

EVALUATION OF BIOACTIVE COMPOUNDS AND MICROBIOLOGICAL PROFILES IN HERBAL MIXTURES MARKETING IN UGEP, CROSS RIVER STATE, NIGERIA

Ndifon Kingsley Assam

Department of Science laboratory technology

*Federal Polytechnic, Ugep, Cross River State, Nigeria

Abstract

Herbal mixtures are widely used in Ugep, Cross River State, Nigeria, for their perceived therapeutic benefits, yet concerns persist regarding their safety and efficacy due to limited regulation. This study evaluated the bioactive compounds and microbiological profiles of ten commonly marketed herbal mixtures using primary data collection methods. Samples were procured from local markets, and phytochemical screening identified bioactive compounds such as alkaloids, flavonoids, tannins, and saponins. Microbial analysis assessed contamination levels using standard plate count techniques, revealing the presence of pathogens like *Escherichia coli*, *Staphylococcus aureus*, and *Salmonella* spp. in varying concentrations. Results showed that 70% of samples contained flavonoids and tannins, while 40% exceeded the World Health Organization (WHO) microbial limit of 10^5 CFU/g for aerobic bacteria. The highest microbial load was 2.3×10^6 CFU/g, indicating significant contamination risks. Theoretical insights from the Biogenic Theory framed the study, emphasizing the natural origin of bioactive compounds and their potential health impacts when compromised by microbial contamination. These findings align with recent literature highlighting variability in herbal product quality across Nigeria. Poor hygiene during preparation and storage likely contributed to contamination, underscoring the need for stricter quality control. Recommendations include enforcing regulatory standards, improving production hygiene, and educating vendors on safe handling practices. This study provides critical data for stakeholders aiming to enhance the safety and efficacy of herbal mixtures in Ugep.

Keywords: Herbal mixtures, bioactive compounds, microbiological profiles, phytochemical screening, microbial contamination, quality control.

Introduction

Herbal mixtures, derived from medicinal plants, have long been a cornerstone of traditional healthcare systems in Nigeria, particularly in rural and semi-urban communities like Ugep in Cross River State. These mixtures, often prepared from locally sourced plants, are prized for their accessibility, affordability, and perceived efficacy in treating a wide range of ailments, including infections, digestive disorders, and chronic conditions (Ekor, 2014). In Ugep, a culturally rich town in Yakurr Local Government Area, the herbal medicine market thrives, with vendors offering diverse concoctions to meet community health needs. This reliance on herbal remedies reflects a broader trend across sub-Saharan Africa, where over 80% of the population depends on traditional medicine for primary healthcare (WHO, 2013). However, the growing popularity of these products has raised critical questions about their safety, quality, and consistency, particularly in unregulated markets like Ugep.

The therapeutic potential of herbal mixtures is largely attributed to their bioactive compounds, such as alkaloids, flavonoids, tannins, and saponins, which exhibit antioxidant, antimicrobial, and anti-inflammatory properties (Mickymaray, 2019). Recent studies have confirmed the presence of these compounds in Nigerian herbal products, underscoring their pharmacological value (Akpan et al., 2020). For instance, flavonoids have been linked to immune system support, while tannins are noted for their astringent and antimicrobial effects (Sofowora, 2013). Despite these benefits, the safety of herbal mixtures is frequently compromised by microbial contamination, a pervasive issue in regions with limited regulatory oversight. Current literature highlights that contamination with pathogens like *Escherichia coli*, *Staphylococcus aureus*, and *Salmonella* spp. is common in herbal products, often due to poor hygiene during harvesting, processing, or storage (Hassan et al., 2023; Oluyomi et al., 2012). A 2022 study by Adebayo et al. reported microbial loads exceeding the World Health Organization (WHO) threshold of 10^5 CFU/g in 65% of herbal samples from Lagos markets, signaling a widespread public health concern.

The problem of this study lies in the dual challenge of ensuring the efficacy of herbal mixtures through their bioactive compounds while mitigating the risks posed by microbial contamination in an unregulated market like Ugep. Unlike pharmaceutical drugs, herbal mixtures in Nigeria are rarely subjected to standardized quality control, leaving consumers vulnerable to substandard or unsafe products (Okunlola et al., 2007). In Ugep, where traditional knowledge drives production, the lack of scientific validation and hygiene protocols exacerbates these risks. Contaminated herbal mixtures can lead to adverse health outcomes, including gastrointestinal infections, systemic toxicity, or reduced therapeutic efficacy, undermining trust in traditional medicine (Esimone et al., 2002). Moreover, the variability in bioactive compound profiles due to factors like plant sourcing, preparation methods, and storage conditions further complicates their reliability as therapeutic agents (Kabera et al., 2014).

Scholars have made notable attempts to address this problem. Okunlola et al. (2007) evaluated herbal products in southwestern Nigeria, advocating for good manufacturing practices (GMP) to reduce contamination. Similarly, Oluyomi et al. (2012) proposed regular microbial testing and vendor education in Anambra State, though implementation remains limited. In Cross River State, Akpan et al. (2020) focused on phytochemical screening of individual plants but did not extend their analysis to marketed mixtures. Globally, Hassan et al. (2023) in Ghana and Shaban et al. (2016) in Kenya have called for stricter regulatory frameworks, yet these efforts have not fully translated to local contexts like Ugep. Despite these contributions, gaps persist in localized studies that combine phytochemical and microbiological assessments to inform context-specific interventions.

In Nigeria, herbal medicine remains a vital healthcare resource, with over 80% of the population relying on it (WHO, 2013). Studies in Cross River State, such as Akpan et al. (2020), have identified bioactive compounds in local plants like *Corchorus olitorius*, but these focus on raw materials rather than marketed mixtures. Ekor (2014) argues that while herbal products are culturally significant, their safety is undermined by limited regulation, a view supported by national surveys showing widespread quality issues (NAFDAC, 2018). Recent advancements, such as Adebayo et al. (2022), use high-performance liquid chromatography (HPLC) to quantify phytochemicals in herbal mixtures, revealing significant variability even within the same region.

Despite this extensive body of work, few studies specifically address the quality of herbal mixtures marketed in semi-urban settings like Ugep, Cross River State. While national and regional studies

highlight contamination and phytochemical variability, localized investigations are scarce, particularly in areas with unique socio-cultural and economic dynamics. Ancient scholars provided foundational insights into plant-based medicine, and modern researchers have advanced analytical techniques, yet the intersection of bioactive compound profiles and microbiological safety in Ugep’s herbal market remains underexplored. This study fills this gap by providing primary data on the phytochemical and microbial quality of herbal mixtures in Ugep, offering a localized perspective to inform public health interventions and regulatory policies in Nigeria’s herbal medicine sector.

This study is anchored on the Biogenic Theory, a framework that explains the origin and function of bioactive compounds in plants as naturally synthesized secondary metabolites. The theory was notably advanced by Justus von Liebig, a 19th-century German chemist, who laid foundational ideas about the role of organic compounds in biological systems (Liebig, 1840). In the context of herbal mixtures marketed in Ugep, the Biogenic Theory provides a lens to evaluate both the presence of bioactive compounds and the impact of microbial contamination. The theory predicts that herbal mixtures should contain secondary metabolites such as flavonoids, tannins, and alkaloids, synthesized by source plants for ecological purposes and retained in prepared products for therapeutic use. This is tested through phytochemical screening, which confirms the natural origin of these compounds in the samples.

However, the theory’s assumption of environmental influence is critical here. The study hypothesizes that while plants produce these beneficial compounds, post-harvest handling such as unhygienic preparation or storage introduces microbial contaminants like *Escherichia coli* and *Staphylococcus aureus*. These contaminants, as external stressors, may degrade bioactive compounds or introduce health risks, negating their therapeutic potential. For instance, tannins’ antimicrobial properties (Mickymaray, 2019) could be undermined by high microbial loads exceeding WHO limits (10^5 CFU/g), as observed in 40% of the samples. The Biogenic Theory thus frames the dual analysis of intrinsic phytochemical value and extrinsic quality threats, linking plant biology to public health outcomes in Ugep’s herbal market.

Method

i. Study Area

The study was conducted in Ugep, a semi-urban town in Yakurr Local Government Area, Cross River State, Nigeria (coordinates: 5.8066° N, 8.0810° E). Ugep is renowned for its vibrant herbal medicine market, serving both local residents and neighboring communities. Sampling occurred in the central market, a hub for herbal vendors, between January and March 2025.

ii. Sample Collection

Ten herbal mixtures (labeled HM1–HM10) were purposively selected based on their popularity and frequency of use, as identified through preliminary interviews with five major vendors. Samples included both liquid (mL) and powdered (g) forms, with three replicates (triplicates) collected per sample to ensure reliability. A total of 100 mL (liquid) or 100 g (powder) per replicate was aseptically collected in sterile polyethylene containers, labeled, and transported in a cold chain (4°C) to the laboratory within 6 hours of collection. Table 3 outlines the sample characteristics.

Table 1
Characteristics of Collected Herbal Mixtures

Sample	Form	Vendor	Reported Use	Collection Date

HM1	Liquid	A	Fever, malaria	15/01/2025
HM2	Powder	B	Digestive issues	18/01/2025
HM3	Liquid	C	Skin infections	22/01/2025
HM4	Powder	D	Respiratory ailments	25/01/2025
HM5	Liquid	E	General wellness	29/01/2025
HM6	Powder	A	Joint pain	05/02/2025
HM7	Liquid	B	Hypertension	10/02/2025
HM8	Powder	C	Diabetes management	15/02/2025
HM9	Liquid	D	Antimicrobial	20/02/2025
HM10	Powder	E	Immune booster	25/02/2025

iii. Phytochemical Screening

Qualitative phytochemical analysis was performed following standard protocols (Sofowora, 2013) to detect key bioactive compounds: alkaloids, flavonoids, tannins, and saponins. For each sample, 5 g (powder) or 5 mL (liquid) was extracted with 50 mL of 70% ethanol, filtered, and subjected to the following tests:

- Alkaloids: Dragendorff's reagent (orange precipitate indicates presence).
- Flavonoids: Shinoda test (red/pink color with magnesium and HCl).
- Tannins: Ferric chloride test (blue-black precipitate).
- Saponins: Froth test (persistent foam >1 cm after shaking with water).

Results were recorded as present (+) or absent (-) based on visual confirmation by two independent analysts to reduce subjectivity. Tests were conducted in triplicate, and outcomes were cross-verified.

Microbiological Analysis

i. Total Microbial Load

Microbial contamination was assessed using the pour plate method (USP, 2016). For each sample, 1 g (powder) or 1 mL (liquid) was homogenized in 9 mL of sterile saline (0.9% NaCl) and serially diluted (10^{-1} to 10^{-5}). Aliquots (1 mL) of each dilution were plated in triplicate on:

- Nutrient agar (NA) for total aerobic bacteria (incubated at 37°C, 24–48 hours).
- MacConkey agar (MAC) for *Escherichia coli* (37°C, 24 hours).
- Mannitol salt agar (MSA) for *Staphylococcus aureus* (37°C, 48 hours).

Colonies were counted using a digital colony counter, and microbial loads were expressed as colony-forming units per gram or milliliter (CFU/g or CFU/mL). The mean of triplicates was calculated.

ii. Pathogen Identification

Presumptive identification of pathogens was based on colony morphology, Gram staining, and biochemical tests (catalase, coagulase, indole, and oxidase). To confirm the presence of *Salmonella* spp., suspected isolates were subjected to PCR analysis. DNA was extracted using a commercial kit (Qiagen DNeasy), and the *invA* gene (specific to *Salmonella*) was amplified using primers:

- Forward: 5'-GTGAAATTATCGCCACGTTTCGGGCAA-3'

- Reverse: 5'-TCATCGCACCGTCAAAGGAACC-3'

PCR conditions included an initial denaturation at 94°C for 5 min, followed by 35 cycles of 94°C (30 s), 55°C (30 s), and 72°C (1 min), with a final extension at 72°C for 7 min. Amplicons (284 bp) were visualized on a 1.5% agarose gel. Positive controls (*Salmonella typhimurium* ATCC 14028) and negative controls (sterile water) were included.

Data Analysis

i. Phytochemical Data

The presence/absence of bioactive compounds was summarized descriptively as percentages across all samples. A contingency table (Table 2) was used to display the distribution.

Table 2

Distribution of Bioactive Compounds Across Samples

Compound	Number of Positive Samples	Percentage (%)
Alkaloids	6	60
Flavonoids	7	70
Tannins	7	70
Saponins	4	40

ii. Microbial Data

Microbial counts were log-transformed ($\log_{10}$ CFU/g or mL) to normalize the data for statistical analysis. One-way ANOVA was applied to determine significant differences in total aerobic counts among the ten samples, with a significance level of $p < 0.05$. The null hypothesis (H_0) was that there were no differences in microbial loads across samples. Post-hoc Tukey's HSD test identified pairwise differences if ANOVA was significant. Analysis was performed using SPSS (v.27).

For pathogen prevalence, a chi-square test assessed associations between sample form (liquid vs. powder) and pathogen detection ($p < 0.05$). Microbial loads were compared to WHO limits (10^5 CFU/g for aerobic bacteria, 10^3 CFU/g for pathogens) to evaluate safety.

iii. Quality Control

All experiments included blanks and controls to ensure reliability. Equipment (autoclaves, pipettes) was calibrated, and media sterility was confirmed before use. Triplicates minimized experimental error, and results were validated by repeating 20% of the assays.

Further Analysis Of Data

i. Phytochemical Results

The qualitative screening revealed variability in bioactive compound profiles (Table 1, original results). Flavonoids and tannins were the most prevalent (70%), suggesting a strong antioxidant potential in these mixtures (Mickymaray, 2019). Alkaloids (60%) and saponins (40%) were less consistent, possibly reflecting differences in plant ingredients or preparation methods.

ii. Microbiological Results

ANOVA results for total aerobic counts showed significant variation among samples ($F(9, 20) = 12.34$, $p = 0.001$), rejecting H_0 . Tukey's HSD identified HM2, HM4, HM7, and HM10 as significantly higher

than HM3, HM6, and HM9 ($p < 0.05$), indicating that contamination levels were not uniform. Table 3 presents the log-transformed means.

Table 3

Log-Transformed Microbial Loads and ANOVA Results

Sample	Log ₁₀ CFU/g or mL (Mean ± SD)	Tukey Group*
HM1	5.18 ± 0.12	b
HM2	6.36 ± 0.15	a
HM3	4.94 ± 0.10	c
HM4	6.28 ± 0.14	a
HM5	5.51 ± 0.11	b
HM6	4.81 ± 0.09	c
HM7	6.04 ± 0.13	a
HM8	5.68 ± 0.12	b
HM9	4.96 ± 0.08	c
HM10	6.23 ± 0.16	a

Note. *Means with different letters differ significantly ($p < 0.05$).

PCR confirmed *Salmonella* spp. in HM4, HM8, and HM10, aligning with biochemical tests. The chi-square test showed no significant association between sample form and pathogen presence ($\chi^2 = 1.25$, $p = 0.26$), suggesting contamination sources were independent of physical state.

Total Results

The analysis of the ten herbal mixtures (HM1–HM10) marketed in Ugep provided comprehensive insights into their bioactive compound composition and microbiological quality. Results are presented in two subsections: phytochemical composition and microbiological profiles, with expanded data and observations.

i. Phytochemical Composition

Phytochemical screening identified the presence of key bioactive compounds, including alkaloids, flavonoids, tannins, and saponins, across the samples. Table 4 below summarizes the qualitative results, with additional notes on intensity based on visual assessment during testing (e.g., strong positive [++], weak positive [+], or absent [-]).

Table 4

Phytochemical Composition of Herbal Mixtures

Sample	Alkaloids	Flavonoids	Tannins	Saponins	Notes on Intensity
HM1	+	++	+	-	Strong flavonoids
HM2	++	+	-	+	Strong alkaloids
HM3	-	++	+	-	Strong flavonoids
HM4	+	-	++	+	Strong tannins
HM5	++	+	+	-	Strong alkaloids

HM6	-	+	-	++	Strong saponins
HM7	+	-	++	-	Strong tannins
HM8	++	+	+	+	Balanced profile
HM9	-	++	-	-	Strong flavonoids
HM10	+	++	+	++	Diverse profile

- **Prevalence:** Flavonoids were detected in 70% of samples (7/10), with strong presence (++) in HM1, HM3, HM9, and HM10, suggesting a common plant base rich in these antioxidants. Tannins were also present in 70% (7/10), with strong intensity in HM4 and HM7. Alkaloids appeared in 60% (6/10), with notable strength in HM2, HM5, and HM8, while saponins were less frequent (40%, 4/10), with HM6 and HM10 showing strong positives.
- **Variability:** HM10 exhibited the most diverse profile, with all four compounds present, while HM9 was limited to flavonoids. This variability likely reflects differences in plant ingredients, preparation methods, or vendor recipes.

ii. Microbiological Profiles

Microbiological analysis assessed total aerobic bacterial counts, yeast and mold counts, and the presence of specific pathogens, with results compared to WHO standards (10^5 CFU/g for aerobic bacteria, 10^3 CFU/g for yeasts and molds, and absence of pathogens in 10 g or mL; WHO, 2007). Table 5 provides an expanded dataset, including additional microbial groups and environmental context.

Table 5

Microbial Load and Pathogens in Herbal Mixtures

Sample	Total Aerobic Count (CFU/g or mL)	Yeast Mold Count (CFU/g or mL)	& E. S. coli aureus spp.		Salmonella spp.	P. aeruginosa spp.	Aspergillus spp.	Sample Form	Storage Condition
HM1	1.5×10^5	2.1×10^3	+	-	-	-	-	Liquid	Open bottle
HM2	2.3×10^6	4.8×10^4	+	+	-	+	+	Powder	Unsealed bag
HM3	8.7×10^4	1.9×10^3	-	+	-	-	-	Liquid	Sealed bottle
HM4	1.9×10^6	3.5×10^4	+	-	+	-	+	Powder	Open container
HM5	3.2×10^5	2.7×10^3	-	+	-	+	-	Liquid	Open bottle
HM6	6.5×10^4	1.2×10^3	-	-	-	-	-	Powder	Sealed bag
HM7	1.1×10^6	5.2×10^4	+	+	-	-	+	Powder	Unsealed bag
HM8	4.8×10^5	3.9×10^3	-	+	+	-	-	Liquid	Open

									bottle
HM9	9.2×10^4	1.5×10^3	-	-	-	-	-	Powder	Sealed bag
HM10	1.7×10^6	4.1×10^4	+	+	+	+	+	Liquid	Open bottle

- **Total Aerobic Count:** Four samples (HM2, HM4, HM7, HM10) exceeded the WHO limit, with HM2 peaking at 2.3×10^6 CFU/g. HM6 and HM9, with counts of 6.5×10^4 and 9.2×10^4 CFU/g, respectively, were the least contaminated, possibly due to sealed storage.
- **Yeast and Mold Count:** All samples surpassed the 10^3 CFU/g limit, with HM7 (5.2×10^4 CFU/g) and HM2 (4.8×10^4 CFU/g) showing the highest fungal loads. *Aspergillus* spp. was identified in four samples (HM2, HM4, HM7, HM10), indicating potential mycotoxin risks.
- **Pathogens:** Seventy percent (7/10) of samples contained at least one pathogen. *E. coli* was present in five samples, *S. aureus* in six, *Salmonella* spp. in three, and *P. aeruginosa* in three. HM10 was the most contaminated, harboring all four pathogens.
- **Form and Storage Impact:** Powders in unsealed bags (HM2, HM7) and liquids in open bottles (HM1, HM5, HM8, HM10) showed higher contamination than sealed samples (HM3, HM6, HM9), suggesting storage conditions as a key factor.

Discussion

The results reveal a dual narrative: the herbal mixtures in Ugep are rich in bioactive compounds with therapeutic potential, yet their microbiological quality poses significant safety risks. Flavonoids and tannins, present in 70% of samples, are known for their antioxidant and antimicrobial properties (Mickymaray, 2019), while alkaloids (60%) and saponins (40%) contribute to anti-inflammatory and immune-boosting effects (Kabera et al., 2014). The strong presence of these compounds in samples like HM10 and HM8 suggests a robust plant-based foundation, likely derived from local species such as *Vernonia amygdalina* or *Ocimum gratissimum*, common in Cross River State (Akpan et al., 2020). However, the therapeutic promise is overshadowed by microbial contamination, with 40% of samples exceeding aerobic bacterial limits and 100% surpassing fungal thresholds.

The microbiological profiles align with regional studies, such as Oluyomi et al. (2012), who found *E. coli* and *S. aureus* in herbal mixtures from Anambra State, and Hassan et al. (2023), who reported fungal contamination in Ghanaian products. The high prevalence of *E. coli* (50% of samples) indicates fecal contamination, likely from untreated water or poor sanitation during processing, a persistent issue in rural Nigeria (Okunlola et al., 2007). *S. aureus* in 60% of samples points to human handling as a contamination source, while *Salmonella* spp. and *P. aeruginosa* in 30% each suggest environmental exposure or cross-contamination in the market. The universal exceedance of yeast and mold limits, with *Aspergillus* spp. in four samples, raises concerns about mycotoxin production, which could degrade bioactive compounds and introduce additional health risks (Sofowora, 2013).

The Biogenic Theory frames these findings by highlighting the natural origin of bioactive compounds and their vulnerability to external microbial interference. While flavonoids and tannins could theoretically inhibit microbial growth, the overwhelming loads (e.g., 2.3×10^6 CFU/g in HM2) likely outstrip their protective capacity, rendering the mixtures unsafe. Storage conditions appear critical: sealed samples (HM6, HM9) had lower counts, while open containers (HM2, HM7, HM10) were heavily contaminated, reflecting exposure to humid tropical conditions and market dust.

These results mirror global trends in unregulated herbal markets (Shaban et al., 2016) but are particularly acute in Ugep due to limited regulatory oversight. The variability e.g., HM6's safety versus HM10's severe contamination suggests that some vendors employ better practices, offering a potential model for improvement. However, the overall picture indicates a public health challenge: consumers relying on these mixtures for ailments may face exacerbated conditions or new infections, especially vulnerable populations like the elderly or immunocompromised.

Conclusion

This study demonstrates that herbal mixtures marketed in Ugep, Cross River State, Nigeria, are a double-edged sword. They contain valuable bioactive compounds flavonoids, tannins, alkaloids, and saponins that underpin their traditional use in healthcare. However, their microbiological profiles reveal widespread contamination, with 40% exceeding aerobic bacterial limits, 100% surpassing fungal thresholds, and 70% harboring pathogens like *E. coli*, *S. aureus*, *Salmonella* spp., and *P. aeruginosa*. These findings confirm the therapeutic potential of these products while exposing significant safety risks due to poor hygiene, inadequate storage, and lack of regulation. The Biogenic Theory underscores this paradox, showing how natural benefits can be undermined by human-induced contamination. The variability in quality highlights both the challenges and opportunities for improving herbal medicine safety in Ugep, emphasizing the urgent need for standardized practices to protect public health.

Recommendations

1. **Regulatory Enforcement:** The National Agency for Food and Drug Administration and Control (NAFDAC) should establish and enforce mandatory microbial and phytochemical standards for herbal mixtures, including regular market inspections and product certification (Ekor, 2014).
2. **Vendor Training:** Implement community-based training programs on Good Manufacturing Practices (GMP), focusing on hygienic processing, use of clean water, and proper storage techniques to reduce contamination risks (WHO, 2007).
3. **Quality Control Testing:** Establish local laboratories or mobile testing units in Ugep to conduct routine microbial and phytochemical analyses, ensuring vendors can verify product safety before sale.
4. **Public Awareness:** Launch educational campaigns to inform consumers about the risks of contaminated herbal mixtures and encourage demand for safer products, empowering community oversight.
5. **Best Practice Replication:** Identify and promote the methods of vendors producing safer mixtures (e.g., HM6, HM9), such as sealed packaging, as scalable models for others in the market.
6. **Research Expansion:** Conduct longitudinal studies to trace contamination sources (e.g., water, soil, or handling) and assess the stability of bioactive compounds under microbial stress, providing data for targeted interventions.

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