern.	White, yellowish, green, or brown.
<b>Mucor spp.</b>	Non-septate, branched hyphae with large	Non-septate hyphae (without	Large spherical sporangium containing	White to grayish, cottony or fluffy	Produces sporangia that release spores when	White to cream, turning darker

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sporangium	internal	numerous	appearance.	ruptured.	(black or
containing	divisions).	round			gray).
sporangia.		sporangia.			

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Fungal contamination in the herbal remedies was also noted, with *Aspergillus spp.* and *Mucor spp.* being the primary fungal isolates. *Aspergillus spp.* is a common environmental fungus that produces conidia in chains, and its colonies are typically green, yellow, or blackish with a velvety appearance. *Mucor spp.*, characterized by non-septate, branched hyphae and large sporangia, produces cottony, white to grayish colonies. These fungi are commonly found in decaying organic material and can pose a risk to human health, particularly in immunocompromised individuals, as they can lead to respiratory infections or other health issues when inhaled.

## DISCUSSION

The Total Heterotrophic Bacterial Count (THBC) values indicated varying levels of bacterial contamination across the herbal remedies. **Goko Cleanser** showed a moderate bacterial contamination ( $1.5 \times 10^4$  CFU/ml), which aligns with other studies where herbal products were reported to harbor significant levels of bacteria due to improper storage and handling (Sornkaet *al.*, 2022). **Agnes Nwamma**, with a lower count of  $7 \times 10^3$  CFU/ml, demonstrated relatively good hygienic conditions, which could be attributed to better preservation practices or packaging (Nwankwo and Olime, 2019). **7 Keys to Power**, on the other hand, exhibited a high bacterial count of  $1.4 \times 10^4$  CFU/ml, indicating a significant level of bacterial growth. This suggests that either the herbal remedy underwent improper handling or was stored in suboptimal conditions, which is consistent with findings from similar studies indicating that herbal medicines can

be prone to bacterial contamination, especially in tropical climates (Anieet *al.*, 2023).

The Total Coliform Count (TCF) provides insight into fecal contamination, with **Goko Cleanser** ( $6 \times 10^{-3}$  CFU/ml) showing a moderate level of contamination. This suggests the possibility of unsanitary conditions during preparation or handling, which can be associated with the handling of raw materials or water quality used during production (Nwokike, 2021). **Agnes Nwamma**, with a very low coliform count ( $1 \times 10^1$  CFU/ml), demonstrated better hygienic practices, while **7 Keys to Power** ( $6 \times 10^3$  CFU/ml) exhibited moderate fecal contamination, raising concerns about the product's safety, especially considering that coliforms are often indicators of poor sanitation and potential contamination with pathogenic bacteria such as *E. coli* (Saad *et al.*, 2022).



The Total Salmonella/Shigella Count (TSS) indicates the presence of potentially harmful enteric pathogens. **Goko Cleanser** tested negative for *Salmonella/Shigella*, which is a favorable result, as these pathogens are responsible for serious gastrointestinal diseases (Akinmoladun *et al.*, 2021). In contrast, **Agnes Nwamma** showed a trace amount ( $1 \times 10^1$  CFU/ml), while **7 Keys to Power** exhibited a significant presence ( $4 \times 10^3$  CFU/ml). The moderate contamination in **7 Keys to Power** raises health concerns as both *Salmonella* and *Shigella* are pathogens associated with foodborne illnesses such as dysentery and salmonellosis, which are particularly dangerous for vulnerable populations, including children and immunocompromised individuals (Ahiabore *et al.*, 2024). Previous studies have also reported *Salmonella/Shigella* contamination in herbal products, often linked to unsanitary production and packaging conditions (Shiaka, *et al.*, 2019).

The Total Fungal Count (TFC) reveals that **Goko Cleanser** exhibited moderate fungal contamination ( $6 \times 10^3$  CFU/ml), which may be due to improper sealing or exposure to moisture. **Agnes Nwamma**, with a low fungal count ( $1 \times 10^1$  CFU/ml), indicated better storage practices or fewer opportunities for fungal growth. **7 Keys to Power** had a high fungal count ( $9 \times 10^3$  CFU/ml), which is concerning as fungi, particularly *Aspergillus spp.* and *Mucor spp.*, can produce mycotoxins that pose serious health risks when consumed. These

fungi are often found in poorly stored or improperly sealed herbal products, and their presence in the samples is consistent with studies showing the risk of fungal contamination in herbal remedies, especially when exposed to humid environments (Ezenwaet *et al.*, 2024). *Aspergillus spp.* and *Mucor spp.*, well-known opportunistic pathogens, can cause respiratory and systemic infections, especially in immunocompromised individuals (Nwokike, 2021).

Bacterial and fungal isolates identified in this study highlight the diversity of microbial contaminants present in the herbal medicines. *Escherichia coli*, a common fecal contaminant, was identified in the samples, indicating possible unsanitary production conditions (Abba *et al.* 2009; Nwankwo and Olime 2019). The presence of *Salmonella* in some samples, particularly **7 Keys to Power**, is a significant concern as it is associated with gastrointestinal illnesses. *Staphylococcus aureus*, another pathogen found in the herbal remedies, is known for its ability to produce toxins and cause food poisoning, especially if the product is not handled properly (Ahiabore *et al.*, 2024). *Aspergillus spp.* and *Mucor spp.*, the fungal isolates identified in the samples, are commonly found in decaying organic matter and can pose a risk to human health, particularly in immunocompromised individuals, as they can lead to respiratory infections or other health issues when inhaled (Morin-Sardin *et al.*, 2017).

The microbial contamination levels in the herbal medicines tested suggest the need for improved hygiene practices during production, handling, and storage. The presence of *E. coli*, *Salmonella*, *Staphylococcus aureus*, and fungi like *Aspergillus spp.* and *Mucor spp.* raises significant health concerns. Consumers should be cautious when purchasing and using herbal remedies, particularly in environments with poor regulatory oversight. Further studies and stricter quality control measures are essential to ensure the safety and efficacy of herbal products in the market.

## CONCLUSION

The study revealed varying levels of microbial contamination in the herbal medicines tested, highlighting significant public health concerns. **Goko Cleanser, Agnes Nwamma, and 7 Keys to Power** exhibited bacterial and fungal contamination, with notable pathogens such

as *Escherichia coli*, *Salmonella spp.*, and *Staphylococcus aureus* identified in some of the products. The levels of contamination ranged from low to moderate, suggesting potential risks if consumed, particularly for vulnerable individuals such as children and immunocompromised persons. The presence of fecal contaminants, as indicated by coliform counts, along with fungal contamination from species like *Aspergillus spp.* and *Mucor spp.*, further reinforces the importance of maintaining stringent hygiene standards during the production, handling, and storage of herbal medicines.

These findings emphasize the need for quality control measures to mitigate microbial contamination in herbal remedies and ensure that products reaching consumers are safe for use. The presence of pathogenic microorganisms, especially those associated with gastrointestinal diseases, underscores the importance of consumer awareness and regulatory oversight in the herbal medicine industry.

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