

ANTIBIOTIC SUSCEPTIBILITY PROFILES OF SELECTED BACTERIA ISOLATED FROM LOCALLY-PREPARED FEMALE APHRODISIACS SOLD IN DUTSE, JIGAWA STATE, NIGERIA

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ABSTRACT

"Kayan mata" are traditional herbal preparations used as aphrodisiacs by women in northern Nigeria. They are widely consumed despite a lack of regulation, linked to socio-cultural factors and perceived benefits for female sexual enhancement. These preparations could pose potential health risks due to microbial contamination. This study aimed to assess the phytochemical profiles, bacteriological quality, and antibiotic susceptibility patterns of bacterial isolates from "kayan mata" sold in Dutse, Jigawa State, Nigeria. Ten distinct samples were collected from five retail outlets, analyzed using standard methods for qualitative phytochemical screening, aerobic mesophilic bacterial counts (AMBC), coliform counts, and isolation/identification of *Escherichia coli*, *Pseudomonas aeruginosa*, *Salmonella* spp., and *Staphylococcus aureus*. Antibiotic susceptibility was determined via Kirby-Bauer disk diffusion. Phytochemical screening revealed variable profiles, with alkaloids and terpenoids most common. Significant microbial contamination was found: 60% of the samples exceeded WHO limits for AMBC (ranging 3.5×10^5 – 7.6×10^6 CFU/mL), and 40% had high coliform counts ($\geq 7.2 \times 10^5$ CFU/mL). Microbial contamination included 50.0% each of *E. coli* and *Salmonella* spp., *P. aeruginosa* was 30.0%, but *S. aureus* was absent. High levels of antibiotic resistance were observed, particularly against Augmentin and most fluoroquinolones, with *Salmonella* isolates showing significant multidrug resistance (MDR). The isolates were most susceptible to Streptomycin and Trimethoprim-Sulfamethoxazole. Samples of "kayan mata" sold in Dutse had high levels of bacterial contamination, with some exhibiting multidrug resistance, which could pose significant public health risks. These findings highlight an urgent need for quality control, public education, and regulation of traditional herbal products.

Keywords: Antimicrobial resistance, Public health, Traditional medicine, Microbial contamination, Antibiotic resistance

INTRODUCTION

Female sexual dysfunction (FSD), encompassing persistent, distressing issues with desire, arousal, orgasm, or pain, represents a significant yet often underdiagnosed public health concern impacting women's quality of life globally (Imam *et al.*, 2024). Epidemiological studies suggest a prevalence ranging from 30% to over 60% of FSD, with rates notably higher among women with cardiovascular risk factors or overt disease (Dike *et al.*, 2020). Despite this high prevalence, FSD is frequently overlooked in clinical settings due to challenges in definition, assessment, limited treatment options (like flibanserin and bremelanotide for hypoactive desire), clinician discomfort, and patient reluctance to discuss sexual health (Edo *et al.*, 2024). The complexity of female sexual response, influenced by a delicate interplay of vascular, neurological, hormonal, psychological, and interpersonal factors, further complicates diagnosis and management. Left unaddressed, FSD can contribute to depression, low self-image, and relationship conflicts (Dike *et al.*, 2020; Mohammed *et al.*, 2022).

In many cultures, traditional practices arise to address perceived sexual difficulties or enhance sexual experiences. In northern Nigeria, "kayan mata"; a Hausa word which literally "women's things", include a wide range of locally-prepared herbal powders, potions, and confections that are widely used by women as aphrodisiacs (Muhammad, 2022). These substances are thought to increase libido, lubrication, partner pleasure, or vaginal tightness (called 'dan matsi In Hausa'), particularly after childbirth or at older age (de Sousa Lima *et al.*, 2020). Traditional gender norms emphasizing male satisfaction and female responsibility for marital harmony, especially in polygynous contexts, drive some

women to use "kayan mata" to secure their husband's affection and maintain stability (Muhammad, 2022). Modern influences, including idealized portrayals of sexuality on social media and in pornography, may also contribute to performance pressures and unrealistic expectations, potentially fueling the demand for aphrodisiacs (Alabi, 2020). While some oral "kayan mata" preparations utilize fruits like dates and tiger nuts, potentially offering nutritional benefits, vaginal preparations raise significant concerns. These can involve inserting substances or using Sit baths with concoctions intended to tighten the vagina or enhance pleasure (Muhammad, 2022).

The widespread use of "kayan mata", particularly vaginal forms, poses considerable health risks primarily due to the complete lack of regulation and standardization in their production and sale. Ingredients, dosages, preparation methods, and potential side effects are typically undisclosed, leaving users vulnerable (Alabi, 2020). A critical concern is microbial contamination, arising from poor hygiene during preparation (e.g., contaminated water, unclean utensils, inadequate sanitation) and informal storage and sales channels. This is particularly alarming as symptoms of genitopelvic pain/penetration disorder (a type of FSD) can overlap with or be exacerbated by infections like urinary tract infections (UTIs), which are highly prevalent among women in Sub-Saharan Africa (Alabi, 2020). Furthermore, oral consumption of contaminated "kayan mata" risks enteric infections. Previous studies have confirmed the presence of pathogenic bacteria, including *Pseudomonas* spp., *Escherichia coli*, *Salmonella* spp., and *Staphylococcus aureus*, samples of "kayan mata" (Imam *et al.*, 2024). The proliferation of these bacteria, many of which exhibit

increasing antimicrobial resistance (AMR), represents a serious public health threat, contributing to the global AMR crisis, especially in resource-limited settings where treatment options are scarce (Imam et al., 2024).

This study aimed to conduct a bacteriological quality assessment of "kayan mata" preparations sold in Dutse, Jigawa State. Specifically, the study sought to isolate and identify pathogenic bacteria including *E. coli*, *Pseudomonas* spp., *Salmonella* spp., and *S. aureus* and evaluate their susceptibility patterns to commonly used antibiotics. This will provide crucial evidence regarding the microbial safety of these widely consumed products, inform consumers, traditional practitioners, and public health authorities to guide interventions and prevent the spread of antimicrobial resistance.

MATERIALS AND METHODS

The investigation was carried out in Dutse, Jigawa State, Nigeria (11.00° N, 8.00–10.10° E). The region has an annual rainfall of ~743 mm with mean daily temperature of ~30 °C (Durumin-Iya et al., 2023).

Collection of Samples

Ten (n = 10) distinct "kayan mata" preparations were purchased in April 2025 from five retail outlets in Dutse metropolis (Hakimi Market, Investment, Rafin sanyi, Yantifa, Modern Market). Two samples were obtained to capture variability in formulation and storage. Each sample was labeled, placed in sterile containers, and transported on ice to the Microbiology Laboratory, at the Department of Microbiology and Biotechnology, Federal University Dutse for immediate analysis.

Phytochemical Screening

Each sample underwent standard qualitative phytochemical assays for alkaloids (Dragendorff's reagent), flavonoids (Shinoda test), saponins (froth test), tannins (ferric chloride test), and glycosides (Bornträger's test) (Harborne, 1998). Results were recorded as present or absent.

Sample Preparation and Enumeration

For ready-to-drink formulations, 1 mL sample was added to 9 mL sterile normal saline to give 10⁻¹ dilution; vacuum-filtered through 0.45 µm membrane. The membrane with entrapped residues in two replica were placed on Nutrient Agar (for total aerobic count) and MacConkey Agar (for coliform count). For concentrated or powdered formulations, each was suspended per label directions, then serially diluted to 10⁻³ in sterile saline. From the 10⁻³ dilution, 0.1 mL aliquots were spread plated on selective media: EMB (for *E. coli*), SSA (for

Salmonella spp.), MSA (for *S. aureus*), Cetrimide Agar (for *P. aeruginosa*). Presumptive isolates were subjected to further biochemical tests as outlined by Cheesbrough (2006). All plates were incubated at 37 °C for 24 h. Colonies were counted when 30–300 colonies were present and expressed as CFU/mL.

Antibiotic Susceptibility Testing

Pure cultures were emulsified in sterile distilled water and adjusted to 0.5 McFarland standard. The Kirby–Bauer disk-diffusion method was performed on Mueller–Hinton Agar using antibiotic discs according to CLSI guidelines. Zones of inhibition were measured (mm) and interpreted as susceptible, intermediate, or resistant (CLSI, 2024). The antibiotic susceptibility testing was performed using commercially prepared Gram-negative antibiotic discs (AbtekBiologicals Ltd) containing Tarivid (OFX), Pefloxacin (PEF), Gentamicin (CN), Augmentin (AU), Ciprofloxacin (CPX), Septrin (SXT), Streptomycin (S), Chloramphenicol (CH), Sparfloxacin (SP) and Ampicillin (AM).

RESULTS AND DISCUSSION

Results

The phytochemical screening of the samples revealed diverse profiles of secondary metabolites across the ten samples. Alkaloids were detected in nine out of ten samples, with only Sample H testing negative. Flavonoids were present in seven samples (B, D, E, F, G, H, I), while Samples A, C, and J lacked this compound. Tannins were identified in six samples (B, D, E, F, G, H), and absent in the remaining four (A, C, I, J). Glycosides were found in five samples (A, B, D, H), with Samples C, E, F, G, I, and J showing negative results. Terpenoids were consistently present in all samples except Sample H. Steroids were detected in six samples (B, C, D, E, F, G), while Samples A, H, I, and J tested negative. Phenols were present in five samples (B, D, E, F, G), and absent in the other five (A, C, H, I, J). Proteins were detected in five samples (A, C, F, G, I), with the remaining five (B, D, E, H, J) showing no presence.

The mean aerobic mesophilic bacterial counts (AMBC) and coliform counts for the ten "kayan mata" samples (CFU/mL) are shown in Table 2. The AMBC ranged between 3.5 × 10⁵ (Sample G) and 7.6 × 10⁶ CFU/mL (Sample B), with six of the ten samples (A–E, H) exceeding the WHO limit of 1 × 10⁵ CFU/mL for oral herbal preparations; only Samples F, G, I and J met or fell below this threshold. Coliforms were undetectable in Samples A, E, G and I; very low (≤ 3 × 10⁰) in F and J; and markedly elevated (≥ 7.2 × 10⁵) in B, C, D and H each far above the WHO maximum of 10 CFU/g or coliforms in oral herbal materials.

Table 1: Phytochemical Compositions of Locally-Prepared Female Aphrodisiacs Sold in Dutse, Jigawa State

Sample Code	Alkaloid	Flavonoid	Tannins	Glycoside	Terpenoid	Steroids	Phenols	Proteins
A	+	-	-	+	+	-	-	+
B	+	+	+	+	+	+	+	-
C	+	-	-	-	+	+	-	+
D	-	+	+	+	+	+	+	-
E	+	+	+	-	+	+	+	-
F	+	+	+	-	+	+	+	+
G	+	+	+	-	+	+	+	+
H	-	+	+	+	+	-	+	+
I	+	+	+	-	+	-	-	+
J	+	-	-	-	+	-	-	-

Keys: Present (+), Absent (-)

Table 2: Mean Aerobic Mesophilic Bacterial and Coliform Counts of Samples

Sample Code	CFU/ml	
	AMBC	Coliform Count
A	3.9x10 ⁶	0
B	7.6x10 ⁶	8.3 × 10 ⁶
C	5.6x10 ⁶	4.7 × 10 ⁶
D	6.0x10 ⁵	7.2 × 10 ⁵
E	1.2x10 ⁶	0
F	4.2x10 ⁵	0.3 × 10 ¹
G	3.5x10 ⁵	0
H	6.0x10 ⁵	5.1 × 10 ⁵
I	4.9x10 ⁵	0
J	5.0x10 ⁵	0.2 × 10 ²

Keys: Aerobic Mesophilic Bacterial Count (AMBC).

Note: WHO Guidelines - AMBC ≤ 10⁵ CFU/g, Coliforms ≤ 10 CFU/g (WHO, 2007)

Table 3: Confirmed Isolates From the "kayan mata" Samples

Sample Code	Organism			
	<i>E. coli</i>	<i>P. aeruginosa</i>	<i>Salmonella</i>	<i>S. aureus</i>
Sample A	+	+	+	-
Sample B	-	-	+	-
Sample C	+	-	-	-
Sample D	-	-	+	-
Sample E	+	-	-	-
Sample F	-	-	+	-
Sample G	+	-	-	-
Sample H	-	+	+	-
Sample I	-	-	-	-
Sample J	+	+	-	-

Key: Present (+), Absent (-)

Across the ten "kayan mata" samples, five (A, C, E, G, J) yielded *E. coli*, three (A, H, J) had *P. aeruginosa*, and five (A, B, D, F, H) harboured *Salmonella*; no sample contained *Staphylococcus aureus*. Notably, Sample A was positive for all three Gram-negative pathogens, whereas Sample I showed no growth of any target organism.

The overall activity of the antibiotics varied markedly across organisms, samples, and drug classes. Ampicillin exhibited moderate efficacy, with 38% of all isolate-antibiotic tests classified as susceptible; these were confined exclusively to the five *E. coli* isolates, whereas the remaining Gram-negative organisms (*P. aeruginosa*, *Salmonella spp*) showed predominantly resistant or intermediate responses. Augmentin (AU) failed to inhibit any of the 13 isolates, yielding 100% resistance. Gentamicin (CN) produced a mixture of outcomes with 31% susceptible, 62 % intermediate, and a solitary resistant result—indicating partial retention of activity against all three species. Fluoroquinolones demonstrated uniformly poor performance: pefloxacin (PEF) was entirely ineffective (0% susceptible), and ofloxacin (OFX) inhibited only 23% of the isolates, with over half classified as resistant.

Streptomycin (S) and trimethoprim-sulfamethoxazole (SXT) were the most active agents, inhibiting 77% and 69% of tests, respectively. However, neither achieved universal coverage: streptomycin had three intermediate readings, while SXT was ineffective against four isolates. Sparfloxacin (SP) showed no susceptible responses and classified 69 % of isolates as resistant, with the remainder intermediate. Ciprofloxacin (CPX) yielded exclusively intermediate or resistant categories

(no susceptible), split roughly 46 % intermediate and 54 % resistant.

When examined by sample, *E. coli* from Sample A was susceptible to ampicillin, gentamicin, streptomycin, and SXT but resistant to all β-lactams and fluoroquinolones. Sample C's *E. coli* displayed susceptibility to amikacin, streptomycin, and SXT, with intermediate responses to gentamicin, sparfloxacin, and ciprofloxacin, and resistance to the remainder. Similar patterns held across other *E. coli* isolates (Samples E, G, J), each uniformly susceptible to ampicillin, streptomycin, and SXT, yet uniformly resistant to augmentin, pefloxacin, ofloxacin, and sparfloxacin; gentamicin and ciprofloxacin produced mixed intermediate results. *P. aeruginosa* from Samples A, H, and J were only reliably inhibited by streptomycin and SXT; gentamicin yielded intermediate zones in one or two isolates, while all other agents—ampicillin, augmentin, pefloxacin, ofloxacin, sparfloxacin, and ciprofloxacin—were resisted by most *P. aeruginosa* strains.

Salmonella isolates demonstrated the highest levels of multidrug resistance. Streptomycin inhibited three of five *Salmonella*, with one intermediate and one resistant reading; SXT was effective against only one isolate. All other antibiotics (ampicillin, augmentin, gentamicin, pefloxacin, ofloxacin, sparfloxacin, ciprofloxacin) exhibited resistance rates of at least 80% among *Salmonella* strains. Sample-level variation was minimal: each *Salmonella*-positive sample resisted the same core group of agents, with only sporadic intermediate responses to gentamicin and ciprofloxacin. The antibiotic susceptibility profiles are outlined in Table 4.

Table 4: Antibiotic Susceptibility Profiles of Bacteria Isolated from the "Kayan mata" Samples

Organism	Isolate Code	Antibiotics/Zones of inhibition in mm (CLSI 2024 Interpretation)								
		AM	AU	CN	PEF	OFX	S	SXT	SP	CPX
<i>E. coli</i>	EC-A	17 (S)	18 (R)	17 (S)	21 (R)	20 (R)	17 (S)	16 (S)	12(R)	13(R)
	EC-C	18 (S)	15 (R)	13 (I)	14 (R)	18 (R)	16 (S)	18 (S)	19(I)	17(I)
	EC-E	18 (S)	15 (R)	14 (I)	19 (R)	19 (R)	16 (S)	18 (S)	17(R)	17(I)
	EC-G	17 (S)	17 (R)	17 (S)	18 (R)	19 (R)	15 (S)	17 (S)	15(R)	15(R)
	EC-J	16 (S)	16 (R)	17 (S)	17 (R)	22 (I)	19 (S)	15 (S)	14(R)	16(I)
<i>P. aeruginosa</i>	PA-A	9 (R)	15 (R)	14 (I)	19 (R)	17 (R)	16 (S)	15 (S)	19(I)	18(I)
	PA-H	9 (R)	12 (R)	15 (I)	13 (R)	12 (R)	14 (I)	17 (S)	16(I)	12(R)
	PA-J	11 (R)	12 (R)	18 (S)	14 (R)	16 (R)	17 (S)	19 (S)	20(I)	12(R)
<i>Salmonella</i>	ST-A	14 (R)	11 (R)	11 (I)	16 (R)	15 (R)	17 (S)	15 (S)	13(R)	12(R)
	ST-B	13 (R)	12 (R)	11 (I)	13 (R)	19 (R)	17 (S)	14 (R)	15(R)	19(I)
	ST-D	13 (R)	11 (R)	9 (R)	12 (R)	17 (R)	13 (I)	13 (R)	16(R)	17(I)
	ST-F	15 (I)	11 (R)	14 (I)	15 (R)	21 (I)	17 (S)	14 (R)	18(R)	13(R)
	ST-H	11 (R)	10 (R)	12 (I)	11 (R)	21 (I)	9 (R)	13 (R)	12(R)	14(R)

Discussion

The analysis of ten "kayan mata" samples revealed significant variations in phytochemical composition and widespread microbiological contamination, raising critical public health concerns. While alkaloids and terpenoids were widely present (90% of samples), other phytochemicals like flavonoids, tannins, steroids, glycosides, phenols, and proteins showed more variable distribution (50-70%). This heterogeneity likely reflects differences in plant ingredients, preparation methods, geographical sourcing, and material handling (Sobeh *et al.*, 2019). This aligns with studies on Nigerian herbal aphrodisiac drinks which also reported the presence of alkaloids, flavonoids, saponins, cardenolides, tannins, and anthraquinones (Dike *et al.*, 2020, Ella *et al.*, 2019). Although some of these phytochemical classes have reported biological activities relevant to sexual function in other contexts (e.g., certain alkaloids affecting hormones, flavonoids influencing blood flow), their specific contributions and safety in "kayan mata" remain unproven due to the lack of standardization. This absence of consistent composition also hinders a clear understanding of potential therapeutic effects or risks, such as endocrine disruption (Ovuru & Okpolor, 2024).

A major finding was the alarming microbial contamination. Aerobic Mesophilic Bacterial Counts (AMBC) ranged from 3.5×10^5 to 7.6×10^6 CFU/mL, with six samples exceeding the 1×10^5 CFU/mL threshold and all ten vastly exceeding stricter limits like 102 CFU/mL for oral aqueous preparations (WHO, 2007). Coliform counts were significantly elevated ($\geq 7.2 \times 10^5$ CFU/mL) in four samples (B, C, D, H). Furthermore, specific bacterial pathogens were detected: *Escherichia coli* in 50% of samples, *Pseudomonas aeruginosa* in 30%, and *Salmonella* spp. in 50%. The co-contamination in Sample A (containing *E. coli*, *P. aeruginosa*, and *Salmonella*) highlights the potential for exposure to multiple harmful organisms. These findings are consistent with widespread reports of high bacterial loads and enteric pathogens in other African herbal medicines, often linked to poor hygiene, inadequate storage, and a lack of regulatory oversight (Ahiabor *et al.*, 2024; Zakari *et al.*, 2023).

Additionally, the bacterial isolates demonstrated concerning levels of antimicrobial resistance (AMR). *Salmonella* spp. exhibited high multidrug resistance (MDR), showing only partial susceptibility to Streptomycin (60%) and Trimethoprim-Sulfamethoxazole (20%), and $\geq 80\%$ resistance to most other tested antibiotics. *E. coli* isolates were uniformly resistant to Augmentin and most fluoroquinolones (Pefloxacin, Sparfloxacin, Ofloxacin), while *P. aeruginosa* showed widespread resistance or intermediate responses.

These patterns, particularly the high MDR in *Salmonella* and resistance to Augmentin and fluoroquinolones, mirror the escalating AMR crisis in West Africa and Nigeria (Diop *et al.*, 2025; Egbule *et al.*, 2024). This suggests that "kayan mata" products could act as reservoirs for drug-resistant pathogens, contributing to the dissemination of AMR within the community, likely driven by the misuse of antibiotics in the region (Mohammed *et al.*, 2022).

The high bacterial loads, presence of coliforms, and detection of pathogens like *Salmonella*, *E. coli*, and *P. aeruginosa* pose a direct risk of infectious diseases to consumers. The consumption of "kayan mata" containing MDR bacteria further serves as a vehicle for introducing these resistant strains, potentially leading to difficult-to-treat infections and exacerbating the broader AMR crisis (Walusansa *et al.*, 2022). While the diverse phytochemicals offer a plausible, though unproven, basis for traditional claims of enhancing female sexual function, these speculative benefits are significantly overshadowed by the clear and substantial risks from microbial contamination and high levels of antimicrobial resistance. The finding that 60% of samples exceeded AMBC limits and 50% contained *Salmonella* underscores serious safety lapses. Addressing these hazards urgently requires improved manufacturing and handling practices, stringent implementation and enforcement of regulatory standards, and increased consumer awareness regarding the dangers of unstandardized herbal preparations (Ahiabor *et al.*, 2024; Zakari *et al.*, 2023, Ndukwu *et al.*, 2021).

CONCLUSION

This study found that "kayan mata" products vary widely in their phytochemical makeup, which shows that there is no consistent standard in how they are made. Even more worrying is the level of microbial contamination we observed. Many samples contained dangerously high amounts of bacteria, including *E. coli*, *Pseudomonas aeruginosa*, and *Salmonella* species and often at levels considered unsafe for consumption. Importantly, some of these bacteria were resistant to multiple antibiotics, making potential infections harder to treat. These results highlight a real public health concern. Without proper regulation, improved hygiene in how these products are made and handled, and better awareness among the public, people could be exposed to serious health risks. Taking immediate action is essential to protect consumers and help prevent the spread of antibiotic resistance.

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