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An investigation of reasons for packaged herbal medicinal product use and safety in Lagos State, Nigeria.

[*1]Amusa Oluwafemi Daniel, [2]Eleyowo Oluwole Olusola.

[1]Department of Cell Biology and Genetics, University of Lagos, Nigeria

[2]Department of Science Laboratory Technology, Lagos State Polytechnic, Nigeria

Received: 05/ September/2018 Accepted: 20/ December/ 2018

ABSTRACT

Background and aims: Herbal medicine is of great importance to man and has been increasingly acceptable worldwide during the last decades as the main form of health care. With this increase, the safety of its products have become a major concern in public health. Hence, the study was aimed to elucidate the reasons for its increased patronage and ascertain the safety of the commonly preferred herbal medicinal product in Lagos State.

Methods: Questionnaires were administered to 200 herbal users randomly selected among the populace of Lagos State. Microbial load and heavy metal analysis were evaluated on 15 most preferred herbal medicinal products for safety evaluation. Results were then compared with WHO permissive limits.

Results: Natural nature (48.5%), effectiveness (17.6%) and affordability (10.3%) were cited as the major reason for the increased preference for herbal medicinal products. These were supported by family (32%) and media (22%) influences. Microbial load of the 15 most preferred herbal medicinal products evaluated were below WHO permissive level while some of them had cadmium, nickel and zinc beyond WHO permissive level. *Baccillus* sp. was observed to be the predominant bacteria (100%) while *Aspergillus niger* was the highest observed fungi (33.33%) among sampled products.

Conclusion: The study revealed that although, the herbal products evaluated in the study had low microbial loads, the presence of cadmium, nickel and zinc beyond permissible limits constitute a major toxic concern to the users.

Keywords: Herbal medicine, Heavy metals, Microbial load

Original article

***Corresponding author**: Amusa O.D. (Ph.D.); Department of Cell Biology and Genetics, University of Lagos, Nigeria; Email: oluwafemiamusa@gmail.com , Phone: +2348065547710

INTRODUCTION

Herbal medicine is of great importance to man and his health. It is perhaps the oldest method of coping with illnesses. Its use has been increasingly accepted worldwide during the last few decades often as the main source of healthcare in low- and middle-income countries (Sa'ad *et al.,* 2006; WHO, 2007). Herbal medicinal product (HMP) encompass a variety of self-prescribed preparations of plant origin that may generally be categorised as food, dietary supplements, cosmetics, and herbal medicinal products. The classification of herbal products is not aligned at either the European Union (EU) or global level, and remains under national competence (AESGP, 2009; WHO, 2005).

However, with their popularity, global market expansion and increased usage, the safety of HMPs has become a major concern in public health (WHO, 2007). They are not completely harmless despite their widespread use and reported benefits (Tracy and Kingston, 2007). There is a common belief by the public that these plants products are safer and more effective than prescription drugs, and that they have fewer adverse effects (Park *et al*., 2010). This has increased the inappropriate and non-regulated use of several HMPs which may put the health of the users at risk of toxicity and infection (Oreagba *et al*., 2011).

We observed recently that there has been an increased preference of package HMPs to hawked or road side herbal products in the Lagos metropolis especially in public places, parks etc. even in the academia. This elicit our interest to evaluate reason(s) for this increase among herbal users and ascertain the safety of the most used HMPs among the sampled populace of Lagos State, Nigeria.

METHODS

Development of Questionnaire for Survey

The questionnaire method was used in the study to evaluate for perception of herbal usage among sampled participants. The questions asked were both open- and closed-ended. Two hundred (200) adult participants who wilfully consented to the study after verbal explanation were selected randomly from the populace of Lagos state, Nigeria for the survey. Each participant was interviewed by the researcher, after the contents of the questionnaire had been explained to them in their native tongue (illiterate participants) or English (literate participants). The structure interview was adopted in this study to allow the explanation of each terminology used to the participants. This was to enable the elimination of bias involving self-administered questionnaire in the study. The questionnaire was divided into four sections; section A dealt with the respondents socio-demographics, section B dealt with the respondents' knowledge about herbal products,

section C dealt with the respondent's attitude towards herbal products and section D dealt with the respondent's perception and beliefs about herbal products. The completed questionnaire was collected and analysed. Data was expressed in counts (responses) and percentages.

Herbal Product sample collection

Fifteen (15) packaged HMPs most used according to sampled respondents for oral administration were bought from local markets in Lagos State, Nigeria. Samples were both liquid and solid, registered and non-registered, local and foreign medication forms. Samples were placed in the sterilized plastic bags and stored accordingly until needed. The sample labels, registration numbers, form, production location and their prescribed usage along with their manufacturers are described in Table 1 below.

Heavy metals evaluation of herbal products

100 ml/10 g of each of the herbal product sample was measured into a 250 ml conical flask and about 2-3 g of glass beads. 5ml of aqua redia (HNO_3:HCl, 1:3) was then added to the content inside the flask and mixed thoroughly. The flask, with the content was later placed on a hot plate to heat for some minutes to evaporate to about 15 ml. The flask was then cooled and another 5ml of conc. HNO_3 was added. The flask was covered with watch glass. Few ml of conc. HNO_3 was added dropwise on till the solution appears light colour and clear. The flask and watch glass were washed with distilled water and the sample was filtered into the Atomic Absorption Spectrometry (AAS) ready for analysis. In the present study, the analysed metals include lead, cadmium, chromium, nickel, copper, manganese, cobalt, iron and zinc. The gases used for reading were acetylene and compressed air in the flame analysis with hollow cathode lamp, a procedure performed in accordance with Association of Analysis Communities (AOAC) guidelines.

Preparation and sterilization of media

Microbial evaluation was done following the method of Eleyowo *et al*. (2016). The following media which include Nutrient Agar (NA), Salmonella Shigella Agar (SSA), MacConkey agar (MCA) and Potato Dextrose Agar (PDA) were used for the enumeration of microbial counts. They were prepared

according to the manufacturer's guide and sterilized in an autoclave at 121^{o}C for 15 min.

Microbial analysis of herbal product samples

Microbial analyses were carried out in Microbiology Laboratory, Department of Microbiology, University of Lagos, Lagos State. The pour plate method was used to cultivate serially diluted portions of the herbal product samples under investigation. Enumeration was carried out on NA, SSA, MCA and PDA for bacteria, coliform and fungi counts. Triplicate plates of appropriate dilutions were prepared. The NA, SSA and MCA plates were incubated at 37^{o}C for 24 - 48 h for bacteria growth while PDA plates were incubated at room temperature (28 ± 2^{o}C) for 48 - 72 h for fungal growth. The developed microbial colonies were counted and computed as colony forming units per millilitre or gram (cfu/ml or cfu/g) of each sample respectively. The colonies were isolated and purified for further identified with biochemical reactions which include gram's staining, coagulase test, catalase test and microscopy (Eleyowo *et al*., 2016).

Statistical analysis

The data generated from evaluations were analysed and expressed as mean values of three replicates of the total heterotrophic bacteria and fungi (cfu/ml or cfu/gm) contained in each herbal product sample screened. All analysis was done using IBM SPSS Statistics v.23 software package.

RESULTS

Socio-demographics of participants

200 participants were evaluated in this study with 50 participants each from the locations of study. Socio-demographics reveals the characteristics of participants accordingly. This also reveals majority of the herbal product users to be between the ages of 18 and 40years (87% of the total sampled). More females (60%) than males (40%) using herbal products were sampled. Herbal product users were also more prevalent among singles (62%) than married (32%). More of Christians (49%) and Muslims (46%) than traditional religious participants (4.5%). The percentage of users in tertiary institutions (69%) was however highest compared to other educational level of users (31%) of herbal products. The percentage of low income earners was highest for herbal users (52.6%) as shown in Table 2

Source of HMP recommendation

Percentage proportion of recommendation sources for sampled herbal users is shown in Figure 1. Recommendations from family on herbal product use was highest (32%) followed by media (22%). The least recommendation came from independent research (5%) followed by pharmacist recommendation (9%).

Evaluation of beliefs and reasons for HMP use

The responses and percentage belief of participants' view of herbal product represented in Table 3. The belief that herbal products are natural (97% believed) and a health promoter (96%) ranked highest among participants' beliefs. The least of the belief was religion inclined (61%), this was followed by packaging authenticity (67.5%).

Various reasons were also given by herbal users sampled for their increased patronage. The most reason for herbal products usage among the evaluated participants was that herbal products are natural products (48.5%), this was followed by herbal products' effectiveness (17.6%) while authenticity was the least reason of herbal product usage (5.1%) (Figure 2). The survey also showed that majority (81.9%) of the participant's preference local herbal products to foreign/imported herbal products (18.1%), with 72.5% of the sampled participants preferring liquid herbal products to the solid or capsule form, while alcohol extract (6.5%) herbal products was least preferred.

Side effects from herbal product usage reported by participants

Majority (61.5%) of the participants revealed that their usage of herbal products was unknown to any health worker. Although, 84% of the participant evaluated revealed no side effects associated with herbal product usage, some participants reported dizziness (25%) and running stomach (25%) to be most prevalent side effects experienced from herbal product usage. This was followed by vomiting (18%) and stooling (18%) (Figure 3).

Evaluation of Heavy metal contents in herbal products sampled

Analysis of heavy metals from herbal products evaluated showed all the herbal products have magnesium more than WHO permissible concentration. All except Hb04 contained cadmium more than permissible limit. Hb02, Hb06, Hb07, Hb08 and Hb12 contained high zinc contents; Hb03 contained high calcium contents; Hb04 and Hb13 contained high nickel; Hb01, Hb10 and Hb11

Reason for herbal use and their safety

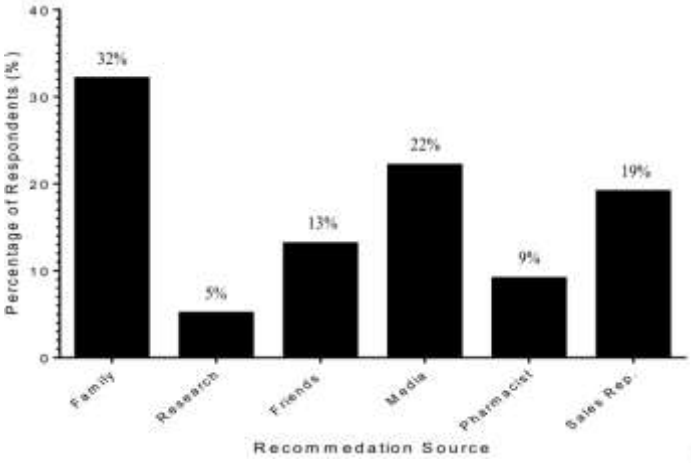

Figure 1: Source of recommendation of herbal product use

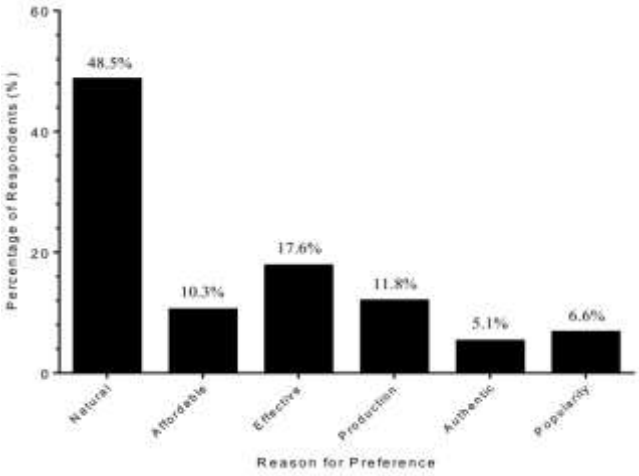

Figure 2: Distribution of reasons for use and preference of herbal products among the respondents evaluated.

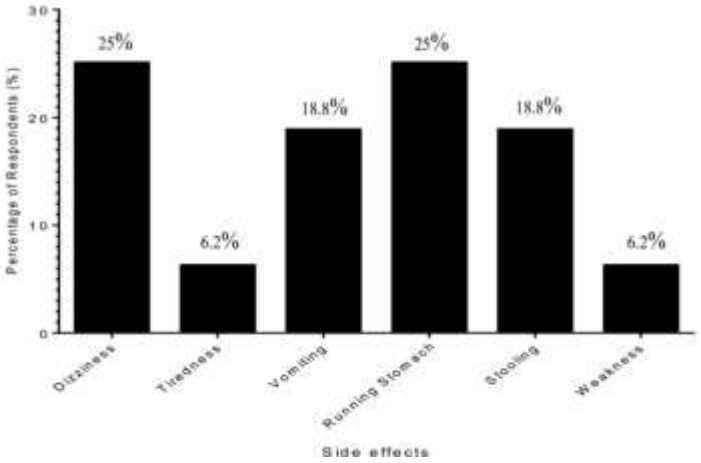

Figure 3: Prevalence of side effects associated herbal product users

contained high chromium contents (Table 4).

Comparative evaluation was done on heavy metal contents in HMP samples based on product's registration status, production location and product's forms in comparison with WHO permissible concentration (Table 5). The evaluation revealed that registered HMP samples contain higher contents of magnesium, cadmium and zinc than both unregistered HMP samples and WHO permissive concentrations. On the other hand, the unregistered brands of HMP samples evaluated contained higher nickel and magnesium contents than both the registered HMP samples and WHO permissive concentrations. In the study, both local and foreign HMP samples contained high nickel, magnesium and zinc contents while local HMPs contained high cadmium contents than WHO permissive concentrations. Also, both liquid and solid forms of HMP samples evaluated contained high nickel and magnesium contents. However, the liquid HMP samples contained high cadmium and zinc contents in addition than the WHO permissive concentrations (Table 5).

Evaluation of microbial load in herbal products sampled

Total bacteria count among evaluated HMPs. No fungi growth was observed with Hb01, Hb02, Hb03, Hb04, Hb07, Hb13 and Hb14. The study also observed no growth in the SSA media used for all HMP samples (Table 6). Prevalent microorganisms isolated include *Bacillus sp.* (100%), *Staphloccocus aureus* (33.33%), *Aspergillus niger* (33.33%), *mucor sp.* (6.67%) and *Rhizopus sp.* (6.67%) were predominat microbes identified in this product (Table 7). Evaluation of registered and non-registered herbal products showed that the registered HMP samples have more bacteria count (1.98 x 10^3cfu) and fungi count (1.17 x 10 cfu) more than the non-registered HMP samples (TBC = 0.92 x 10^3cfu, TFC = 1 x 10^2cfu) (Figure 4). The study also observed more bacteria and fungi colonies (TBC = 2.21 x10^3cfu, TFC = 1.2 x 10 cfu) in local HMP samples than the foreign HMP samples evaluated (TBC = 0.92 x10^3cfu, TFC = 1.0 x10 cfu) (Figure 5). On the other hand, the solid forms of HMPs sampled evaluated harboured more bacteria and fungi colonies (TBC = 3.10 x 10^3cfu, TFC = 1.2 x 10 cfu) than the liquid forms HMPs sampled in the study (TBC = 1.03 x 10^3cfu, TFC = 1.0 x 10 cfu) (Figure 6).

DISCUSSION

There has been significant increase in the production, distribution, marketing and consumption of herbal products in developing countries like Nigeria (WHO, 2007). The study revealed various reasons for the increase HMP use among sampled herbal users from the populace of Lagos State. Majority of herbal users cited the natural nature, effectiveness and affordability of the herbal products as

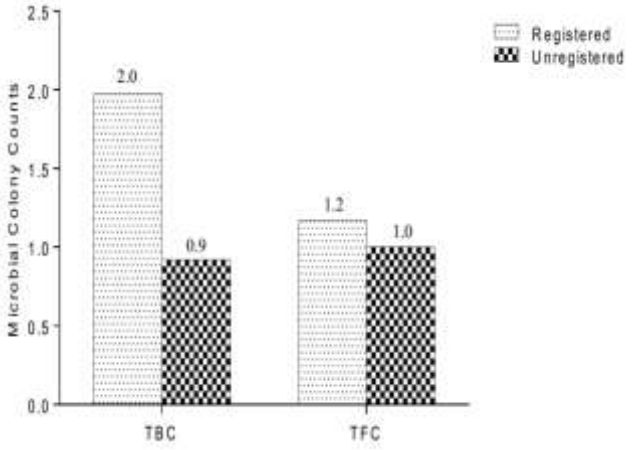

Figure 4: Microbial load of local registered and non-registered herbal products evaluated (TBC: Total Bacteria Count x10^3; TFC: Total Fungi Count x10)

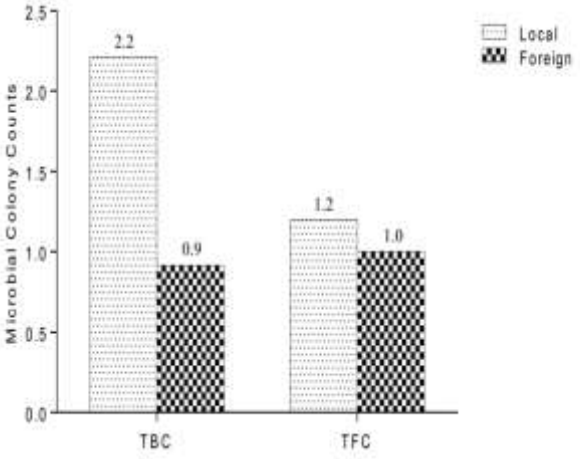

Figure 5: Microbial load of local and foreign herbal products evaluated (TBC: Total Bacteria Count x10^3; TFC: Total Fungi Count x10)

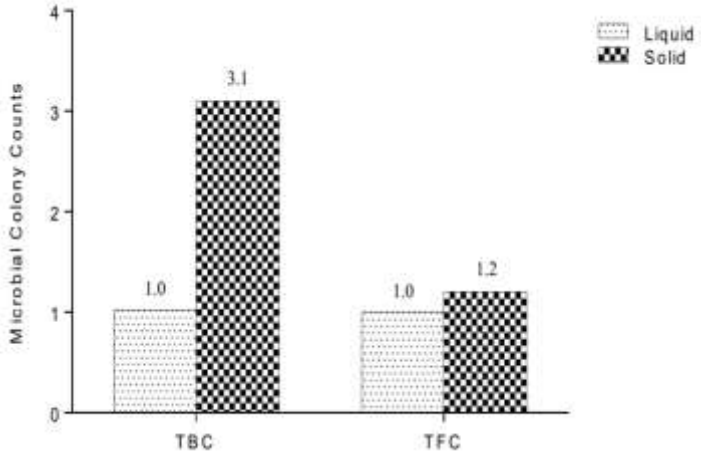

Figure 6: Microbial load of liquid and solid herbal product evaluated (TBC: Total Bacteria Count x 10^3; TFC: Total Fungi Count x 10)

the main reasons for their use. This was supported by the belief that "natural product is safe and affordable" when compared to the unorthodox medicine. This corroborates with the work of Himesh *et al*. (2012) who stated that the demand for herbal products was due to the fact that it is natural and not likely to cause serious side effects compared to conventional drugs. These beliefs were shared by family members, as recommendation by family was observed to the highest influential means of increasing packaged herbal product preference as shown in this study. The media also played a key role in increasing the preference of packaged HMP use among sampled participants as many herbal companies today have taken the media advantage in reaching more customers than the conventional hawking methods. One additional factor that foster the use of packaged HMPs was its availability as reported by the respondents in this study. This coupled with affordability have been known to increase consumer product relationship.

Despite these, herbal users should not be ignorant of the possible contamination of these products by microorganisms especially bacteria and fungi as well as heavy metals contamination (Himesh *et al*., 2012). The study evaluated the safety of 15 most preferred packaged HMPs as stated by respondents in the metropolitan city of Lagos. Some of these products were registered and not registered, liquid or solid forms, majorly local but a few were foreign. The national agency in charge of HMP safety compliance is the National Agency for Food and Drug Administration and Control (NAFDAC). This agency is charge with registering and management of various types of drugs, herbal product inclusive, in Nigeria. This agency is also responsible for regulating and controlling the manufacture, importation, exportation, advertisement, distribution, sale and use of food, drugs, cosmetics, medical devices, chemical and packaged water in Nigeria. They are also charge of making sure that drugs introduced into the general public are of high quality and safe for public use. The registration number of product signifies the certification of the product by this agency. The presence of a certified number from NAFDAC on these products signifies 'safe' and puts confidence on the end user or consumer as regards the product's use. However, the presence of cadmium and zinc metals beyond permissive limits in some registered packaged HMPs in this study is of high concern and questions the integrity of this licensing agency. Although microbial contamination among studied HMPs were below WHO permissive limits, registered packaged HMPs had both higher bacteria and fungi counts than the unregistered packaged herbal product. This did not corroborate with the reports of Archibong *et al*. (2017) who reported high microbial contamination in unregistered HMPs than the registered HMPs. Etuk *et al*. (2008) and Idu *et al*. (2015) also reported a high microbial

contamination in random samples of HMPs purchased in Lagos State contrary to the results obtained in this study. This might be due to improvement in HMP preparation and production procedures from the herbal product companies.

The preference of local packaged HMPs by respondents against foreign HMPs is an indication of the respondents' trust on their native HMPs. The study showed that local packaged HMPs did not differ in safety level with packaged foreign HMPs. Although, both local and foreign packaged HMPs evaluated were having nickel, magnesium, and zinc contaminations more than WHO permissive limits, the presence of cadmium in local packaged HMPs more than permissive limit is an indication of the poor handling of local packaged HMPs when compared to foreign HMPs. Similarly, local packaged HMPs were observed to harbour more microbial load than their foreign counter parts.

The study showed that solid packaged HMPs contained more heavy metal contaminations than the liquid forms. Although most of the respondents preferred the liquid form of HMPs to the solid forms, this is of high concern. This preference might be due to the belief that the liquid forms are more effective than the solid forms although, there was no data to back this reason up. Nickel and magnesium were observed in both liquid and solid forms higher than WHO permissive limits, cadmium and zinc beyond permissive limits were observed in the liquid forms which was lacking in the solid forms of packaged HMPs. The presence of these heavy metals above permissive limits may cause bioaccumulation of these toxic metals in regular consumers which may result in health complications issues. On the other hand, total bacteria and fungi counts were observed to higher in solid packaged herbal forms than the liquid packaged HMP forms but below permissive level. *Bacillus sp.* (100%), *Staphloccocus aureus* (33.33%), *Aspergillus niger* (33.33%), *mucor sp* (6.67%) and *Rhizopus sp* (6.67%) were predominant microbes identified in this study. Previous studies have shown high diversity of microbial contaminations in herbal products both registered and unregistered, liquid or solid forms (Oyetayo, 2008; Archibong *et al*., 2017).

Herbal preparations are used in different forms and may carry a large number of microbes originating from soil, usually adhering to various parts of the herb. The contaminants that present serious health hazard are pathogenic bacteria such as *Salmonella*, *Escherichia coli*, *Staphylococcus aureus*, *Shigella* species and other Gram positive and Gram negative strains of bacteria (Ngari *et al*., 2013). *Salmonella, E. coli* and *Shigella* species were not found among all evaluated HMPs in this study. Although, *Staphylococcus aureus* growth was observed in some HMPs, it was below

significant level when compare to WHO permissive limit. The presence of *Bacillus* species may be as a result of inadequate heat processing, improper handling of products and contaminated processing equipment (Frazier and Westhoff, 2003). The presence of the fungal contaminant shows the possibility of poor storage conditions. This is a serious contaminant since some common species of fungi produce toxins like aflatoxins if allowed to build up in the product. According to the WHO**,** aflatoxins in herbal drugs can be dangerous to health even if they are absorbed in minute amounts (Ngari *et al*., 2013).

The contamination of herbal remedies with heavy metals may be due to soil and atmospheric contamination which poses a threat to its quality and safety. Medicinal plants used in HMPs preparation are normally contaminated with toxic metals during growth, development and processing (Nwoko and Mgbeahuruike, 2011). Some arise from past or present use of agents that pollute the environment and subsequently medicinal plants, such as factory emissions or persistent chemical residues. Due to their excessive use and disposal, contaminants from environmental sources may even be present where an herb is organically grown (WHO, 2007). Microbial contamination of herbs and/or products may result from improper handling during production and packaging. The most likely sources of contamination are microbes from the ground and processing facilities (contaminated air, microbes of human origin). Cross contamination is also possible from extraneous materials such as plastics, glass, and other materials which come in contact with medicinal herbs, herbal preparations or products. Hypothetically, sources of biological contamination could be human excrement, animal manure and faeces used as fertilizers (Kosalec *et al*., 2009).

CONCLUSIONS

The natural nature and affordability coupled with the safety believe in the natural products of HMPs is shown to be the major factors determining the increase use of HMPs. This was largely influenced by family ties as revealed in this study. While most of the HMPs used by herbal users evaluated showed some certain level of safety as believed by users, the presence of some certain heavy metals beyond permissible limits calls for concern. More efforts need to be made by manufacturers to ensure product safety is not compromised at any level of production.

CONFLICT OF INTEREST

The authors declare that there is no conflict of interest. The authors alone are responsible for the content of the paper

Authors' Contributions

Amusa, O.D. Project conception, Questionnaire design and development, Data collection and preparation, Analysis of data, Interpretation of results, Manuscript review

Eleyowo, O.O. Project conception, Questionnaire development, Data collection and preparation, Interpretation of results, Manuscript preparation and review

ACKNOWLEDGEMENT

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Table 1: List of Herbal products evaluated

Code	Brand Label	Form	Production	NAFDAC	Applications
Hb01	Eroxil 5000	Liquid	Nigeria	AF-1300L	Chronic pile, waist pain and haemorrhoid, fatigue, indigestion, low libido.
Hb02	Oroki herbal mixture	Liquid	Nigeria	None	Menstrual disorders and man power
Hb03	Asheitu	Liquid	Ghana	None	Cure for typhoid, paratyphoid fevers, cleans kidney, empty the bowel gently.
Hb04	Fijk Flusher	Liquid	Nigeria	A7-1313L	Tummy problems, improve digestion, reduce excess wright, activate the flow of bile, prevent kidney and bladder infection, purify blood, increase man power, ease menstrual period.
Hb05	Super Bitters	Liquid	Nigeria	A7-0303L	Management of stomach disorders, indigestion, peptic/duodenal ulcers, detoxify the body, cleanses colon impurities, reduce fats/cholesterol level etc.
Hb06	Yoyo bitters	Liquid	Nigeria	A7-1055L	Blood purification, aid digestion, relief menstrual pains, reduce body fat/weight, prevent and cure pile, effective treatment for bones, erectile dysfunction, blood circulation, general body pains.
Hb07	Swedish bitters	Liquid	Germany	A7-0126L	Activates the flow of bile, tones the liver, improves digestion,.
Hb08	Yoyo bitters	Solid	Nigeria	A7-1051L	Blood purification, aid digestion, relief menstrual pains, reduce body fat/weight, prevent and cure pile, effective treatment for bones, erectile dysfunction, blood circulation, general body pains.
Hb09	M&T capsule	Solid	Nigeria	A7-0294L	Malaria
Hb10	Extramune	Solid	India	None	Strengthens immune system,
Hb11	Manfx	Solid	India	A7-0953L	Erectile dysfunction, fertility
Hb12	Kofol	Liquid	None	None	None
Hb13	Essentiale forte	Solid	Germany	None	Hepatoprotectives, bile stabilization, regulation balance of blood lipids, acceleration and support of liver cell regeneration, normalization of the impaired functions and enzymatic activities of a liver cell.
Hb14	Bio-Strath	Solid	South Africa	None	Dietary supplement
Hb15	Sopa A1	Solid	Nigeria	A7-0374L	Blood related diseases, jaundice, anaemia, blood purification.

NAFDAC: National Agency for Food and Drug Administration and Control

Table 2: Characteristics distribution of respondents' socio-demography

Variables	Number of Response	Percentage Response (%)	Valid Percentage response (%)
Age (Years)			
18-25	126	63.0	63.0
26-40	48	24.0	24.0
41-60	23	11.5	11.5
61 and above	3	1.5	1.5
Total	200	100.0	100.0
Gender			
Male	80	40.0	40.0
Female	120	60.0	60.0
Total	200	100.0	100.0
Marital Status			
Single	132	66.0	66.0
Married	64	32.0	32.0
divorced/widowed	4	2.0	2.0
Total	200	100.0	100.0
Religion			
Christianity	99	49.5	49.5
Islamic	92	46.0	46.0
Traditional	9	4.5	4.5
Total	200	100.0	100.0
Education			
None	8	4.0	4.0
Primary	6	3.0	3.0
Secondary	48	24.0	24.0
Tertiary	138	69.0	69.0
Total	200	100.0	100.0
Occupation			
Student	121	60.5	60.8
civil servant	24	12.0	12.1
private enterprise	28	14.0	14.1
Employed	18	9.0	9.0
Unemployed	7	3.5	3.5
Retired	1	.5	0.5
Total	199	99.5	100.0
Income (x #1,000)			
1-15	70	35.0	52.6
16-25	24	12.0	18.0
26-35	11	5.5	8.3
36-45	8	4.0	6.0
46 and above	20	10.0	15.0
Total	133	66.5	100.0

Table 3: Perception and Beliefs of herbal users evaluated

Belief about herbal products	**Number of Respondents**	**Percentage (%)**
They are affordable	193	95.5
They are always available	184	92.0
They are authentic in packaging	135	67.5
It is supported by religion	123	61.5
They are harmless (safe)	145	73.6
They are natural	194	97.0

Table 4: Heavy metal prevalence in herbal product samples evaluated

Samples	Cr	Ni	Mn	Ca	Mg	Na	K	Pb	Cd	Cu	Zn
	mg/l										
Hb01	1.429±0.009*	0.011±0.01	0.029±0.009	49.42±0.020	5.012±0.002*	3.042±0.002	4.072±0.002	0.014±0.023	ND	0.306±0.006	1.692±0.520
Hb02	ND	0.134±0.018*	0.019±0.009	32.11±0.010	4.770±0.070*	3.012±0.002	6.001±0.001	ND	ND	0.282±0.002	4.014±0.004*
Hb03	ND	0.016±0.006	0.042±0.002	61.01±0.010*	5.601±0.001*	2.904±0.004	5.014±0.005	ND	ND	0.199±0.009	2.067±0.007
Hb04	ND	0.027±0.007*	0.019±0.009	28.11±0.010	8.110±0.010*	3.551±0.001	3.046±0.006	ND	0.009±0.001*	0.301±0.001	1.994±0.004
Hb05	ND	ND	0.036±0.006	31.04±0.040	5.220±0.020*	4.901±0.001	4.441±0.001	0.014±0.023	ND	0.292±0.002	3.012±0.002
Hb06	ND	0.012±0.002	0.032±0.002	32.65±0.595	4.631±0.467*	7.012±0.002	3.661±0.001	0.001±0.001	ND	0.330±0.030	11.040±0.040*
Hb07	ND	ND	0.041±0.001	21.42±0.020	3.994±0.004*	6.022±0.002	2.990±0.090	0.009±0.009	ND	0.098±0.008	10.060±0.060*
Hb08	ND	0.003±0.003	0.057±0.007	22.04±0.040	6.012±0.002*	6.110±0.010	2.701±0.001	ND	ND	0.196±0.006	7.012±0.002*
Hb09	ND	ND	0.026±0.006	27.94±0.040	7.012±0.002*	5.701±0.001	4.121±0.001	ND	ND	0.172±0.002	1.099±0.009
Hb10	1.831±0.001*	0.007±0.007	0.033±0.003	24.70±0.700	8.011±0.001*	6.008±0.008	5.672±0.002	ND	ND	0.283±0.003	2.014±0.004
Hb11	0.441±0.001*	0.002±0.001	0.047±0.007	32.01±0.010	6.421±0.001*	5.110±0.010	3.941±0.001	ND	ND	0.311±0.001	2.099±0.009
Hb12	ND	0.001±0.001	0.029±0.009	31.09±0.090	9.011±0.001*	4.201±0.001	1.911±0.006	ND	ND	0.261±0.001	2.114±0.004
Hb13	ND	0.129±0.183*	0.019±0.009	29.30±0.300	4.201±0.001*	4.167±0.007	2.072±0.002	ND	ND	0.421±0.001	6.012±0.002*
Hb14	ND	0.014±0.004	0.016±0.006	26.07±0.070	5.201±0.001*	3.811±0.001	3.016±0.006	ND	ND	0.182±0.002	1.098±0.008
Hb15	ND	0.012±0.002	0.018±0.008	26.91±5.739	4.887±0.090*	3.041±0.001	4.210±0.010	ND	ND	0.142±0.002	2.419±0.009
WHO	0.05	0.02	0.2	50	0.2	20		0.01	0.003	1.0	3.0

* value exceeding permissible permitted; values are represented in mean ± SD standard deviation of replicate (n = 3) determinations; ND: Not detected; WHO: World Health Organization Maximum Permitted Concentration; Cr: Chromium; Ni: Nickel; Mn: Manganese; Ca: Calcium; Mg: Magnesium; Na: Sodium; K: Potassium; Pb: Lead; Cd: Cadmium; Cu: Copper; Zn: Zinc

Table 5: Heavy metal prevalence in herbal product samples based on registration status, production location and form

Group	Cr	Ni	Mn	Ca	Mg	Na	K	Pb	Cd	Cu	Zn
	mg/l										
Product Status											
Registered (n = 9)	ND	0.011±0.009	0.034±0.014	30.171±8.078	5.700±1.258*	4.943±1.383	3.687±0.600	0.010±0.015	0.009±0.009*	0.239±0.084	4.492±3.693*
Unregistered (n = 6)	ND	0.060±0.114*	0.026±0.012	34.638±13.910	5.557±1.358*	3.981±1.159	4.355±1.596	ND	ND	0.273±0.088	3.041±1.826
Product Type											
Local (n = 8)	ND	0.033±0.077*	0.030±0.014	31.278±7.909	5.707±1.206*	4.546±1.526	4.032±0.953	0.010±0.017	0.009±0.009*	0.253±0.0.069	4.035±3.231*
Foreign (n = 6)	ND	0.034±0.085*	0.033±0.013	32.418±13.603	5.572±1.404*	4.670±1.182	3.784±1.278	0.009±0.009	ND	0.249±0.107	3.892±3.265*
Product Form											
Liquid (n = 8)	ND	0.034±0.077*	0.031±0.010	35.857±12.712	5.794±1.712*	4.331±1.464	3.892±1.227	0.010±0.015	0.009±0.009*	0.259±0.073	4.499±3.649*
Solid (n = 7)	ND	0.028±0.078*	0.031±0.016	26.995±3.558	5.964±1.246*	4.850±1.132	3.676±1.129	ND	ND	0.244±0.094	3.108±2.273
WHO	0.050	0.020	0.200	50.000	0.200	NA	NA	0.010	0.003	1.000	3.000

* value exceeding permissible permitted; values are represented in mean ± standard deviation of samples in group; n; number of samples in group; ND: Not detected; WHO: World Health Organization Maximum Permitted; Cr: Chromium; Ni: Nickel; Mn: Manganese; Ca: Calcium; Mg: Magnesium; Na: Sodium; K: Potassium; Pb: Lead; Cd: Cadmium; Cu: Copper; Zn: Zinc; NA: Not available

Table 6: Microbial load evaluation from herbal products sampled

Samples	Form	TBC	TFC	Prevalent Organisms Identified
		cfu/ml or cfu/gm		
Hb01[a]	Liquid	$1.1x10^2$	NG	*Bacillus sp., Staphloccocus aureus*
Hb02[a]	Liquid	$2.1x10^2$	NG	*Bacillus sp., Staphloccocus aureus*
Hb03[b]	Liquid	$4x10^2$	NG	*Bacillus sp.*
Hb04[a]	Liquid	$1x10^2$	NG	*Bacillus sp.*
Hb05[a]	Liquid	$3x10^2$	1x10	*Bacillus sp., Aspergillus niger*
Hb06[a]	Liquid	$2x10^2$	1x10	*Bacillus sp., Mucor sp*
Hb07[b]	Liquid	$1x10^2$	NG	*Bacillus sp.*
Hb08[a]	Solid	$8x10^3$	2x10	*Bacillus sp., Aspergillus niger*
Hb09[a]	Solid	$4.1x10^3$	1x10	*Bacillus sp., Staphloccocus aureus, Aspergillus niger*
Hb10[b]	Solid	$2x10^3$	1x10	*Bacillus sp., Rhizopus sp.*
Hb11[b]	Solid	$1x10^3$	1x10	*Bacillus sp., Aspergillus niger*
Hb12	Liquid	$6.1x10^2$	1x10	*Bacillus sp., Staphloccocus aureus, Mucor sp.*
Hb13[b]	Solid	$1x10^3$	NG	*Bacillus sp.*
Hb14[b]	Solid	$1x10^3$	NG	*Bacillus sp.*
Hb15[a]	Solid	$4.1x10^3$	1x10	*Bacillus sp., Staphloccocus aureus., Aspergillus niger*
WHO		10^5	10^2	

a and b superscript denote local and foreign herbal products; * value exceeding maximum permitted; NG: No growth; cfu/ml: colony forming unit per millilitre; cfu/gm: colony forming unit per gram; TBC: Total Bacteria Count; TFC: Total Fungi Count; WHO: World Health Organization maximum colony count permitted.

Table 7. Frequency occurrence of isolated microorganisms

Microbial Isolates	Frequency	Occurrence (%)
Bacillus sp.	15	100
Staphloccocus aureus	5	33.33
Aspergillus niger	5	33.33
Mucor sp.	1	6.67
Rhizopus sp.	1	6.67