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Microbiological, parasitological and lead contamination of herbal medicines consumed in Enugu, Nigeria.

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Abstract

Background/purpose: Herbal medicines are made from mixtures of different plant components used by traditional medical practitioners for the treatments of various diseases in Nigeria in unhygienic conditions. The present study investigates microorganisms contaminating these concoctions and also assessing any contamination with heavy metal with limited reference to lead poison from samples consumed in Enugu, Nigeria.

Method: Eighty herbal preparations were randomly purchased from different locations and dealers in Enugu according to their therapeutic claims. The samples were analysed and examined microscopically for evidence of parasite. Serial dilutions of the preparations were aseptically prepared, while the total viable count was determined for the isolates which were identified using standard culture and biochemical techniques. The method of Association of Analytical Chemists (AOAC) was adopted for estimating the lead content of the samples.

Results: Three species of protozoan ((*Entamoeba histolytica*, *Entameoba coli*, *E. coli*, *Giardia intestinalis*), and three species of helminth parasite (*Toxocara canis* hookworm, *Ascaris lumbricoides*) were recovered with a contamination rate of 51.3 percent. *A. lumbricoides* was the highest recovered parasitic organism (53.7%), *Bacillus* spp. (38.2%), *Staphylococcus aureus* (7.3%), *Escherichia coli* (18.2) and *Pseudomonas aeruginosa* (20%), were the commonest bacterial isolates while *Salmonella* spp. and *Klebiella* spp. yielded 3.6% each. Fungi species were also isolated from the various samples (*Aspergillus flavus* (22.7%), *A. niger* (10.7), *Geotrichum candidum* (17.3), *Trichophyton mentagrophytes* (10.7%), *Microsporium canis* (6.7%), *Rhizopus* (17.3), *Mucor* spp. (5.3%), *Penicllium* spp. (5.3%), *Syncephalastrum racemosus* ranking the least (4%) while lead was dictated with a range of 0.027-1.081 mg/L in the positive samples.

Conclusion: There was a huge contamination of the herbal concoctions in Enugu with microorganisms, and some with lead poison. This could be a source of many enteric and other infections that abound in the expanse, and stress the pressing need for standardization and regular tests of these provisions by appropriate agencies.

Keywords: Bacteria, Fungi, Lead, Herbal medicine, Nigeria.

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Introduction

According to World Health Organization (WHO) [1], herbal medicines are medications prepared from one or more plant parts (roots, stem, bark, seeds and or fruits). The practice of ethno medicine is a complex multidisciplinary system constituting the use of plants, spirituality and the natural environment as had long been the source of healing for people for millennia [2]. Long term before the advent of modern medicine, herbs were the mainstream remedies for nearly all ailments [3]. Over one-third of the population in developing countries lack access to essential medicines, hence the use of herbal medicinal products and supplements has increased tremendously over the past three decades with not less than 80% of people worldwide relying on them for some part of primary healthcare [1].

In Nigeria, like in other developing nations of the globe, more and more people are using herbal medications. The conventional drugs are generally fake or good ones remain out of reach to the poor masses. People also have the erroneous feeling that when the medicine is 'natural', that spells 'safety'. Some traditional herbalists in Enugu have also attached 'prizes' to each bottle of herbal medicine purchased. Vouchers are issued upon each purchase made and these will eventually form tickets for a 'lucky dip' at the end of the year with prizes like brand new vehicles, motorbikes, sewing machines and free holiday tickets attached. The more you buy and consume, the more chances you have to win. Hence, the use of herbal medicine is now a household word in Enugu and more people in the society, young and old, educated and uneducated have embraced herbal medicines.

The growing popularity has also been linked with its typical low side effect profiles, low cost and high level of acceptance by patients and the majority of the population [4,5].

The increasing use of these concoctions raises concerns about their safety to users. The herbs used in making herbal medicines are normally soaked in water, which further compounds the problems of safety, including the general environmental pollution and filth that abound in most parts of the study area. Water is also a scarce commodity and most people use water from local ponds and streams, or hand dug wells as pipe-borne water is almost unavailable.

Previous studies in the area show a huge contamination of both streams and well water sources [6,7]. Oyetayo [8] noted that some of the shortcomings of herbal medicines are scientific proof, imprecise dosage, imprecise diagnosis and unhygienic conditions under which the herbal products are produced as well as the contaminated environment (soil and air) where the plants are grown. Herbal preparation may therefore carry large and varied number and types of microorganisms originating from water, air and soil or from the unhygienic practices of the manufacturers, dealers as well as utensils and containers.

The contamination of herbal medicines with heavy metals has also been reported as medicinal plants have been shown to be contaminated with toxic metals in the environment for growth in Nigeria [9,10]. Both pathogenic microbes and lead presence in herbal preparations meant for treatment can give rise to serious health hazards, damage and deterioration of the general health status of the partakers. The aim of the present study therefore is to investigate bacterial, fungal, parasitic and lead contamination of herbal medicines largely consumed in Enugu, Enugu State, Nigeria.

Materials and Methods

Samples of herbal medicines examined in the current study have already been treated with all pre-manufacturing processes, including boiling if need be (depending on methods used by the manufacturer) have been concluded prior to purchase. The samples have been bottled and need no further treatment or processing prior to use. The labels on each bottle include instructions like 'keep in a cool, dry place or avoid direct sunlight or shake before drinking including dosage'. A total of 80 samples of herbal medicines were purchased from traditional herbalists, dealers, distributors and hawkers in the Enugu East, Enugu South and Enugu North Local Government Areas of Enugu, in Enugu State Nigeria. For each sample, the therapeutic claims were noted as the herbalists would not release the identity of the herbs, roots and other plant parts used for the preparation (Table 1). Labels of each sample bottle (which gave information on company names and therapeutic claims) were removed prior to laboratory studies, just immediately after purchase from dealers, and replaced with numbers. These numbers were rhymed with the labels in a privately kept laboratory notebook till the end of the entire study to avoid any manner of bias.

Microbiological study

Standard bacterial and fungal cultures on *Salmonella*, Shigella agar, Mackonkey agar, nutrient agar and Eosin methylene blue agar, Potato Dextrose Agar (PDA) and Biochemical techniques were used for the isolation and identification of the various species. After the preparation of the culture media, the sterility was confirmed by incubating blindly selected plates of different types at 37°C for overnight.

One ml of each sample was aseptically introduced into 9 ml of sterile distilled water and properly mixed and a 10 fold serial dilution up to 10^{-6} was done. Pour plate method was used for microbial enumeration. 0.1 ml from the 10^{-6} dilution was pipetted into the sterile petri dish and 20 ml of freshly prepared cooled to 50°C, nutrient agar and PDA each was dispensed into the various plates, mixed well and allowed to solidify. Plates for bacteria were incubated at 37°C for 24 h and those for fungi were kept at room temperature for up to 72 h. Developed colonies were counted to obtain total viable count and discrete colonies were sub cultured onto fresh agar plates for proper identification. Each sample was cultured and total viable counts done in triplicates for both bacteria and fungi.

Identification of organisms

Bacterial isolates were identified based on their cultural characteristic and biochemical tests (Gram reactions, motility, coagulase, catalase, oxidase, indole, urease, citrate and carbohydrate fermentation tests while fungal isolates were examined macroscopically using their cultural characteristics, pigmentation, and microscopically using lactophenol cotton blue mounts and examined under 40X objective.

Parasitological study

Wet mount preparations with physiologic saline and iodine were prepared from sediments after centrifuging the herbal medicines. The formalin-ether concentration techniques were used for the detection of helminth egg and protozoan cysts. Each sample was prepared in triplicates for parasitological studies.

Analysis for lead

The methods of the Association of Analytical Chemists (AOAC) were adopted [11]. Analysis was done after chemical degradation (wet washing with nitric acid) (1:2) and extracted with deionized water. The extract was aspirated into the spectra AA 220 FS spectrophotometer after inserting lead hollow cathode lamp. The result of the lead content of the various samples is then measured and each sample was also treated in triplicates.

Results

Microbiological study

A total of 7 bacterial species was encountered in the study. *Bacillus* spp. ranked highest (38.2%) while *Klebsiella* and

Salmonella spp. were the least encountered with 3.6% occurrence each.

The general rate of occurrence of bacterial organisms was 68.8% of the total 80 samples screened (Table 2).

Nine species of fungi were recorded in the study with a total occurrence rate of 93.8%. The highest was *Aspergillus flavus* (22.7%), with *Syncephalastrum* spp. as the least with only 4% occurrence rate (Table 3).

Parasitological study

A total of 41 (53%) parasitic occurrence was regarded in the 80 herbal medicine sample studied. *Ascaris lumbricoides* 53.7% recorded the highest, hookworm ova 19.5% was the next in occurrence followed by *Toxocara canis* 12.2%. The least were *Entamoeba coli*, *E. histolytica*/*dispar* as *Giardia intestinalis* bracketing at 4.9% each (Table 4).

The mean total microbial counts are shown in Table 5 according to therapeutic claims. Samples were collected in batches of 10 each according to the therapeutic claims randomly selected from the 3 local government areas in Enugu.

Though more fungi were recorded in the study from the 80 samples of herbal medicine studied, followed by bacterial and the parasites being the least, there was however no statistically significant differences in the occurrence of these organisms in the different samples investigated.

Lead content

The concentration of lead ranged from <0.001-1.082 ppm with the highest detected in malaria and typhoid preparations, followed by blood purifier preparations, with the least recorded for irregular menstruation preparations (Table 6).

Table 1. *Distribution of herbal medicine analysed in Enugu.*

Herbal medicines	Indications	Dosage
1-10	Diabetes, ulcer, hypertension	3 table spoons daily
11-20	Malaria and typhoid	One cup 3 × daily
21-30	Sexually transmitted disease	2 table spoons 3 × daily
31-40	Irregular menstruation	3 table spoons daily
41-50	Stomach pain and dysentery	3 table spoons daily
51-60	Infertility/low sperm	One cup 3 × daily
61-70	Pile, haemorrhoids, waist pain	One cup 2 × daily
71-80	Blood purifier	Adult 2 table spoons 4 × daily

Table 2. *Bacterial isolates from 80 samples of herbal medicine.*

Bacteria	Occurrence	%
Bacillus spp.	21	38.2
Pseudomonas aeruginosa	11	20
Escherichia coli	10	18.2
Enterobacter spp.	5	9.1
Staphylococcus aureus	4	7.3
Klebsiella spp.	2	3.6
Salmonella spp.	2	3.6
Total	55	68.8

Table 3. *Fungal isolates from the 80 samples of herbal medicines.*

Fungi	Occurrence	%
Aspergilus flavus	17	22.7
Rhizopus spp.	13	17.3
Geotrichum candidum	13	17.3
Aspergillus niger	8	10.7
Trichophyton rentagrophytes	8	10.7
Microsporium canis	5	6.7
Penicillium spp.	4	5.3
Mucor spp.	4	5.4
Syncephilastrum racemosus	3	4
Total	75	93.8

Table 4. *Parasites detected in 80 samples of herbal medicines.*

Parasites	Occurrence	%
Ascaris lumbricoides	22	53.7
Hookworm	8	19.5
Toxocara canis	5	12.2
Entamoeba coli	2	4.9
Giardia infestinalis	2	4.9
Entamoeba hisdytica/dispar	2	4.9
Total	41	51.3

Table 5. *Mean total bacterial and fungal counts of all herbal medicine samples according to therapeutic claims.*

Herbal medicine	Therapeutic claims	Total bacterial counts (cfu/ml)	Total fungal counts (cfu/ml)
1-10	Diabetes, ulcer hypertension	2.5×10^3	7.7×10^4

11-20	Malaria and typhoid	8.0×10^4	8.0×10^5
21-30	Sexually transmitted diseases	4.5×10^6	3.5×10^5
31-40	Irregular menstruation	$2,2 \times 10^6$	1.1×10^3
41-50	Stomach pain and dysentery	3.2×10^5	4.3×10^4
51-60	Infertility/low sperm count	7.2×10^7	7.0×10^4
61-70	Pile, haemorrhoids and waist pain	2.1×10^3	1.2×10^5
71-80	Blood purifier	9.0×10^6	3.0×10^7

Table 6. *Level of lead detected in the herbal medicines according to therapeutic claims.*

S. no	Types of herbal medicine	Mean lead levels detected (mg/l)
1	Diabetes, ulcer hypertension	0.061
2	Malaria and typhoid	0.027
3	Sexually transmitted diseases	0.179
4	Irregular menstruation	0.179
5	Stomach pain and dysentery	0.245
6	Infertility, low sperm count	0.173
7	Pile, haemorrhoids and waist pain	1.081
8	Blood purifier	0.097

Numbers reported are means of triplicate determination. Detection limit for AHPA/WHO, 0.005 mg/l

Discussion

From the results, it could be seen that the herbal concoctions consumed in Enugu, Nigeria are largely contaminated by bacteria, fungi, parasitic organisms and lead. The total bacterial counts ranged from 2.1×10^3 in the pile, haemorrhoids and waist pain preparations to blood purifier with 9.0×10^6 CFU/ml. Fungi ranged from 1.1×10^3 CFU/ml in the irregular menstruation preparations to 8.0×10^5 CFU/ml in the malaria and typhoid preparations. This is quite high in many of these herbal concoctions when compared with the specific standard limit of 1×10^5 CFU/ml for bacteria and fungi as recommended by the World Health Organization [12].

Almost all the herbal medicines analysed were seriously contaminated with various species of bacteria including *Bacillus* spp, *Pseudomonas aeruginosa*, *E. coli*, *Enterobactes* spp, *Staphylocoocus aureus*, *Klebsiella* and *Salmonella* species, while the fungi included 9 species (*A. flavus*, *Rhizopus* spp, *Geotrichum candidum* as highest with *Syncephalastrum* spp. as the least. Many of these bacteria are enteric suggesting inadequate hygienic practices on the side of producers and marketers suggesting the access of unwholesome materials like faecal material/wastes into these herbal medicines. Coliforms are also used as indicators for faecal pollution of water supplies, also suggesting that access to the herbs might have been by way of a polluted environment, contaminated water used in the preparations, contaminated utensils, and contaminated herbs/plants as well as directly from handlers. *Bacillus* spp. ranked highest and this may be as a result of inadequate heat processing, improper handling of products and contaminated processing equipment [13], probably through the soil.

Microbial contaminants such as bacteria, fungi and parasites pose a general health hazard. Herbal medicines harbour various types of microorganisms. This is because the trees and plants from which they are made have microorganisms adhered to their stems, barks, leaves, flowers, fruits and roots. Also, the microbial contamination of herbal medicines depends on several environmental factors, including the gross pollution in our environment, coupled with the sub-standard processing methods of the concoctions. Microbial contamination may also come from dust, atmosphere and contaminated utensils, water and those preparing them. This therefore encourages an almost similar distribution of these organisms, both qualitatively and quantitatively which differences were not statistically significant among the batches of tested herbal medicines.

Studies abound associating microbial and heavy metal contamination of herbal medicines and plants in different parts of Nigeria. The results obtained from the current series are in accordance with the work of Idu et al. [14]. In Benin City, Edo State, Nigeria where some similar bacteria were isolated from medicinal plants, including *Staphylococcus aureus*, *E. coli* and *Bacillus subtilis*. The fungi encountered in that study were also found in this study except that *Aspergillus niger* (85%) and *Penicillium* spp. (50%) were the highest fungal isolates in the current series, they were only 10.7% and 5.3% respectively in this study which may be associated with the extent and type of environmental contamination involved in these herbal preparations. However, in the study of Akure, Ondo State of Nigeria, the herbal products yielded a completely different set of fungi (*Basidiobotrytis* spp., *Oedocephalum* spp., *Varicosporium* and *Articulospora* spp. and three *Bacillus* species only [8]. The current series also agreed with the study done in Lagos [15], where apart from *Flavobacterium* spp., almost similar bacteria were encountered and apart from *Aspergillus oryzae* and *Fusarium* spp., the fungal isolates encountered in the current series largely rhymed. This may also be related to the nature and type of environmental contamination. In Ado-Ekiti, Western Nigeria, 20 bacterial spp. and 3 fungal strains were isolated from herbal products studied [16]. In this study, 60% of the samples were contaminated by *E. coli*, 40% of *Salmonella* and *Pseudomonas* species, 30% by

Staphylococcus aureus, 20, *Serratia marcescens*, 30% *Klebsiella pneumoniae* and 10% was contaminated by *Proteus mirabilis*. This is not in accordance to the current study where these isolates were much less in the herbal products analysed. Here, *E. coli* contaminated 18.2% of the samples, *Pseudomonas* spp. 20%, *Staphylococcus aureus* 7.3%, *Klebsiella* and *Salmonella* species, each only in 3.6%. No *Serratia* spp. nor *Proteus* spp. were encountered. Our work recorded *Bacillus* spp. (38.2%) while no *Bacillus* spp. was recorded in the Ado-Ekiti study [17]. Similar distribution, but with varying percentages were also recorded in some Nigerian States for fungi and bacteria with levels of aflatoxin that were objectionable in herbal medicines. Our study also shows that parasites are possible contaminants of herbal medicines. This is line with the work of Posadzki et al. [18] who implicated parasites as contaminants and adulterants of herbal medicinal products. *Cryptosporidium* species, *Taenia*, *Blastocystis*, *Giardia* and *Entamoeba* species were similarly detected in fresh packaged herbs in Iran and also in fresh unwashed herbs [19]. Thus, it is likely that parasitic contamination of herbal medicines found in our study may have come from contaminated water or herbs and may be contributing to the many parasitic ailments that abound in the area.

The study also revealed that the herbal medicines were contaminated with mean lead levels ranging between 0.027-1.08 mg/L in the different groups analysed. These values were above that of WHO [12], of 0.005 mg/L for detection limit of lead for herbal preparations. The concentration of lead in the herbal medicines ranges from 0.027-1.081 mg/L. The concentration of lead is not significantly different among the herbal medicines tested $P<0.05$. The WHO, Malaysia, China, and Thailand set the limit for lead in medicinal herbs at 10 ppm. Based on the set standards, the concentration of the tested herbal medicines is below the permissible limit. However, lead is considered one of the most toxic elements, causing both acute and chronic poisoning with adverse effects on various body systems such as kidney, liver, digestive, brain damage and disorder of the central nervous system [20], and there may be a future cumulative effect for those that consume a lot of these concoctions. The WHO sets maximum acceptable limits of $<10^5$-$<10^7$ CFU/g for general microbiological contamination of herbal medicines. Specific limits for herbal medicines meant for internal use range between 10-10^3 for *E. coli* and other *Enterobacteria* and maximum of 10^5 for aerobic bacteria while there is a total absence of pathogenic bacteria including *Shigella*, *Salmonella* and *Clostridium* spp, while yeasts and moulds is 10^3 per gram. The Lead level was observed to be different between the batches of herbal medicines though not statistically significant. Lead is introduced into the environment through gasoline from automobiles and trucks, gradual losses of fine metal particles. Additionally, lead is introduced into the biosphere through the burning of coal, through industrial production of metal products such as steel and brass, as well as lead for batteries and its addition to paints and solder. It's therefore very likely that herbs obtained from bushes/trees close to roadsides with high traffic and from around mechanics workshops, industrial zones and paint factories, including areas where houses are being renovated or painted and close to welder's workshops will be more prone to higher lead contamination of both raw and finished products. The highest lead levels in this study were detected from herbal medicines manufactured and purchased in Emene zones of Enugu East local Government area, which is the industrial layout of Enugu State, Nigeria. The values obtained in the current study are much higher than the WHO maximum acceptable limits for herbal medicines, which suggests a serious need for an instituting an urgent regulatory and quality standards for maximum limits in Nigeria. However, there may be need to use much more numbers of samples of herbal medicines from different parts of the country in order to have a true representation and to appreciate the exact degree of variability.

This poses a public health challenge, realizing that lead content beyond this value in food, drug and water are implicated in hepatic, renal, cardiovascular diseases, blood, nervous and bone disease as reported by Martin et al. [21] and Jarup [22]. A previous study in Enugu [9], revealed a lead level of 0.11 mg/kg-0.80 mg/kg of some road side tree leaves suggesting that tree leaves on high traffic routes had the highest concentration of lead showing some implication for human health, plant biochemical status and phytopotency. In Enugu, many herbalists harvest tree leaves, roots and bark for herbal medicines from trees found along the roads and the detection of high lead levels in herbal medicines as detected in this study is therefore, not very surprising. By virtue of this finding, it is not unlikely that phytopotency of these preparations must have been affected too.

A key and growing source of heavy metal contaminants in the urban atmosphere is the automobile [23,24]. In automobiles, lead is mainly a component of engine oil, other lubricants and fuel [25] and leaded fuel is still widely used in most developing countries because it is less expensive. In countries where leaded fuel is used, the addition of alkyl-lead additives accounts for eighty percent of lead in ambient air [26].

The present results indicate the need for regular monitoring for microbial contaminants and other unwholesome materials in the herbal medicines widely consumed with this part of the world including standardization of practice and instituting a regulatory system for these preparations and their consumption. Education and regular re-training of the traditional herbalists is also seriously recommended.

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