

ASSESSMENT OF HEAVY METAL AND MICROBIAL QUALITY OF FIVE HERBAL MEDICINAL CONCOCTIONS SOLD IN ANAMBRA STATE

ABSTRACT

The increasing popularity and widespread use of herbal remedies as alternative medicine in Nigeria has raised genuine concern for their efficacy and public safety. In this study, five herbal concoctions sold in Anambra State claimed to be beneficial in the treatment of sexually transmitted diseases, diabetes, pneumonia, typhoid and malaria, for boosting Immune and sexual enhancement, were procured from herbal vendors and evaluated for possible contaminants. The pH of the herbal concoctions were checked using digital pH meter. Heavy metals analysis was carried out using Varian AA 240 Flame Atomic Absorption Spectrophotometer (AAS). The microbial quality test was carried out using standard methods. The pH of the samples, showed that they are all acidic. The metal analysis revealed presence of Lead, Cadmium, Chromium, Zinc and Copper at concentrations below the WHO maximum permissible limit for herbal preparations. The concentration of Iron in the five herbal Samples and Manganese in sample **A** and **D** are above the maximum permissible limit. Microbial quality test shows that two of the herbal preparations contained bacteria with total viable bacterial count range of 3×10^2 to 4×10^2 cfu/ml and one contained fungi with total viable fungal count of 1×10^2 cfu/ml. All within the World Health Organization (WHO) acceptable limits. The result of this study shows that the concentrations of heavy metals in the herbal concoctions and the microbial load are not a matter of concern from the toxicity point of view. Therefore it is ascertained that the studied herbal

concoctions are safe for human consumption. However, caution should be placed on the strict regulation and registration of such products.

Keywords: Herbal concoction, Contaminants, Bacterial counts, Fungal counts, Regulation, Popularity.

1. INTRODUCTION

Herbal medicine, also called botanical medicine or phytomedicine, refers to herbs, herbal materials, herbal preparations, and finished herbal products that contain parts of plants or other plant materials as active ingredients [1]. The plant materials include seeds, berries, roots, leaves, bark or flowers[2]. Since ancient times, herbal medicines have been used by many different countries throughout the world to treat illnesses and to assist bodily functions and their uses have increased substantially both in developed and developing countries [3]. Over 80% of the populations in some Asian and African countries depend on traditional medicine for primary health care [1]. In Nigeria, there appears to be an overwhelming increase in usage of herbal medicinal products in the treatment and prevention of diseases due to their availability and affordability [4]. Despite the widespread use of herbal medicines globally and their reported benefits, they are not completely harmless [1]. Recent studies have shown fluctuating amounts of contaminations ranging from heavy metals, micro-organisms and other adulterants in herbs and herbal products from different parts of the world [4]. Medicinal herbs have been reported to be contaminated with microorganisms indigenous to the soil and plants where they are grown. Also, poor conditions during harvesting and post-harvest handling of the herbs and herbal products are predisposing factors to contaminations [5,6]. Studies have reported contamination of herbal medicinal products with several microorganisms such as *Bacillus* spp., *E.coli*, *Salmonella* spp.,

Staphylococcus aureus, *Trichosporium spp*, *Candida spp*, *Shigella spp*, *Penicillium spp* and *Aspergillus spp* among others [7-9]. Moreover, Plants take in water with minerals and heavy metals from soil and in this way, toxic or non-toxic heavy metals deposits on different parts of the plants[10]. Some heavy metals like manganese, selenium, iron, zinc and copper, are essential in very minute amounts to maintain the metabolism of the human body while some like cadmium, arsenic, lead and mercury are toxic even at low concentrations [11-14]. Contamination of herbal medicines with toxic metals such as cadmium, lead, arsenic and mercury has also been reported [15]. Hence, with the ever increasing use of herbal medicines and the global expansion of the herbal medicines market, safety of the consumer has become a concern [9,15]. It is therefore of utmost importance that the microbiological status and potential toxicity as a result of heavy metal contamination of herbal medicines be ascertained before they are used. Therefore, this present study evaluated five herbal concoctions for their heavy metal content and microbial load.

2. MATERIALS AND METHODS

2.1 Sample Collection:

Five (5) different herbal concoctions were randomly selected for this study from their different producers in Anambra state. The Herbal concoctions were selected based on high patronage and usage. The herbal product samples selected were identified and coded sample A to D as indicated in Table 1. Some of the herbal medicine had NAFDAC registration number and some do not have NAFDAC registration number.

Table 1. Herbal Concoctions and their Indications:

Product Code	Product Name	Form	Compositions	Indications	NAFDAC Reg.no
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A	Otika herbal flusher	Liquid	<i>Veronia amygdalina</i> , <i>Xylopia aethiopica</i> , <i>Zingiber officinale</i> , <i>Cymbopogon Citrtus</i> , <i>Morinda Lucida</i> , Aloe vera	Immune booster, diabetes, Sexually transmitted disease(Syphilis)	Present
B	No name	Liquid	Not indicated	Pneumonia	Absent
C	African Iba mixture	Liquid	<i>Kigelia Africana</i> , <i>Nauclea latifolia-linn</i>	Malaria and Typhoid	Present
D	Key starter for men	Liquid	Saffron leaf, Panax ginseng, Lutea root, Mondia whitei, Cinnamon, Rosea leaf	Sexual Enhancement	Absent
E	Cold RC	Liquid	Not indicated	Pneumonia	Absent

2.2 pH Determination:

A digital pH Model ME-962P was used for the pH determination. The pH of the samples were determined directly as described by Onwordi *et al.*,[3]. The pH of the herbal samples was monitored at time intervals of 24hrs, 48hrs, 72hrs and 120hrs.

2.3 Heavy Metal Analysis:

The heavy metal analysis was done using Varian AA 240 Atomic Absorption Spectrophotometer [16]. Each of the herbal samples were thoroughly mixed by shaking and 100ml of each was transferred into a glass beaker of 250ml volume to which 5ml of concentrated nitric acid was added and heated to boil until the volume is reduced to about 15-20ml with addition of

concentrated nitric acid in increments of 5ml. The mixture is cooled, transferred and made up to 100ml using metal free distilled water. The sample is aspirated into the oxidizing air-acetylene flame. When the aqueous sample is aspirated, the sensitivity for 1% absorption is observed.

2.4 Microbial load analysis:

This was carried out using pour plate method as described by [17]. Nutrient Agar (NA) for enumeration of bacteria and Sabouraud Dextrose Agar (SDA) for enumeration of fungi were prepared based on manufacturer's directives and sterilized at 121°C for 15 min. The media were allowed to cool to 45°C. Ten fold serial dilutions of the herbal concoctions were prepared by first dispersing 100 mg (powder solid sample) and 1ml (for liquid samples) in 9 ml of sterile distilled water in sterile test tubes and the resulting solutions diluted further to 10^{-10} . Using a sterile pipette, 1 ml of the diluted samples was taken and aseptically transferred into sterile petri dishes. Then 15 ml of molten sterile nutrient agar was poured into the seeded Petri dishes and swirled to distribute the medium homogeneously. This was allowed to stand for 5 minutes to diffuse and solidify and then incubated for 24 hours at 37°C. Colonies which develop on the plates between 30 and 300 were counted and recorded as colony forming units per millilitre (Cfu/ml).

3. RESULTS

3.1 pH result:

The pH values of the five herbal concoctions are shown in **Table 2**. It ranged from strongly acidic of 3.43 to slightly acidic of 6.56. Sample **D** has the highest pH value of 6.56 and sample **E** has the lowest pH value of 3.43.

3.2 Heavy Metals Analysis Result:

The mean concentrations of heavy metals detected in the five herbal concoctions are shown in **Table 3** and **4**. The result showed that the concentration of lead in the samples ranges from

0.2727 to 2.0130mg/L. Sample **C** had the highest concentration of lead (2.0130mg/L) while Sample **E** had the least concentration (0.2727mg/L). The concentration of Cadmium ranges from 0.1121 to 0.1302mg/L. Sample **B** had the highest concentration of Cadmium (0.1302mg/L) and Sample **E** had the least concentration (0.1121mg/L). The concentration of Chromium ranges from 0.0683 to 0.5528mg/L. Sample **C** had the highest concentration of Chromium (0.5528mg/L) and Sample **A** had the least concentration (0.0683mg/L). The concentration of Zinc in the herbal Samples ranges from 0.1518 to 0.4287mg/L. Sample **A** had the highest concentration of Zinc (0.4287mg/L) and Sample **E** had the least concentration (0.1518mg/L). The concentration of Iron in the herbal Samples ranges from 0.4149 to 7.7552mg/L. Sample **A** had the highest concentration of Iron (7.7552mg/L) and Sample **C** had the least concentration (0.4149mg/L). The concentration of Copper in the herbal Samples ranges from 0.0103 to 0.0651mg/L. Sample **A** had the highest concentration of Copper (0.0651mg/L) and Sample **E** had the least concentration (0.0103mg/L). The concentration of Manganese in the herbal Samples ranges from 0.1951 to 2.7044mg/L. Sample **A** had the highest concentration of Manganese (2.7044mg/L) and Sample **C** had the least concentration (0.1951mg/L).

3.3 Microbial Quality:

The result of the microbial quality of the ten herbal samples is shown in **Table 5**. It shows that Sample **B** and **E** are contaminated with bacteria, with bacterial count of 4×10^2 cfu/ml and 3×10^2 cfu/ml respectively. It also shows that Sample **B** is contaminated with fungi, with fungal count of 1×10^2 cfu/ml.

Table 2. pH Values of the Herbal Concoctions:

Sample	A	B	C	D	E
pH	5.08±0.07	5.15±0.14	5.78±0.14	6.56±0.14	3.43±0.07

Table 3. Mean Concentrations of Heavy Metals in the Herbal Concoctions:

Sample	Pb (mg/L)	Cd (mg/L)	Cr (mg/L)	Zn (mg/L)	Fe (mg/L)
A	0.2987±0.0006	0.1277±0.0021	0.0683±0.0005	0.4287±0.0059	7.7552±0.0041
B	0.3506±0.0002	0.1302±0.0007	0.2236±0.0019	0.2439±0.0004	5.7672±0.0031
C	2.0130±0.0000	0.1167±0.0001	0.5528±0.0002	0.2104±0.0029	0.4149±0.0012
D	0.3377±0.0005	0.1277±0.0002	0.1429±0.0008	0.3659±0.0028	5.3582±0.0041
E	0.2727±0.0002	0.1121±0.0004	0.0807±0.0001	0.1518±0.0039	1.9881±0.0030

Table 4. Mean Concentrations of Heavy Metals in the Herbal Concoctions:

Sample	Cu (mg/L)	Mn (mg/L)
A	0.0651±0.0007	2.7044±0.0027
B	0.0459±0.0004	0.2633±0.0008
C	0.0493±0.0012	0.1951±0.0039
D	0.0212±0.0000	0.8629±0.0045
E	0.0103±0.0003	0.2748±0.0027

mg/L = milligram litre

Table 5. Total Viable Bacterial and Fungal Counts in the Herbal Samples:

Product code	Bacteria load (cfu/ml)	Fungal load (cfu/ml)
A	No growth	No growth
B	4 × 10 ²	1 × 10 ²
C	No growth	No growth
D	No growth	No growth

E	3×10^2	No growth
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4. DISCUSSION

The pH of the herbal samples are suitable although Zamir *et al.*, [18]. noted that pH 5–8.5 may support bacterial growth. The heavy metals of major concern to the human health in this study include lead, cadmium and chromium. Metals such as zinc, copper, iron and manganese are essential nutrients and important for the physiological and biological functions of the human body. However, an increase in their intake above certain permissible limits can make them toxic [13]. Zinc is required for numerous processes in the body; protein synthesis, DNA synthesis, immune function, nerve function, accelerating wound healing, decreasing inflammation, treating diarrhea, reducing oxidative stress, preventing osteoporosis, lowering blood sugar, reducing acute respiratory infections (like pneumonia) and many enzymatic reactions [19-20]. Whittaker [20] and Besong *et al.*, [21] noted that zinc increases testosterone levels and improves blood flow to the genital area thereby enhancing sexual function. The concentrations of zinc in the herbal samples were all below the maximum permissible limit of 50mg/L set by WHO [22] for herbal preparations. The presence of zinc in sample **A** and **D** could have contributed to their use in treating diabetes and enhancing sexual performance respectively. Also zinc in sample **B** and **E** could have contributed to their use in treating pneumonia. Moreover, since zinc boosts immune system, its presence in an antimalarial drug will help fight malaria. The concentrations of Iron in the herbal samples are above the maximum permissible limit of 0.1mg/L set by WHO [22] for herbal preparations. However, Iron is an essential nutrient which helps the body to make hemoglobin and myoglobin, promote energy production, reduce fatigue, synthesize hormone, support immune system and cognitive function. Samali *et al.*, [23] identified iron in the anti-

malarial herbal preparations analyzed and noted that it boosts optimum hemoglobin supply which suppress severity of malaria condition by meeting the body requirement. He also reported that iron identified in the analyzed sex-drive herbal preparations is required to increase blood volume for optimal sex performance [23]. The concentrations of copper in the herbal samples are below the maximum permissible limit of 20mg/L set by WHO [22] for herbal preparations but copper is an essential mineral which plays a role in making red blood cells, maintaining nerve cells and immune system [24]. It has also been reported that copper acts as an antioxidant, reduce inflammation, lower cholesterol and blood pressure. Sample **A and D** had concentration of manganese above the maximum permissible limit of 0.3mg/mL set by WHO[22] for herbal preparations. Manganese helps the body form connective tissue, bones, blood clotting factors and sex hormones [25].Manganese has also been reported to reduce inflammation [26-27] and regulates blood sugar [27-28] and just like zinc increases testosterone level, thereby enhancing sexual function [29]. Manganese just like zinc will also contribute to the use of sample **A and D** in treating diabetes and enhancing sexual performance respectively. Lead is a highly poisonous metal affecting every organ in the body mostly the nervous system [30]. Ingestion of lead has been reported to cause severe damage to the brain and kidney, anaemia, miscarriage in pregnant women, reduce fertility in men and even death [30]. Cadmium can also lead to a variety of adverse health effects including cancer [14]. Cadmium exposure can result to renal and hepatic dysfunction [14]. Chromium has been reported to cause severe respiratory, cardiovascular, gastrointestinal, hepatic and renal damage [31]. However, The concentrations of Lead, Cadmium and Chromium in the Samples were all below the maximum permissible limit of 10mg/L, 0.30mg/L and 2mg/L respectively set by WHO [22] for herbal preparations and therefore not a threat from toxicity point of view. The results of this study is related to that of

Turkson *et al.*,[32] who also reported the presence of iron, zinc, copper, manganese, lead and chromium all below WHO permissible limit in two polyherbal products sold in Ghana and contrary to the results of this study, Abdullahi *et al.*,[13] reported the presence of cadmium, lead, copper, chromium and zinc, with chromium and lead above WHO permissible limit in anti-malarial concoctions marketed in Kaduna. For the microbial quality, according to WHO [22], the maximum acceptance limit/safe level for total aerobic bacteria and fungi count in herbal preparations is 10^7 and 10^4 (cfu/mL) respectively. European Pharmacopeia (EP) gave maximum acceptance limit for aerobic bacteria and fungi count as 10^5 and 10^4 (cfu/mL) respectively while United States Pharmacopeia (USP) gave maximum acceptance limit for aerobic bacteria and fungi count as 10^4 and 10^3 (cfu/mL) respectively [18]. The results obtained compared to allowable limit of acceptability according to WHO, USP and EP showed that the bacteria growth in Sample **B** and **E** are still below the acceptable limit and also the fungal count of Sample **B** is below the acceptable limit. The result of this study corroborates with that of Turkson *et al.*,[32] who also reported total bacterial count range of $1.27 \times 10^3 - 2.17 \times 10^3$ and total fungal count range of $1.09 \times 10^3 - 1.83 \times 10^3$ cfu/ml below WHO permissible limit in two polyherbal products analyzed. Contrary to this results, Hamza *et al.*,[33] reported bacterial contamination of herbal concoctions marketed in Gombe having total bacterial count range of 1.0×10^9 to 2.4×10^9 cfu/ml above WHO permissible limit. Microbial contaminants can cause serious health Hazards. Yusuf *et al.*,[34] cited some infectious bacterial agents as the causes of dysentery, travellers' diarrhoea, abdominal disorder and pains, sore throats, staphyloenterotoxemia, salmonellosis, fever and many others. Ibrahim *et al.*,[35] reported that *S. aureus* has been an agent of food poisoning. The presence of *S. typhi* in the herbal concoctions can cause enteric fever, and gastroenteritis when ingested at very low doses [36]. The presence of fungi in herbal preparations under certain

conditions may lead to the secretion of toxic metabolites such as mycotoxins and mycotoxins possess substantial risk of carcinogenic, neurotoxic, immunotoxic, and mutagenic effects [18].

5. CONCLUSION

The result of this study shows that the concentrations of heavy metals in the herbal samples are not a matter of concern from the toxicity point of view. Also microorganism detected in two of the herbal concoctions had counts below WHO permissible limit. Therefore it is ascertained that the studied herbal concoctions are safe for human consumption. However, it should be noted that presence of microorganism is a possible threat to human health especially if the drugs are not properly stored and also heavy metals have tendency to accumulate having low excretion rates through the kidney which could result in damaging effects on humans even at very low concentrations. Hence periodical assessment of these herbal preparations would go a long way toward predicting the quality assurance and safer use of these herbal products. Manufacturers of herbal products especially sample **B** should be compelled to adhere to strict quality control measures in order to ascertain the quality and safety of their finished products. National Agency for Food Drug Administration and Control (NAFDAC) should enforce strict regulation and registration of these commercial herbal drugs. Moreover, beyond compliance, it is also essential to monitor establishments that market these herbal medicines by checking that they have a license from appropriate health authority for this trade.

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