Original Research Article

Assessment of the Microbial Quality of Mekocin, Goko Cleanser and Omega Roots Commercially Sold as Liquid Herbs in Nigerian Markets

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ABSTRACT

The importance of herbs in trado-medicine cannot be overemphasized; however, some herbs prepared in liquid form, and sold in Nigeria markets may be contaminated by microorganisms, fumigants, endotoxin, pesticides and toxic metals, and that the presence of contaminants in herbal products can reduce or even destroy the therapeutic activity of the product, as well as causing adverse effects. The aim of this study was to determine the microbial load of some liquid herbal products sold in Port Harcourt Metropolis. Three (3) different liquid herbal products (Goko cleanser, Mekocin and Omega roots) were used for the study, and were coded as HEB B, HEB C and HEB D respectively. The liquid herbal products were analyzed for their microbiological qualities by testing for the presence of total bacterial load, isolation and identification of pathogenic microorganisms of the herbal products using various culture media. The microbial isolates were identified based on cultural and morphological characteristics, biochemical testing and Gram staining. The predominant bacteria isolate from Goko cleanser (HEB B) is *Bacillus* spp, from Mekocin (HEB C) are *Bacillus* spp and *Aspergillus* spp, and from Omega roots (HEB D) is *Shigella* spp. The descriptive analyses for average bacteria in colony-forming unit/ml from Goko cleanser (HEB B), Mekocin (HEB C) and Omega roots (HEB D) were 24.60±2.24, 48.00±9.08 and 50.70±44.60 respectively. The presence of these microorganisms may be attributed to the source of raw materials and possible contamination in the manufacturing process. It is therefore recommended that herbal medicine producers be properly educated on the dangers associated with intake of microbial contaminants. Also regulatory agencies such as NAFDAC should periodically access the quality of herbal products particularly in Port Harcourt Metropolis and the Nigerian markets in general.

Keywords: Mekocin, Omega roots, Goko cleanser, microorganism, microbial isolates

1.0 INTRODUCTION

Human existence has recorded plants to be a major source of treatment for several medical conditions, and thus the use of plants for therapeutic purpose is still practiced today, and used as a form of alternative medicine. About 85% of the populations of the third world countries

rely on herbal medicine due to affordability, and the avoidance of antibiotic resistance which is commonly associated with the use of antibiotics of western origin (Okeke *et al*., 1999; Hack, 2005). Similarly, a great percentage of Asian and African countries population as estimated by the World Health Organization (WHO) currently use trado-medicine as part of their primary health care (Edgar *et al*., 2002).

Rios and Receo (2005) reported that plants which had healing potentials and contained antimicrobial principles were accepted years ago before microorganisms were discovered by mankind. The healing properties of plants are often related to the amount of secondary metabolites, which vary from one plant to another. Recent studies reported that extract of various parts of herbal plants possess broad spectrum antimicrobial activities when used against pathogenic organisms (Sudhakar *et al*., 2006; Khan *et al*., 2006; Oyetayo and Oyetayo, 2006).Therefore, the world health organization recommended the use of non-toxic herbal preparations in medical establishments of developing countries for the treatment of various illnesses (WHO, 2007).

In Nigeria however, herbal medicines are usually processed from different parts of plants, such as roots, root bark, stems, stem bark, leaves, flowers and fruits, and both the rural and urban markets are flooded with commercial herbal preparations available in different forms including tinctures, teas, tablets, bulk herb or fluid extracts such as authentic bitters and shake off (Knox and Gaster, 2007).

Presently, so many extracts of herbal plants has undergone thorough quality control measures for marketing as drugs (Sittie, 2005). However, these herbal drugs are not free of microbial contamination, and incorrect preparation and/or dosage (Lau *et al*., 2003). Chan (2003) reported that some cultivated herbal medicinal crops are contaminated by microorganisms,

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fumigants, endotoxin, pesticides and toxic metals, and that the presence of contaminants in herbal products can reduce or even destroy the therapeutic activity of the product, as well as causing adverse effects (Khailing *et al*., 1966).

The objective of this study was to assess the microbial quality of Mekocin, Goko Cleanser and Omega roots commercially sold as liquid herbs in Nigerian markets.

2.0 MATERIALS AND METHODS

2.1 Study Area

This study was carried out in Port-Harcourt, Rivers State, Nigeria.

2.2 Study Design/Sample Collection

A total of 15 samples of 3 commercially produced liquid herbal products namely Goko Cleanser, Mekocin and Omega Roots (n=5) were randomly purchased from herbal drug dealers at Garrison, Rumuokoro, and Mile 1 markets, respectively. These herbal products Goko Cleanser, Mekocin and Omega Roots were represented with alphabets HEB B, HEB C and HEB D respectively. Similarly, *Moringa oleifera* seed was also purchased, washed and used to prepare a liquid extract in a sterile environment and labeled as the control.

2.3 Information Listed on the Leaflets of the Liquid Herbal Drugs under Study

1. **Goko Cleanser**

The manufacturers claim this liquid herbal drug contains *Zingiber officinale,* caramel, *Cajanus cojan, Veronia amygdalina, Allium sativium,* and *Saccharum officinarium.* NAFDAC registration number is absent.

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2. **Mekocin**

The manufacturers claim this liquid herbal drug contains Aloe vera and Ginseng. NAFDAC registration number is absent.

3. **Omega Roots**

The manufacturers claim this liquid herbal drug contains Ginseng, *Carica papaya, Magnifera indica, Nebouidia leavis, Azadirachta indica, Jasminium officinli,* and *Aloe barbadensi*. NAFDAC registration number is present.

2.4 Preparation of Media

Media such as nutrient agar and other appropriate selective media which consisting of Sabouraud Dextrose agar, Salmonella-Shigella Agar, Mannitol Salt Agar, and MacConkey Agar obtained from Lab M, TM Media and Biotec were used to culture and isolate the pathogens. The purchased dehydrated media were suspended in distilled water and heated to boil. They were then sterilized in an autoclave at 121^{o}C for 15 minutes. Then 20mls of the sterile media were poured into Petri plates and cooled. The sterility of the prepared media was confirmed by incubating some randomly selected plates at 37^{o}C for 24hours (Prescott *et al.*, 1999).

2.5 Sample Analysis

The spread plate technique as described by Kathryn (2013) was used for the study, in which 1ml of each sample including the control (Moringa) was aseptically introduced into sterile McCartney bottles containing 9ml sterile distilled water which served as the diluents, they were properly shaken, followed by the preparation of a 10-fold serial dilution. Then 0.1ml of each sample dilutions were poured on the surface of the plates containing 20ml nutrient agar,

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and was labeled. Aliquots from the control sterile distilled water which served as the negative control were also plated and spread using sterile bent glass rod. All plates were incubated at 37^{o}C for 24 hours for total heterotrophic bacteria counts. After incubation, plates with 30-300 colony forming units were accepted because this range was considered statistically significant and the counts were taken using a hand tally counter and lens to determine the total heterotrophic bacteria, the arithmetic mean counts were also derived from each samples. In determining the colony forming units (CFUs), the number of colonies counted on a plate was multiplied by the dilution factor to obtain the colony forming unit (CFUs).

The number of CFUs per ml of sample = the number of colonies x volume plated x the dilution factor of the plate counted.

Each of the test organisms were then purified by re-isolating on freshly prepared nutrient agar for bacteria isolates using the streak method and then incubated for 18 hours. The bacterial isolates were identified and characterized on the basis of their cultural characteristics using differential and selective media, Gram staining and biochemical reactions (Cheesebrough, 2002) as follows:

2.5.1 Isolation of Bacteria

I. Isolation of *Shigella* spp.

A pure culture from nutrient agar was sub-cultured onto the surface of a Salmonella-Shigella agar which was incubated at 37^{o}C for 24 hours. Growth of red-pink circular colonies was observed indicating the presence of *Shigella* spp. The bacterial isolates was identified and confirmed by Gram staining and subjected to further biochemical identification such as

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Indole, Citrate utilisation, Triple Sugar Iron (TSI), Motility, and Urease (Cheesebrough, 2002).

II. Isolation of *Bacillus* spp.

A pure culture from nutrient agar was sub-cultured onto the surface of freshly prepared MacConkey (MCA) and Blood agar plates incubated for 24 hours at 37^{o}C. Growth of pink irregular shaped colonies on MacConkey agar and cream colonies with heamolysis on Blood agar was observed indicating the presence of *Bacillus* spp. The bacterial isolates were identified and confirmed by Gram staining and subjected to further biochemical identification such Gram staining technique, motility test and carbohydrate utilization test (Cheesebrough, 2002).

III. Isolation of *Apergillus* spp.

A colony of the test organism was cultured by streaking onto the surface of freshly prepared Saboraud Dextrose agar plates. It was incubated at 250C for 5 days. Growth of yellowish-green colonies was considered to be the presence of *Apergillus* spp. Gram stain and further microscopic studies was done and was compared and confirmed with the coloured charts as documented by Cheesebrough, (2002).

IV. Gram Staining Reaction

This was carried out to differentiate gram positive from gram-negative organisms. Organisms which retained the colour of the initial stain are called gram positive while those that do not retain the primary stain when decolorized are gram negative. The non-retention of the stain is due to the cell composition (Cheesebrough, 2002). All the tests were performed in triplicate, and the mean values presented.

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3.0 RESULTS

3.1 Total Bacterial Counts

The distribution of bacteria in the different herbal samples based on the average colony counts on triplicate plates, dilutions used, and the total bacteria counts in colony forming units per ML (CFU/ML) as indicated by the different sample codes (HEB A-C) and their sub groups are shown in table 1 below.

Table 1: Total Bacterial Counts (CFU/ML)

Sample Codes	Average Colony Count on Triplicate Plates	Dilutions Used	Total Bacteria Counts (CFU/ML)
HEB B			
B1	69	10^{-2}	$6.9x10^{4}$
B2	NS	NS	NS
B3	44	10^{-3}	$4.4x10^{5}$
B4	44	10^{-2}	$4.4x10^{4}$
B5	35	10^{-2}	$3.5x10^{4}$
HEB C			
C1	52	10^{-3}	$5.2x10^{5}$
C2	54	10^{-3}	$5.4x10^{5}$
C3	55	10^{-3}	$5.5x10^{5}$
C4	46	10^{-3}	$4.6x10^{5}$
C5	33	10^{-3}	$3.3x10^{5}$
HEB D			
D1	55	10^{-2}	$5.5x10^{4}$
D2	84	10^{-5}	$8.4x10^{7}$
D3	68	10^{-5}	$6.8x10^{7}$
D4	NS	NS	NS
D5	NS	NS	NS
Moringa seed extract **(Control)**	–	10^{-1}	–
Water	–	–	–

KEYS:
NS = Not Significant
– = No growth

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CFU/ML = Colony Forming Unit per ml
HEB B = Goko Cleanser
HEB C = Mekocin
Omega Roots = HEB D

3.2 Average Bacterial Counts

The average bacterial counts in each of the liquid herbs are shown below in table 2.

Table 2: Average Bacterial Counts

SAMPLE CODES	AVERAGE BACTERIAL COUNTS (CFU/ML)
HEB B	2.46×10^5
HEB C	4.8×10^5
HEB D	5.07×10^7
CONTROL (MORINGA AND WATER)	-

HEB B = Goko Cleanser
HEB C = Mekocin
Omega Roots = HEB D

3.3 Descriptive Analyses for Average Bacteria

The descriptive analyses for average bacteria in the liquid herbs is shown below in table 3.

Table 3: Descriptive Analyses for Average Bacteria (CFU/ML)

HERBAL PRODUCTS	Descriptive analysis (Mean± SD) for Average Bacteria Counts (CFU/ml)
HEB B	24.60±2.24
HEB C	48.00±9.08
HEB D	50.70±44.60

KEYS:
± = (Mean± Standard Deviation)
– = No growth
CFU/ML = Colony Forming Unit per ml
HEB B = Goko Cleanser
HEB C = Mekocin
Omega Roots = HEB D

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3.4 Herbal Samples and Predominant Microbial Isolates

The different liquid herbs with their respective bacterial isolates are shown in table 4 below.

Table 4: Herbal Samples and Predominant Microbial Isolates

Samples	Predominant Bacteria Isolates
HEB B	*Bacillus spp.*
HEB C	*Bacillus spp., Aspergillus spp.*
HEB D	*Shigella spp.*

HEB B = Goko Cleanser
HEB C = Mekocin
Omega Roots = HEB D

4.0 DISCUSSION

Of the liquid herbs used for this study, only Omega roots had a NAFDAC registration number, while Goko cleanser and Mekocin had no such number; this is not in accordance with the prohibition law of the manufacturer, advertisement, sale and distribution of herbal medicinal products in Nigeria without proper registration by NAFDAC (Herbal medicine and related products regulations, 2004).

The liquid herbs had their contents stated on their labels, and this is in agreement with the European Agency for the Evaluation of Medicinal Products (EMEA) and World Health Organization (WHO), which emphasized on the importance and need for the various constituents of drugs to be stated clearly on their labels (EMEA, 1998; WHO, 1993).

The results from this study shows that both the total and mean counts were generally higher than the accepted values as given by WHO norms which states that the total bacteria count should be 10^5 CFU/ml; the results reveals that the herbal products were grossly contaminated

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with different bacteria. The predominant bacteria isolate from HEB B is *Bacillus* spp., from HEB C are *Bacillus* spp and *Aspergillus* spp., and from HEB D is *Shigella* spp.

This study is in line with the results obtained by Oyetayo (2008) and Bibha and Nabaraj (2012) who analyzed herbal medicines and reported that Bacillus spp. which is commonly found in soil, air and dust was the major contaminants in almost all herbal medicines tested. Similarly, Esimone (2007) reported *Bacillus subtillis* as being predominant in herbal medicines. The result is also comparable with Adeleye *et al*. (2005) who they reported the presence of *Bacillus subtillis* and *Bacillus cereus* in herbal medicines. However, the presence of these microbes may be due to poor handling and the unhygienic condition of the environment. This result is also in line with a similar study carried out by Idu *et al*. (2008) who reported that the bacteria populations isolated from liquid herbs include *Staphylococcus aureus* (50%), *Bacillus subtillus* (40%) and fungal isolates consists of *Aspergillus Spp* (85%) and *Penicillum* spp (50%).

Furthermore, this study is also similar to the study carried out by Coulibaly *et al*. (2009) on traditional medicines where he found out that the herbal drugs bought from herbalists were contaminated with *Escherichia coli* and *Staphylococcus aureus*. Due to the habitat of these two organisms which are the human digestive tract and genitals, there is a possibility of humans contaminating these samples reflecting a lack of strict hygienic measures. Also in agreement with this study, is that carried out by Oyetayo (2008), where the microbial load of two Nigerian herbal remedies from Ondo state, Akure was analyzed resulting in the isolation of 3 bacteria (*Bacillus cereus*, *Bacillus subtilis* and *Bacillus coagulans*).

Furthermore, the microbial agents isolated in this study are known to be predominant in air and water as a result, their presence in the herbal products may be due to the raw materials,

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water, methods of preparation used and the sanitary conditions of the environment were the herbal products were produced. Considering the following facts regarding the increased usage of herbal drugs in the society along with poor quality control measures taken by the manufacturers and vendors, it leaves a great question mark on the safety of consumer health (Deshpande *et al*., 2010).

The Moringa seed liquid extract that was prepared using aseptic conditions and good manufacturing procedures showed no growth of organism when analyzed. This has proved the fact which still remains that the herbal medicine producers are ignorant of the sanitary conditions to which these herbal medicines should be manufactured or prepared. Most of them go to an extent of using bottles with fancy labels to attract their consumers. These bottles may not have been properly washed or sterilized resulting in the high levels of microbial load isolated in this study. Microbial contamination can render plant material toxic through the production of toxic compounds which goes a long way to pose more problems for the consumers of these products and so rather than treating or curing the ailments for which they are taken, it creates new health risks or aggravates it thereby making them to not get better.

5.0 CONCLUSION

This study has shown that most of the herbal products sold in Port Harcourt metropolis are not sterile. In other words, they are not free from contaminants, and when consumed, they can serve as a possible source of infection thereby creating more harm than good to the consumers of these products. Therefore there is need for health officials and regulatory agencies to perform at regular intervals a more detailed analysis of these herbal preparations.

COMPETING INTERESTS DISCLAIMER:

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Authors have declared that no competing interests exist. The products used for this research are commonly and predominantly use products in our area of research and country. There is absolutely no conflict of interest between the authors and producers of the products because we do not intend to use these products as an avenue for any litigation but for the advancement of knowledge. Also, the research was not funded by the producing company rather it was funded by personal efforts of the authors.

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