

## Microbiological Screening Of Some Herbal Drinks Hawked In Some Parts Of Rivers State.

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**Abstract:** The microbiological quality of five indigenous herbal drinks (Afato, Opaeyin, Emagon, Munru, and Dokita Igbo) from three different hawkers, in Port Harcourt Metropolis was investigated. The assessment of the microbial contamination on the herbal products were carried out using standard method; total bacteria counts, coliform counts and fungal counts. The average results per total bacterial counts in Dikta igbo, Emagon, Afato, Opaeyin and Munru herbal drink were  $9.2 \times 10^7$  CfU/ml,  $4.1 \times 10^7$  CfU/ml,  $8.8 \times 10^7$  CfU/ml,  $7.8 \times 10^7$  CfU/ml and  $9.6 \times 10^7$  respectively while the average counts for coliforms in  $3.5 \times 10^5$  CfU/ml,  $3.8 \times 10^5$  CfU/ml,  $3.5 \times 10^5$  cfu/ml,  $3.7 \times 10^5$  cfu/ml and  $4.3 \times 10^5$  CfU/ml also the average fungal counts for the five herbal drinks were  $4.4 \times 10^5$  CfU/ml,  $3.5 \times 10^5$  cfu/ml,  $5.6 \times 10^5$  CfU/ml,  $4.5 \times 10^5$  CfU/ml and  $3.6 \times 10^5$  CfU/ml respectively. The overall resistance pattern by the gram positive and gram negative isolates to standard antibiotics were; ampiclox (100%), Zimacef (100%), amoxicillin (100%), gentamycin (40%), streptomycin (40%), septrin (30%), peilogivafloxacin (20%), sparifloxacin (20%) and ofloxacin (20%). The results of this work showed the presence of some pathogenic microorganisms, such as *Escherichia coli* (18%), *Staphylococcus spp* (29%), *Salmonella spp* (21%), *Klebsiella spp* (4%), *Shigella spp* (14%), *Serratia spp* (6%), and *Proteus spp* (8%), that could impair this herbal products, which may be a source of infection to consumers, therefore, production of these herbal drinks by manufacturers should be done under hygienic condition, water should be tested continuously for microbial growth, raw materials should be tested before use especially those of natural origin and only thoroughly monitored herbal drinks, whose microbiological quality are government or NAFDAC approved should be allowed for sale to the public.

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### Introduction

Herbal drinks are preparation made from plants by means of simple processes involving harvesting, drying and storage and are used for the treatment and prevention of diseases (EMEA, 2005).

In Nigeria, there appears to be an overwhelming increase in the public awareness and usage of herbal medicinal products in the treatment diseases (Okumola *et al.*, 2007), with this interested usage, the safety, efficacy and quality of these medicinal products have been an important concern for health authorities and health professionals.

Herbal remedies are often perceived as being natural and therefore safe, but they are not free from adverse effects, which may be due to factors such as adulterations, contamination, misidentification, lack of standardization, incorrect preparation and for dosage, inappropriate labeling and or advertisement.

Herbal drinks are often promoted to the public as being “natural” and completely “safe alternatives to conventional medicines. African plants used medicinally are widely assumed to be safe, but many are potentially toxic (Fennel *et al.*, 2004). All around the world, there is talk about “health for all” but it has been realized that modern pharmaceuticals are and

will remain out of reach of a large proportion of human population for the foreseeable future. This necessitates the use of other sources of human knowledge to provide common health benefits. Thus, herbal medicine is now regarded as important but under utilized tool against disease. The World Health Organization (WHO) recognized this fact in the early 1970s and encouraged governments to effectively utilize local knowledge of herbal medicine for disease prevention and health promotion (Gupta *et al.*, 2010).

In most African countries including Nigeria herbal drinks is recognized as an important component healthcare system, especially among rural dwellers that constitute about 70% of the population (Esinome *et. al*, 2002). The World Health Organization (WHO 2000). Survey indicate that about 70-80% of the world’s population particularly in developing countries rely on non-conventional medicines mainly of herbal origin for their primary healthcare. Medical plants collected in the wild area may be contaminated by other species or plants through misidentification accidental contamination, an intentional adulteration, all of which may have unsafe consequences. (Fennal *et al.*, 2004). Aerobic bacteria and fungi are normally present in plant materials and to increase due to

uncontrolled growing, harvesting storing and processing.

Some pathogenic bacteria such as *Salmonella* sp, *Escherichia coli*, *Staphylococcus* sp, *Shigella* and other gram positive and gram negative bacteria have been found from previous studies to be microbial contaminants of herbal drinks and as such, present serious health hazard. There are many documented reports of the effects of microbial proliferation within these herbal products with attend not odour and visible spoilage (Esimone *et al.*, 2002). This study was aimed at;

- Evaluating the bacteriological and mycological quality of some the herbal drinks.
- Comparing the level of contamination of five different herbal drinks from three (3) different identified hawkers in strategic parts of Rivers State.
- And to check the susceptibility of isolated microorganisms to a given antibiotics.

### Materials And Methods

- Five different herbal drinks (Dikita Igbo, Afato, Munru, Ewe Mangon, Opaeym) were purchased randomly from three different hawkers in strategic parts (Choba, Rumuomasi and Mile 3 market) in Port Harcourt Metropolis. All samples collected from the different identified hawkers, were then stored in sterile container and transferred to the microbiology laboratory in University of Port Harcourt for Microbiological analysis.

#### Media Used

The media used for the microbial analysis were, plate count (PCA) MacConkey agar (MA), Mannitol Salt agar MSA, Salmonelle Shigella agar, Potato Dextrose agar, and Mueller Hinton agar. All the media were prepared and sterilizes following the manufacturers instructions.

### Determination Of Microbial Quality Of The Herbal Drinks

- Exactly 10ml of each sample was aseptically transferred into a corresponding sterile tube containing 90ml of sterile peptone water and were serially diluted to  $10^{-1}$  to  $10^{-6}$  From the appropriate dilution ( $10^{-5}$  and  $10^{-6}$ ) 0.1ml was inoculated separately onto Plate count agar, while dilution ( $10^{-2}$  and  $10^{-3}$ ) was inoculated separately onto, MacConkey agar, Manitol Salt agar and Salmomella Shigella agar, and spread evenly using a sterile bent glass rod for each media. Each experiment has carried out duplicates. All of the inoculated media were incubated at  $37^{\circ}\text{C}$  for 48 hours. After the period of incubation, the colonies formed on the Media were counted and recorded as colony forming units per mililitres (cfu/ml).

Then, pure cultures were obtained from the plates and stored on agar plants and kept in the refrigerator until when needed for further biochemical tests. The biochemical tests conducted on the isolates were citrate utilization, indole production, catalase, Hydrogen Sulphide production, oxidase test, Methyl red test, Vogers proskaver test and Gram staining. Fungi counts was carried out by pipetting from the appropriate dilution of the sample ( $10^{-2}$  and  $10^{-3}$ ) 0.1ml and placed on the Potato Dextrose agar c containing 0.4% lactic acid. The plates were then incubated for 3-4 days at  $25^{\circ}\text{C}$ . After period of incubation, the colonies formed on plates were counted and recorded as colony forming units per mililitres (cfu/ml). The fungi colonies are checked for their cultural and morphological characteristics.

The antibiotic susceptibility tests were performed on Muller Hinton agar plate. The bacteria isolates from the samples were reactivated by sub culturing from agar slant onto plate count agar plates and was incubated for 18-24 hours.

The inoculums was standardized by transferring three distinct and separate colonies of the pure culture of the test organism using sterile wire loop into 5ml of nutrient broth. The broth was incubated for 3 hours at  $37^{\circ}\text{C}$  to allow for the growth of test organisms; till the quality was equivalent to the turbidity of 0.5 Mc Farland  $\text{BaSO}_4$ . The standardized inocula were swabbed on Mueller Hinton agar plate. Antibiotic dirc containing the following, pefloxacin ( $5\mu\text{s}$ ) cofloxacin ( $5\mu\text{s}$ ) Ciprofloxacin ( $5\mu\text{s}$ ), norfloxacin ( $10\mu\text{s}$ ), nalidixic acid ( $30\mu\text{s}$ ) and nitrofurantoin ( $300\mu\text{s}$ ), were placed on the inoculated plates and presses firmly on the agar complete contact. The plates were inverted and left on the work table for 30 minutes to allow for diffusion of antibiotics into the agar. The plates were incubated at  $37^{\circ}\text{C}$  for 18 to 24 hours.

### Results

The results of the total bacterial counts for the five herbal drinks samples from three different hawkers are shown in table 1. The average bacterial counts for Dokita Igbo, Emagon, Afato, Opaeyin and Munru herbal drinks were  $9.2 \times 10^7$  Cfu/ml,  $4.1 \times 10^7$  Cfu/ml,  $8.8 \times 10^7$ ,  $7.8 \times 10^7$  and  $9.6 \times 10^7$  respectively. The total bacterial counts (TBC) of all the test herbal sample ranged from  $9.9 \times 10^7$  to  $3.9 \times 10^7$  Cfu/ml. A total of 32 bacterial and fungi (28 bacteria and 4 fungi strains) were isolated from herbal products examined. Five (50%) of the sample were contaminated with *Escherichia coli*, which is an intestinal bacterium and an, indication of faecal contamination. Five (50%) were contaminated with *Salmonella spp*. Four (40%) were contaminated with *Staphylococcus spp*, one (10%) were contaminated

with *Klebsiella* and *Proteus spp* and two (20%) were contaminated with *Serratia spp*.

According to the antibiogram conducted all the gram positive strains of *Staphylococcus spp* and the gram negative bacteria *Escherichia coli*, *Salmonella*

*spp*, *Shigella spp*, *Klebsiella spp*, *Proteus spp*, and *Serratia spp* were sensitive to perfloracin, gentamycin, rocephin, ciprofloxacin, streptomycin, septrin, erythromycin, chloramphenicol, and ofloxacin.

**Table 1: Bacteria Population of Herbal Products**

Herbal Sample Code	Bacteria Counts In CFU/ml			
	TBC	ST	SA	TCC
HD1	9.4X10 <sup>7</sup>	8.8X10 <sup>5</sup>	7.8X10 <sup>5</sup>	5.5X10 <sup>5</sup>
HD2	8.9X10 <sup>7</sup>	8.8X10 <sup>5</sup>	7.2X10 <sup>5</sup>	5.8X10 <sup>5</sup>
HD3	9.4X10 <sup>7</sup>	8.0X10 <sup>5</sup>	7.4X10 <sup>5</sup>	5.1X10 <sup>5</sup>
HE1	3.7X10 <sup>7</sup>	3.9X10 <sup>5</sup>	7.2X10 <sup>5</sup>	3.9X10 <sup>5</sup>
HE2	4X6X10 <sup>7</sup>	3.6X10 <sup>5</sup>	4.2X10 <sup>5</sup>	3.8X10 <sup>5</sup>
HE3	3.9X10 <sup>7</sup>	4.0X10 <sup>5</sup>	3.7X10 <sup>5</sup>	3.6X10 <sup>5</sup>
HA1	8.6X10 <sup>7</sup>	6.1X10 <sup>5</sup>	3.7X10 <sup>5</sup>	3.6X10 <sup>5</sup>
HA2	9.0X10 <sup>7</sup>	5.4X10 <sup>5</sup>	3.3X10 <sup>5</sup>	3.4X10 <sup>5</sup>
HA3	8.9X10 <sup>7</sup>	6.0X10 <sup>5</sup>	3.7X10 <sup>5</sup>	3.6X10 <sup>5</sup>
HP1	7.0X10 <sup>7</sup>	7.2X10 <sup>5</sup>	6.1X10 <sup>5</sup>	3.9X10 <sup>5</sup>
HP2	8.3X10 <sup>7</sup>	6.0X10 <sup>5</sup>	5.7X10 <sup>5</sup>	3.4X10 <sup>5</sup>
HP3	8.1X10 <sup>7</sup>	7.2X10 <sup>5</sup>	6.1X10 <sup>5</sup>	4.0X10 <sup>5</sup>
HM1	9.9X10 <sup>7</sup>	6.8X10 <sup>5</sup>	3.6X10 <sup>5</sup>	4.8X10 <sup>5</sup>
HM2	9.7X10 <sup>7</sup>	6.0X10 <sup>5</sup>	3.6X10 <sup>5</sup>	3.6X10 <sup>5</sup>
HM3	9.4X10 <sup>7</sup>	6.5X10 <sup>5</sup>	3.4X10 <sup>5</sup>	4.4X10 <sup>5</sup>

Total bacteria counts (TBC), *Salmonella spp.* (ST), *Staphylococcus spp.* (SA)

KEY: HD – Herbal (Dokita igbo), HE -Herbal (Emagon), HA – Herbal (Afato), HP – Herbal (Opayin), HM – Herbal (Munru)

**Table 2: The Average Total Bacterial Counts For Five Herbal Drink Samples From Three Different Hawkers**

Sample	H1 (cfu/ml)	H2 (cfu/ml)	H3 (cfu/ml)	Average cfu/ml
Dokita igbo	9.4 X10 <sup>7</sup>	8.9 X10 <sup>7</sup>	9.4 X10 <sup>7</sup>	9.2 X10 <sup>7</sup>
Emangon	3.7 X10 <sup>7</sup>	4.6 X10 <sup>7</sup>	3.9 X10 <sup>7</sup>	4.1 X10 <sup>7</sup>
Afato	8.6 X10 <sup>7</sup>	9.0 X10 <sup>7</sup>	8.9 X10 <sup>7</sup>	8.8 X10 <sup>7</sup>
Opneyin	70 X10 <sup>7</sup>	8.3 X10 <sup>7</sup>	8.1 X10 <sup>7</sup>	7.8 X10 <sup>7</sup>
Munru	9.9 X10 <sup>7</sup>	9.7 X10 <sup>7</sup>	9.4 X10 <sup>7</sup>	9.6 X10 <sup>7</sup>

• KEY: H1 – First set of herbal drinks, H2 – Second set of herbal drinks, H3 – Third set of herbal drinks.

**Table 3 :The Average Total Coliform Counts For Five Herbal Drink Samples From Three Different Hawker**

Sample	H1 (cfu/ml)	H2 (cfu/ml)	H3 (cfu/ml)	Average cfu/ml
Dokita igbo	5.5 X10 <sup>5</sup>	5.8 X10 <sup>5</sup>	5.1 X10 <sup>5</sup>	5.5 X10 <sup>5</sup>
Emangon	3.9 X10 <sup>5</sup>	3.8 X10 <sup>5</sup>	3.6 X10 <sup>5</sup>	3.8 X10 <sup>5</sup>
Afato	3.9 X10 <sup>5</sup>	3.8 X10 <sup>5</sup>	3.6 X10 <sup>5</sup>	3.5 X10 <sup>5</sup>
Opneyin	3.9 X10 <sup>5</sup>	3.4 X10 <sup>5</sup>	4.0 X10 <sup>5</sup>	3.7 X10 <sup>5</sup>
Munru	4.8 X10 <sup>5</sup>	3.6 X10 <sup>5</sup>	4.4 X10 <sup>5</sup>	4.3 X10 <sup>5</sup>

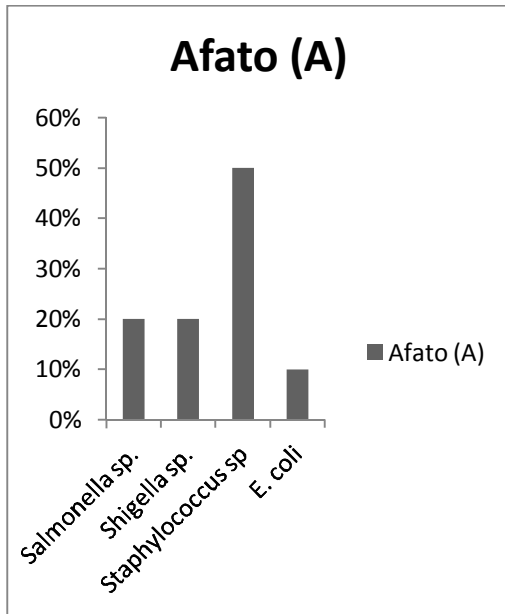
**Table 4: The Fungi counts for five herbal drink sample from three different hawkers**

Sample Code	Fungal Counts (Cfu/MI)	Average Count (Cfu/MI)
D1	4.1 x 10 <sup>5</sup>	
D2	4.7 x 10 <sup>5</sup>	4.4 x 10 <sup>5</sup>
D3	4.4 x 10 <sup>5</sup>	
E1	3.3 x 10 <sup>5</sup>	
E2	3.4 x 10 <sup>5</sup>	3.5 x 10 <sup>5</sup>
E3	3.7 x 10 <sup>5</sup>	
A1	6.0 x 10 <sup>5</sup>	
A2	5.3 x 10 <sup>5</sup>	5.6 x 10 <sup>5</sup>
A3	5.4 x 10 <sup>5</sup>	
P1	4.9 x 10 <sup>5</sup>	
P2	4.8 x 10 <sup>5</sup>	4.5 x 10 <sup>5</sup>
P3	3.9 x 10 <sup>5</sup>	
M1	3.7 x 10 <sup>5</sup>	
M2	3.5 x 10 <sup>5</sup>	3.6 x 10 <sup>5</sup>
M3	3.7 x 10 <sup>5</sup>	

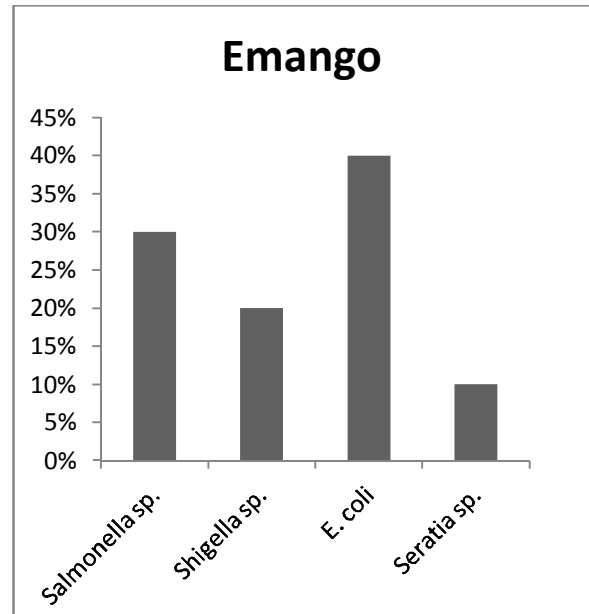
• KEY: HD – Herbal (Dokita igbo), HE -Herbal (Emagon), HA – Herbal (Afato), HP – Herbal (Opaeyin), HM – Herbal (Munru)

**Table 7: Percentage Occurrence of Bacteria in the Five Herbal Drinks Samples**

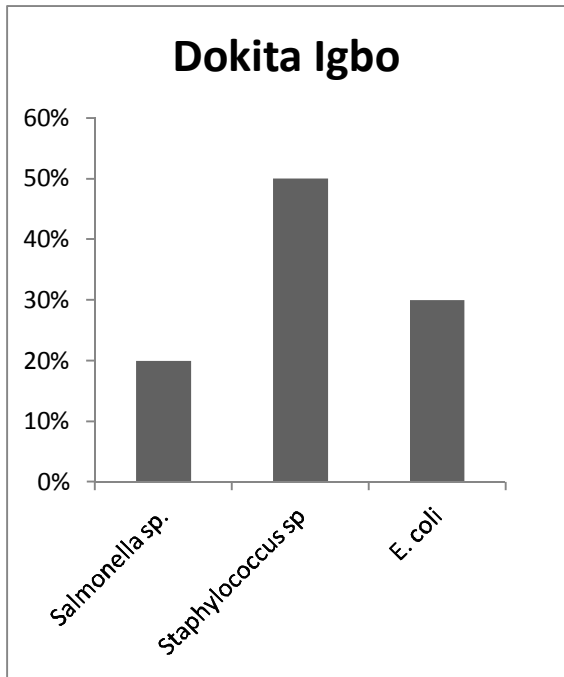
Herbal Drinks	<i>Salmnella sp</i>	<i>Shigella sp</i>	<i>Staphylococcus sp</i>	<i>Klebsiella sp</i>	<i>Escherichia coli</i>	<i>Proteus sp</i>	<i>Serratia sp</i>
Afato (A)	2(20%)	2(20%)	5(50%)	0	1(10%)	0	0
Emango (E)	3(30%)	2(20%)	0	0	4(40%)	0	1(10%)
Dokita Igbo (D)	2(20%)	0	5(50%)	0	3(30%)	0	0
Opaeyi (P)	3(30%)	0	4(40%)	1(10%)	1(10%)	0	1(10%)
Munru	1(10%)	2(20%)	5(50%)	0	1(10%)	1(10%)	0



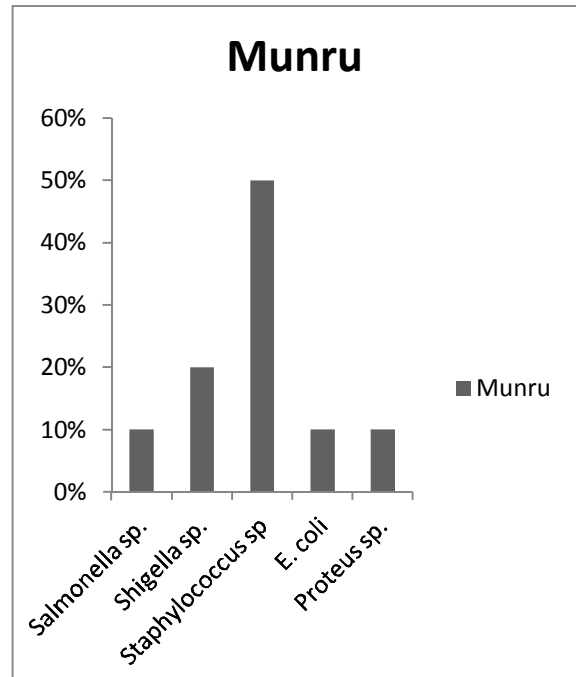
**Figure 1:** Frequency of occurrence of bacteria in Afato herbal drink



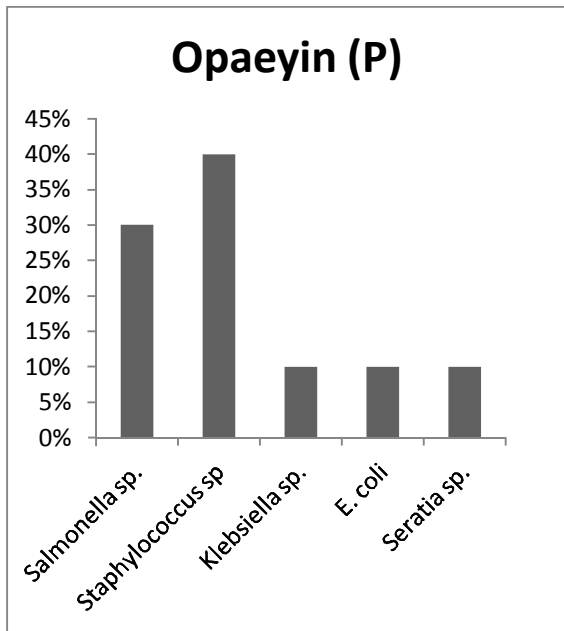
**Figure 2:** Frequency of occurrence of bacteria in Emango herbal drink



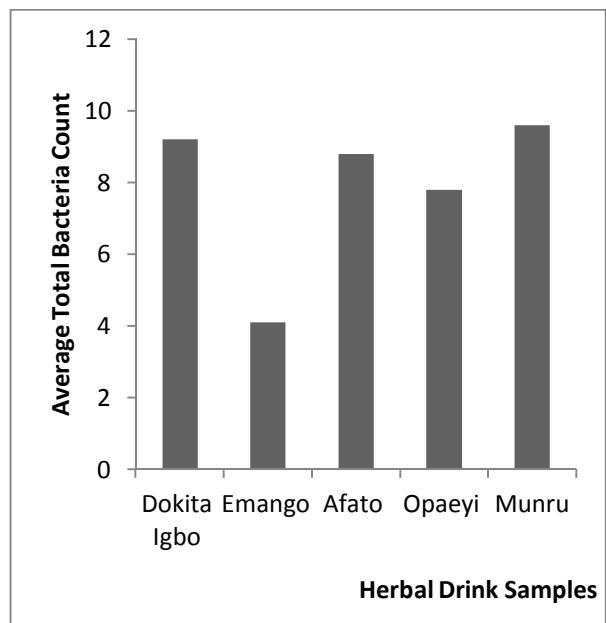
**Figure 3:** Frequency of occurrence of bacteria in Dokita Igbo herbal drink



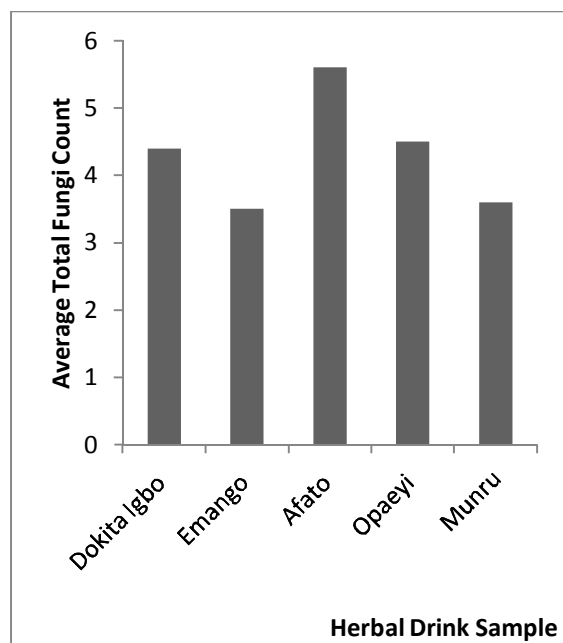
**Figure 5:** Frequency of occurrence of bacteria in Munru herbal drink



**Figure 4:** Frequency of occurrence of bacteria in Opaeyin herbal drink



**Figure 6:** A bar chart showing the average Total bacteria count for each of the five herbal drink samples



**Figure 7:** A bar chart showing the average Total fungi count for each of the five herbal drink samples

### Discussion

The presence of microbial contaminant in non-sterile herbal products can reduce or even inactivate the therapeutic activity of the product and has potential to adversely affect patient taking these herbal products (Nakajina *et al.*, 203). Some infectious disease outbreaks have been associated with the use of heavily contaminated raw materials of natural origin, since the microbial quality of the herbal drinks is influenced by the environment and quality of the raw materials used during formulation, the manufacturers should ensure that the microbial load is brought to a minimal safety level in the raw materials, finished forms and the packaging components, to maintain appropriate quality, safety and efficacy of the products.

Most of these products get contaminated through improper handling by these local hawkers thereby reducing the effectiveness of the product and also posing a potential health hazard to the consumers. The bacterial contaminants isolated from these test herbal drinks were *Escherichia coli* (18%), *Staphylococcus spp* (29%), *Shigella spp* (14%), *Salmonella spp* (21%), *Klebsiella spp* (4%), *Serratia spp* (6%) and *Proteus spp* (8%), which showed wide resistance to ampiclox, zimacep, amoxicillin, augumentin, ofloxacin, gentamycin, and septrin, suggesting that they could be producers of B-lactamase. The presence of B-lactamase enzymes in these pathogenic microorganisms tend to prevent the activity of these antibiotics from functioning properly, thereby causing a resistance to these antibiotic in the human body

system, thus, making the individual susceptible to the activities of these pathogenic microorganisms, which is a major health implication.

The presence of *Escherichia coli*, *Salmonella spp*, *Shigella spp*, *Klebsiella spp*, *Serratia spp*, *Proteus spp*, and *Staphylococcus spp* in these herbal drinks poses a serious health problem to the consumers of these herbal products. For instance: *Escherichia coli*, *Salmonella spp*, *Shigella spp*, *Serratia spp*, *Klebsiella spp*, and *Proteus spp* are gram negative, rod shape organisms, which are enteric organisms and when found in the human system, affect the gastrointestinal tract (GIT), leading to health problems such as dysentery, diarrhea, *Salmonellosis*, to consumers of these herbal drinks, instead of doing good to the immune system of the individual. *Staphylococcus spp* is a gram positive cocci, catalase positive organism, which is also a normal flora of the skin. This pathogenic microorganism poses a serious health implication, when consumed into the body, thereby infecting the urinary tract, causing urinary tract infection and other skin infection such as rash, pimples e.t.c, to the consumers of these herbal drinks. However, results of this study exceed the standard limit of  $10^5$  cfu/g for total aerobic count given in European Pharmacopoeia as reported by Okumlola *et al.*, 2010. But the average coliform count of this work does not conform to the standard limit that coliforms should be absent in herbal preparation as given in European Pharmacopoeia and according to the world health organization (WHO) guidelines and standard for assessing the microbiological quality of herbal medicinal products; that no *Salmonella spp* or *Escherichia coli* strain should be present in herbal products. This result renders these herbal drinks under study unfit for human consumption. Since coliforms are the most reliable indicators of faecal contamination, the presence of coliforms in these herbal drinks is an index of the degree of faecal contamination which may indicate a possible presence of harmful disease causing organisms. The presence of fungi could be as a result of the fungal spores suspended in dust present where the herbs are kept since the herbal drinks were prepared with water, the fungi could proliferate, spoil the products and possible produce mycotoxins, which in turn makes the drink dangerous on consumption. The general contamination of these herbal products under this study may be due to the preparation and materials used in preparing the herbal products. It could also be from the hawkers that were involved in the process of production. (Egbebi, 2011) It has been observed from this study that most traditional prepared herbal drinks in Port Harcourt metropolis and Nigeria as a whole are likely to be contaminated with a wide variety of potentially pathogenic bacteria, there is therefore need

for constant monitoring and control of the herbal medicinal products, manufactured, sold, advertised, and used in Nigeria by appropriate agencies. Also the government should take adequate control measures to set specific standards for quality and dosage of traditional medicine. Otherwise, a lot of harm may be done to the health of those who patronize herbalists for medical care. Hawkers of herbal medicinal products also contributes immensely to the poor quality of the products, due to their poor and hygiene practices. Hawking of herbal medicines should be dissuaded and only thoroughly monitored herbal drink (i.e those whose microbiological quality and other qualities are government or NAFDAC approved should be allowed for sale to the public.

### Recommendations

1. Production of these herbal drinks should be done under hygienic condition.
2. Water must be tested continuously for microbial growth. It might be necessary to sterilize deionized water to obtain a sufficient purity.
3. Care must be taken to select good herbs, while infected herbs should be discarded.
4. Raw materials should be tested before use, especially those of natural origin.
5. It is necessary to reassess production processes to ensure that technique capable of reducing microbial contaminations are employed

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