

# Microbiological Quality of Herbal Formulation used for the treatment of Typhoid Fever Sold in Makurdi Metropolis, Central Nigeria

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## ABSTRACT

Herbal formulation has been used in recent years to treat various diseases including malaria, typhoid, dysentery, and cholera. To investigate the microbiological quality of herbal formulations. Herbal formulations were purchased from four different markets (Wadata market, Wurukum market, Modern market, and North bank market) in Makurdi metropolis. Microbiological analysis was carried out using pour plate method. Identification of isolated microorganisms was based on their cultural, morphological, and biochemical characteristics using standard microbiological procedures. Microbiological analyses showed that the total bacterial counts (TBC) of all the test herbal samples obtained from the various markets ranged from  $1.8 \times 10^3$  to  $9.3 \times 10^3$  cfu/ml and the total fungal count in the herbal mixture had a range of  $1.0 \times 10^3$  to  $2.5 \times 10^3$ . Four bacterial species were identified and they include; *Bacillus* spp, *Escherichia coli*, *Staphylococcus aureus* and *Enterobacter* spp. The least occurring bacterial isolate was *Bacillus* spp (12.5%), while the highest occurring was *Staphylococcus aureus* (37.5%). Four fungal isolates were identified and they include, *Aspergillus niger*, *Penicillium* spp, *Scedosporium* spp, and *Phialophora parasiticum*. *Aspergillus niger* and *Phialophora parasiticum* were the least occurring fungal isolate (12.5%) while *Scedosporium* spp and *Phialophora parasiticum* were the most occurring fungal isolate (37.5%). Since applications of herbal medicines for curative purposes is on the increase, there is a need for risk assessment of the microbial load of the medicinal plants at critical control points during processing. Furthermore, the danger associated with the potential toxicity of herbal therapies employed over a long period of time demand that the practitioners be kept abreast of the reported incidence of renal and hepatic toxicity resulting from the ingestion of medicinal herbs.

**Keywords:** Herbal formulation; typhoid fever; Bacterial counts; *Bacillus* spp; Toxicity

## INTRODUCTION

It is estimated that approximately 80% of the population in developing countries uses herbal medicine as part of their primary health care. The use of herbs dates back to the time of the early man, who had the crudest tools as his implements and used stones to start his fire [1]. Medicinal science came into

existence through a Greek man called Hippocrates and as such he earned his reputation as the father of medicine (Heber, 2008). Most savage people believed that disease was due to the presence of evil spirits in the body and could be driven out only by the use of poisonous and disagreeable substances calculated to make the body an unpleasant place to remain. The knowledge regarding the source and use of the various products suitable for the purpose was usually restricted to medicine men of the tribe [2].

Contamination of herbal medicinal products is defined as the "undesired introduction of impurities of a chemical or microbial nature or of foreign matter, into or onto a starting material, intermediate product or finished product or finished flavored product throughout production, sampling, packaging, or repackaging, storage or transport"

The introduction of micro-organisms in herbal medicinal products can alter the physicochemical characteristics of the products, which may lead to harmful effects on the quality of the herbal medicine products. The organisms he said were *Staphylococcus aureus*, *Escherichia coli*, *Rhizopus stolonifer*, *Candida* species, etc [3]. Bacteria are ubiquitous in nature, therefore because of the ubiquitous nature of bacteria, they contaminate almost every material, herbal juice no exception [4].

Among the main safety risks related to medicine is contamination by micro-organisms of various kinds that may be adherent in leaves, stems, flowers, seeds, and roots from which herbal medicines are prepared. Alternatively, microorganisms can be introduced during harvesting, handling, open-air drying, preserving, and manufacturing.

Although medicinal plants with their chemical constituents carry huge applications in the treatment or prevention of various diseases. The plant materials are exposed to various contaminants like toxic elements, pesticides, residues, etc. but the chief contaminants mainly responsible for the deterioration of herbal products are the microbe. Several studies showed that herbal plants are associated with broad variety of microbial contaminants [5]. When herbal products contaminated with these micro-organisms are consumed by people, they can cause serious health issues, one of the most serious issues surrounding the safety of herbal formulations

concerns regulating the level of mycotoxins found in herbal preparations. Mycotoxins are a group of around 400 toxic secondary metabolites produced by fungi such as *Aspergillus*, *Penicillium*, *Fusarium*, *Claviceps* and *Alternaria*. Recently [6] showed that 22% of herbal plant samples studied failed to comply with the quality requirement for traditional medicine. Herbal medicine is most often polyherbal, being prepared from mixtures of different plant parts obtained from various plant species and families and may contain multiple bioactive constituents that could be difficult to characterize [7]. The bioactive principles in most herbal preparations are not always known and there could be possibilities of interaction with each other in solution. The quality as well as the safety criteria for herbal drugs may be based, therefore, on a clear scientific definition of the raw materials used for such preparations. Also, herbal medicine may have multiple physiological activities and could be used in the treatment of a variety of disease conditions [8]. It could be administered in most disease states over a long period of time without proper dosage monitoring and consideration of toxic effects that might result from such prolonged usage [9].

The usages of herbs as medicines are determined mostly by the community and environment in which one grows up. [10] carried out a study to determine the socio-demographic characteristics and pattern of use of herbal medicines by women admitted to the Obstetrics and Gynaecology Department in the Komfo Anokye Teaching Hospital (KATH), a teaching hospital serving the Northern part of Ghana and made the following observations: More than fifty percent (50%) of patients used herbal medicines which were mostly unknown to the attending health workers. The less educated as well as the unskilled/ semi-skilled used herbal medicines more frequently compared to their more skilled and educated counterparts. Herbal medicine use is thus more prevalent in the groups who usually have poor socio-economic facilities and carry most of the burden of social deprivation. It is possible that their disease conditions may be adversely affected.

To achieve the desired benefit from herbal preparations, an individual must take the required dose over a certain length of time. Although it is generally believed that most herbal preparations are safe for consumption, some herbs like most biologically active substances could be toxic with undesirable side effects [11].

## MATERIALS AND METHOD

### Study Area

The study was carried out in Makurdi, Benue State. It has a population of 226,198 (1991 census) with a landmass of 16km radius. It has major markets like the North Bank market, Wurukum market, High-level market, Modern market, and Wadata market.

### Sample Collected

The commercially sold herbal formulation was purchased from four different markets (North bank market, Wurukum market, Modern market, and Wadata market). Two samples were purchased from each market and were immediately transported to the Department of Microbiology laboratory of the Federal University of Agriculture, Makurdi for microbiological analysis.

## PREPARATION OF CULTURE MEDIA

### Nutrient Agar

This is an all-purpose medium, very rich for the support of bacterial growth, 28 g of Nutrient agar was weighted and dissolved in 1000 ml of de-ionized water and autoclaved, it was allowed to cool before it was aseptically poured into sterile Petri dishes.

### Potato dextrose agar (PDA)

65g of potato dextrose agar was dissolved in 1000 ml of de-ionized water, the powder was allowed to dissolve and the tube was plugged with cotton wool, covered with aluminum foil, and autoclaved at 121C for 15 minutes. It was allowed to cool aseptically and poured into a sterile plate. The surface was flamed to remove air bubbles. The plates were allowed to solidify and then dried in the hot air dryer 10% lactic acid (0.5ml) was incorporated to prevent the growth of bacteria and other organisms apart from fungi.

### Isolation of Microorganisms

One milliliter (1ml) of each sample was measured into a sterile test tube containing 9ml of sterilized distilled water. The 10-1 suspension was serially diluted using tenfold serial dilution up to 10-4. Aliquot of 1ml of the appropriate dilution was plated in nutrient agar for the isolation of bacteria while Potato dextrose agar was used for fungi isolation. The inoculated nutrient agar plates were incubated at 37°C for 24-48 hours, while PDA plates were incubated at room temperature (28°C) for 3-5 days. After incubation, the number of discrete colonies was counted in terms of colony-forming units. The viable counts were obtained by reference to the serial dilution used.

## MICROBIOLOGICAL EVALUATION OF SAMPLE

### Culture of samples

Each of the herbal formulation was shaken vigorously and inoculated into two different media: Nutrient agar for the cultivation of microbes, and PDA for the isolation and cultivation of pathogenic fungi and yeast.

The liquid herbal formulation was diluted by dissolving 1g of the sample in 9 ml of normal saline, the aliquots (0.1ml) of the herbal preparations were inoculated into plates containing different culture agar and incubated at 37°C for 24-48 hrs for visible colonies. The plates containing potato dextrose agar were incubated at room temperature for 5 days. After incubation, visible colonies that developed were enumerated and recorded as colony-forming units/ml (cfu/ml) of cfu/g.

### Identification of colonies on the different media

The following parameters were used to identify colonies on Nutrient Agar and potatoes dextrose Agar. The different parameters used include; Colour, Elevation, opacity, size, and surface.

### Subculture

This was done in order to obtain a pure culture for the identification of organisms, A discrete colony of the organism to be identified was collected from cultured plates, using a sterile wire loop and subcultured on mannitol salt agar, salmonella shigella agar, EMBA, using a streaking method and incubated for 24 hrs at 37C for bacteria.

Again a discrete colony from the fungi culture was sub cultured into another PDA and allowed to grow for 72 hours, after which identification of the organisms were done.

served during consumption of the crop, medicinal uses of the crop ruminant ownership of the farmers and their fodder uses of breadfruit parts.

### 2.3 Sampling Method

## CHARACTERIZATION OF ISOLATES

### Gram Staining

This is a differential staining that classifies bacteria into gram-positive or negative. Thin smears of fresh, pure bacteria cultures were made on clean grease free glass slides. The slides were allowed to air dry and heat-fixed by passing them over a Bunsen burner flame. The slides were placed on a rack over a sink and flooded with crystal violet for 60 seconds, the slides were rinsed with clean running tap water and flooded with iodine for 60 seconds, the slides were rinsed with water and decolorized with 95% alcohol for 30 seconds, the slides were counter stain with safranin 60 seconds, they were rinsed with clean running water and allowed to air dry after which they are viewed under the microscope using oil immersion lens (100x). Gram-positive bacteria stained purple while gram-negative bacteria stained red.

## BIOCHEMICAL TEST FOR IDENTIFICATION OF BACTERIA

### Indole Test

This test is carried out to test the ability of an organism to break down tryptophan, an amino acid to pyruvate and indole. The test organism is inoculated into 5 ml of sterile peptone water and incubated aerobically at 37C for 24 hrs. The production of indole was tested by adding 0.5 ml of Kovac's reagent to the broth culture and shaken gently. A positive result shows a red colour in the alcohol layer which indicates that indole was produced while a negative result shows the absence of red colour.

### Catalase Test

The test demonstrates the presence of catalase which is an enzyme that catalyzes the release of oxygen from hydrogen peroxide (H<sub>2</sub>O<sub>2</sub>). A colony of 24 hours old culture was picked using a sterile loop and then emulsified in a few drops of hydrogen peroxide on a clean slide. The presence of effervescence indicated catalase positive reaction where as a negative reaction showed no effervescence

### Coagulase Test

The slide method described by [11] was used for the test. A drop of normal saline was placed on each end of a slide. An 18-24 hours old culture of the test organism was emulsified in each of the drops to make two thick suspensions. Thereafter, a drop of human plasma was added to one of the suspensions. The mixture was stirred for about 5 seconds. Clumping of the organism within 10 seconds is a positive test. No plasma was added to the second suspension which is the control to differentiate any granular appearance of the organism from true coagulase clumping.

### Urease Test

Isolates were inoculated into liquid urea agar supplemented with urea and aseptically dispensed into sterile test tubes, and slanted to solidify. They were incubated at 37C for 24-48 hours. The development of bright pink or red color indicates a positive urea reaction [12].

### Citrate Utilization Test

Simmon's citrate agar was prepared based on the manufacturer's instruction, sterilized, poured, and allowed to solidify at a slant angle of 45C. The test organism was streaked on the surface of the agar slant and then incubated at 37C for 48 hours. A change in the color of the agar medium from green to blue following the growth of the organism on the slant indicated a positive test, while no color change indicated a negative test.

### Fungal Identification

The fungal isolates were identified microscopically using lactophenol cotton blue test. The identification was achieved by placing a drop of the stain on the clean slide with the aid of a wire loop. A small portion of mycelium from the fungal culture was removed and placed in a drop of lactophenol. The mycelium was spread on a slide with the aid of the wire loop. A cover slip was gently applied with little pressure to eliminate air bubbles. The slide was then mounted and observed with x10 and x40 objective lenses respectively.

## RESULTS

**Table 1** Presents the Morphological and bacterial counts of each sample in 4 different markets in Makurdi metropolis. It shows that MM1 (Modern market 1) had the highest counts of 12.5x10<sup>3</sup>cfu/ml, while WD2 (Wadata 2) had the least count of 1.7x10<sup>3</sup>.

**Table 1:** Morphological and colony count of bacteria isolated from herbal formulation from 4 different markets in Makurdi metropolis.

Sample Source (cfu/ml)	Markets	Counts
Herbal formulation	NB1	9.3X10 <sup>3</sup>
Herbal formulation	NB2	3.4X10 <sup>3</sup>
Herbal formulation	WD1	4.2X10 <sup>3</sup>
Herbal formulation	WD2	1.7X10 <sup>3</sup>
Herbal formulation	WK1	2.5X10 <sup>3</sup>
Herbal formulation	WK2	1.8X10 <sup>3</sup>
Herbal formulation	MM1	12.5X10 <sup>3</sup>
Herbal formulation	MM2	1.9X10 <sup>3</sup>

**Table 2** Shows the average counts of fungal isolates which shows that NB1 (North bank 1) had the highest count of 2.5x10<sup>3</sup> cfu/ml, while the least count was obtained from WD1 (Wadata 1) with 1.0x10<sup>3</sup> cfu/ml.

**Table 2:** Average colony count of fungal isolates from the herbal formulation in 4 different markets in makurdi metropolis

Sample Source (cfu/ml)	Markets	Counts
Herbal formulation	NB1	2.5 X10 <sup>3</sup>
Herbal formulation	NB2	2.0 X10 <sup>3</sup>
Herbal formulation	WD1	1.0 X10 <sup>3</sup>
Herbal formulation	WD2	2.0 X10 <sup>3</sup>
Herbal formulation	WK1	2.3 X10 <sup>3</sup>
Herbal formulation	WK2	1.4 X10 <sup>3</sup>
Herbal formulation	MM1	1.5 X10 <sup>3</sup>
Herbal formulation	MM2	1.8 X10 <sup>3</sup>

**Table 3** and **4** show the occurrence of bacterial isolates from the sample collected from four different markets. *Staphylococcus aureus* had the highest frequency of 37.5% of the isolates in the sample. *Bacillus* spp had the lowest frequency of 12.5% of the bacterial isolates in the sample.

(North Bank 1) and WK2 (Wurukum 2) had all the bacterial isolates recorded in this study from herbal formulation, while WD1 (Wadata 1) had only 1 bacterial isolate.

**Table 3:** Occurrence of bacterial isolates from the herbal formulation in 4 different markets in makurdi metropolis

Isolates	Frequency	Percentage
<i>Staphylococcus aureus</i>	3	37.5%
<i>Entermoeba spp</i>	3	25%
<i>Escherichia coli</i>	2	25%
<i>Bacillus spp</i>	1	12.5%

**Table 4:** Occurrence of the bacterial isolates from the different markets

Sample Source	Markets	<i>Staphylococcus aureus</i>	<i>Enterobacter spp</i>	<i>Escherichia coli</i>	<i>Bacillus spp</i>
Herbal formulation	NB1	+	+	+	+
	NB2	+	+	-	-
	WD1	+	-	-	-
	WD2	-	+	+	+
	WK1	+	-	-	+
	WK2	+	+	+	+
	MM1	+	-	+	+
	MM2	+	-	+	+

- = Absent      + = Present

The percentage occurrence of fungal isolates from the herbal formulation, shows that *Scedoporiumpspand* *Penicillium spp* both had the same frequencies of occurrence representing 37.5% of the total fungal isolates. While *Aspergillus spp*, had a percentage frequency 12.5% of the total fungal identified. (Table 5)

**Table 5:** Percentage occurrence of fungal isolates from the herbal formulation in 4 different markets in Makurdi metropolis

Isolates	Occurrence	Percentage
<i>Scedosporium spp</i>	3	37.5%
<i>Penicillium spp</i>	3	37.5%
<i>Aspergillus niger</i>	1	12.5%
<i>Phialophora parasiticum</i>	1	12.5%

On table 6 the occurrence in percentage of fungal isolates in four different markets is presented. The study shows that WK2 (Wururkum 2) had all the fungal isolates in the herbal formulation identified representing the highest frequency of occurrence, while Mm1, (Modern market 1) had had the least fungal isolates.

**Table 6:** Occurrence of fungal isolates from the herbal formulation in 4 different markets in Makurdi metropolis

Sample Source	Markets	<i>Scedosporium spp</i>	<i>Penicillium spp</i>	<i>Aspergillus spp</i>	<i>Phialophora spp</i>
Herbal formulation	NB1	+	-	-	+
	NB2	-	+	+	+
	WD1	-	-	-	+
	WD2	+	+	+	-
	WK1	+	-	+	+
	WK2	+	+	+	+
	MM1	-	+	-	-
	MM2	+	+	+	-

- = Absent      + = Present

## DISCUSSION

This study has shown that there are varieties of microorganisms present in our various herbal formulations which could have resulted from contaminated soils, plants and its products, preparation processes, quality of water, containers, and processing equipment. However, these microorganisms exhibit multi-resistance to many antibiotics. Since herbal formulations are mainly prepared for human

consumption, there is a very high chance of passing the antibiotics resistant microorganisms into the human ecosystem. This poses a great danger to human health. Since herbal concoctions are prepared using varieties of medicinal plants that contain active constituents that are cheap and effective against common bacterial infections.

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. Microorganisms such as *Escherichia coli*, and *Scedosporium spp* reported in this study are generally known to proliferate in potable, distilled, and de-ionized water while *Bacillus spp*, *Staphylococcus*, *Aspergillus* and penicillin are commonly isolated from air. The most common source of post-preparation herbal contamination is the packaging vessels. In order to enhance consumer acceptability, most herbalists in Nigeria have adopted the use of bottles and plastic containers as packaging vessels for their preparations. Unfortunately, these vessels are not subjected to any form of sterilization after washing them. Contamination of the preparation coupled with the humid tropical environments may result in the proliferation of microbial contaminants in the herbal remedies (WHO, 2007); this probably explains the high microbial counts recorded in this study. Such high levels of microbial contamination have been shown to result in spoilage and degradation of the products or may constitute a health hazard to the user [14]. Most herbal preparations are made up of different components of various plant species and these preparations are not standardized with respect to color, taste, consistency, etc. Unlike orthodox drugs, changes in the appearance, odor, taste, etc of herbal formulations due to spoilage are hardly readily detected by the patients [13]. Among the microorganisms isolated from Herbal Medicine, *Bacillus*, *Staphylococcus*, *E. coli*, and penicillin were the major contaminants. Although the pathogenicity of these organisms was not assessed, species of these agents have been incriminated in serious human infections. *Bacillus spp* are widely distributed in the soil, dust, air, and water and they are resistant to environmentally destructive factors. Apart from the unacceptable microbial loads observed in the samples, the presence of contaminants considered to be completely unacceptable in herbal preparations was demonstrated. The most common isolates in the tested samples were Gram-positive organisms belonging to the genera *Bacillus* and *Staphylococcus aureus* are normal commensals of the mammalian skin, hands, and mucous membranes. Upon consideration of the extent of human contact involved in the preparation of herbal medicinal samples, it is most likely that sources of the contaminating *Staphylococcus spp*. are the producers of the herbal formulations this suggests that the level of hygiene of persons involved in the preparation of the tested samples may be low. Similar studies carried out on herbal samples include work by [15-20] have all reported that the pathogens frequently isolated in herbal products were *S. aureus*, *E. coli*. Except for *Scedosporium* which was not isolated in some reports mentioned above. This work varies by reporting a higher count of *Bacillus spp*. Contamination by *Bacillus spp* could have arisen during growth of the herbs as the bacterium is commonly found in soils. *Escherichia coli*, a major faecal coliform may have been introduced from the water used during the processing of the herbs. Microbial contamination of

herbal mixture as shown in this study, may also be a result of the plant materials, utensils used during preparation, poor hygiene of the manufacturer, or even the packaging vessel after processing as also suggested by many authors earlier reported. Microorganisms are present everywhere and can easily contaminate any substrate. Considering the packaging materials, it is worthy of note that this contributes greatly to microbial contamination as the final stage of the processing is the packaging. Most of the packaging cans used are not sterilized and are usually picked up where they are found littered along the road or in public places and barely washed before being used to package finished herbal products. It was observed that fungal growth was more than bacterial growth and this is attributable to the low pH value of all the herbal formulation samples which is favorable for fungal growth. The high microbial load and presence of specific pathogens in the tested herbal formulation have serious clinical as well as pharmaceutical implications. Clinically, consumers of any of these products are at risk of contracting infections by the different pathogens which may be of great consequence if not identified and treated appropriately. The presence of *Escherichia coli* in the sample indicates poor hygiene practices and a lack of adequate handling of the products. According to the European pharmacopoeia 2007, no *Salmonella* spp or *Escherichia coli* strain should be present in samples [21]. The intake of a high concentration of accumulated toxins produced by organisms such as *Bacillus cereus*, *Staphylococcus aureus*, and *Aspergillus niger* may lead to undesirable reactions in consumers. [22] reported that the presence of *Bacillus* spp. in herbal preparations is an indication that the water used in the preparation of the products is not from a good source. The risk is greater if the consumer is a young child with undeveloped immunity, an elderly with diminished immunity, or the immunocompromised patients. Incidentally, these groups of consumers are the most in need of herbal medicines for the treatment of many diseases to which they are susceptible

## CONCLUSION

*Staphylococcus aureus*, *Escherichia coli*, *Enterobacter* spp, *Bacillus* spp *Scedosporidium* spp *Penicillium* spp, *Aspergillus niger*, and *Phialophora parasiticum* spp were isolated and identified as micro-organisms associated with contamination of herbal formulations sold in markets within Makurdi metropolis, with *Staphylococcus aureus* having the highest bacterial colony count and *Scedosporium* spp having the highest fungal count.

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