

## Bacterial Contamination in some selected Herbal Medicinal Preparations Marketed in Kano Metropolis, Nigeria

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### ABSTRACT

According to a World Health Organization (WHO) report, almost 80% of people rely on traditional medicines, which are primarily made from plants, and the use of medicinal plants is continuously increasing globally. However, during harvesting or air-drying, as well as long-term storage in dried form, raw materials from medicinal plants are easily contaminated with pathogens (from the plants themselves, the soil, water, air, and dust). This might result in microbial degradation. This research aims to evaluate the bacterial load on the (10) randomly selected herbal medicinal preparations (powdered and liquid samples) collected from five markets of Kano metropolis, Kano State Nigeria and to isolate and identify the bacterial load of contaminants using cultural, morphological and biochemical techniques. The Plate technique was utilized to examine the samples for the presence of bacterial contamination on nutrient agar plates. The bacteria load was quantified in terms of colony-forming units per gram (CFU/g) for powdered samples labeled A<sub>p</sub> to E<sub>p</sub> and colony-forming units per milliliter (CFU/ml) for liquid samples labeled A<sub>l</sub> to E<sub>l</sub>. The result revealed substantial variations in bacterial counts, the least contamination was observed in powdered sample C recording  $1.70 \times 10^7$  CFU/g, while the highest contamination recorded  $4.14 \times 10^{15}$  CFU/ml for liquid sample D. No contamination was seen in liquid sample E. A total of six bacterial isolates were identified: *Escherichia coli*, *Staphylococcus aureus*, *Enterobacter aerogenes*, *Klebsiella pneumoniae*, *Streptococcus faecalis* and *Salmonella typhi*. This study concludes that herbal medicinal products sold in Kano metropolis show varying levels of bacterial contamination, with liquid preparations containing higher bacterial loads than powdered forms, the presence of certain bacteria could be as a result of unhygienic handling during harvesting, preparation, and storage.

**Keywords:** Bacterial count, ethno-medicinal plants, microbial contamination, microorganism, herbal medicinal samples.

### INTRODUCTION

Herbal medicinal preparations (HMPs) are widely consumed in Nigeria and other

African countries as accessible, affordable alternatives or complements to conventional medicines due to cultural acceptance and ease of access (Ahiabor, 2024). Bacterial contamination of herbal products has been repeatedly documented in Nigeria and across Africa, with studies reporting the presence of clinically important bacteria including *Escherichia coli*, *Staphylococcus aureus*, *Salmonella* species, and other coliforms in locally sold herbal medicine (Hamza, 2023).

Approximately 80% of the world's population relies on traditional medicines, many of which are plant-based (World Health Organization, 2019). The use of medicinal plants is steadily increasing worldwide, medicinal plants play a crucial role in the pharmaceutical, agricultural, and food industries (Dennis, 2024). Factors contributing to contamination include the use of unregulated preparation techniques, unhygienic handling, and inadequate storage conditions. In addition, microbial counts in several studies frequently exceed acceptable limits set by international safety standards, indicating significant public health risks to consumers of these unmonitored products (Adewumi *et al.*, 2025).

Most bacterial contaminants found exceeding the World health limits are associated with gastrointestinal infections, systemic illnesses, and increased antibiotic resistance, adding further concern to the safety of hawked herbal formulations (Ekor, 2013).

Furthermore, some herbal medicinal samples have been shown to contain both pathogenic bacteria and heavy metals, creating a dual-risk exposure scenario for consumers who rely on these products for routine health management (Czech, 2001). These findings underscore the potential for herbal medicinal preparations to serve as vehicles for gastrointestinal and systemic infections, particularly when contaminated with enteric pathogens capable of causing foodborne illness.

Kano is one of Nigeria's largest commercial hubs, with a high density of herbal vendors operating in open markets and along major streets, where environmental conditions may promote microbial proliferation. Given the absence of regulatory oversight and the widespread consumption of these products, localized surveillance becomes essential.

## **MATERIALS AND METHODS**

### **Study area**

This cross-sectional study was carried out on locally available herbal medicines from some various markets in Kano State Metropolis which were Kurmi market, Kofar Wambai market, Kofar mata road called Rimi market and Sabongari market as well as those traditional sellers hawking on the roadside of Emir's Palace road.

### **Experimental site**

The powdered and liquid herbal medicinal preparations were taken to the Department of Microbiology, Bayero University Kano laboratory for microbial determination and identification of microbes.

### **Collection of Samples**

Ten samples were purchased from traditional herbal medicine vendors across five different markets within the survey area, comprising five liquid and five powdered

preparations. The merchants were chosen at random from Kano metropoly markets according to the availability of plants. Liquid samples were taken as supplied by the producers/sellers, whereas powdered samples were obtained using sterile hand gloves to avoid contamination and were then placed in sterile autoclaved nylon bags.

### **Determination of bacterial load**

The total colony-forming unit was determined by the spread plate technique described by Abba *et al.*, (2008). Serial dilutions was made using five successive dilutions, this was made by combining one milliliter of liquid samples and one gram of powdered sample with nine milliliters of sterile peptone water, both of which were weighed.

For the enumeration of bacteria, aliquots from the proper dilutions were plated on nutrient agar after a five-fold serial dilution. To determine the bacterial burden, plates that produced 30–300 colonies were used. CFU/mL was calculated using the fourth serial dilution ( $10^{-1}$ ), which yielded countable colonies. Exactly 1ml of the solutions from the forth serial dilutions were aseptically poured unto the Petri dishes filled with nutritional agar. Agar plates were cooled and allowed to solidify and then incubated at 37°C for 24hrs. Following incubation, the numbers of colonies on the plates were counted to determine the bacterial burden. CFU per gram was determined for each powdered sample while CFU/ ml were determined for each liquid sample. One milliliter of the solution was aseptically applied to Petri dishes with nutritional agar in order to count the bacteria (Sing *et al.*, 2008). The World Health Organization's guidelines for therapeutic plant materials were compared to the levels of bacterial contamination. (WHO, 2019). Acceptable colony counts range is  $1.0 \times 10^5$  per gram and per ml of the sample depending on the method of plant processing, storage, and product administration to the user (WHO, 2019).

### **Isolation of bacterial species**

The isolation of suspected bacterial species from samples was done by using different selective media, Eosine methylene blue agar (EMB) was used to isolate *Escherichia coli*. Exactly 38g/l of the media was suspended into 1000ml of sterile distilled water, the mixture was allowed to dissolve and autoclaved at 121°C for 15minutes. After cooling, 5% of the media was incorporated unto the plates, each diluted sample was streaked onto the labeled plates containing the media. The plates were further incubated at 37°C to isolate suspected bacterial species.

*Salmonella-Shigella* agar (SSA) was used to isolate *Salmonella* and *Shigella*. One liter (100 ml) of sterile distilled water was used to suspend precisely 60 g/l of the medium. The liquid was then aseptically poured onto multiple labeled plates, equally streaking the diluted samples on each plate. The putative bacterial species were then found by incubating the plates ( Merlin *et al.*, 2019).

Mannitol salt agar (MSA) was used to test for the presence of *Staphylococcus aureus*, 111g/L of the media was suspended in 100ml sterile distilled water, this was autoclaved at 121°C for 15minutes and allowed to cool. The mixture was poured aseptically into the labeled plates whereas diluted samples were evenly streaked on the surface of the plates. The plates were incubated at 37°C to detect the presence of suspected bacteria.

MacConkey agar was used for the isolation of *Klebsiella pneumoniae* and *Enterobacter aerogenes*. However, blood agar was used to detect the presence of *Streptococcus faecalis*. exactly 2.8g of nutrient agar was weighed and suspended into 100ml of sterile distilled water, the mixture was stirred and autoclaved at 121°C for 15minutes and allowed to cool at 45°C. five (5) ml of rabbit blood was aseptically added into the medium, swirled and poured into sterile plates containing diluted samples, this was allowed to solidify. The plates were incubated at 37°C for 24hours, after incubation, colonies that showed  $\alpha$ -hemolysis produced 1 to 3mm greenish zones of inhibition and was regarded as incomplete hemolysis. The presence of a clear transparent zone of inhibition confirms  $\beta$ - hemolysis which is also regarded as complete hemolysis. It was concluded that *Streptococcus faecalis* is an  $\alpha$ - hemolysis organism (forbes *et al.*, 2020).

### **Identification of pure colonies using morphological characteristics**

Based on morphological differences, colonies were isolated from their pure cultures (Mensa *et al.*, 2019). Petri-dishes were labelled and sub culturing was carried out by streaking. Streaked plates were incubated and cultural appearance of colonies of plates after incubation were observed and recorded, Colony morphology observations formed a major identifying criterion for bacteria. The characteristics that were observed included (circular, irregular, spreading), elevation (flat, slightly raised or markedly raised), pigmentation (red, black white, purple pink, colorless), size (pinpoint, small, medium, large) and texture (Hamza, 2023).

### **Identification of bacteria using Gram staining and microscopy**

Bacterial isolates were first sub-cultured onto nutrient agar plates and incubated at 37 °C for 24 hours to obtain fresh colonies suitable for staining (Stefan *et al.*, 2021). Pure, well-isolated colonies were selected using a sterile inoculating loop to avoid contamination during smear preparation (Willey *et al.*, 2022). A clean grease-free microscope slide was labeled and a thin bacterial smear was prepared by emulsifying a small colony in a drop of sterile distilled water, followed by air-drying (Onyekwu *et al.*, 2024). The smear was then heat-fixed by passing the slide briefly through a flame to ensure adhesion of cells to the slide surface (Forbes *et al.*, 2020).

Gram staining was performed using primary stain, the smear was flooded with crystal violet for 60 seconds to allow penetration of the stain into the bacterial cells. The slide was then treated with Gram's iodine for 60 seconds, forming a crystal violet-iodine complex that enhances dye retention (Stefan *et al.*, 2021). Decolorization was carried out using acetone-alcohol for 15 seconds until runoff appeared clear. Over-decolorization was avoided to prevent false Gram-negative results (Willey *et al.*, 2022). Finally, the smear was counter stained with safranin for 60 seconds to stain decolorized Gram-negative bacteria. After staining, the slide was gently rinsed with water between each step and blotted dry using absorbent paper.

Microscopic examination was conducted using a bright-field compound microscope at 100× oil immersion objective (Forbes *et al.*, 2020). Bacterial cells were differentiated based on Gram reaction and morphology (Stefan *et al.*, 2021).

### **Identification of bacteria using biochemical test**

Biochemical tests were performed to differentiate bacterial isolates based on their metabolic, enzymatic, and physiological characteristics, following standard clinical microbiology procedures (Forbes *et al.*, 2020). These tests were used in combination with Gram staining results to achieve accurate bacterial identification at the genus or species level (Willey *et al.*, 2022). Fresh overnight cultures were obtained by sub-culturing isolates on nutrient agar and incubating at 37 °C for 24 hours (Cappuccino & Welsh, 2020). Pure colonies were necessary to prevent mixed biochemical reactions and ensure reliable test outcomes (stefan *et al.*, 2021).

#### **Indole test**

This was accomplished by inoculating a wire loop full of the suspected bacteria from EMB agar into a 10 ml sterile peptone water tube, which was then cultured for 24 hours. A turbid tube was obtained after incubation, and one to three drops of Kovac's reagent were applied. This caused the surface of the tube to turn pink, signifying the presence of *E. coli*. Without any organism or inoculation, the control tube was incubated.

#### **Methyl red test**

To do this, isolates of *Salmonella*, *Klebsiella*, *E. Coli*, and *Shigella* were cultured overnight in methyl broth medium tubes. After autoclaving 10 ml of methyl red medium apiece, the isolates were loaded into the tubes with a loop full of them, and they were cultured for 24 hours at 37°C. To verify the presence of the organisms, one to three drops of methyl red indicator were added to each tube, and a red hue showed the presence of *E. coli*. Without any organism or inoculation, the control tube was incubated. Voges-Proskauer test.

VP medium tubes that had been autoclaved and incubated were used for this. After adding 5% alpha-naphthol and 40% potassium hydroxide (KOH), isolate cultures were inoculated into the tubes overnight. A color shift was noticed and noted. Without any organism or inoculation, the control tube was incubated.

#### **Citrate Utilization test**

Using Simmons citrate agar slants, bacterial isolates were streaked and incubated for 48 hours. Development of a blue color signified ability to utilize citrate as a sole carbon source, a characteristic of *Klebsiella* and *Enterobacter* species (Cappuccino & Welsh, 2020).

#### **Hydrogen sulphide test**

This was accomplished by dispensing Salmonella Shigella agar into sterile tubes and inoculating test organisms with a sterilized wire loop. Eight grams of the media were dissolved in one hundred milliliters of distilled water and autoclaved at 121 degrees Celsius. Following a 24-hour incubation period at 37°C, a black gas generation indicated the production of hydrogen sulfide gas by *Salmonella* species, while a yellow color shift indicated fermentation by *Shigella* species.

#### **Motility test**

The test organisms were suspended into the peptone water tubes and cultured at 37°C for 24 hours, thereafter exactly 0.5g of peptone was dissolved in sterile distilled water and autoclaved at 121°C. In order to verify the organism's motility, a drop of the overnight culture was applied using the stab procedure to a sterile cover slide. The organism's visible motile red colored flagellum was then examined under a microscope. An organism's darting zigzag movement denotes a positive result.

#### **Catalase test**

The test organism was streaked into a sterile test tube using a sterilized inoculating loop, and a drop of hydrogen peroxide was applied to the bacteria. Approximately 0.5g of peptone was dissolved in sterile distilled water and autoclaved at 121°C. A successful reaction where hydrogen peroxide breaks down into hydrogen and oxygen gas is indicated by the formation of bubbles.

#### **Coagulase test**

A clean glass slide was coated with approximately 0.5g of normal saline and 5% human serum. The test organism was then emulsified into the glass slide solution. The slide was repeatedly rocked, and the creation of agglutination denotes a positive result.

#### **Mannitol test**

Test organisms were streaked on sterile plates containing mannitol salt agar, which had been prepared and autoclaved at 121°C. The plates were then incubated for 24 hours at 37°C. A positive test result is when the color shifts from red to yellow, signifying an acidic pH.

### **RESULTS**

Nine out of ten of the samples tested were contaminated to varying degrees with different bacterial species which is in agreement with previous works on microbial quality of herbal medicine which indicates that samples analyzed showed bacterial contamination by Umair *et al.* (2017). Results obtained were in numbers in terms of average mean values of colony-forming units per gram (CFU/g) and ml (CFU/ml) for powdered and liquid samples respectively of microorganisms present. For both powdered and liquid samples, mean aerobic bacterial count, safe limits, and standard deviations are shown in Table 1.

**Table 1:** Mean Bacterial counts in cfu/g and ml ( $\pm$ SD) for powdered and liquid samples sold at some major markets in Kano State.

<b>Samples</b>	<b>Markets collected</b>	<b>Bacterial counts (<math>\pm</math>SD)</b>	<b>cfu/g</b>	<b>W.H.O Limits</b>
A <sub>P</sub>	Abubakar rimi	$2.86 \times 10^8 \pm 0.02$		$1.0 \times 10^5$
B <sub>P</sub>	Kurmi	$3.42 \times 10^7 \pm 0.01$		$1.0 \times 10^5$
C <sub>P</sub>	Kurmi	$1.70 \times 10^7 \pm 0.03$		$1.0 \times 10^5$
D <sub>P</sub>	Roadside sellers	$2.30 \times 10^6 \pm 0.05$		$1.0 \times 10^5$
E <sub>P</sub>	Sabon gari	$4.50 \times 10^8 \pm 0.05$		$1.0 \times 10^5$
<b>Liquid samples</b>		<b>Bacterial count (<math>\pm</math>SD)</b>	<b>cfu/ml</b>	<b>W.H.O Limits</b>
A <sub>L</sub>	Kofar Wambi	$3.86 \times 10^8 \pm 0.01$		$1.0 \times 10^7$
B <sub>L</sub>	Kurmi	$4.03 \times 10^6 \pm 0.02$		$1.0 \times 10^7$

C <sub>L</sub>	Abubakar Rimi	$3.60 \times 10^6 \pm 0.05$	$1.0 \times 10^7$
D <sub>L</sub>	Sabongari	$4.60 \times 10^8 \pm 0.01$	$1.0 \times 10^7$
E <sub>L</sub>	Roadside sellers	$1.00 \times 10^4 \pm 0.00$	$1.0 \times 10^7$

Key A<sub>P</sub> to E<sub>P</sub>: powdered sample A to E

A<sub>L</sub> to E<sub>L</sub>: liquid sample A to E

**Table 2:** Colony and microscopic characteristics of bacteria from traditional herbal medicine samples (HM's)

S/N	Samples	Colony characteristic	Microscopic view	bacterial species
1	A <sub>L</sub> C <sub>P</sub>	Green metallic shiny colonies were formed on EMB agar plates	Short thick rods stained uniformly	<i>Escherichia coli</i>
2	E <sub>P</sub> C <sub>L</sub> B <sub>L</sub>	Black colonies were grown on SSA agar after incubation	Short plump rod cells seen under the microscope	<i>Salmonella typhi</i> , <i>Enterobacter aerogenes</i>
3	D <sub>L</sub> B <sub>P</sub> A <sub>P</sub>	Light pink colony was produced on macconkay agar plates	Long non motile bacilli cells uniformly stained	<i>Enterobacter aerogenes</i>
4	C <sub>L</sub> B <sub>P</sub>	Smooth mucoid flat colonies was grown on macconkay agar	Short non motile capsulated	<i>Klebsiella pneumonia</i>
5	D <sub>P</sub>	yellow colony was produced on mannitol salt agar plates	uniformly gram+ve cocci cells in pairs	<i>Staphylococcus aureus</i> ,
6	E <sub>P</sub>	produced incomplete haemolysis (green cells) on blood agar	uniformly gram+ve cocci cells	<i>Streptococcus faecalis</i> ,

Key: A<sub>L</sub> to E<sub>L</sub>: liquid samples

A<sub>P</sub> to E<sub>P</sub>: powdered samples

**Table 3:** Biochemical identification of bacterial species isolated from traditional herbal medicines sold at some major markets in Kano State.

TESTS	Organisms						
	<i>E.coli</i>	<i>S. tyhphi</i>	<i>S. dysenteriae</i>	<i>K pneumoniae</i>	<i>E.eurogenes</i>	<i>S. aureus</i>	<i>S. faecalis</i>
Indole	+	—	—	—	—	—	—
Methyl red	+	+	+	—	—	—	—
Voges proskver	—	—	—	+	+	—	—

Citrate utilization	—	—	—	+	+	—	—
Hydrogen sulphide	—	+	—	—	—	—	—
Motility	+	+	—	—	+	—	—
Catalase	—	—	—	—	—	+	+
Coagulase	—	—	—	—	—	+	+
Mannitol	—	—	—	—	—	+	+

Key + presence of  
 \_ Absence of

## DISCUSSION

The total aerobic bacterial counts for all the samples ranged from  $1.00 \times 10^4$  to  $4.60 \times 10^8$ , this study showed that nine of the herbal medicinal preparations analyzed were contaminated and as well are unsafe for human consumption, while only one liquid sample (E<sub>L</sub>) had no contamination and is less than the acceptable limits by W.H.O (2021). These substances also operate as natural barriers against bacteria, limiting their growth and stability as reported by (Twari *et al.*, 2009). The liquid samples showed higher levels of bacterial contamination, whereas the powdered sample showed the lowest levels of bacterial contamination, this is due to the liquid samples containing higher amount of free water, which is essential for bacterial survival, metabolism, and multiplication. Liquids often provide a uniform medium that allows nutrients to dissolve easily, making them readily available for bacteria (Abba *et al.*, 2008) this experiment disagreed with Salwa *et al.*, (2026) which indicated that powdered samples analyzed are more contaminated with bacteria. The highest viable aerobic bacterial count was observed in liquid sample D ( $10^8$ ) which was collected from Sabon gari market while the lowest aerobic bacterial count recorded was observed in powdered sample C ( $10^7$ ) which was collected from Kurmi market. The high bacterial load recorded in this study also suggests the possible presence of pathogenic or opportunistic microorganisms, which may pose serious health risks to consumers, particularly immunocompromised individuals, children, and the elderly (Abdulai *et al.*, 2015). Consumption of heavily contaminated herbal preparations has been associated with gastrointestinal infections, toxin production, and reduced therapeutic efficacy due to microbial degradation of bioactive compounds Tsai *et al.*, (2012). From these findings, contamination may be trader-specific rather than species-specific, as the traders may have employed unhygienic methods for harvesting, preparing, handling, and storing the completed herbal preparations Salwa *et al.*, (2026). It is also likely that inadequate sanitation of storage materials (bottles and containers) used to hold the herbal preparations prior to patient intake is the cause of the contamination of the liquid herbal sample. (Ahiabor, 2024).

From this study majority of the herbal medications that were examined had bacterial contamination. The bacteria present were *Enterobacter aerogenes*, *Escherichia coli*,

*Staphylococcus aureus*, *Streptococcus faecalis*, *Klebsiella pneumoniae* and *Salmonella typhi*. These are commonly transmitted through physical contact, the use of dirty equipment, and contaminated water, all of which are common in our communities and the facilities where these herbal medicine items are manufactured (Adegoke *et al.*, 2020). This study demonstrated that there are bacterial contaminations in the herbal medications commonly sold at some markets of Kano State which may be caused by improper preparation techniques. Microorganisms may come into contact with the products during manufacturing, handling raw materials, and product packaging. In addition, medicinal plants are contaminated by the conditions and environments in which they are grown and collected (Abba *et al.*, 2008). Microbial contaminations have been reported to originate from unsanitary materials and instruments (Nelson *et al.*, 2004). *Salmonella typhi*, *Staphylococcus aureus*, and *Enterobacter aerogenes* are known for causing a variety of complications, particularly in people with compromised immune systems. Additionally, many bacteria commonly associated with contaminated herbal medicinal products possess virulence factors that enable them to cause disease in humans. These organisms can produce a wide range of proteins and enzymes, such as proteases, hemolysins, and lipases, which actively damage host tissues and disrupt normal cellular structures (Todar, 2020). Tissue damage facilitates bacterial invasion and colonization while reducing the effectiveness of the host's physical barriers (Abba *et al.*, 2008). The findings of this study, which is similar to that of Oyetayo, (2008), indicated that *Enterobacter aerogenes* and *Escherichia coli* were found more in herbal medicinal samples. Contaminating bacteria may produce virulence-associated proteins and exotoxins capable of damaging host tissues, suppressing immune responses, and inducing gastrointestinal disorders such as diarrhea and abdominal discomfort, thereby posing significant health risks to consumers.

## **CONCLUSION**

This research indicates that numerous locally hawked herbal medicinal products available in Kano State markets are significantly tainted with certain bacteria, rendering them potentially unsafe and possibly harmful to the health of consumers. The herbal samples contained *Escherichia coli*, *Enterobacter aerogenes*, *Staphylococcus aureus*, *Klebsiella pneumoniae*, *Streptococcus faecalis*, and *Salmonella typhi*. However this study has shown that locally already packed liquid herbal medicines contained higher bacterial load contamination compared to powdered samples marketed in Kano State Nigeria. The study's conclusion highlight the critical need for the implementation of good manufacturing practices in the production, handling, and distribution of herbal medicinal products, public education of herbal medicine producers and vendors on basic hygiene and quality assurance principles is also essential. Regular microbiological monitoring should be employed before herbal products are been sold to consumers.

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