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Research Article

Bacteriological Quality and Antibiotic Resistance Profile of Liquid Herbal Concoctions Sold in Ado-Ekiti, Nigeria

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Abstract

Introduction: Herbal medicine is an integral part of traditional healthcare systems around the world. It is still commonly used to treat various illnesses, especially in developing countries. In Nigeria, many people use herbal products. However, most of these herbal formulations do not have proper regulation or microbiological quality control, which raises serious public health issues. This study aimed to assess the bacterial quality and antibiotic resistance of liquid herbal mixtures sold in Ado-Ekiti, Nigeria.

Methods: A total of thirty (30) liquid herbal concoction samples were collected using the convenience sampling method from herbal vendors within Ado local government area in Ado-Ekiti, Ekiti State, Nigeria. The samples represented five locally used herbal formulations, each purportedly used for specific ailments.

These included: T (Typhoid concoction), I (IBA concoction), IR (Inurirun concoction), J (Jedi concoction), and A (Arariro concoction), with six samples collected per category and analyzed them using standard microbiological methods. This included the Most Probable Number (MPN) method, colony counts, and biochemical tests. We evaluated antibiotic susceptibility using the disc diffusion method according to CLSI guidelines. Results showed that 27 samples (90%) were contaminated with bacteria, with counts exceeding 10^5 CFU/mL. Of these, 19 (63.3%) exhibited bacterial loads exceeding 1 MPN/100 mL, while 4 samples each (13.3%) recorded 1 MPN/100 mL and 2 MPN/100 mL. The most prevalent bacterial isolates were *Salmonella typhimurium* (25.9%), *Klebsiella pneumoniae* (22.2%), *Staphylococcus aureus*, and *Escherichia coli* (18.5% each). Alarmingly, 88.9% of isolates displayed multidrug resistance (MDR), with Proteus vulgaris exhibiting 100% resistance to seven antibiotics. The Multiple Antibiotic Resistance (MAR) index ranged from 0.41 to 1.0, indicating high-risk contamination sources.

Conclusion: These findings underscore the urgent need for improved regulation, routine microbiological screening, and public education on the safety of herbal medicines. Strengthening quality control measures is crucial to protect consumers and prevent the spread of antibiotic-resistant bacteria through traditional medicinal products.

Key words: Herbal concoctions; Bacterial contamination; Multidrug resistance; mar index; Nigeria

1.Introduction

Herbal medicine, also known as phytotherapy or botanical medicine, has long been an integral part of traditional healthcare systems across many nations. For centuries, local communities have relied on the therapeutic potential of various plant parts including roots, leaves, seeds, bark and flowers to manage ailments. These remedies, developed through indigenous knowledge systems and passed down through generations, remain vital in meeting the primary healthcare needs of many populations. The evolution of

herbal medicine is closely linked with cultural practices, environmental factors and historical developments unique to each region [1, 2].

Archaeological findings suggest the use of medicinal plants dates back to the Paleolithic era, approximately 60,000 years ago. Furthermore, early documentation of herbal practices can be traced to ancient civilizations in Egypt, China, Greece, and India [3]. In recognition of its global relevance, the World Health Organization [4] has emphasized the importance of integrating traditional medicine into modern healthcare systems, promoting the safe and effective use of herbal therapies through rigorous guidelines and quality assessments. In Nigeria, herbal medicines continue to play a significant role in the management of common diseases such as malaria, typhoid fever, diarrhea, hypertension, and various skin infections. Despite their widespread use, regulatory oversight remains minimal. Many herbal preparations are produced without standardized protocols or microbiological testing, raising concerns about product safety and efficacy [5]. The lack of formal training among producers further exacerbates risks associated with contamination and inconsistent dosing. Given the reliance of a significant proportion of Nigeria's population on traditional remedies, ensuring the microbiological safety of herbal products is of paramount importance. This study was conducted to assess the bacteriological quality and antibiotic resistance profiles of liquid herbal concoctions sold in Ado-Ekiti, Ekiti State, Nigeria.

2.0 Materials and Methods

2.1. Study Area

The study was conducted in Ado Ekiti, the capital city of Ekiti State, Nigeria. Ado Ekiti is an ancient city with a rich cultural heritage, located in the southwestern region of Nigeria.

2.2. Sample Collection

A total of thirty (30) liquid herbal concoction samples were collected using the convenience sampling method from herbal vendors within Ado local government area in Ado-Ekiti, Ekiti State, Nigeria. The samples represented five locally used herbal formulations, each purportedly used for specific ailments. These included: T (Typhoid concoction), I (IBA concoction), IR (Inurirun concoction), J (Jedi concoction), and A (Arariro concoction), with six samples collected per category. All samples were aseptically collected into sterile universal sample bottles and labeled accordingly. The samples were transported under appropriate conditions to the Microbiology Laboratory, Ekiti State University, Ado-Ekiti, for microbiological analysis

2.3 Microbiological Analysis

2.3.1 bacterial Isolation

Bacterial colonies were initially isolated using MacConkey broth and subsequently sub-cultured onto MacConkey agar plates. Plates exhibiting bacterial growth were selected for further analysis. Distinct colonies were then identified and sub-cultured onto both Eosin Methylene Blue (EMB) agar and MacConkey agar using the streak plate method under aseptic conditions. The sub-cultured plates were incubated aerobically at 35°C for 24 hours. After incubation, a well-isolated colony from each plate was selected for further examination.

2.3.2Identification of Bacterial Isolates

The bacterial isolates obtained from the samples were characterized using a combination of colonial, morphological, and biochemical analyses. The characterization process was based on standard microbiological techniques as described by Chesbrough [6].

2.3.3 Determination of Bacterial Count Using the Most Probable Number (MPN) Method

The total bacterial count of the herbal concoction samples was determined using the most probable number (MPN) method.

Preparation of MacConkey Broth

Double-strength MacConkey broth was prepared according to the manufacturer's instructions. The broth was dispensed into sterile bottles in 10 mL and 50 mL volumes. To ensure sterility, the bottles were autoclaved at 121°C for 15 minutes.

Sample Preparation and Incubation

10 mL and 50 mL of the herbal concoction samples were dispensed into the prepared MacConkey broth bottles. Durham tubes were inserted into each bottle to collect gas produced by bacterial fermentation. The bottles were incubated at 37°C for 24 hours. During this period, the tubes were observed for signs of effervescence (gas production) and color change, indicating bacterial growth.

Interpretation

The number of positive tubes showing gas production and/or color change was recorded and the MPN value was calculated using a statistical table and expressed as colony-forming units (CFU) per milliliter of sample.

2.3.3 Determination of Total Bacterial Count

The drop plate method was used to enumerate the total bacterial count. Serial dilutions of the herbal concoction samples were prepared using sterile phosphate-buffered saline (PBS). 10 µL of each dilution was pipetted onto a sterile agar plate and spread evenly using a sterile spreader. The plates were incubated at 37°C for 24-48 hours. Colonies were counted using a colony counter, and the total bacterial count was calculated using the following formula: Total Bacterial Count (CFU/mL) = (Number of colonies × Dilution factor) / Volume of sample plated

2.4Antibiotic Susceptibility Testing

Antibiotic susceptibility of all bacterial isolates was determined using the standard disc diffusion method, in accordance with Clinical and Laboratory Standards Institute (CLSI) guidelines [7]. Nine commercially available antibiotic discs were tested: Amikacin (30 µg), Ceftriaxone (30 µg), Cefoperazone (30 µg), Ciprofloxacin (5 µg), Cotrimoxazole (25 µg), Gentamicin (10 µg), Meropenem (30 µg), Cefuroxime (30 µg), and Tetracycline (30 µg). Mueller-Hinton agar (Oxoid, UK) was prepared following the manufacturer's instructions and poured into sterile Petri dishes. Pure colonies of Escherichia coli were transferred aseptically into 5 mL of nutrient broth and incubated at 35°C. The turbidity of the inoculum was adjusted to match the 0.5 McFarland standard. Using the pour plate method, Mueller-Hinton agar plates were inoculated uniformly with the bacterial suspension. Antibiotic discs were applied to the agar surface using a multidisc dispenser (Oxoid, Basingstoke, UK). Plates were incubated at 37°C for 24 hours in an inverted position. Controls were included using E. coli ATCC 25922. Following incubation, zones of inhibition around each disc were measured in millimeters. The results were interpreted as susceptible, intermediate, or resistant based on CLSI 2020 interpretive criteria [7].

3.0. Results

Out of the 30 herbal concoction samples analyzed, 27 (90%) demonstrated bacterial contamination, with counts exceeding 105 CFU/mL, while only 3 samples (10%) showed no detectable bacterial presence. Among the contaminated samples, 19 (63.3%) exhibited bacterial loads greater than 1 MPN/100 mL. Additionally, 4 samples (13.3%) had a bacterial load of 1 MPN/100 mL, and another 4 samples (13.3%) recorded 2 MPN/100 mL. Importantly, all contaminated herbal concoctions exhibited bacterial growth within the range of 1–2 MPN/100 mL, as presented in Table 1. The bacterial isolates identified are presented in Table 2. Salmonella typhimurium was the most frequently isolated organism, detected in 7 samples (25.9%), followed by Klebsiella pneumoniae with 6 isolates (22.2%). Staphylococcus aureus and Escherichia coli were each identified in 5 samples (18.5%). Proteus vulgaris was the least frequently isolated bacterium, found in 4 samples (14.8%). The distribution of bacterial isolates across the various herbal concoction types is summarized in Table 3. Salmonella typhimurium was predominantly found in Jedi (50%) and Inu Rirun (33.3%) concoctions. Staphylococcus aureus appeared most frequently in the Typhoid and Arariro concoctions (33.3% each), while Proteus vulgaris was most common in Typhoid concoctions (33.3%). The antibiotic susceptibility patterns of the isolates are summarized in Figure 1. amikacin, meropenem, and gentamicin. Furthermore, resistance to cefuroxime and chloramphenicol was observed in 85% of the isolates, while ceftriaxone and cefoxitin showed resistance rates of 74% each. The lowest level of resistance was observed for gentamicin (40%). Proteus vulgaris demonstrated the highest level of multidrug resistance, with 100% resistance to seven antibiotics: ceftriaxone, tetracycline, cotrimoxazole, cefuroxime, chloramphenicol, amikacin and meropenem. Similarly, Salmonella typhimurium exhibited 100% resistance to four antibiotics: cotrimoxazole, amikacin, and meropenem. Of the 27 bacterial isolates, 24 (88.9%) exhibited multiple antibiotic resistance (resistant to ≥5 antibiotics). The lowest recorded Multiple Antibiotic Resistance (MAR) index was 0.41, observed in Salmonella typhimurium isolated from the Arariro concoction. However, isolates of Salmonella typhimurium, Escherichia coli, Klebsiella pneumoniae, and Proteus vulgaris showed a MAR index of 1.0, indicating resistance to all antibiotics tested, as presented in Table 4.

SAMPLES						
Bacterial Counts	Typhoid Concoction	Iba concoction	Inu Rirun concoction	Jedi concoction	Ara Riro Concoction	Total
0(-)(MPN/100mls)	0	1	0	2	0	3 (9.4%)
< 1(MPN/100mls)	4	3		3	6	19 (16.3%)
1(MPN/100mls)	1	1	2	0	0	4 (13.3%)
2(MPN/100mls)	1	1	1	1	0	4 (13.3%)
TOTAL	6	6	6	6	6	30(60%)

Table 1: Percentage of Bacteria Load in Herbal Concoction (MPN/100mls).

Name of isolates	Number of strains isolated
Staphylococcus aureus	5(18.5%)
Escherichia coli	5(18.5%)
Proteus vulgaris	4(18.8%)
Salmonella typhirium	7(25.9%)
Klebsiella pneumoniae	6(22.2%)
TOTAL	27

Table 2: Bacterial Profile from Hawked Herbal Concoctions in Ado-Ekiti.

ORGANISMS	SAMPLES				
	T	I	IR	J	A
Staphylococcus aureus	2(33.3%)	-	1(16.6%)	-	2(33.3%)
Escherichia coli	-	1(20%)	1(16.6%)	1(25%)	2(33.3%)
Proteus vulgaris	2(33.3%)	1(20%)	-	-	1(16.6%)
Salmonella typhirium	-	2(40%)	2(33.3%)	2(50%)	1(16.6%)
Klebsiella pneumoniae	2(33.3%)	1(20%)	2(33.3%)	1(25%)	-
TOTAL	6	5	6	4	6

Table 3: Percentage Distribution of Pathogenic Isolates Recovered from Herbal Concoction Based on Samples.

Key: T-Typhoid herbal concoction; I- IBA herbal concoction; IR-Inurirun herbal concoction; J- Jedi herbal concoction
A- Arariro herbal concoction

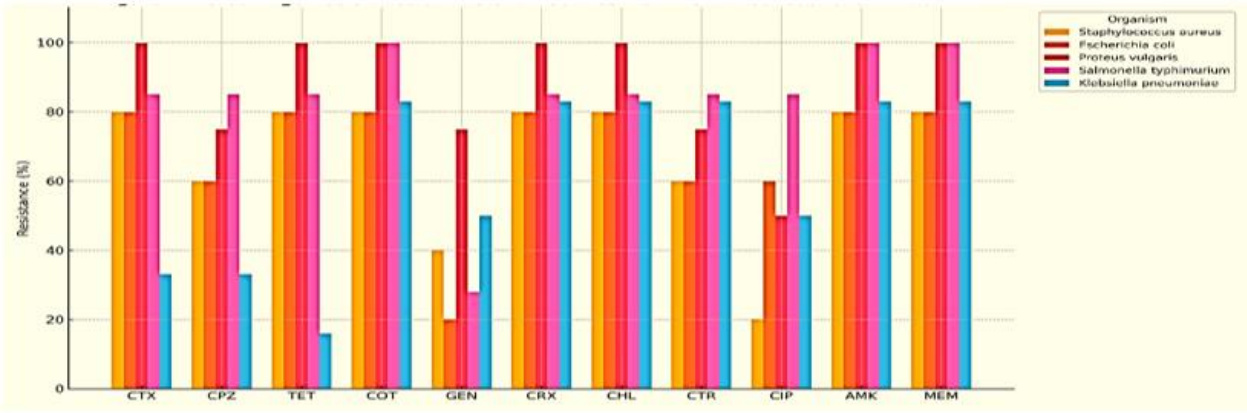

Figure 1: Percentage Resistance of Bacterial Isolates from Herbal Concoction in Ado-Ekiti.

Percentage resistance patterns of bacterial isolates against commercial antibiotics, showing varying resistance profiles across species. High resistance was observed particularly in Proteus vulgaris and Salmonella typhimurium, notably against ceftriaxone, amikacin and meropenem, indicating the presence of multidrug-resistant strains in herbal preparation

Key: n- number of isolates; CTX-ceftriaxone; CPZ-cefoperazone; TET-tetracycline; COT-Cotrimoxazole; GEN-gentamycin; CRX- cefuroxime; CIP- ciprofloxacin; AMK- amikacin; MEM- Meropenem.

Isolate	Source Code	No. of Antibiotic Classes	MARI
Staphylococcus aureus	T5	8	0.91
	IR1	9	0.91
	A3	7	0.83
	A5	8	0.75
Salmonella typhimurium	I3	8	0.91
	I5	9	0.91
	IR2	9	0.91
	IR5	9	0.91
	J2	9	0.91
	J5	9	1.0
	A6	5	0.41
Escherichia coli	I2	9	1.0
	IR4	8	0.83
	J1	8	0.83
	A4	8	0.75
Klebsiella pneumoniae	T3	6	0.75
	T6	7	0.75
	I6	9	1.0
	IR3	6	0.58
	IR6	8	0.83
Proteus vulgaris	T2	9	1.0
	T4	8	0.83
	I4	7	0.83
	A2	9	0.91

Table 4: Multiple Antibiotic Resistance Index (MARI) of Bacterial Isolates.

Multiple Antibiotic Resistance Index (MARI) of bacterial isolates obtained from herbal concoctions sold in Ado-Ekiti. Each isolate is listed with its source code and the number of antibiotics to which it exhibited resistance. MARI was calculated as the ratio of antibiotics to which the isolate was resistant to the total number tested. A MARI value ≥0.2 is indicative of a high-risk contamination source.

***Key:** T – Typhoid concoction; I – IBA concoction; IR – Inurirun concoction; J – Jedi concoction; A – Arariro concoction

4.0 Discussion

This study presents compelling evidence of significant bacterial contamination in locally marketed herbal concoctions in Ado-Ekiti, Nigeria. Of the 30 samples examined, 90% (n = 27) harbored bacterial loads ranging from 1.0×10^2 to 1.0×10^5 CFU/mL a significant bacterial count above acceptable microbial limits for herbal products for human consumption as established by international standards [4]. Only three samples (10%) were found to be microbiologically sterile. Comparable contamination levels were observed by De Sousa-Lima et al. (2020), who reported that 31.8% of herbal medicines in Brazil exceeded 10^5 CFU/g [8]. These findings are consistent with previous studies in Nigeria, where herbal remedies are regularly produced and distributed under unregulated and unhygienic conditions [9,

10]. The high bacterial contamination rate observed in this study may be due to poor handling practices during preparation, storage, and distribution. The isolation of indicator organisms such as Escherichia coli, Staphylococcus aureus, and Salmonella typhimurium which are recognized human pathogen further emphasizes the inadequacies in the sanitary standards for handling the local herbal preparations. Possible sources of contamination include the use of untreated water, unhygienic handling, and the exposure of raw plant materials to fecal matter, insects, or soil. These lapses pose significant public health risks, especially in communities that depend heavily on traditional medicine as a primary or sole healthcare option. Antibiotic susceptibility profiling revealed that all bacterial isolates exhibited resistance to multiple commonly used and last-resort antibiotics, including amikacin, meropenem, ceftriaxone, and cefuroxime. Multidrug resistance (MDR), defined as resistance to three or more classes of antibiotics, was observed in 88.9% (24/27) of isolates. Alarmingly, isolates of Proteus vulgaris, Salmonella typhimurium, Escherichia coli, and Klebsiella pneumoniae recorded Multiple Antibiotic Resistance Index (MARI) values of 1.0, indicating resistance to all antibiotics tested. MARI values exceeding 0.2 are suggestive of environments with high antibiotic usage or misuse [11]. The presence of pathogenic bacteria in these samples corroborates findings from earlier studies [12, 13,14]. Many of the isolated organisms are environmental in origin—residing in soil, water, air, and vegetation—but their ability to cause disease in humans is well established. For instance, Staphylococcus aureus produces enterotoxins implicated in foodborne illness, toxic shock syndrome, and scalded skin syndrome [6]. The health risks are particularly acute for individuals who are already immunocompromised, such as those self-medicating with herbal products for chronic conditions [15]. The resistance profiles of Proteus spp. and Salmonella typhi—which showed 100% resistance to eight and four antibiotics, respectively—mirror trends seen in clinical settings [14, 16]. The widespread resistance observed may result from horizontal gene transfer, with Staphylococcus spp. playing a significant donor role in plasmid-mediated resistance [12]. Furthermore, the resistance patterns observed in Klebsiella pneumoniae and Escherichia coli are likely due to both plasmid acquisition and chromosomal mutations [17]. These findings are in agreement with Dashen et al. (2020), who reported bacterial contamination in 45 liquid herbal products, with isolation rates of 4.67% for Escherichia coli, 17.78% for Staphylococcus aureus, 13.33% for Salmonella spp., 15.56% for Bacillus spp., and 6.67% for Proteus vulgaris [18]. However, it is noteworthy that Bacillus spp. was not detected in this study.

4.1 Conclusion

This study highlights the urgent need for regulatory oversight in the production and distribution of herbal medicinal products in Nigeria. The widespread bacterial contamination and high levels of antimicrobial resistance observed pose a serious public health threat. Regulatory agencies should implement and enforce Good Manufacturing Practices (GMP), require regular microbiological quality assessments, and establish stricter control measures for herbal drug vendors. Public health education campaigns are also essential to raise awareness among consumers and traditional healers about the risks associated with unregulated herbal products. Given the increasing reliance on traditional medicine, ensuring the microbiological safety and therapeutic integrity of these products is critical for safeguarding public health.

Availability Of Date and Materials

The authors declare that all the data supporting the findings are provided within the manuscript.

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Competing Interests

The author has declared that no competing interests exist.

Authors' Contribution

This work was carried out in collaboration between all authors. Author IJO, AFA, and OAO designed the study, and wrote the protocol, Authors AAA, EOA, DOS, and ECO wrote the first draft of the manuscript. Authors IJO and AFA managed literature searches and the analyses of the study. All authors read and approved of the final manuscript.

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