



(RESEARCH ARTICLE)



## Bacteriological examination of herbal mixtures consumed in Awka, Anambra State, Nigeria

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International Journal of Science and Research Archive, 2025, 16(03), 209–218

Publication history: Received on 20 July 2025; revised on 28 August 2025; accepted on 03 September 2025

Article DOI: <https://doi.org/10.30574/ijrsra.2025.16.3.2507>

### Abstract

Herbal mixtures are widely consumed in the world over for therapeutic and health maintenance purposes. However their quality and safety are often unregulated, raising concerns about microbial contamination. This research investigated the bacteriological quality of 10(ten) herbal mixture samples purchased from various retailers in Awka. The research aimed to determine the bacterial loads, isolate, characterize and identify bacterial species present using standard microbiological methods. The results revealed a significant and concerning level of microbial contamination across most herbal samples, with several exceeding the World Health Organization's (WHO) permissible limits of  $10^4$  cfu/ml for non-sterile herbal medicinal products. The mean pH values of the samples ranged from  $2.93 \pm 0.076$  to  $5.72 \pm 0.076$ . The study identified high bacterial loads, with THBC (Total heterotrophic bacterial count) reaching up to  $2.45 \times 10^3$  log cfu/ml. The presence of coliforms was widespread, with TCC (Total coliform Count) and TFCC (Total faecal coliform count) loads reaching up to  $1.99 \times 10^3$  and  $1.88 \times 10^3$  log cfu/ml, respectively. Furthermore, key pathogens were detected, which include *Staphylococcus aureus* (up to  $1.77 \times 10^3$  log cfu/ml), *Salmonella* (up to  $1.96 \times 10^3$  cfu/ml), and *Shigella* (up to  $1.75 \times 10^3$  cfu/ml). The identified isolates, which include *Salmonella* spp. and *Escherichia coli*, were characterized as Gram-negative rods or Gram-positive cocci. In vitro pathogenic profiling confirmed their virulence, whereby all tested strains showed  $\beta$ -hemolysis on blood agar and a positive reaction to the Congo red binding assay. The consistent absence of essential labeling information, such as manufacturing and expiry dates, across all samples further revealed a critical lack of quality control and regulatory oversight. Collectively, these findings showed a significant risk associated with the consumption of these herbal mixtures in Awka, necessitating stricter regulatory oversight and quality control measures for these consumed products.

**Keywords:** Herbal mixtures; Bacterial isolates; Awka metropolis; Bacteriological quality.

### 1. Introduction

Herbal mixtures, derived from plants or plant extracts, serve as vital therapeutic substances and dietary supplements globally, particularly in developing nations like Nigeria (Nontokozo and Simelane, 2018). These mixtures, available in diverse forms such as pills, teas, extracts, and powders, are extensively utilized to manage various ailments, including chronic conditions (Oladimeji, 2022). World Health Organization (WHO) acknowledges herbal mixtures as products containing plant parts or materials as active ingredients for disease prevention or treatment (Rojas *et al.*, 2022). In Nigeria, popular herbal products like Yoyo bitters, Weifa body defense, and Goko cleanser are widely consumed. Despite the advent of chemically synthesized drugs, a significant portion of the global population, particularly in developing countries, continues to rely on traditional practitioners and herbal medicines for primary healthcare. In Africa, up to 90% of the population depends on traditional medicine, and its use is also increasing in industrialized nations due to affordability, alignment with personal ideologies, concerns about synthetic drug side effects, and a desire for personalized healthcare (Msomi and Simelane, 2019; Aznar-Lou *et al.*, 2019).

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The therapeutic efficacy of many herbal mixtures is often linked to biotransformation by gut microbiota, emphasizing the role of fermentation (Ahtesham, 2016). Despite their perceived benefits, herbal mixtures are highly susceptible to bacterial contamination. Direct contact with soil during cultivation and harvesting, coupled with unhygienic practices during preparation, storage, and transportation, can render them prone to bacterial contamination (Olaniran *et al.*, 2022). Numerous studies have investigated the bacterial quality of herbal mixtures globally, Oluwatoyin and Adebayo (2016) reported that 90% of unregistered herbal oral liquid medicines in Ile-Ife, southwestern Nigeria, exceeded permissible microbial limits ( $10^4$  cfu/ml), with *Escherichia coli* and *Salmonella* species detected. Samuel and Mathew (2024) found significant bacterial counts (e.g., *Staphylococcus aureus*, *E. coli*, *Salmonella spp* and *Shigella spp*) in unregistered HMPs (Herbal medicinal products) in Kaduna Metropolis, all exceeding World Health Organization's (WHO) permissible limits of  $10^4$  cfu/ml for non-sterile herbal medicinal products, and noted high antibiotic resistance among isolates. Ezenwa (2024) examined herbal preparations in South-West and South-South Nigeria, finding some popular brands free from pathogens, but most unbranded herbal mixtures were contaminated with *E. coli*, *Staphylococcus aureus*, and *Bacillus sp.* Such contamination can alter the physical, chemical, and organoleptic properties of herbal products, adversely affecting their therapeutic potential and posing serious health risks, especially to vulnerable populations, hence the need to investigate and assess the bacterial quality of herbal mixtures sold in Awka, Metropolis of Anambra state, Nigeria

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## 2. Materials and Methods

### 2.1. Sample Collection

Ten (10) samples of various herbal mixture preparations were randomly purchased from different retail shops across Awka Metropolis, Anambra State, Nigeria. Samples were grouped as, A1 to A10, whereby A1, A2, A3, A4, A5, A6, A7, A8, A9 and A10 represent Herbal diarrhea, Herbal cough and catarrh, Herbal stomach ulcer, Herbal pile pain management, Herbal arthritis, Herbal Man power, Herbal hypertension, Herbal washing and setting, Herbal Herbal infection cure and Herbal malaria and typhoid respectively. which were herbal mixtures that were randomly purchased from different sellers from places (Amawbia, Ifite and Unizik temporary sites) in Awka, Metropolis.

### 2.2. Sterilization of Glassware

All glassware, including test tubes, conical flasks, beakers, pipettes, knives, and spatulas, were thoroughly washed, sterilized in a hot air oven at 180°C for 2 hours, and stored at 4°C (Sangari *et al.*, 2022).

### 2.3. Media Preparation

Culture media were prepared according to manufacturers' instructions. These included Nutrient agar, Mannitol salt agar, MacConkey agar, and Eosin Methylene Blue (EMB) agar. Media were measured, thoroughly mixed, and sterilized by autoclaving at 121°C for 15 minutes. After sterilization, they were cooled to 45°C using water bath before being aseptically dispensed into sterile Petri dishes (Sangari *et al.*, 2022).

### 2.4. Examination of the Herbal Mixtures

#### 2.4.1. Macroscopic Examination

Physical evaluation of the herbal mixtures were performed as described by Sharif *et al.* (2017). This involved careful examination of the color, odor, consistency, method of packaging, and the presence of manufacturing and expiry dates.

#### 2.4.2. pH Determination

The pH values of samples were determined using a digital pH meter, following the protocol by Vijayakumar and Adedeji (2017). Ten milliliters (10 ml) of each homogenized herbal mixture were measured into separate test tubes. The pH meter was standardized using standard buffers (pH 4.0 and pH 7.0). The pH electrode was then immersed into the homogenized mixture for 2 minutes, and the displayed pH reading was recorded. Measurements were repeated at least three times with fresh samples, and the average pH value was taken. The electrode was rinsed with distilled water after each measurement and stored properly to maintain accuracy.

#### 2.4.3. Microbial Examination of the Samples

Determination of Bacterial Loads of the Herbal Samples

Bacterial loads were determined using the method of Osuntokun *et al.*, (2022) by first vigorously shaking the samples. Using pour plate method,  $10^{-3}$  dilution was transferred into pre-labelled Petri dishes and mixed with 20 ml of sterile molten media (Nutrient agar for total bacteria), previously cooled to 45°C. The plates were swirled to ensure proper mixing, allowed to set, and incubated. Incubation was performed aerobically at 37°C for 24 hours. After incubation, growth colonies were counted using a colony counter.

## 2.5. Isolation of the Isolates

Developed colonies from the plates were subcultured by streaking on appropriate selective media to obtain pure cultures. MacConkey agar was used for gram-negative enteric bacteria (lactose and non-lactose fermenters), Eosin Methylene Blue (EMB) agar for *Escherichia coli*, Mannitol Salt agar for *Staphylococcus* species (especially *Staphylococcus aureus*), *Salmonella-Shigella* (SSA) agar for *Salmonella* and *Shigella* species. Single colonies from mixed culture plates were picked using a sterile wire loop and streaked onto solidified and cooled media in Petri dishes. The bacterial plates were incubated aerobically at 37°C for 24 hour. Pure cultures were obtained and stored on agar slants at 4°C (Osuntokun *et al.*, 2022).

## 2.6. Characterization and Identification of Isolates

Isolates were characterized and identified based on colonial morphology, microscopic examination, and biochemical tests, according to the method of Osuntokun *et al.*, (2022), the following methods of Identification were employed

## 2.7. Bacterial Characterization and Identification of Isolates

### 2.7.1. Gram Staining

Thin smears of fresh, pure cultures were made on clean, grease-free glass slides, air-dried, and heat-fixed. Slides were flooded with crystal violet for 30 seconds, rinsed with tap water, flooded with iodine for 60 seconds, rinsed, decolorized with 95% alcohol for 30 seconds, and counterstained with safranin for 30 seconds. After rinsing and air-drying, slides were viewed under a microscope using an oil immersion lens (100x).

### 2.7.2. Spore Staining

Smears of isolates were made on clean, grease-free glass slides, air-dried, and heat-fixed. Slides were flooded with Malachite green and steamed over boiling water for 15 minutes. They were then washed with clean water, counterstained with safranin for 30 seconds, rinsed, dried, and viewed under the microscope.

### 2.7.3. Citrate Test

This test identified organisms capable of utilizing citrate as their sole carbon source. Simmon's citrate agar slants were inoculated with the test organism using a sterile wire loop and incubated at 37°C for 24 hours.

### 2.7.4. Indole Test

This test assessed the ability of an organism to break down tryptophan into pyruvate and indole. The test organism was inoculated into 5 ml of sterile peptone water and incubated aerobically at 37°C for 24 hours. Indole production was checked by adding 0.5 ml of Kovac's reagent to the broth culture and gently shaking. A red coloration in the alcohol layer observed.

### 2.7.5. Motility Test

Motility was determined by inoculating the test organism into SIM (Sulphide Indole Motility) medium by stabbing the butt with a sterile needle. Spread growth throughout the media was checked.

### 2.7.6. Catalase Test

This test differentiated catalase-producing *Staphylococci* from non-catalase-producing bacteria like *Streptococci*. A drop of distilled water was placed on grease-free glass slide, the test organism emulsified in it, and 3% hydrogen peroxide was added. Presence of bubbles was checked.

### 2.7.7. Sugar Fermentation

The ability of isolates to utilize various sugars (glucose, lactose, sorbitol, inositol, xylitol, and mannitol) as sole carbon sources, with the production of acid, gas, or both, was investigated. One gram of peptone was dissolved in 220 ml of de-

ionized water, and 0.3 ml of Bromocresol purple indicator was added. Equal quantities of this peptone water were distributed into six sterile conical flasks. Sugars (2.2 g each) were respectively dissolved into the peptone water, and 10 ml of each sugar-peptone water mixture was dispensed into test tubes containing inverted Durham tubes. These were sterilized by autoclaving at 121°C for 3 minutes. Respective isolates were aseptically inoculated into the sterilized sugar solutions and incubated for 48 hours at 37°C. A color change was checked.

#### 2.7.8. *In vitro* Pathogenicity Test

This was done using the method of Ayyash *et al.*, (2018). Hemolytic activity was measured using Blood Agar and Congo red fortified media. The isolates were streaked over blood and congo agar red fortified plates and incubated at 37°C for 24 hours to observe the zone of inhibition. Hemolytic activity was characterized as  $\alpha$ -hemolytic (green halo zone),  $\beta$ -hemolytic (clear zone), and  $\gamma$ -hemolytic (no zone).

### 3. Results

The result of the Bacteriological Examination of Herbal Mixtures Consumed in Awka, Anambra State, Nigeria are shown below, whereby Table 1 showed the macroscopic examination, organoleptic properties (color, odor, consistency) and labeling deficiencies (absence of manufacturing and expiry dates) of the ten herbal samples. This is followed by figure 1, which showed pH determination that quantifies the acidity of each sample, which is an important factor for assessing the chemical composition and its suitability for microbial growth. Table 3 quantifies the bacterial loads in logarithmic colony-forming units per milliliter (log cfu/ml), revealing the overall microbial contamination level (THBC) and the presence of significant indicator organisms, including Total Coliforms (TCC), Total Faecal Coliforms (TFCC), and specific pathogenic bacteria such as *Staphylococcus aureus* (TSAC), *Salmonella* spp. (TSAL), and *Shigella* spp. (TSC). Table 4 presents the morphological and biochemical characterization of the isolated bacterial strains, revealing their Gram reaction, shape, and key metabolic activities, which were used for presumptive identification. Finally, Table 5 showed the *in vitro* pathogenic profiles of the isolates, specifically demonstrating their hemolytic activity and Congo red binding ability as indicators of potential virulence.

#### 3.1. Macroscopic Examination of the Herbal Samples.

The table below showed the Macroscopic Examination of the Herbal Samples. The examination focused on colour, odour, consistency, method of packaging, manufacturing date, and expiry date. In terms of Colour, a prevalent observation across all samples was various shades of brown, including "Light brown" shown by A1, A6, "Dark brown" (A3, A5, A9, A10, ). Other colours noted were "Colourless" (A2), "Yellow" (A4), "Light red" (A7), and "Light yellow" (A8, ).

The Odour profiles were diverse, with "Pungent herbal" being the most frequently reported characteristic (A1, A5, A6, A9, A10). Other distinct odour included "Peppermint herbal" (A2, ), "Menthol herbal" (A3 and A7), "Strawberry herbal" (A4), "Apple herbal" (A8).

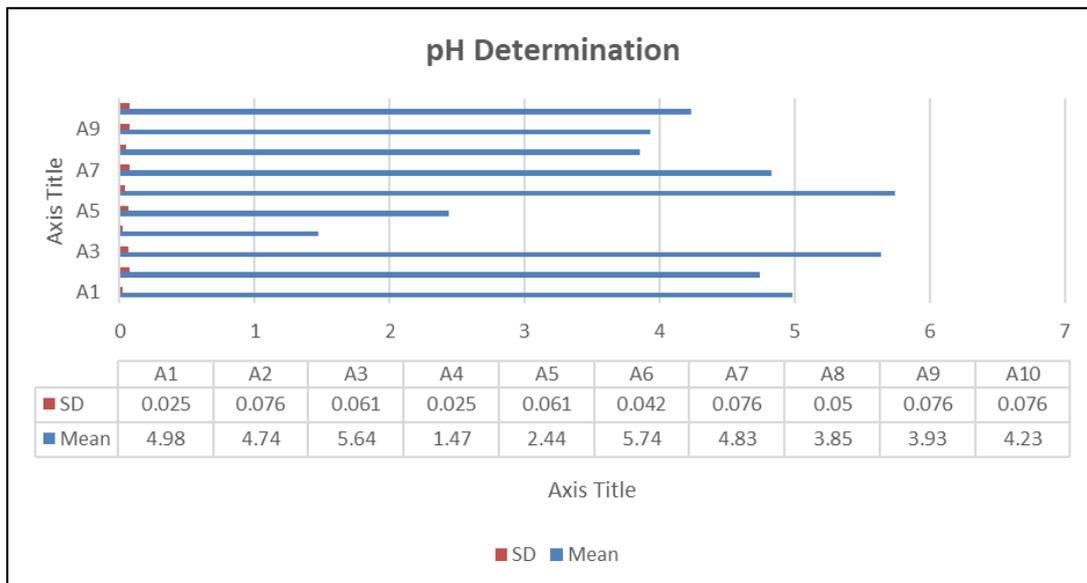
The Consistency of the samples predominantly fell into "Thin-liquid" (A1, A2, A3, A6, and A10), with some samples being "liquid" (A5, A8, and A9, ). "Gel" (A7) and some were also observed. "Syrupy" (A4) indicated thicker liquid consistency. Then the Method of packaging, "Plastic bottle" was used across most samples (A1, A2, A5, A9, and A10), while "Nylon" was also used (A3, A4, A6, A7 and A8). A critical observation across all herbal samples (A1-A10) is the consistent notation of "NA" (Not Available) for both "Manufacturing date" and "Expiry date." This pervasive lack of dating information has significant implications for consumer safety and product quality control. Without these essential details, it is impossible to assess the age of the product, its potential for microbial spoilage or growth of pathogenic organisms over time, or the stability of its active compounds.

**Table 1** Macroscopic Examination of the Herbal Samples.

Colour	Odour	Consistency	Method packaging	of	Manufacturing date	Expiry date
Light brown	Pungent	Thin-liquid	Plastic bottle		Not Available	Not Available(NA)
	herbal				(NA)	
Colourless	Peppermint herbal	Thin- liquid	Plastic bottle		Not Available (NA)	Not Available (
Dark brown	Menthol	Thin-liquid	Nylon		NA	NA
	herbal					
Yellow	Strawberry herbal	Syrupy	Nylon		NA	NA
Dark brown	Pungent herbal	liquid	Plastic bottle		NA	NA
Light brown	pungent	Thin -liquid	Nylon		NA	NA
Light red	Menthol herbal	Gel	Nylon		NA	NA
Light yellow	Apple herbal	Liquid	Nylon		NA	NA
Dark brown	Pungent herbal	Liquid	Plastic bottle		NA	NA
Dark brown	Pungent	Thin-liquid	Plastic		NA	NA
	herbal		Bottle			

Key;NotAvailable; A1 =Herbal diarrhoea; A2 = Herbal cough and catarrh;A3 = Herbal stomach ulcer; A4 = Herbal pile pain management; A5 = Herbal antritis; A6 = Herbal Man power; A7 = Herbal hypertension; A8 = Herbal washing and setting; A9 = Herbal infection; A10 = Herbal malaria and typhoid;

**3.2. pH Determination**



**Figure 1** The pH of the herbal Mixtures purchased from Awka, Metropolis.

Key; NA = Not Available; A1 =Herbal diarrhoea; A2 = Herbal cough and catarrh;A3 = Herbal stomach ulcer; A4 = Herbal pile pain management; A5 = Herbal antritis; A6 = Herbal Man power; A7 = Herbal hypertension; A8 = Herbal washing and setting; A9 = Herbal infection; A10 = Herbal malaria and typhoid

The result of the pH determined from the herbal mixtures purchased were shown below (Figure 1). The bar chart presents the pH of samples (A1 to A10), indicating their mean pH values and standard deviations. Sample A6 showed the highest mean pH of 5.74, closely followed by A3 at 5.64. Samples A1 and A7 showed moderately acidic pH values of 4.98 and 4.83, respectively. Sample A9 had a mean pH of 3.93, while A8 was slightly more acidic at 3.85. Samples A5 and A2 were quite acidic with mean pH values of 2.44 and 4.74 respectively. Notably, sample A4 displayed the lowest mean pH value of 1.47, indicating strong acidity. The standard deviations (SD) were relatively low across all samples, ranging from 0.025 to 0.076, which suggests good precision and reproducibility in the pH measurements for each sample, hence there is a statistically significant difference in the results among the herbal samples.

### 3.3. Determination of the bacterial loads of the herbal samples (Bacterial Load(logcfu/ml))

The table (table 2) below showed the bacterial loads of the herbal samples collected from Awka metropolis. Whereby the Total Heterotrophic Bacterial Count (THBC) values ranged from  $1.22 \pm 0.01$  log cfu/ml in sample A4 to  $2.45 \pm 0.03$  log CFU in sample A3. Similarly, Samples A3, A6, A7, A8, and A10 consistently showed higher THBC. Total Coliform Count (TCC) was detected in 9 out of 10 samples, with values ranging from  $0.00 \pm 0.00$  log cfu/ml (A4) to  $1.99 \pm 0.01$  log cfu/ml (A6). Total Fecal Coliform Count (TFCC) was detected in 8 out of 10 samples, with values from  $0.00 \pm 0.00$  log CFU (A2, A4, A5) to  $1.88 \pm 0.03$  log CFU (A6). Samples A3, A6, A7, and A10 showed higher levels of both TCC and TFCC, indicating significant potential for fecal contamination. Statistical analyses further confirmed significant differences in bacterial loads across samples and analysis ( $p < 0.05$ )

**Table 2** Bacterial loads of herbal samples collected

Sample	THBC	TCC	TFCC	TSAC	TSAL	TSC
A1	$1.85 \pm 0.01$	$1.29 \pm 0.01$	$1.22 \pm 0.01$	$1.22 \pm 0.01$	$0.99 \pm 0.02$	$0.46 \pm 0.01$
A2	$1.67 \pm 0.01$	$0.47 \pm 0.01$	$0.00 \pm 0.00$	$0.90 \pm 0.01$	$0.69 \pm 0.00$	$1.07 \pm 0.01$
A3	$2.45 \pm 0.03$	$1.73 \pm 0.01$	$1.59 \pm 0.01$	$1.77 \pm 0.01$	$1.71 \pm 0.01$	$1.58 \pm 0.04$
A4	$1.22 \pm 0.01$	$0.00 \pm 0.00$	$0.00 \pm 0.00$	$0.00 \pm 0.00$	$0.00 \pm 0.00$	$0.00 \pm 0.00$
A5	$1.77 \pm 0.06$	$1.29 \pm 0.01$	$0.99 \pm 0.01$	$0.29 \pm 0.01$	$0.47 \pm 0.02$	$0.00 \pm 0.00$
A6	$2.24 \pm 0.01$	$1.99 \pm 0.01$	$1.88 \pm 0.03$	$0.59 \pm 0.01$	$1.71 \pm 0.06$	$1.07 \pm 0.01$
A7	$2.17 \pm 0.02$	$1.67 \pm 0.18$	$1.56 \pm 0.04$	$0.99 \pm 0.01$	$1.77 \pm 0.07$	$1.60 \pm 0.07$
A8	$2.29 \pm 0.20$	$1.07 \pm 0.01$	$0.69 \pm 0.01$	$1.72 \pm 0.06$	$1.96 \pm 0.26$	$1.75 \pm 0.04$
A9	$1.93 \pm 0.02$	$1.19 \pm 0.01$	$0.97 \pm 0.01$	$0.94 \pm 0.02$	$1.45 \pm 0.17$	$1.07 \pm 0.01$
A10	$2.27 \pm 0.01$	$1.83 \pm 0.20$	$1.77 \pm 0.02$	$0.58 \pm 0.03$	$1.90 \pm 0.26$	$1.08 \pm 0.04$
THBC-Total Heterotrophic Bacterial Counts; TCC-Total coliform counts;						
TFCC-Total faecal coliform counts;			1 faecal coliform counts			
TSAC-Total <i>Staphylococcus aureus</i> counts						
TSAL-Total <i>Salmonella</i> Counts; TSC-Total <i>Shigella</i> Counts;						

### 3.4. The Bacteria Isolation, Identification And Characterization Of The Isolates From The Herbal Mixtures

The morphological and biochemical characterization of the isolates from the herbal mixtures as shown in table 3, revealed the presumptive presence of significant food borne pathogens such as *Salmonella spp.*, *Staphylococcus aureus*, and *Escherichia coli*. Isolate 1 (*Salmonella spp.*), showed as Gram-negative rods, motile, and colorless colonies with a dark center, indicating it as a non-lactose fermenter and an H<sub>2</sub>S producer. These traits were highly consistent with *Salmonella spp.* Isolate 2 and Isolate 3 (*Staphylococcus aureus*), showed typical golden yellow colonies, were Gram-positive cocci, and non-motile. The golden yellow color on MSA signifies mannitol fermentation, a key characteristic of *Staphylococcus aureus*. Isolate 4 and 5 (*Escherichia coli*), were isolates obtained from Eosin Methylene Blue (EMB) Agar which showed a distinctive green metallic sheen, were Gram-negative rods, and were also found to be motile. The metallic sheen is a strong indicator of vigorous lactose fermentation, which is also a characteristic of *Escherichia coli*.

**Table 3** Morphological and Biochemical Characteristics of the Bacterial Isolates

Isolate 1	Isolate 2	Isolate 3	Isolate 4	Isolate 5
Colourless but dark centred	Golden yellow colour	Golden yellow colour	Green metallic sheen	Green metallic sheen
Raised	Raised	Raised	Raised	Raised
Smooth	Smooth	Smooth	Smooth	Smooth
Smooth	Smooth	Smooth	Smooth	Smooth
-	+	+	-	-
Rods	Cocci	Cocci	Rods	Rods
+	-	-	+	+
+	+	+	+	+
-	+	+	-	-
-	-	-	+	+
+	-	-	+	+
+	+	+	-	-
+	-	-	-	-
+	+	+	+	+
-	+	+	+	+
-	+	-	-	+
+	+	-	-	-
-	-	+	+	-
+	-	-	+	-
<i>Salmonella spp</i>	<i>S.aureus</i>	<i>S.aureus</i>	<i>E.coli</i>	<i>E.coli</i>

### 3.5. The In vitro Pathogenic Profile of The Isolates.

The Table 4 below showed the in vitro pathogenic profile of the bacteria isolates from the herbal mixtures, whereby a consistent characteristic across all five isolates (Isolate 1-5) was their ability to induce beta-haemolysis on blood agar, a strong indicator of their capacity to completely lyse red blood cells. This haemolytic activity suggests a common virulence factor among the isolates. However, a notable heterogeneity was observed in the Congo Red agar test, which serves as a proxy for the production of exopolysaccharides (EPS) and biofilm-forming ability. Isolates 1, 2, and 4 showed a strong positive reaction by forming red colonies, suggesting robust EPS production. In contrast, Isolate 3 displayed a weaker positive result with the formation of pink colonies, while Isolate 5 showed a negative result, forming gray/light gray colonies.

**Table 4** In vitro Pathogenic Profile of The Isolates

Isolate	Blood Agar	Congo Red
Isolate 1	$\beta$ -haemolysis	Formation of red colonies
Isolate 2	$\beta$ -haemolysis	Formation of red colonies
Isolate 3	$\beta$ -haemolysis	Formation of pink colonies
Isolate 4	$\beta$ -haemolysis	Formation of red colonies

Isolate 5	β-haemolysis	Formation of gray/light gray colonies
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#### 4. Discussion

The Bacteriological Examination Of Herbal Mixtures Consumed In Awka, Anambra State, Nigeria was investigated whereby the findings of the study provided a comprehensive bacteriological assessment of herbal mixtures sold in Awka metropolis. The macroscopic examination (Table 1), revealed a lack of essential labeling information, such as manufacturing and expiry dates, for all samples. This absence of critical data is a major concern, as it hinders consumer awareness regarding product shelf-life and safety, a common issue in unregulated traditional medicine markets (Ekor, 2014). The wide range of pH values observed in figure 1 (0.025 to 5.74) suggests diverse formulations, which could influence bacterial survival and growth, this variability in pH suggests inconsistent preparation methods or uncontrolled fermentation processes. pH is a critical intrinsic factor influencing microbial growth and survival. While extremely low pH can inhibit many pathogenic bacteria, a wide range of pH values within a product indicates poor standardization (Siddiqui *et al.* 2023). Such heterogeneity can either favor the growth of acid-tolerant spoilage organisms or, at higher pH, create a permissive environment for a broader spectrum of microbial contaminants, including potential pathogens. This lack of control can selectively enrich for certain microbial populations, potentially including those that are detrimental, or allow for the rapid proliferation of contaminants if conditions become favorable as pH is a crucial factor in microbial ecology. The bacterial load analysis revealed widespread contamination across the herbal samples, with several exceeding the WHO permissible limits ( $10^4$  cfu/ml) for non-sterile herbal medicinal products (De Sousa Lima *et al.*, 2020). The mere presence of any coliforms (which are indicators of fecal contamination) or specific enteric pathogens like *Salmonella* and *Shigella* is inherently unacceptable in products intended for human consumption, especially those not subjected to terminal sterilization. The presence of *Staphylococcus aureus* is concerning due to its potential to produce enterotoxins, while *Salmonella* and *Shigella* are well-known causes of severe gastrointestinal infections (Bhandari, 2023; Lampel, 2018). This finding is consistent with Oluwatoyin and Adebayo (2016), who reported that the total aerobic count in most marketed herbal mixtures in India exceeded the WHO bacteriological limit, indicating poor microbiological quality. Similarly, Samuel and Mathew (2024) found significant bacterial counts ( $\geq 3.0 \times 10^7$  CFU/ml) of *Staphylococcus aureus*, *Escherichia coli*, *Salmonella* species, and *Shigella* species in unregistered herbal medicinal products in Kaduna Metropolis, all exceeding the WHO limit of  $10^4$  CFU/ml.

The confirmed presence of these pathogenic bacteria directly translates to a serious health hazard, with the potential for causing severe gastrointestinal infections (e.g., gastroenteritis, dysentery), systemic illness (e.g., sepsis), and even death, particularly in vulnerable populations such as children, the elderly, and immuno-compromised individuals. This seriously contradicts the common perception that herbal remedies are natural and safe. This widespread contamination is attributable to inadequate hygiene throughout the supply chain, including cultivation, harvesting, processing, packaging, transport, and storage. Specific contributing factors include direct contact with soil during harvesting, unhygienic preparation methods, and the use of non-sterile water (Samuel and Mathew, 2024).

The morphological and biochemical characterization of bacterial isolates from herbal mixtures (Table 4), confirmed the presence of significant bacterial pathogens in the herbal mixtures. Specifically, *Salmonella* species, *Staphylococcus aureus*, and *Escherichia coli* were identified. These identifications are consistent with known microbial contaminants of herbal products reported by Oluwatoyin and Adebayo (2016) which similarly identified *Staphylococcus aureus*, *Escherichia coli*, *Pseudomonas aeruginosa*, *Shigella* species, and *Salmonella* species in marketed herbal mixtures in India. Samuel and Mathew (2024) also reported the presence of *Staphylococcus aureus*, *Escherichia coli*, *Salmonella* species, and *Shigella* species in unregistered herbal medicinal products in Kaduna Metropolis. Ezenwa (2024) found *E. coli*, *Staphylococcus aureus*, *Bacillus sp.*, *Aspergillus sp.*, *Proteus sp.*, *Rhizopus sp.*, and *Penicillium* in some herbal mixtures in Nigeria, noting that these organisms were consistent with findings from other researchers. Dashen *et al.* (2020) also reported the presence of *Escherichia coli*, *Staphylococcus aureus*, and *Salmonella sp.* in liquid herbal preparations in Jos metropolis, Nigeria. While Alegbeleye (2018) found *Staphylococcus aureus* and *Escherichia coli* in herbal preparations in Gombe main market, they notably reported the absence of *Salmonella typhi* and *Shigella dysenteriae* in all samples, which contrasts with the findings of this study and others.

Further in vitro pathogenic profiling (Table 4) showed that all these identified pathogenic isolates exhibited β-hemolysis on blood agar. Beta-hemolysis is a critical virulence factor, indicating the complete lysis of red blood cells by bacterial hemolysins. This enzymatic activity is directly associated with the ability of pathogens to cause tissue damage, acquire nutrients (e.g., iron from hemoglobin), and contribute to the severity of infections in vivo.

This finding elevates the concern from mere bacterial presence to the confirmed presence of bacteria with demonstrated pathogenic potential. It suggests that these contaminated herbal mixtures are highly unhygienic which is

capable of causing infections when consumed. This aligns with prior research (e.g., Oluwatoyin and Adebayo, 2016; Samuel and Mathew, 2024) which consistently reports the presence of these specific pathogens in unregistered herbal products in Nigeria, further validating the findings of this study .

Therefore the findings of significant pathogenic contamination in the herbal mixtures showed a critical challenge in traditional medicine. The occurrence of harmful bacteria necessitates medical intervention. The lack of proper manufacturing and expiry dates further complicates quality assurance. This research showed the need for regulatory bodies in Nigeria to implement and enforce stringent quality control measures, including microbiological testing and good manufacturing practices (GMP), for herbal mixtures in other to safeguard health.

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## 5. Conclusion/ Recommendation

This study provides compelling evidence of significant bacterial contamination, including various pathogenic bacteria, in herbal mixtures sold in Awka metropolis. The consistent absence of manufacturing and expiry dates further highlights a critical lack of quality control and regulatory oversight, posing substantial public health risks to consumers. These findings showed the need for stringent quality assurance and regulatory enforcement for herbal products in Nigeria.

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## Compliance with ethical standards

### *Disclosure of conflict of interest*

No conflict of interest to be disclosed.

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