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Antimicrobial Efficacy of Selected Herbal Mixtures from Ibadan, Nigeria against Microorganisms Associated with Sexually Transmitted Infections: Phytochemical Analysis and *In Vitro* Assessment

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ABSTRACT

Introduction: The global rise of antimicrobial-resistant pathogens exacerbates challenges in treating sexually transmitted infections (STIs), driving the search for alternative therapies. In Ibadan, Nigeria, herbal mixtures are widely used for STIs without scientific validation. This study evaluated the *in vitro* antimicrobial efficacy of six commercial herbal mixtures against STI-associated pathogens and characterized their phytochemical profiles to assess their therapeutic potential. **Methods**: Six commercial herbal preparations (A–F) traditionally used for STIs were tested against *Candida albicans*, *Staphylococcus aureus*, *Escherichia coli*, and *Klebsiella pneumoniae* using agar well diffusion assays, with standard antimicrobials as controls. Qualitative and quantitative spectrophotometric and titrimetric assays characterized their primary phytochemical constituents. **Results**: Most mixtures showed no antimicrobial activity. Only Mixture A exhibited concentration-dependent activity exclusively against *E. coli*, with zones of inhibition increasing from 14.5 mm at 50% (*v/v*) to 19.0 mm at 100% (*v/v*). No mixture inhibited *C. albicans*, *K. pneumoniae*, or the multidrug-resistant *S. aureus*. Standard antibiotics remained highly active against most isolates. Phytochemical profiling detected tannins, saponins, alkaloids, flavonoids, terpenoids, glycosides, and steroids in varying concentrations, with flavonoids and tannins being most abundant, whereas chalcones were consistently below detection limits. **Conclusion**: The tested herbal mixtures demonstrated limited broad-spectrum efficacy, failing to validate manufacturers' claims. While current formulations are not promising alternative therapies, Mixture A warrants further research to isolate its active compounds, optimize formulations, and validate safety *in vivo*. These findings underscore the critical need for rigorous scientific evaluation and stronger regulatory oversight of traditional remedies.

INTRODUCTION

Sexually transmitted infections (STIs) constitute a major global health challenge, with approximately 1 million new infections acquired daily [1, 2]. The growing threat of antimicrobial resistance further underscores the urgent need for innovative prevention and treatment strategies. Notably, Sub-Saharan Africa is disproportionately affected, accounting for approximately 50% of the global STI burden, particularly regarding HIV transmission [1, 3]. Within this region, epidemiological studies in Nigeria have reported a prevalence of curable STIs of approximately 18% among low-risk populations and as high as 23% among sex workers [4]. Pathogenic bacteria and fungi are the major etiological agents of STIs. These pathogens can lead to severe and prolonged infections, substantial healthcare costs, increased morbidity and mortality, and the development of antimicrobial resistance [2, 5].

Additionally, antimicrobial resistance (AMR), a critical global health threat, is compounded by high-risk sexual behaviors that facilitate the transmission of drug-resistant pathogens causing STIs [6].

Controlling STIs presents significant challenges, particularly in low- and middle-income countries (LMICs) where healthcare infrastructure is often limited

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[1]. The often asymptomatic presentation of STIs further hinders control efforts and predisposes untreated individuals to serious complications and onward transmission [7]. In response to the persistent high prevalence of STIs and the escalating threat of AMR among STI-associated pathogens, local manufacturers have formulated various herbal preparations, rooted in traditional medicine practices, as potential alternatives to conventional antibiotics for STI management. Additionally, some manufacturers claim that their products not only treat STIs but also act as sexual performance-enhancing agents [8].

Phytochemicals are plant-derived secondary metabolites with established pharmacological activities. Studies have shown that phytochemicals possess a wide spectrum of biological activities, including antimicrobial, anti-inflammatory, and antioxidant properties, among others [9]. Given this therapeutic potential, Ibadan, one of the most populous cities in Nigeria, was selected as the study location because of the high prevalence of herbal preparation use among its large population. Specific herbal preparations were selected based on manufacturers' claims that they could enhance sexual performance and treat STIs. However, despite their widespread use and purported efficacy in Ibadan, there is a lack of empirical evidence validating their antimicrobial properties and phytochemical profiles. Rigorous scientific investigation is essential to validate these commercial claims and determine the potential of these herbal preparations as efficacious and safe therapeutic options.

Therefore, the specific objectives of this study were to: (i) characterize the phytochemical profiles and (ii) evaluate the *in vitro* antimicrobial efficacy of commonly marketed herbal mixtures from Ibadan. This assessment focused on clinically relevant microbial isolates associated with STIs, specifically *C. albicans*, *S. aureus*, *E. coli*, and *K. pneumoniae*. This study provides valuable scientific data on traditional herbal remedies for STIs and potentially informs the development of novel therapeutic strategies to combat the growing challenge of antimicrobial resistance in STI management.

MATERIAL AND METHODS

Source of microbial isolates and ethical approval. Clinical isolates of four microorganisms (*Candida albicans*, *Staphylococcus aureus*, *Escherichia coli*, and *Klebsiella pneumoniae*), originally isolated from high vaginal swabs, were sourced from the Microbiology Laboratory of University College Hospital (UCH), Ibadan, Nigeria. Prior to commencing the study, ethical approval was obtained from the Research Ethics Committee of Lead City University, Ibadan (Approval No. LCURE1245). All procedures involving the use of clinical isolates were conducted in accordance with institutional guidelines and ethical standards. As the study used archived isolates without patient identifiers, informed consent was waived by the ethics committee.

Phenotypic characterization and microbial identification. The microbial panel comprised *C. albicans*, *S. aureus*, *E. coli*, and *K. pneumoniae*. These stock cultures were preserved in glycerol at -20 °C and resuscitated using standard microbiological procedures. For resuscitation, each bacterial stock culture was streaked onto sterile Mueller-Hinton agar slants, while *C. albicans* was streaked onto Sabouraud Dextrose Agar (SDA). The bacterial cultures were incubated at 37°C for 18–24 h, while *C. albicans* was incubated at 35 ± 2 °C for 24–48 h. Microbial identity was confirmed using biochemical tests. For Gram-negative bacteria (*E. coli* and *K. pneumoniae*), the indole, methyl red, Voges-Proskauer, and citrate (IMViC) battery was employed. *S. aureus* was confirmed using the coagulase test, Gram staining, and characteristic growth on Mannitol Salt Agar (MSA). Gram-negative isolates were further differentiated by observation of characteristic growth patterns on Eosin Methylene Blue (EMB) agar and MacConkey agar. Additionally, each isolate was cultured on blood agar to assess hemolytic activity.

Sample collection and preparation of herbal mixtures. Six commercially available herbal preparations were procured from local markets in Ibadan, Nigeria. Prior to opening, the sealed containers of these preparations were externally decontaminated by wiping with 70% (*v/v*) ethanol. Under aseptic conditions, the preparations were transferred into sterile universal bottles. To preserve their chemical integrity, the preparations were stored at 4 °C until further use. All subsequent assays were conducted under sterile conditions within a Class II biological safety cabinet. For the antimicrobial assay, the liquid herbal preparations were evaluated both in their undiluted form (100% *v/v*) and at graded volume-by-volume concentrations (66.7%, 50%, and 40% *v/v*) prepared with sterile distilled water, allowing for the observation of concentration-dependent microbial inhibition. All dilutions were freshly prepared immediately prior to testing to ensure stability of active constituents. The reporting of volume-to-volume *(v/v)* dilution ratios adheres to established procedures for evaluating crude liquid herbal formulations in antimicrobial susceptibility testing [10].

Culture media preparation. MacConkey agar and Mueller Hinton agar were prepared according to the manufacturers' instructions [9]. For sterilization, the prepared media were autoclaved at 121 °C and 103 kPa (15 psi) for 15 min. After autoclaving, the media were allowed to cool to approximately 47–50 °C before being aseptically poured into sterile Petri dishes and solidified. The solidified agar plates were stored at 4 °C until use.

Evaluation of antimicrobial activity by agar well diffusion assay. The antimicrobial activity of each herbal preparation was quantitatively assessed using the agar well diffusion method. For inoculum preparation, pure cultures of each test organism were emulsified in sterile peptone water, and the turbidity was adjusted to match a

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0.5 McFarland standard. Mueller Hinton agar plates were prepared for bacterial testing. After solidification, each plate was uniformly inoculated by swabbing the entire surface with 0.1 mL of the standardized microbial suspension. The inoculated plates were then air-dried for approximately 15 min under sterile conditions. Five equidistant wells (6 mm diameter) were aseptically punched into each Mueller-Hinton agar plate using a sterile cork borer. Four wells were designated for testing the herbal preparations at concentrations of 100%, 66.7%, 50%, and 40% (*v/v*), respectively [10]. Each well was filled with 100 µL of the corresponding herbal preparation dilution. The fifth well, serving as a negative control, received 100 µL of sterile distilled water [11]. In accordance with Clinical and Laboratory Standards Institute (CLSI) disk diffusion guidelines, standard antimicrobial disks were applied to separate inoculated plates as positive controls. All disks were applied at their specified potencies. To facilitate diffusion of the herbal preparations, the plates were pre-incubated at room temperature for 30 min. Subsequently, the plates were incubated at 37 °C for 24 h for bacteria and at 35 ± 2 °C for 48 h for *C. albicans*. Following incubation, antimicrobial activity was quantified by measuring the diameter (in millimeters) of the clear zone of inhibition surrounding each well. All assays were performed in duplicate on separate occasions, with zone diameters measured in triplicate for each well, to ensure reproducibility [2]. Zone diameters were interpreted according to CLSI guidelines for antimicrobial susceptibility testing [12].

Antimicrobial susceptibility testing. The susceptibility of the bacterial isolates (*S. aureus*, *E. coli*, and *K. pneumoniae*) to a panel of conventional antibiotics was determined using the Kirby–Bauer disk diffusion method according to CLSI guidelines on freshly prepared Mueller–Hinton agar plates [2].

Prior to testing, isolates were subcultured from stock cultures onto appropriate media to confirm purity. Inocula were prepared as described previously (0.5 McFarland standard, approximately 1.5×10^8 CFU/mL) [13, 14].

The diameters of the zones of inhibition on the agar plates were measured and documented. These measurements were used to determine susceptibility profiles, based on the interpretive criteria of the CLSI guidelines [15]. The antibiotic disks used in this study included: pefloxacin (10 µg), amoxicillin (30 µg), ceftriaxone (25 µg), trimethoprim–sulfamethoxazole (30 µg), chloramphenicol (30 µg), ciprofloxacin (10 µg), sparfloxacin (10 µg), amoxicillin–clavulanate (30 µg), gentamicin (10 µg), cefuroxime (20 µg), erythromycin (10 µg), ampicillin–cloxacillin (30 µg), and ofloxacin (10 µg). The selection of these antibiotic disks and interpretation of inhibition zones were guided by CLSI guidelines [16].

Phytochemical screening of the herbal mixture. Comprehensive screening was performed to identify and quantify major phytochemical classes in the herbal preparations. Validated methods described in standard pharmacognosy and phytochemistry texts were employed for the qualitative and quantitative detection of secondary metabolites. The phytochemicals analyzed included tannins, saponins, alkaloids, flavonoids, steroids, terpenoids, glycosides, chalcones, and phenols. Qualitative and quantitative analyses were carried out using spectrophotometric and titrimetric techniques to determine the presence and concentrations of these compounds in the herbal mixtures [2]. Qualitative screening confirmed the presence or absence of each phytochemical class, while quantitative analysis determined their levels in the mixtures.

Qualitative phytochemical analyses. The accuracy of each assay was confirmed using commercially available reference standards as positive controls. The presence of key phytochemicals in the herbal preparations was determined by observing characteristic color changes or precipitate formation using the following standard procedures:

- **Tannins**: A 1 mL aliquot of each herbal preparation was boiled in 20 mL of distilled water for 5 min and filtered. The presence of tannins was indicated by the formation of a blue-black (hydrolyzable tannins) or green (condensed tannins) coloration immediately after adding 2–3 drops of 0.1% (*w/v*) ferric chloride ($FeCl_3$) solution to the filtrate.
- **Phlobatannins**: 2 mL of each herbal preparation were boiled in 10 mL of 1% (*v/v*) aqueous HCl solution for 2–5 min. The presence of phlobatannins was indicated by the formation of a red to crimson precipitate upon cooling.
- **Saponins**: Approximately 5 mL of each herbal preparation was boiled in 20 mL of distilled water for 5 min, cooled to room temperature, and filtered. A 10 mL aliquot of the filtrate was mixed with 5 mL of distilled water and vigorously shaken. The presence of saponins was indicated by a stable, persistent froth that emulsified upon addition of 3 drops (approximately 0.15 mL) of olive oil.
- **Flavonoids**: The presence of flavonoids was evaluated using two methods. First, using the aluminum chloride colorimetric test, 3 mL of 1% (*w/v*) $AlCl_3$ solution was added to 5 mL of the herbal preparation; the mixture was shaken and observed for the development of a yellow coloration within 5 min. Second, a separate 5 mL aliquot was treated with 1–2 mL of dilute (10%) ammonia solution followed by careful addition of 1 mL of concentrated H_2SO_4 along the tube wall; a positive result was indicated by an initial yellow coloration that faded upon standing for 2–5 min.
- **Steroids (Liebermann-Burchard Test)**: 2 mL of each herbal preparation were dissolved in 2 mL of chloroform and mixed with 2 mL of acetic anhydride, followed by careful addition of 2 mL of concentrated

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H_2SO_4 along the side of the tube. The mixture was allowed to stand at room temperature. The presence of steroids was indicated by the development of a blue-green coloration within 5–10 min.

- **Terpenoids (Salkowski Test)**: 2 mL of each herbal preparation were mixed with 2 mL of chloroform, and 3 mL of concentrated H_2SO_4 was carefully layered down the side of the test tube. The presence of terpenoids was indicated by a reddish-brown coloration at the interface within 2–5 min.
- **Cardiac Glycosides and Cardenolides (Keller-Kiliani Test)**: 5 mL of each herbal preparation were mixed with 2 mL of glacial acetic acid containing 1 drop (approximately 0.05 mL) of 1% (*w/v*) $FeCl_3$ solution. This mixture was then carefully layered with 1 mL of concentrated H_2SO_4. A positive test was indicated by the formation of a brown ring at the interface (characteristic of deoxysugars in cardenolides) and a violet ring in the upper acetic acid layer.
- **Alkaloids:** A 1 mL aliquot of each herbal preparation was mixed with 5 mL of 1% (*v/v*) aqueous HCl, heated on a steam bath at 40–60 °C for 3–5 min, and then filtered while hot. The filtrate was divided and treated with several alkaloid-detecting reagents. The presence of alkaloids was indicated by the formation of a precipitate: cream-colored with Mayer's reagent (potassium mercuric iodide), or reddish-brown with either Wagner's reagent (iodine in potassium iodide) or Dragendorff's reagent (potassium bismuth iodide).
- **Anthraquinones:** 5 mL of each herbal preparation were mixed with 10 mL of benzene and filtered. The filtrate was shaken with 5 mL of 10% (*w/v*) NH_3 solution. The presence of anthraquinones was indicated by the development of a pink, red, or violet color in the lower ammoniacal phase after the layers settled.
- **Chalcones**: 2 mL of 10% (*w/v*) ammonia solution were added to 5 mL of each herbal preparation. The presence of chalcones was indicated by the formation of a reddish tint.
- **Phenols:** 5 mL of each herbal preparation were mixed with 10 mL of distilled water, 2 mL of concentrated ammonium hydroxide solution, and 5 mL of concentrated amyl alcohol. After 30 min, the appearance of a blue-green tint indicated the presence of phenols.

Tannin quantification. For tannin quantification, 0.20 g of each herbal preparation was extracted with 20 mL of 50% (*v/v*) methanol by incubating in a covered beaker for 1 h at 80 °C in a water bath. The resulting extract was filtered through Whatman No. 41 filter paper into a 100 mL volumetric flask. Subsequently, 20 mL of distilled water, 2.5 mL of Folin-Denis reagent, and 10 mL of 17% (*w/v*) sodium carbonate (Na_2CO_3) solution were added, and the mixture was allowed to stand for 15–20 min for color development. A standard curve was prepared using tannic acid solutions (0–10 ppm), which were processed identically to the samples. The absorbance of all solutions was measured at 760 nm using a Spectronic 2D spectrophotometer, which was calibrated using the blank and tannic acid standards.

Calculation of Tannin content. The total tannin content was first determined as tannic acid equivalents (TAE) using the standard curve. The percentage of tannin (% *w/w*) in each herbal preparation was then calculated using the following equation:

% Tannin (*w/w*) = [(C × V × DF) / W] × 100

Where:

- C = Concentration of tannins in the diluted extract (mg/mL), determined from the linear regression equation of the tannic acid standard curve.
- V = Total volume of the extract (100 mL or 0.1 L).
- DF = Dilution factor used during the procedure.
- W = Initial weight of the herbal preparation sample (in mg).

Flavonoid quantification. For flavonoid quantification, 0.50 g of each herbal preparation was extracted with 80 mL of 95% (*v/v*) ethanol. The solution was filtered (Whatman No. 1), and the final volume of the extract was adjusted to 100 mL with ethanol. For flavonoid quantification, the total flavonoid content was determined using the aluminum chloride colorimetric method. Briefly, 1 mL of the extract was mixed with 1 mL of 2% (*w/v*) $AlCl_3$ in methanol. The mixture was incubated at room temperature for 10 min, and the absorbance of the resulting yellow complex was measured at 415 nm using a Jenway V6300 spectrophotometer. A standard curve was generated using quercetin (0–5 ppm), which were processed identically to the samples.

Calculation of Flavonoid content. The total flavonoid content was first determined as quercetin equivalents (QE) using the standard curve. The percentage of total flavonoid content (% *w/w*) in each herbal preparation was then calculated using the following equation:

% Flavonoids (*w/w*) = [(C × V) / W] × 100

Where:

- C = Concentration of flavonoids in the extract (mg/mL), interpolated from the linear regression equation of the standard curve.
- V = Total volume of the stock extract (100 mL).
- W = Initial weight of the sample (in mg).

Statistical Methods. All experiments were conducted in triplicate (n = 3 per group) unless otherwise stated; antimicrobial assays were performed in duplicate on separate occasions, with zone diameters measured in triplicate for each well, as described above. Quantitative phytochemical data were expressed as mean ± SD; for

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non-parametric data, median and interquartile range (IQR) were reported. Microbial counts were reported as colony-forming units per milliliter (CFU/mL). The diameters of zones of inhibition from antimicrobial susceptibility testing were also presented as mean ± SD.

Data were first assessed for normality using the Shapiro–Wilk test. Homogeneity of variance was evaluated using Levene's test. Where data met the assumptions of parametric analysis, one-way analysis of variance (ANOVA) was used to determine statistically significant differences among the herbal preparations. Tukey's HSD post hoc test was then applied to identify significant pairwise differences among group means.

For datasets that did not satisfy parametric assumptions even after log transformation (*e.g.,* where several values were below detection limits, treated as censored data), non-parametric analysis using the Kruskal–Wallis test was applied, followed by Dunn's post hoc test with Bonferroni correction.

The significance threshold was set at $P < 0.05$; 95% confidence intervals (CIs) were calculated where applicable. All statistical analyses were performed using IBM SPSS Statistics version 26.0 (IBM Corp., Armonk, NY, USA) and GraphPad Prism version 9.0 (GraphPad Software Inc., San Diego, CA, USA).

RESULTS

***In vitro* antimicrobial activity of herbal mixtures**. The six herbal preparations, three of which were registered with the National Agency for Food and Drug Administration and Control (NAFDAC) (Table 1), demonstrated minimal to no inhibitory effects against the tested microorganisms (*C. albicans*, *S. aureus*, *E. coli*, and *K. pneumoniae*). Of all the preparations, only the unregistered Mixture A showed antimicrobial activity, which was specific to *E. coli* (Tables 2–4). This activity was concentration-dependent, producing mean zones of inhibition of 19 mm, 17.5 mm, and 14.5 mm at concentrations of 100%, 66.7%, and 50% (*v/v*), respectively. No activity was observed at the lowest concentration of 40% (*v/v*). Furthermore, no other preparations showed activity against *C. albicans*, *S. aureus*, or *K. pneumoniae*.

Table 1. Characteristics of herbal medicinal products evaluated for antimicrobial activity

Mixture code	Product name	NAFDAC registration No.
A‡	Legend Std Mixture	NA*
B	Prebens General Disease Herbal Tonic	NA
C	Med-Bunch STD Eradication Flusher	A7-2124L
D	Bakaida Flusher	A7-4328L
E	Kambest STD Infection Flusher	A7-4829L
F	Weakness Private Parts Mixture	NA

Abbreviations: NA, Not available (product not registered with NAFDAC); NAFDAC, National Agency for Food and Drug Administration and Control (Nigeria); STD, sexually transmitted disease.
‡Mixture A was sourced from Ghana; all other mixtures were sourced from Nigeria.

Table 2. *In vitro* antimicrobial activity of registered herbal preparations against selected clinical isolates

Test organism	Mixture code	100% (*v/v*)	66.7% (*v/v*)	50% (*v/v*)	40% (*v/v*)
K. pneumoniae	C	R	R	R	R
	D	R	R	R	R
	E	R	R	R	R
C. albicans	C	R	R	R	R
	D	R	R	R	R
	E	R	R	R	R
E. coli	C	R	R	R	R
	D	R	R	R	R
	E	R	R	R	R
S. aureus	C	R	R	R	R
	D	R	R	R	R
	E	R	R	R	R

Abbreviations: R, Resistant (no zone of inhibition observed; zone diameter = 0 mm). **Note:** Zone diameters are expressed in millimeters (mm). All registered herbal preparations (Mixtures C, D, and E) showed no antimicrobial activity against any tested organism at all concentrations.

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Table 3. *In vitro* antimicrobial activity of unregistered herbal preparations against selected clinical isolates

Test organism	Mixture code	100% (*v/v*)	66.7% (*v/v*)	50% (*v/v*)	40% (*v/v*)
K. pneumoniae	A	R	R	R	R
	B	R	R	R	R
	F	R	R	R	R
C. albicans	A	R	R	R	R
	B	R	R	R	R
	F	R	R	R	R
E. coli	A	19.0 ± 0.5	17.5 ± 0.5	14.5 ± 0.5	R
	B	R	R	R	R
	F	R	R	R	R
S. aureus	A	R	R	R	R
	B	R	R	R	R
	F	R	R	R	R

*Data represent the mean diameter ± SD of the zone of inhibition (mm) from triplicate measurements. R, Resistant (no zone of inhibition observed; zone diameter = 0 mm). Zone diameters ≥14 mm indicate antimicrobial activity

Table 4. Summary of *In vitro* susceptibility patterns of clinical isolates to the herbal preparations

Test organism	Mixture(s) showing activity	Susceptibility pattern
K. pneumoniae	None	R (all mixtures)
C. albicans	None	R (all mixtures)
E. coli	A only	S at ≥50% (*v/v*)†
S. aureus	None	R (all mixtures)

Abbreviations: S, Sensitive (zone ≥14 mm); R, Resistant (no zone observed).
†Mixture A was the only preparation showing antimicrobial activity, and this was concentration-dependent and specific to *E. coli*.

Susceptibility of clinical isolates to conventional antibiotics. The susceptibility profiles of the clinical isolates to conventional antibiotics are presented in Table 5. Overall, the isolates showed variable susceptibility patterns to the tested antibiotics. *E. coli* was susceptible to pefloxacin, gentamicin, amoxicillin-clavulanate, chloramphenicol, ceftriaxone, sparfloxacin, and ciprofloxacin. *K. pneumoniae* was susceptible to ofloxacin, erythromycin, and ciprofloxacin. In contrast, *S. aureus* was resistant to all 13 antibiotics tested, suggesting possible multidrug resistance.

Antifungal susceptibility of *C. albicans*. The *C. albicans* isolate was susceptible to both antifungal agents tested, exhibiting mean zones of inhibition of 20 mm for ketoconazole and 25 mm for fluconazole (Table 6).

Table 5. *In vitro* susceptibility of clinical isolates to conventional antibiotics

Antibiotic (abbreviation)	Disk potency (µg)	Zone of inhibition (mm)*		
		E. coli	*K. pneumoniae*	*S. aureus*
Amoxicillin (AM)	30	-	-	-
Trimethoprim-Sulfamethoxazole (SXT)	30	-	-	-
Pefloxacin (PEF)	10	18	-	-
Cefuroxime (CXM)	20	-	-	-
Gentamicin (GN)	10	20	-	-
Ofloxacin (OFX)	10	-	14	-
Erythromycin (E)	10	-	17	-
Ampicillin-Cloxacillin (APX)	30	8	-	-
Amoxicillin-Clavulanate (AMC)	30	15	-	-
Chloramphenicol (CH)	30	17	8	-
Ceftriaxone (CRO)	25	16	-	-
Sparfloxacin (SP)	10	20	-	-
Ciprofloxacin (CPX)	10	19	11	-

*Data are presented as the mean zone of inhibition diameter (mm). A dash (-) indicates no zone of inhibition observed. The *S. aureus* isolate was tested against all listed antibiotics and showed no zone of inhibition (0 mm) for any of them.

Table 6. *In vitro* susceptibility of the *C. albicans* isolate to standard antifungal agents

Antifungal agent	Disk potency (µg)	Zone of inhibition (mm)‡	Interpretation
Ketoconazole	10†	20.0 ± 0.5	S
Fluconazole	10	25.0 ± 0.5	S

‡Data are presented as mean diameter ± SD from triplicate measurements. †Ketoconazole is not included in current CLSI antifungal guidelines; disk potency as provided by manufacturer. ***Abbreviations*:** S, susceptible. Fluconazole interpretation based on CLSI breakpoints (susceptible: ≥19 mm).

Microbial load of herbal preparations. Microbiological analysis revealed microbial contamination in all herbal preparations tested (Tables 7 and 8). The identified contaminants included *Bacillus*

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spp., *S. aureus*, *Pseudomonas aeruginosa*, *E. coli*, and *Salmonella* spp. Mixture E showed the greatest microbial contamination, with a total count of 12 CFU/mL, whereas Mixtures A and D had the lowest counts at 2 CFU/mL each.

Phytochemical analysis. The qualitative phytochemical profiles of the herbal preparations revealed the presence of several major secondary metabolite classes (Table 9). Tannins, saponins, alkaloids, flavonoids, steroids, and phenols were detected in all six preparations. In contrast, other phytochemical classes showed variable distribution across the samples. For example, glycosides were detected in some preparations (Mixtures A, B, E, and F) but were absent in others (Mixtures C and D), while terpenoids were found only in Mixtures A, E, and F. Chalcones were not detected in any preparation.

Table 7. Microbial load (CFU/mL) of registered herbal preparations

Microorganism	Mixture C	Mixture D	Mixture E
Bacillus spp.	3.0 ± 0.6	1.0 ± 0.6	3.0 ± 0.6
S. aureus	2.0 ± 0.7	1.0 ± 0.8	7.0 ± 0.7
P. aeruginosa	1.0 ± 0.6	0.0 ± 0.0	0.0 ± 0.0
E. coli	2.0 ± 0.8	0.0 ± 0.0	1.0 ± 0.6
Salmonella spp.	1.0 ± 0.9	0.0 ± 0.0	1.0 ± 0.9
Fungi	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0
Total microbial count	9	2	12

Note: Values are expressed as mean ± standard deviation from three independent experiments (n = 3). Zero values indicate no detectable microbial growth. Total microbial counts are presented as absolute values.

Table 8. Microbial load (CFU/mL) of unregistered herbal preparations

Microorganism	Mixture A	Mixture B	Mixture F
Bacillus spp.	0.0 ± 0.0	1.0 ± 0.6	3.0 ± 0.6
S. aureus	2.0 ± 0.6	0.0 ± 0.0	1.0 ± 0.6
P. aeruginosa	0.0 ± 0.0	0.0 ± 0.0	1.0 ± 0.8
E. coli	0.0 ± 0.0	2.0 ± 0.7	2.0 ± 0.6
Salmonella spp.	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0
Fungi	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0
Total microbial count	2	3	7

Note: Values are expressed as mean ± standard deviation from three independent experiments (n = 3). Zero values indicate absence of detectable microbial growth. Total microbial counts are presented as absolute values.

Table 9. Qualitative phytochemical profile of the herbal preparations

Mixture	Steroids	Tannins	Saponins	Alkaloids	Flavonoids	Terpenoids	Glycosides	Chalcones	Phenols
A	++	+	+	++	+	+	+	-	+
B	+	+	++	++	++	-	++	-	+
C	+	+++	++	++	+	-	-	-	+
D	+	++	++	++	+	-	-	-	+
E	++	++	++	++	+	+	+	-	+
F	+	+	++	++	+	+	++	-	+

Note: +++, high concentration; ++, moderate concentration; +, low concentration; –, not detected.

The quantitative phytochemical analysis revealed notable variations in the concentrations of the detected compounds across the different herbal preparations (Table 10). For example, flavonoid content was highest in Mixture B (0.525 mg/g), while saponin content was most abundant in Mixture F (0.321 mg/g). In contrast, alkaloids and terpenoids were generally present at lower concentrations across most preparations, with chalcones falling below the detection limit in all samples.

Table 10. Quantitative phytochemical composition (mg/g of liquid preparation of herbal mixture)

Mixture	Tannins	Saponins	Alkaloids	Flavonoids	Terpenoids	Glycosides	Chalcones	Phenols	Steroids
A	0.145 ± 0.007	0.013 ± 0.002	0.041 ± 0.003	0.207 ± 0.012	0.032 ± 0.004	0.022 ± 0.002	BDL	0.084 ± 0.006	0.027 ± 0.003
B	0.142 ± 0.006	0.143 ± 0.010	0.064 ± 0.004	0.525 ± 0.035	BDL	0.500 ± 0.003	BDL	0.042 ± 0.003	0.030 ± 0.003
C	0.345 ± 0.020	0.194 ± 0.012	0.051 ± 0.004	0.402 ± 0.025	BDL	BDL	BDL	0.047 ± 0.004	0.021 ± 0.002
D	0.223 ± 0.013	0.154 ± 0.009	0.043 ± 0.003	0.374 ± 0.022	BDL	BDL	BDL	0.037 ± 0.003	0.014 ± 0.002
E	0.250 ± 0.015	0.143 ± 0.010	0.031 ± 0.003	0.308 ± 0.018	0.030 ± 0.003	0.019 ± 0.002	BDL	0.022 ± 0.002	0.029 ± 0.003
F	0.156 ± 0.009	0.321 ± 0.020	0.014 ± 0.002	0.420 ± 0.025	0.041 ± 0.004	0.034 ± 0.003	BDL	0.069 ± 0.005	0.015 ± 0.002

† All values are presented as mean ± SD (n = 3). BDL, below detection limit.

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DISCUSSION

In Nigeria, herbal preparations are widely accessible to the public [17]. Widespread consumption of these products is driven by a combination of cultural practices and manufacturers' claims of efficacy against microorganisms associated with STIs [18]. This study therefore aimed to validate these claims by procuring and analyzing six commercially available products. Specifically, Mixture A originated from Ghana, while the remaining five were Nigerian products. Notably, three of the Nigerian products (Mixtures C, D, and E) were registered with NAFDAC, which suggests they were deemed suitable for consumption. Conversely, the remaining three products (Mixtures A, B, and F) were sold without NAFDAC approval. This observation aligns with findings from previous research, where only five out of nine sampled herbal products had obtained NAFDAC registration [18]. The sale of unregistered products raises concerns regarding regulatory compliance and adherence to quality control standards. Consequently, this highlights the need for regulatory bodies such as NAFDAC to strengthen their efforts to ensure that all herbal preparations on the market are either appropriately registered or removed from sale.

The *in vitro* antimicrobial activity of the herbal preparations was evaluated against the selected microbial isolates. Overall, the majority of the tested microorganisms, including *K. pneumoniae*, *C. albicans*, and *S. aureus*, were not inhibited by the herbal preparations. A notable exception was Mixture A, which demonstrated efficacy against *E. coli* at concentrations of 100%, 66.7%, and 50% (*v/v*). Notably, the zone of inhibition produced by Mixture A at its highest concentration (19 mm) was comparable to that of the standard antibiotic gentamicin (20 mm), indicating preliminary *in vitro* activity against *E. coli*. Furthermore, the complete resistance of *S. aureus* to all tested herbal preparations underscores the therapeutic challenge posed by this pathogen in STI management.

These findings largely contradict the manufacturers' claims of broad-spectrum antimicrobial activity for their herbal preparations. Conversely, these results are partially consistent with studies conducted by other researchers, where extracts from *Aristolochia bracteolata* L., *Ageratum conyzoides* L., *Calliandra portoricensis* (Jacq.) Benth., *Curculigo pilosa* (Schum. & Thonn.) Engl., *Gladiolus dalenii* van Geel, and *Securidaca longepedunculata* demonstrated efficacy against *E. coli* and other microorganisms implicated in STIs [16]. A study showed that the aqueous extract of *Cymbopogon citratus* leaf demonstrated antimicrobial activity against several microorganisms linked to genital infections and male infertility [19]. This comparison suggests that while some plant extracts hold antimicrobial promise, claims of efficacy cannot be generalized. Therefore, rigorous *in vitro* and *in vivo* investigations, coupled with comprehensive microbial analysis, are crucial for identifying promising extracts with therapeutic potential.

To contextualize the herbal preparations' activity, the susceptibility of the clinical isolates to conventional antibiotics was also evaluated to provide a benchmark. These findings underscore the ongoing challenges of antimicrobial resistance. While conventional antimicrobials like gentamicin and pefloxacin were effective against *E. coli*, the isolates of *K. pneumoniae* and *S. aureus* showed varied susceptibility patterns to the tested antibiotics (Table 5). Therefore, the broad-spectrum anti-STI claims made by the manufacturers could not be substantiated by this study. The limited efficacy, with only Mixture A showing activity against *E. coli*, suggests that the specific formulations tested have minimal therapeutic potential as currently prepared.

This study also determined the microbial load of both registered (Mixtures C, D, and E) and unregistered (Mixtures A, B, and F) liquid herbal preparations. All six herbal preparations were found to be contaminated with bacteria, with total viable counts ranging from 2 to 12 CFU/mL, indicating low-level contamination (Tables 7 and 8). The most frequently isolated contaminants were *S. aureus* and *Bacillus* spp., which were detected in four of the six preparations (67%). Other contaminants identified included *E. coli*, *P. aeruginosa*, and *Salmonella* spp., while no fungal contaminants were found. The presence of microbial contaminants in herbal preparations is a widely documented concern [20], and other studies have reported similar findings, including parasitic and heavy metal contamination [21]. These findings are consistent with previous reports. For example, studies in Nigeria have identified *Bacillus* spp., *E. coli*, *S. aureus*, and *Enterobacter* spp. in herbal formulations for typhoid fever [22]. Furthermore, fungal isolates such as *Aspergillus niger*, *Penicillium* spp., *Scedosporium* spp., and *Phialophora parasiticum* have also been reported in similar herbal products [22].

Notably, none of the evaluated registered products showed antibacterial activity against any of the clinical isolates, although registered herbal products are expected to adhere to safety and efficacy guidelines. On the other hand, unregistered Mixture A showed notable activity against *E. coli*, whereas the three NAFDAC-registered products were inactive. This disparity highlights that registration does not guarantee therapeutic efficacy and underscores the need for post-market surveillance and rigorous scientific validation of all herbal products, regardless of their regulatory status.

The presence of microbial contaminants in the herbal preparations analyzed in this study raises significant concerns regarding their microbial safety and quality control, likely stemming from inadequate handling and manufacturing practices. This finding also questions the efficacy of the regulatory oversight that permitted these products to enter the market. Consequently, these results highlight a critical need for regulatory agencies to

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implement more stringent quality control standards to prevent such contamination throughout the production and distribution chain of these herbal preparations.

All herbal preparations were distributed in plastic bottles, and inadequate handling of these containers during manufacturing may have contributed to contamination [23]. The phytochemical analysis † All values are presented as mean ± SD (n = 3). BDL, below detection limit.

confirmed the presence of key bioactive classes in all preparations. Notably, Mixture A, the only active preparation, contained terpenoids, which were absent in four of the five inactive preparations. It is plausible that terpenoids, either alone or in synergy with other detected compounds such as flavonoids and alkaloids, contributed to the observed activity against *E. coli*. Many of these phytochemicals are known to exhibit antimicrobial or synergistic effects [24, 25]. However, the general lack of efficacy observed in most preparations suggests that the concentrations of active compounds may have been insufficient to inhibit microbial growth under the tested conditions. Their presence in herbal preparations consumed across Nigeria has been extensively documented [26, 27]. This observation may also explain the observed variability between registered and unregistered products, highlighting potential inconsistencies in ingredient quality and manufacturing processes that warrant stricter regulatory oversight [28].

Further research is warranted to identify the specific bioactive compounds in Mixture A responsible for the observed activity and to explore potential synergistic interactions. Furthermore, *in vivo* studies are essential to comprehensively evaluate the safety and efficacy of these herbal preparations. Ultimately, this work underscores the importance of integrating traditional herbal medicine with modern medical practices to develop alternative therapeutic strategies for STIs. Achieving this requires effective collaboration among traditional practitioners, the pharmaceutical industry, and regulatory agencies to guarantee the safety, efficacy, and consistent quality of herbal products utilized in the management of STIs [10].

Comparing the activity of Mixture A to that of standard antibiotics provides context for its potential as an alternative or complementary therapy. Although the present findings do not strongly support the tested preparations' efficacy, herbal preparations theoretically offer the advantage of chemical complexity, potentially enabling synergistic interactions against multiple microbial targets in the management of complex infections. Further isolation and characterization of bioactive compounds from promising preparations like Mixture A could lead to the discovery of novel therapeutic agents and help establish standardized quality control measures for herbal medicine.

Despite these insights, this study has several limitations. Notably, it did not correlate specific bioactive compounds with antimicrobial activity. Furthermore, the reliance on *in vitro* assays, rather than *in vivo* studies, limits the translational applicability of the findings. Methodologically, the study was limited to agar diffusion and did not determine minimum inhibitory concentrations (MICs) using broth dilution, which would have provided more quantitative data on potency. Future research should prioritize *in vivo* studies, clinical trials, and pharmacokinetic investigations to validate efficacy and safety, as well as to address potential adverse effects and optimize dosage regimens. Additionally, the small sample size (six herbal preparations and four microbial isolates) limits the generalizability of the findings, and a larger sample would be needed to draw more robust conclusions.

In conclusion, this study evaluated the antimicrobial potential of six commercially available herbal preparations from Ibadan, Nigeria, against key STI-associated pathogens. The findings demonstrate a significant lack of broad-spectrum antimicrobial activity in the tested products, contradicting the manufacturers' claims of efficacy. Only one preparation, Mixture A, showed notable activity, and this was limited to *E. coli*. The activity of Mixture A is likely linked to its unique phytochemical profile, underscoring the importance of such analysis. Therefore, while traditional remedies may hold potential, this study highlights that rigorous scientific validation is essential. Future collaborative research between traditional practitioners, scientists, and regulatory bodies is crucial for developing safe and effective integrative therapies for STIs.

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CONFLICTS OF INTEREST

The authors declare that there are no conflicts of interest associated with this manuscript.

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AI DISCLOSURE

No artificial intelligence tools were used during manuscript preparation. All scientific content, data interpretation, and conclusions were developed independently by the authors, who assume full responsibility for the accuracy and integrity of the research.

DATA AVAILABILITY

Data supporting this study are available from the corresponding author upon reasonable request.

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AUTHORS' CONTRIBUTIONS

NAE: Conceptualization, Methodology, Investigation, Formal analysis, Writing – original draft, Writing – review & editing, Project administration. FCA: Supervision, Validation, Resources, Writing – review & editing. ETU: Conceptualization, Methodology, Formal analysis, Writing – review & editing. OAO: Investigation, Data curation, Writing – review & editing. All authors † All values are presented as mean ± SD (n = 3). BDL, below detection limit.

have read and agreed to the published version of the manuscript.

ETHICS STATEMENT

Ethical approval for this study was obtained from the Research Ethics Committee of Lead City University (Approval No.: LCURE1245). All procedures were conducted in accordance with institutional and national research guidelines.

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