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# MICROBIAL QUALITY OF LIQUID HERBAL PRODUCTS HAWKED WITHIN KADUNA METROPOLIS

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**ABSTRACT**

Herbal medicine refers to the use of plants for the treatment of illness. The aim of this study was to assess the microbial quality of liquid herbal products hawked within Kaduna metropolis. A total of 28 herbal samples were collected from different markets and were analyzed for the presence of bacteria and fungi using standard method of spread plate method. The isolated bacteria were further tested for antibacterial susceptibility to commonly used antibiotics using disc diffusion technique. The result showed Kawo market had the lowest bacterial count of $3.17\pm2.16 \times10^6$, while Unguwan rimi market had the highest bacterial count of $4.45\pm2.49 \times10^6$. Unguwan Rimi market had the lowest fungal count of $2.27\pm1.76\times10^3$, while Sabo market had the highest fungal count of $3.18\pm1.82\times10^3$. Seventeen 17(60.71%) of the products were contaminated with different bacterial species including *Escherichia coli*, *Salmonella spp*, *Staphylococcus aureus* and *Pseudomonas sp* with percentage occurrence of 100%, 35.29%, 76.47 and 58.82% respectively. Fifteen 15(53.57) of the products were contaminated with different fungal species including *Aspergillus niger*, *A. flavus*, *A fumigatus*, *Mucor sp* and *Rhizopus sp* with percentage occurrence of 20.0%, 26.67%, 13.33%, 20.0% and 13.33% respectively. All the bacterial isolates were resistant to ampicillin and ceftazidine, 59% of *E. coli* were susceptible to gentamicin and nitrofurantoin. *Staphylococcus aureus* had a 100% susceptibility to ciprofloxacin and ofloxacin. Eighty (80%) of *Pseudomonas sp* were susceptible to gentamicin, while 83% of *Salmonella spp* were susceptible to gentamicin.

**Keywords**: Bacteria, Fungi, Herbal medicine, Market, Susceptibility

## INTRODUCTION

Herbal medicines are the most ancient form of health remedies known to humankind. Despite the great advances achieved in modern medicine, plants still make an important contribution to health care and several specific herbal extracts have been demonstrated to be efficacious for specific conditions. Medicinal plant has been defined by World Health Organization (WHO) consultative group as any plant which in one or more of its organs contains substances that can be used for therapeutic purposes or which are precursors for the synthesis of useful Drugs (Anie *et al.*, 2022).

Medicinal plants, which include vegetables, herbs, and spices, are used in a variety of ways in traditional medicine, and they contain chemical entities that can be used to generate new pharmaceuticals. In addition, they contain multitudes of naturally occurring chemical compounds (Adeosun *et al.*, 2022).

Seventy to eighty percent of the world population particularly in the developing countries rely on non-conventional medicines mainly of herbal sources in their primary healthcare (Darkwa *et al.*, 2022). World Health Organization has described tradition as one of the surest means to achieve total health care coverage of the world's population. In pursuance of its goal of providing accessible and culturally acceptable health care for the global population, WHO has also encouraged the rational use of traditional plant-based medicines by member states and has developed technical guidelines for the assessment of herbal medicine (WHO, 2000).

Natural products are rarely evaluated in the well-controlled clinical trials that are required to receive approval by regulatory bodies; therefore, they tend to have less scientific evidence to support their efficacy. However, all medicinal compounds are chemicals, whether synthesized in plants, animals or in manufacturing laboratories, therefore, all medicinal chemical compounds should be held accountable to similar standards of quality (identity, purity and stability), clinical effectiveness and safety; irrespective of their source. Reliable and consistent quality is the basis of efficacy and safety of herbal medicine products (Walther *et al.*, 2016).

In view of unabated increase in microbial resistance to available antibiotics, many people have found the use of herbal preparations as the solution to their various ailment. However, many of these herbal preparations are not adequately prepared to avoid microbial contamination (Anie *et al.*, 2022). The concern over quality of the products is mainly due to their potential contamination, considering their natural origin. Herbal medicines in Nigeria are used in form of various preparations to treat various types of ailments including asthma, eczema, irritable bowel syndrome, premenstrual syndrome, rheumatoid arthritis, migraine, menopausal symptoms, chronic fatigue and cancer among others (Achibong *et al.*, 2020).

With the increasing use of herbal medicines and the global expansion of the medicines market, safety has become a concern for both health authorities and the public in many counties. This is because many contaminants and residues that may cause harm to the consumers have been reported. The quality assessment of herbal medications is therefore very important in order to justify their acceptance in modern system of medicines (Achibong *et al.*, 2020).

The therapeutic efficacy of plants depends on their active constituents which are effective against the specific ailments. In the case of high microbial load in plants, it may be possible that active constituents of the plants may deteriorate. The contamination of herbal drugs by microbes is a very common phenomenon as report as available on the occurrence of microbial contamination in herbal drugs (Abba *et al.*, 2009).

The aim of the research was to assess the microbial quality of some liquid herbal products hawked within Kaduna Metropolis.

## MATERIALS AND METHOD

### Sample collection

A total of 28 herbal preparations were collected from four different markets (Central market, Kawo market, Unguwan Rimi market and Sabo market) within Kaduna metropolis, 7 samples from each market.

### Sample preparation

Ten (10mL) of each sample were measured and homogenized in 90mL of sterile peptone water. Ten-fold serial dilutions of the homogenates were made. Aliquot of 0.1mL of $10^{-4}$ dilutions of the homogenate were plated in duplicate on Plate Count Agar and Sabouraund Dextrose Agar for detection of total aerobic mesophilic bacterial count and total fungal count respectively. The plates were incubated at $37^0$C for 12-24 hours for bacterial count and $25^0$C for 2-4 days for fungal count. The plates were observed after the incubation period and colonies were counted using colony counter, counts were recorded and presented as colony forming unit per mL (CFU/mL).

### Identification of the isolated organisms

Discrete colonies were sub-cultured on to freshly prepared Nutrient agar for bacteria and Sabouround Dextrose agar for fungi for the isolation of pure culture using streak plate method. The bacterial isolated were identified using gram staining and biochemical test (catalase test, coagulase test, indole test, urease test, citrate utilization test, motility test, Triple sugar Iron test and methyl red test) as described by Cheesbrough, (2006). The fungal isolates were identified based on macroscopic (colony morphology) and microscopic characteristics confirmed and authenticated with the use of mycological atlas.

### Standardization of inoculum

A standard inoculum was prepared using overnight broth culture of each of the bacterial isolate by diluting with sterile saline solution to match 0.5 McFarland standard ($1x10^8$cells/mL). The standardized inoculum was used for the antibacterial activity testing (Olaniran *et al.,* 2022).

### Susceptibility testing (Kirby-Bauer agar disc diffusion method)

The standardized inoculum of each of the bacterial isolate were inoculated onto a freshly prepared Mueller Hinton Agar plate using spread plate method. The inoculum was allowed to stand for 5 minutes and then appropriate antimicrobial discs were placed on the surface of the seeded agar plate very gently with a sterile forcep and incubated at $37^0$C for 24 hours. After the incubation the plated were observed for zones of inhibition and the results were presented as mean zone of inhibition ± standard deviation.

## RESULTS AND DISCUSSION

The mean bacterial and fungal count of the herbal products shows that samples from Unguwan Rimi market have the highest bacterial count of $4.45x10^6$±2.49 while samples from Kawo market with $3.17x10^6$±2.16 have the least mean bacterial count. On the other hand, Sabo market had the highest fungal count of $3.18x10^3$±1.82 and Unguwan rimi market had the lowest fungal count of $2.27x10^3$±1.76

**Table 1: Mean bacterial and fungal count of herbal product hawked within kaduna metropolis**

| Market | Bacterial count x $10^6$ CFU/mL | Fungal count x $10^3$ CFU/mL |
|---|---|---|
| Central | 3.67±1.04 | 2.30±0.99 |
| Kawo | 3.17±2.16 | 2.78±0.93 |
| U/rimi | 4.45±2.49 | 2.27±1.76 |
| Sabo | 3.58±2.61 | 3.18±1.82 |

A total of 46 bacterial species were isolated and identified, out of which *Escherichia coli* had the highest occurrence 17(36.96%), while *Salmonella* sp had the lowest frequency of occurrence 6(13.04%).

**Table 2: Percentage occurrences of bacteria isolated from herbal medicine**

| Market | *E. coli* | *Salmonella* | *S. aureus* | *Pseudomonas* | Total |
|---|---|---|---|---|---|
| Central | 3(17.65) | 0(0.00) | 3(23.08) | 1(10.00) | 7(36.96) |
| Kawo | 4(23.53) | 2(33.33) | 3(23.08) | 3(30.00) | 12(26.09) |
| U/rimi | 5(29.41) | 2(33.33) | 4(30.77) | 3(30.00) | 14(30.43) |
| Sabo | 5(29.41) | 2(33.33) | 3(23.08) | 3(30.00) | 13(28.26) |
| Total | 17(36.96) | 6(13.04) | 13(28.26) | 10(21.74) | 46(100.00) |

A total of 11 fungal species were isolated out of which *Aspergillus flavus* had the highest frequency of occurrence of 5(45.46%), while *Aspergillus fumigatus* and *Rhizopus* Sp had the lowest frequency of occurrence of 2(18.18%) each

**Table 3: Percentage occurrence of fungi isolated from herbal medicine**

| Market | *A. niger* | *A. flavus* | *A. fumigatus* | *Rhizopus Sp* | *Mucor* | Total |
|---|---|---|---|---|---|---|
| Central | 1(33.33) | 2(66.67) | 0(0.00) | 0(0.00) | 0(0.00) | 3(27.27) |
| Kawo | 0(0.00) | 1(25.00) | 1(25.00) | 1(25.00) | 1(25.00) | 4(36.36) |
| U/rimi | 1(33.33) | 1(33.33) | 0(0.00) | 0(0.00) | 1(33.33) | 3(27.27) |
| Sabo | 1(20.00) | 1(20.00) | 1(20.00) | 1(20.00) | 1(20.00) | 5(45.46) |
| Total | 3(27.27) | 5(45.46) | 2(18.18) | 2(18.18) | 3(27.27) | 11(100.00) |

All the bacterial isolates were 100% resistant to Ampicillin and Ceftazidime, while *Staphylococcus aureus* was 100% susceptible to Ciprofloxacin and Ofloxacin antibiotics. *Salmonella* Sp and *Pseudomonas* were 83% and 80% susceptible to Gentamicin respectively. *Escherichia coli* was 58% susceptible to Gentamicin and Nitrofurantoin each.

**Table 4: Percentage susceptibility of bacterial isolates to antibiotics**

| Antibiotic | Amp | Ceft | Cef | Gent | Cipr | Ofl | Aug | Nitr |
|---|---|---|---|---|---|---|---|---|
| | % Susceptibility | | | | | | | |
| **Organism** | | | | | | | | |
| *E. coli* (n=17) | 0.00 | 0.00 | 0.00 | 58.8 | 0.00 | 29.4 | 41.2 | 58.8 |
| *Salmonella* (n=6) | 0.00 | 0.00 | 33.33 | 83.3 | 0.00 | 50.0 | 16.7 | 16.7 |
| *S. aureus* (n=13) | 0.00 | 0.00 | 0.00 | 30.8 | 100.0 | 100.0 | 38.5 | 31.8 |
| *Pseudomonas* (n=10) | 0.00 | 0.00 | 20.0 | 80.0 | 70.0 | 50.0 | 0.00 | 30.0 |

Key: Amp= Ampicillin, Ceft= Ceftazidime, Cef= Cefuroxime, Gent=Gentamicin, Cipr=Ciprofloxacin, Ofl=Ofloxacin, Aug= Augmentin, Nitri=Nitrofurantoin

**Discussion**

Microorganisms of different types are able to adhere to the leaves, stems, floral, seeds, and root systems from which herbal medicine can be prepared, and potential pathogens may be introduced during harvesting and processing (Olaniran *et al.,* 2022). According to the WHO report, there is widespread availability and usage of herbal preparations by a large percentage of persons in many developing countries (Igbeneghu and Lamikanra, 2016). The results of this study have shown that the herbal preparations examined were contaminated by bacteria and fungi. the mean bacterial counts were generally higher than the accepted limit ($10^5$ CFU/mL for bacteria and $10^3$ CFU/mL for fungi) of herbal medicine specification (WHO, 2007). The microbial count obtained in this study is in agreement with those of Esimone *et al.* (2002), Abba *et al.* (2009), Archibong *et al.* (2017), Anie *et al.* (2022). in South East, Kaduna, Awka and Abraka, Nigeria respectively, also Noor *et al.* (2013) in Bangladesh. A number of sources of contamination of herbal preparation especially during preparation have been identified. The microflora of the final product may represent contaminants from the raw materials, equipment, water, and atmosphere and from personnel. Therefore, the process of harvesting, drying, storage, handling and the soil influence the bacteriological quality of raw material which in turns affects the entire quality of the herbal preparation. The highest bacterial counts obtained in liquid preparations could be as a result of hydrated nature which is known to favour microbial growth.

The bacteria isolated in this study include *Escherichia coli Pseudomonas spp Salmonella sp* and *Staphylococcus aureus* similar bacteria were also isolated by Igbeneghu & Lamikanra (2016) in Ile-Ife and Hamza *et al.* (2023) in Gombe. Microorganisms such as *Escherichia coli*, and *Pseudomonas spp* are generally known to proliferate in potable water while *Staphylococcus*, *Aspergillus* and *Mucor* are commonly isolated from air (Esimone *et al.,* 2002). The most common source of post-preparation herbal contamination is the packaging vessels. The presence of these microorganisms in herbal remedies do not only make them hazardous, but might also change the physical, chemical and natural properties of the herbal remedies by altering the contents of active ingredients or converting them to toxic products (Chinakwe *et al.,* 2023). These pathogenic contaminants could have various health implications on the users of these products. *Escherichia coli* are organisms associated with gastrointestinal tract and its presence indicate the likelihood of fecal contamination. *Staphylococcus spp* has been associated with a number of complications especially to immune compromised individuals, this is in agreement with report of Archibong *et al.* (2017). It can produce proteins that disable the immune system and damage tissues. *Staphylococcus aureus* produces potent enterotoxins associated with food borne intoxication, toxic shock syndrome and staphylococcal scalded skin syndrome, septicemia and arthritis. *Pseudomonas* is known to cause UTI. This organism can constitute serious health hazards, especially in immune-compromised patients. Most *Pseudomonas species* are implicated in UTI, respiratory system infection, dermatitis, soft tissue infection, bacteremia, bone and joint infection, gastroenteritis and systemic infections. *Salmonella spp* are known to cause gastrointestinal infection which is characterized by diarrhea (Achibomg *et al.,* 2017).

The fungal isolates identified in this study are also detected by other researches Esimone *et al.* 2002. The presence of these fungal isolates in the herbal preparations could be as a result of contamination from soil and organic matter, which are medically important pathogens of human invasive aspergillosis. *Aspergillus spp* are associated with food poisoning and may be responsible for infections particularly in immuno-compromised individuals, moulds are responsible for biodeterioration of a number of substrates including raw materials and thereby decreases the medicinal potency of herbal drugs. *Aspergillus* spp in herbal drugs could mean that there was some growth of these organisms established before the complete drying of drugs. *Aspergilla* are capable of growing at low water content; in order to avoid such growth and the possible production of toxic metabolites, care should be taken to dry the product quickly before these moulds have the chance to establish any significant growth. Members of the genera *Aspergillus* are reported to produce the widest range of mycotoxins, such as aflatoxin which are reported to be nephrotoxic and carcinogenic (Archibong *et al.,* 2017).

The high level of microbial contamination observed in this study may be attributed to the methods of their preparation. The soil, harvesting, drying, improper handling and storage conditions influence the microbial quality of herbal drugs. The presence of microbial contaminant in non-sterile pharmaceutical products can reduce or even inactivate the therapeutic activity of the products and has the potential to adversely affect patients. The production and consumption of herbal remedies should be properly supervised and monitored to ensure that only good quality products get to the consumers (Chinakwe *et al.,* 2023).

Antibiotic susceptibility studies on the bacterial contamination of the liquid herbal products indicated that the isolates were susceptible to some of the antibiotics tested and resistant to others. The almost uniform antibiotic susceptibility of the isolates could be as a result to their environmental origins and agrees with observation of Archibong *et al.* (2017) that some environmental bacterial isolates are sometimes susceptible to antibiotics because resistance transfer is thought to be much less efficient in the environment where microorganisms are widely separated by distance. *Escherichia coli* was 100% resistant to 4 of the antibiotics used in this study and could pose a great threat to consumers. The high degree of antibiotic resistance observed in this study may be due to the transfer of drug resistance plasmids among the isolates. The high level of resistance to many antimicrobial agents shown by *Escherichia coli* may also be due to mutation in addition to plasmids acquisition (Esimone *et al.*, 2007).

## CONCLUSION

The microbial quality of the sampled herbal formulations was evaluated with a count range of 3.17±2.16 x$10^6$ - 4.45±2.49 x$10^6$ and 2.27±1.76x$10^3$ - 3.18±1.82x$10^3$ for bacteria and fungi respectively. Seventeen 17(60.71%) of the products were contaminated with different bacterial species including *Escherichia coli*, *Salmonella spp*, *Staphylococcus aureus* and *Pseudomonas sp* with percentage occurrence of 100%, 35.29%, 76.47 and 58.82% respectively. Fifteen 15(53.57) of the products were contaminated with different fungal species including *Aspergillus niger*, *A. flavus*, *A fumigatus*, *Mucor sp* and *Rhizopus sp* with percentage occurrence of 20.0%, 26.67%, 13.33%, 20.0% and 13.33% respectively. All the bacterial isolates were resistant to ampicillin and ceftazidine, 59% of *E. coli* were susceptible to gentamicin and nitrrofurantoin. *Staphylococcus aureus* had a 100% susceptibility to ciprofloxacin and ofloxacin. Eighty (80%) of *Pseudomonas sp* were susceptible to gentamicin, while 83% of *Salmonella spp* were susceptible to gentamicin. Producers of herbal liquid preparations should be educated on the need to enforce good manufacturing practices, effective harvesting practices, and safe handling and storage of liquid herbal medicinal products

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