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Heavy Metal and Bacteriological Contamination of Herbal Medicines Sold Over the Counter in the Municipality of WA of the Upper West Region-Ghana.

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Abstract—Analysed in the present study was heavy metal and microbial presence in fifteen (15) different herbal drugs sold at various herbal and chemical licensed shops across the Wa municipality of Ghana. Levels of the metals- Fe, Pb, Zn and Cu were analysed using Flame Atomic Absorption Spectrophotometer. Fe, Pb, Zn and Cu levels were below their respective WHO permissible limits. Fe emerged the most abundant metal, followed by Zn, Cu and Pb for all tested herbal drugs. Fe, Pb, Zn and Cu levels recorded respectively ranged from 1.530±0.007 to 6.774±0.012 ppm, 0.012±0.001 to 0.036±0.002 ppm, 0.935±0.008 to 3.612±0.006 ppm and 0.086±0.013 to 0.440±0.575 ppm. Microbiological analyses were performed by serial dilution and streak plating techniques. Total viable count was performed followed by isolation and enumeration of microbial species including *Staphylococcus aureus*, total coliforms and *Salmonella*, using appropriate selective media in each instance. Three (3) out of the fifteen (15) samples tested, representing 20% showed no microbial presence. The remaining twelve (12) samples representing 80% were found to contain microorganisms. They contained at most two (2) of the three (3) microbial species tested for their presence. *Staphylococcus aureus* emerged the most common microbial isolate being detected in six (6) out of the total samples tested (thus in 40% of samples). Total coliforms were detected in four (4) samples representing an estimated 27% whiles in three (3) of the samples tested, *Salmonella* spp. were detected (thus in 20% of samples).

Keywords—Herbal medicines, Heavy metal contamination, Microbial contamination, Atomic absorption spectrophotometry, Wa Municipality.

I. INTRODUCTION

Herbal medicines are essentially derived from plant parts and often used with virtually no form of chemical or biological processing for the treating various health conditions [1]. Predominantly, plant parts including roots, leaves, barks, fruits as well as seeds are used in the preparation of herbal drugs. Most people have accepted these medicines to be relatively safe compared to western medicines owing to their natural origin/attributes. More than 80% of the population of Africa and developing countries rely on herbal drugs to meet their healthcare needs [2]. The use of herbal medicines has seen grand patronage in both developing and developed countries [3]. The high costs associated with western medicines invariably places contemporary health care out of the reach of the majority of Africans causing them to chiefly rely on traditional herbal formulations to meet their basic health care needs. Then and again, the fact that foreign medicines are mostly beyond the reach of most people in Africa has given grounds to the use of herbal drugs as the preferred alternative for the treatment of various ailments [4]. Thus, for most people living in remote and poor areas, herbal medicines may turn out to be the only available medicinal therapy and thus serving as the first line and basic health care remedy [5, 6]. In Ghana for instance, the use of traditional/herbal medicine has been fused into the conventional health care delivery system [7]. The quality of any herbal drug/medicine not only affects its efficacy but also the very safety of the consumer [5]. Several plants of medicinal value are cultivated across Ghana. These plants are to a greater extent exposed to heavy metal contamination owing to the surge in mining activities in most parts of Ghana. In addition, herbal plants may be exposed to heavy metal contaminants through the process of irrigation, as well as via the application of pesticides and fertilizers [8]. Microbial contamination may also occur through soil, the manure used for planting as well as when persons infected with pathogenic bacteria come into contact with medicinal plants or finished herbal medicines in the course of harvesting, collection and post-harvest and manufacturing processes [9, 10].

The application of herbal medicines in the treatment of various diseases has reached astronomical levels so to speak [3]. There are notable problems that come with the use of herbal medicines ranging from the lack of precise doses to unhygienic methods of preparation that often result in microbial contamination [5]. Taking into account the rise in the use of traditional herbal medicines in Ghana, the quality and safety of herbal products should be of paramount to concern to stakeholders including the health authorities, pharmaceutical companies and the general public at large [3, 5].

The paper has been structured under the following headings: section I which contains a brief introduction of the subject matter , section II which briefly describes related work of other researchers, section III which details the methods and materials used, section IV which contains the results and discussion of findings and section V which concludes research work.

II. RELATED WORK

The presence of diverse heavy metals and microbial contaminants have been reported in various herbal plants and drugs sold on Ghanaian markets in several studies.

Nkansah et al. [8] assessed the heavy metal levels of fifteen (15) medicinal plants sold in Kumasi Central market. Heavy metals of interest included iron (Fe), cadmium (Cd), lead (Pb) and zinc (Zn). All the metals analysed except Cd recorded levels within the WHO maximum permissible limits. The study also established a positive correlation between Cd and Zn levels. The study concluded that the medicinal plants were unsafe for human consumption.

Annan et al. [11] in a similar study also found differences in the levels of heavy metals for the same species of medicinal plants that were growing in different environments. They assessed the levels of Pb, Cd, Hg, Al and As in 10 herbal plants sampled from 5 different geographical locations to ascertain the relationship between the geographical locations and the heavy metal contents of the various herbal plants.

Odonkor et al. [5] also investigated the microbial quality of ten (10) herbal medicinal products obtained from traditional medicine distributors and retail pharmacy shops in Accra, Ghana. They found the microbial loads of the products to considerably vary. The least microbial count was 2.2 x 10^3 CFU/mL with the highest count being 6.2 x 10^3 CFU/mL. Predominant organisms identified included *Staphylococcus aureaus* and *Bacillus* spp. Only one sample had fungi isolated from it. They concluded on the need for continuous monitoring and the need for the standardization of herbal medicines sold on the Ghanaian market.

Similarly, Dei-Tutuwa et al. [12] also found *Escherichia coli* and *Staphylococcus aureaus* in liquid herbal drugs using the polymerase chain reaction (PCR) technique. The numbers of *Escherichia coli* and *Staphylococcus aureus* detected in overnight enriched samples were respectively 10 CFU/mL and 10^3 CFU/mL.

The present study seeks to assess heavy metals and microbial contaminants in some herbal medicines sold at various herbal and chemical licensed shops across the Wa municipality of Ghana. The majority of people within this part of Ghana heavily depend on herbal drugs in managing a wide array of diseases. In Ghana and broadly in Africa, the use of herbal medicines or plants as sources of medicines is more of a tradition passed on from generation to generation [13]. Herbal medicines can in fact be seen as the only alternative medicine for individuals within the very remote areas of Ghana. The outcome of the study is expected to influence policy directions in the aspects of manufacturing/production and monitoring and evaluation of the quality of herbal drugs on the Ghanaian market.

III. MATERIALS AND METHODS

Study Area

The Wa Municipality has its administrative capital at Wa. It is one of the 9 Municipalities and District Assemblies in Ghana's Upper West Region. Its population comprise of 52,996 males and 54,218 females totaling 107,214. It has an estimated landmass area of 234.74 square kilometers, representing approximately 6.4% of the region [14, 15]. It is bordered by Nadowli District to the North and Sawla-Tuna-Kalba District of the Northern Region to the South. Majority of its inhabitants are Moslems in addition to few Christians and Traditionalists [15]. Few immigrants from neighbouring Burkina Faso can be found here predominantly are involved in cattle herding with some also working as farm labourers and others engaged in illegal mining.

Sampling

A total of fifteen (15) different herbal drugs samples were purchased from herbal and chemical licensed shops across the Wa municipality randomly for the study. Sample drugs purchased for the study comprised drugs commonly used in the treatment of illnesses such as malaria, typhoid and menstrual complications. These drugs essentially dominate the Ghanaian market. Samples were assigned the following codes: AB, ABT, ADB, AKB, AKM, ALB, ALM, BMM, BLT, GHM, MFR, MHE, MMW, NHM and RH. Sample codes do not in any way reflect or represent acronyms of herbal drugs used in the study.

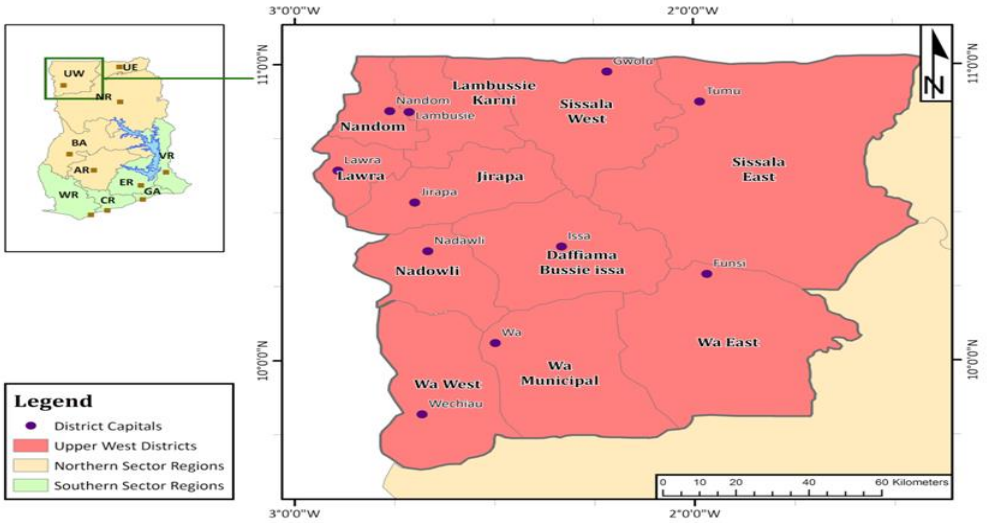

Figure 1: Wa Municipal Map [15]

Apparatus

Glassware used in various experiments were thoroughly cleaned and rinsed. They were initially soaked in a detergent solution for 15 mins rinsed and soaked overnight in 10% (v/v) HNO_3. They were subsequently rinsed with deionized distilled followed by 0.5% (w/v) $KMnO_4$. They were subsequently rinsed with distilled water; oven dried and allowed to cool prior to use [16]. 500 mL digestion tubes, digestion blocks and a hot plate were used. The flame atomic absorption spectrophotometer (FAAS) was used in measuring the metals- iron (Fe), lead (Pb), zinc (Zn) and copper (Cu).

Calibration of Atomic Absorption Spectrophotometer (AAS)

Commercial stock solutions of, Fe, Pb, Zn and Cu were used in preparing the respective standard solutions that were used in calibrating the Atomic Absorption Spectrophotometer (AAS) in each instance. A series/range of concentrations was prepared by serial dilution in each instance for the calibration of the AAS machine [17].

Sample Digestion

Samples were wet digested prior to elemental analysis. In each instance, the sample herbal drug was thoroughly mixed by shaking and 50 mL transferred into a 200 mL Erlenmeyer flask. To the content of the Erlenmeyer flask was added 10 mL of concentrated HNO_3 followed by few boiling chips. The mixture was placed on a hot plate and heated until the volume reduced to about 10-20 mL and complete digestion was indicated by a light coloured solution. The digested sample was filtered using a glass funnel and a Whatman 1 acid-washed filter paper into a 50 mL volumetric flask and topped up to the mark with deionized distilled water [18]. Digested samples were stored at 4°C, ready for AAS analysis. Samples were analyzed at the Environmental Quality Laboratory of Anglo Gold Ashanti- Obuasi.

Heavy Metals Determination

The levels of the metals- Fe, Pb, Zn and Cu were analysed in triplicate using FAAS. A blank was initially run through the AAS machine followed by a series of calibration (standard) solutions. The respective responses were then measured and used to plot a calibration curve. The AAS was adjusted to read zero (0) for the blank solution. For each element, a calibration graph was plotted after which the test samples were atomized and their responses measured. The level of each element in each digest was determined in triplicate. Metal concentrations were obtained from the calibration curve in relation to the absorbance obtained for the test solutions [14].

Microbiological (Bacteriological) Analysis

Total viable count was performed followed by the identification of specific bacterial populations including *Staphylococcus aureus*, total coliforms and *Salmonella* spp. Microbiological analysis was performed at the Microbiology Laboratory of the Food and Drugs Authority, Ghana.

Total Viable Count

Total viable count was performed by the plate count technique. 1 mL of sample was serially diluted through to 10-9 using physiological saline solution. 0.1 mL of each dilution was spread on top of a plate of tryptone soy agar (TSA) medium. Each dilution was plated in triplicate. Plates were left to stand in an upright position for 10 min to facilitate absorption of the inoculum into the agar. Plates were inverted and incubated at 32.5°C for 24h [19]. Average microbial colonies per mL of plated dilutions were calculated from the relation below:

Colony Forming Unit (CFU/mL) = (Number of colonies x Dilution factor)/Volume plated……..…. (1) [19].

Identification and Enumeration of Staphylococcus aureus

10 mL of sample was placed in a sterile container containing 90 mL of buffered saline peptone water (BSPW) and mixed for a minute. This was followed by preparation of three decimal dilutions of the sample homogenate. For each dilution, 10 mL was pipetted into 90 mL of tryptone soy broth (TSB) and incubated at 32.5°C for 24 h. This was followed by streaking a volume of 1 mL of the obtained culture on mannitol soy agar (MSA) and incubating the plate at 32.5°C for 24 h. Resulting colonies were further plated on DNase agar and incubated at 32.5°C for 24 h [20].

Identification and Enumeration of Coliforms

Total coliforms were enumerated to include *Escherichia coli* per the method adopted. 25 mL of sample herbal medicine was pipetted into a sterile container containing 225 mL of sterile buffered dilution water and mixed thoroughly for a minute. Three decimal dilutions of the homogenate were prepared. Each decimal dilution was plated in triplicate. 100 mL of each dilution was filtered using a 47 mm, 0.45 µm pore size cellulose ester membrane filter. The filter was then transferred onto a plate of MI agar. The plate in each instance was incubated for 48 h at 35°C. Colonies appearing blue in colour or fluorescing under ultraviolet light (366 nm) were counted to reflect the total coliforms [21]. Appearing colonies were each transferred into tubes of lauryl tryptose broth (LSB) and incubated at 35°C for 48 h. LSB tubes that tested positive for gas were sub-cultured in brilliant green bile lactose broth (BGLB) for 48 h at 35°C. Gas production in BGLB confirmed coliforms presence [22].

Identification and Enumeration of Salmonella spp.

Salmonella spp., were detected following the method described by Nair et al. [23]. 25 mL of sample was added to 225 mL of buffered peptone water (BPW) and subjected to pre-enrichment at 37°C for 18 h. Three decimal dilutions of pre-enriched sample homogenate were prepared for enumeration of *Salmonella* spp. after incubation. Each decimal dilution was replicated twice. To 10 mL of Rappaport-Vassiliadis broth, 0.1 mL of a dilution of pre-enriched inoculum was added and incubated at 42°C for 24 h. This was followed by the streaking of a loopful (10 µl) of each inoculum on xylose lysine deoxycholate (XLD) agar and incubation at 37°C for 24 h. Presumptive colonies for *Salmonella* appeared slightly transparent red halo with a dark center surrounded by pinkish to reddish zone. Colonies were confirmed by inoculation onto triple sugar iron (TSI) agar slant and incubating for 24 h at 35°C. TSI agar slant was checked for the production of hydrogen sulphide (H_2S) gas and alkalinity to confirm the presence of *Salmonella* spp.

Analysis of Data

Concentrations of metals were presented as mean ± standard deviation (SD) using Minitab (17) statistical software. One-way ANOVA was performed to compare mean concentrations of the various metals measured across the herbal drugs tested. Tukey simultaneous tests for differences in mean metal concentrations for the individual herbal drugs were also conducted using same software. Graphs and bar charts were generated using Microsoft excel (2016) software.

IV. RESULTS AND DISCUSSION

The general premonition that herbal drugs sold in various herbal shops, chemical licensed and pharmaceutical shops across Ghana possibly contain varying levels of various heavy metals as well as diverse species and numbers of microorganisms largely influenced the conduct of this study in an attempt to confirm the assertion.

Heavy Metal Contaminants in Tested Herbal Drugs

Fe, Pb, Zn and Cu were found in the herbal drugs tested in the present study. Shown in Table 1 are the mean concentrations of the aforementioned metal contaminants detected in the fifteen (15) herbal drugs tested in the present study with Figure 2 depicting graphically the levels of these metals in the various herbal drugs tested.

Fe, Pb, Zn and Cu levels measured were found to be lower their respective WHO permissible limits of 15.0, 10.0, 50.0 and 20.0 ppm [22, 23]. Fe emerged the most abundant metal, followed by Zn, Cu and Pb in that order across all tested samples. Ranges of Fe, Pb, Zn and Cu concentrations detected were respectively 1.530±0.007 to 6.774±0.012 ppm, 0.012±0.001 to 0.036±0.002 ppm, 0.935±0.008 to 3.612±0.006 ppm and 0.086±0.013 to 0.440±0.575 ppm (Table 1).

One-way ANOVA analysis to compare mean concentrations of detected metals across the herbal drugs tested showed significant differences ($p < 0.05$). Tukey simultaneous tests for differences in concentration means for the different herbal drugs tested were significant for almost all pairwise tests with regards to Fe and Zn. In the case of Fe, a total of 101 pairwise tests out of the total 105 differed significantly in their mean concentrations ($p < 0.05$). Same was the case for Zn. In the case of Pb, 69 pairwise tests out of the total 105 differed significantly in their mean concentrations ($p < 0.05$). For Cu however, Tukey simultaneous tests for differences in concentration means did not differ significantly ($p > 0.05$) for all 105 pairwise tests.

Table 1: Fe, Pb, Zn, and Cu levels measured for the various herbal drugs tested

SAMPLE CODES	Metal (Mean concentrations in ppm ± SD)			
	Fe	Pb	Zn	Cu
AB	6.774±0.012	0.017±0.001	3.431±0.018	0.281±0.005

ABT	2.175±0.013	0.036±0.002	0.935±0.008	0.123±0.003
ADB	3.358±0.032	0.024±0.002	1.047±0.006	0.149±0.006
AKB	3.741±0.041	0.023±0.001	1.644±0.007	0.215±0.051
AKM	5.033±0.019	0.026±0.003	2.825±0.007	0.440±0.575
ALB	5.666±0.020	0.029±0.001	1.246±0.004	0.267±0.007
ALM	2.642±0.017	0.022±0.002	2.542±0.006	0.163±0.007
BMM	1.792±0.007	0.018±0.001	1.007±0.005	0.158±0.006
BLT	6.556±0.010	0.012±0.001	3.612±0.006	0.207±0.005
GHM	1.767±0.014	0.021±0.002	1.274±0.008	0.086±0.013
MFR	1.530±0.007	0.026±0.002	1.307±0.003	0.163±0.009
MHE	3.120±0.005	0.023±0.002	2.018±0.004	0.245±0.007
MMW	2.965±0.006	0.014±0.001	1.726±0.034	0.142±0.011
NHM	3.016±0.004	0.020±0.001	1.782±0.013	0.181±0.003
RH	1.766±0.036	0.017±0.001	1.078±0.011	0.180±0.013
WHO LIMITS	15.0	10.0	50.0	20.0

*SD = Standard Deviation

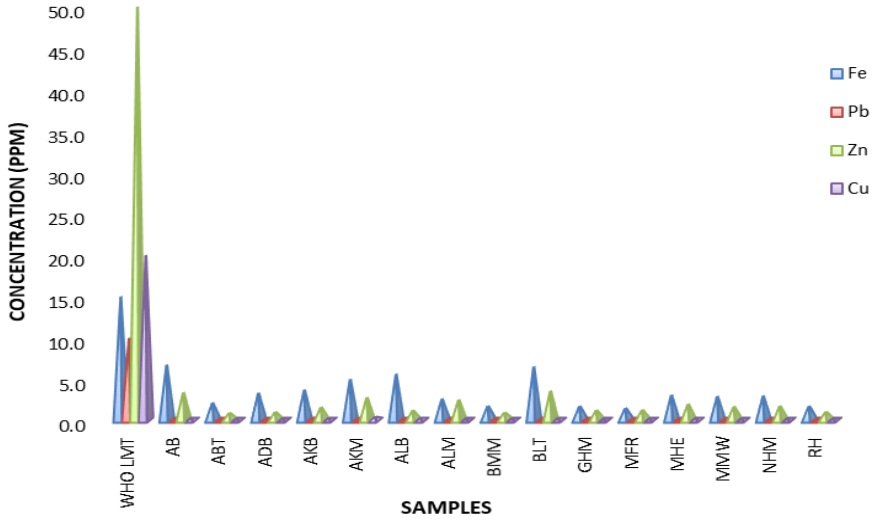

Figure 2: Metal levels of the various herbal drugs studied

Heavy metals are known occur naturally (non-anthropogenic) at background levels with their presence in soil media mainly attributable to breakdown of parent rocks [26, 27]. Elevated background levels/concentrations in soils have been due to anthropogenic activities. Thus, as a result of anthropogenic activities, there has been a high prevalence of heavy metals in soils which end up reaching and being assimilated into medicinal/herbal plants [27, 28]. Fe, Pb, Zn and Cu levels detected in the present study could thus possibly come from the very herbal plants used in preparing the drugs. Several plants of medicinal value are cultivated in various parts of Ghana. These medicinal plants are to a greater extent exposed to heavy metal contamination from the surge in anthropogenic activities including mining (particularly galamsey), irrigation, application of synthetic fertilizers, organic manures and pesticides, contamination of agro ecosystems by industrial effluents or transportation and unhygienic storage conditions, among others [8, 24]. Other possible means by which heavy metal contamination of the tested herbal drugs may have occurred are via contamination during harvesting, cross contamination during processing as well as through the intentional addition of metals for alleged health benefits [29].

Despite the fact that very small amounts of some metals are of health benefit to humans [30], the ability of medicinal or herbal plants to bioaccumulate and bioconcentrate heavy metals in their tissues essentially increases their harmful potential on consumption [8, 31]. Thus, the consumption of trace amounts of metal contaminants above certain concentrations could pose danger human health causing acute and/or chronic poisoning [30]. In respect of the present study, though the levels of Fe, Pb, Zn and Cu detected in the various herbal drugs tested were below their respective WHO permissible limits and do not in any way explicitly imply ‘contamination’, their consumption could still be injurious once they exceed certain thresholds. The order of abundance of the various heavy metals detected was Fe > Zn > Cu > Pb. This could be explained in relation to the herbal plant specie and the rate of uptake of these metals influenced by the level of prevalence/abundance of these elements within the environments where they grow or are grown. Annan et al. [11] in a study found differences in the levels of heavy metals for the same species of medicinal plants grown in different environments.

The presence of Fe, Pb, Zn and Cu in the various herbal drugs as established in the present study, corroborates the

findings of many studies that have reported diverse heavy metal contaminants in various medicinal plants and commercial herbal drugs sold on Ghanaian markets and in various chemical licensed shops across Ghana [11, 32-34]. In the above cited studies, heavy metal contaminant levels detected in herbal drugs were ascribed to the tendency of the medicinal/herbal plants to accrue metals in their tissues. Fe levels (1.530±0.007 to 6.774±0.012 ppm) measured in the present study for instance was consistent with Fe levels of 2.51±0.03 to 7.06±0.03 ppm recorded by Nkansah et al. [8] in their study of heavy metals in medicinal herbs sold in Kumasi Central market. Fe levels recorded for the present study were however lower compared to the range of 20 to 753 ppm reported by Annan et al. [32]. Nkansah et al. [8] in their study also recorded Pb levels in the range of 0.44±0.01 to 0.89±0.01 ppm. The present study in comparison recorded lower Pb levels in the range of 0.012±0.001 to 0.036±0.002 ppm. Pb levels for the present study were however consistent with the range of 0.001 to 1.510 ppm recorded by Sarpong and Boateng [34] in their study. Zn levels in the range of 0.935±0.008 to 3.612±0.006 ppm recorded in the present study were higher compared to the range of 0.21±0.02 to 1.07±0.02 reported by Nkansah et al. [8]. Cu levels in the range of 0.086±0.013 to 0.440±0.575 ppm recorded in the present study were also lower compared to the range of 8.0 to 114.5 ppm recorded by Annan et al. [32] in their profiling of heavy metals in some medicinal plants.

Health Implications Associated with Fe, Pb, Zn and Cu Contaminants in Herbal Drugs

Fe is a major component of haemoglobin and essential for human growth and development. It functions in the transfer of oxygen and electrons in humans. Fe deficiency would thus impede oxygen transfer to cells and cause tiredness, abysmal work performance and dwindled immunity [14]. Low Fe levels are also known to cause gastrointestinal infection, nose bleeding and myocardial infection [35]. Fe in excess is dangerous in children and may cause gastrointestinal and skin problems [36] as well as toxicity and Fe overload as the human body can excrete very little Fe [14].

Pb coupled with the fact that it has no nutritive value is also very toxic [37]. Continuous exposure to Pb can impair nervous system performance and interfere with renal clearance [38]. Inorganic Pb is carcinogen. It is known to cause miscarriage in pregnant women [8]. Pb consumption/exposure above a certain concentration, can result in high blood pressure with adverse effects on key organs such as the kidney and the brain [36].

Another essential element Zn plays a vital role in growth, enzyme structure, enzyme catalytic and regulatory actions in more than three hundred (300) enzymes. Its deficiency is often associated with growth retardation, bone metabolism and hypogonadism. Zn though an essential element, can cause fever, nausea and general weakness when consumed in excess [8].

Cu is an essential element to the human body being a component of many enzyme systems. It facilitates the absorption of Fe and its incorporation into haemoglobin [32, 39]. Cu, though essential in trace amounts is responsible for hyperactivity in autistic children and in low doses can cause anaemia. In high doses it can result in liver and kidney damage, as well as stomach and intestinal irritation [14].

Bacteriological Contaminants in Tested Herbal Drugs

The results of microbial analysis performed are shown in Table 2. Microbial analysis performed included total viable count (TVC) followed by the isolation and enumeration of selected microbial species including *Staphylococcus aureus*, coliforms and *Salmonella*. Three (3) out of the fifteen (15) tested herbal drugs, representing 20% showed no microbial presence. The remaining twelve 12 samples representing 80% however were found to contain microbes. They contained at most two (2) of the three (3) microbial species tested for their presence. *Staphylococcus aureus* emerged the most common microbial isolate being detected in six (6) out of the fifteen (15) herbal drugs tested (thus in 40% of samples). Coliforms were detected in four (4) samples representing an estimated 27% whiles in three (3) out of the fifteen (15) herbal drugs tested, *Salmonella* spp. were detected (thus in 20% of samples) (Table 2). Figure 3 shows a plot of the logarithm of microbial concentrations in the different herbal drugs tested.

One-way ANOVA analysis conducted to compare microbial load means across the herbal drugs tested showed significant differences ($p < 0.05$). Tukey simultaneous tests for differences in microbial load means for the different herbal drugs tested were significant ($p < 0.05$) for 20 out of 66 pairs with regards to TVC. In respect of *Staphylococcus aureus*, microbial load means differed significantly ($p < 0.05$) for 12 out of the 15 pairwise tests. For total coliforms, microbial load means differed significantly ($p < 0.05$) for 5 out of 6 pairwise tests. In the case of *Salmonell*a spp., microbial load means were significantly different ($p < 0.05$) for 2 out of 3 pairwise tests.

Table 2: Microbial counts for the various herbal drugs tested

SAMPLE CODES	MICROBIAL ANALYSIS (CFU/mL)			
	TVC	S. AUREUS	COLIFORMS	SALMONELLA SPP.
AB	$4.4 \pm 0.10 \times 10^4$	$1.1 \pm 0.06 \times 10^3$	ND	ND
ABT	ND	ND	ND	ND
ADB	$1.6 \pm 0.20 \times 10^5$	ND	$2.2 \pm 0.07 \times 10^1$	ND
AKB	$2.3 \pm 0.30 \times 10^6$	ND	$2.0 \pm 0.02 \times 10^1$	$1.4 \pm 0.02 \times 10^1$

AKM	$3.4 \pm 0.10 \times 10^3$	$1.4 \pm 0.03 \times 10^1$	ND	ND
ALB	$2.2 \pm 0.17 \times 10^6$	ND	$1.6 \pm 0.07 \times 10^2$	$1.2 \pm 0.03 \times 10^1$
ALM	$2.3 \pm 0.06 \times 10^5$	$2.2 \pm 0.16 \times 10^1$	ND	ND
BMM	$4.4 \pm 1.00 \times 10^1$	ND	ND	ND
BLT	$3.1 \pm 0.16 \times 10^2$	$2.3 \pm 0.06 \times 10^2$	ND	$1.8 \pm 0.02 \times 10^2$
GHM	$1.0 \pm 0.02 \times 10^1$	ND	ND	ND
MFR	$3.4 \pm 0.03 \times 10^4$	ND	$3.4 \pm 0.07 \times 10^1$	ND
MHE	$4.1 \pm 0.06 \times 10^3$	$1.2 \pm 0.03 \times 10^2$	ND	ND
MMW	ND	ND	ND	ND
NHM	ND	ND	ND	ND
RH	$1.2 \pm 0.07 \times 10^3$	$3.0 \pm 0.04 \times 10^1$	ND	ND

*ND = None Detected

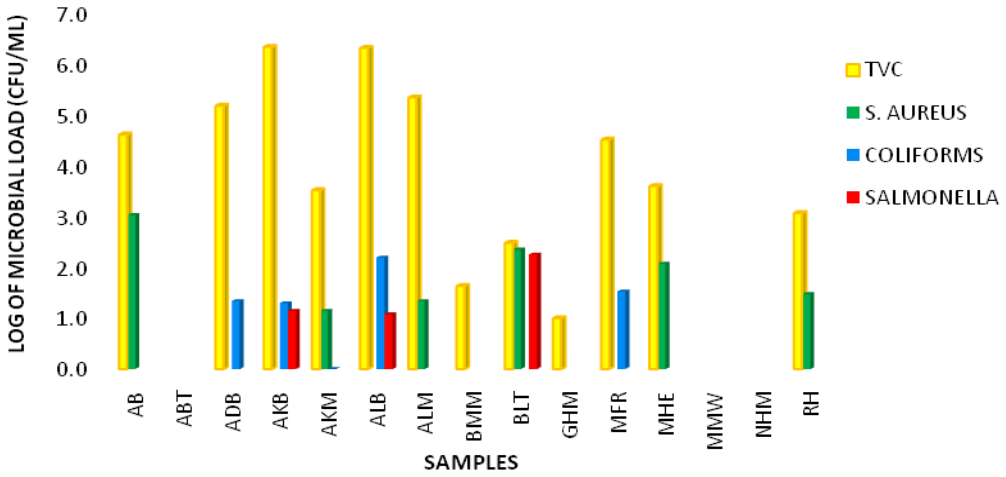

Figure 3: Logarithmic plot for microbial load of the various herbal drugs studied

Total viable counts essentially reflected the total number of viable microbial entities present in the tested herbal drugs. Total viable counts across tested herbal drugs ranged from $1.0 \pm 0.02 \times 10^1$ to $2.3 \pm 0.30 \times 10^6$ CFU/mL. The respective recorded counts for the individual isolates ranged from$1.4 \pm 0.03 \times 10^1$ to $1.1 \pm 0.06 \times 10^3$ CFU/mL for *Staphylococcus aureus*, $2.0 \pm 0.02 \times 10^1$ to $1.6 \pm 0.07 \times 10^2$ CFU/mL for total coliforms and $1.2 \pm 0.03 \times 10^1$ to $1.8 \pm 0.02 \times 10^2$ CFU/mL for *Salmonella* spp.

The presence of the above-mentioned microbial isolates in essence reflects poor practices during harvesting and production of these herbal drugs tested. Their presence is undesirable and poses public health threat taking into account the fact these drugs are intended for internal use. The threat to health is heightened by the probable proliferation of microbial populations due to water activity as most of these herbal drugs are water-based formulations. According to WHO [25] guidelines, herbal medicines that are meant for internal use should be devoid of *Salmonella* and *Shigella* spp. at any stage. That is to say herbal drugs that were found to contain *Salmonella* spp. ($1.2 \pm 0.03 \times 10^1$ to $1.8 \pm 0.02 \times 10^2$ CFU/mL) in the present study are unwholesome and unfit for consumption. Compared to the present study, tested herbal drugs were found to contain *Staphylococcus aureus* in the range of $1.4 \pm 0.03 \times 10^1$ to $1.1 \pm 0.06 \times 10^3$ CFU/mL, well below the WHO [25] guideline of 10^5 CFU/mL and essentially presents no health threat on consumption. In a study by Odonkor et al. [5], *Staphylococcus aureus* emerged the most predominant microbial isolate in tested herbal medicinal products with numbers ranging from 2.2 to 5.3×10^3 CFU/mL. Total coliforms numbers recorded in the present study ($2.0 \pm 0.02 \times 10^1$ to $1.6 \pm 0.07 \times 10^2$ CFU/mL) were also below the WHO [25] guideline of 10^3 CFU/mL for Enterobacteria but above the guideline of 10^1 CFU/mL for *Escherichia coli*. Thus, from the present study, herbal drugs that contained coliforms (predominantly enterobacteria) other than *Escherichia coli* present no health threat on consumption. Herbal drugs that showed no microbial presence pose no health treat on consumption except possibly in relation to the presence of heavy metal levels measured that could be injurious on exceeding certain thresholds.

Introduction of microbial isolates identified in the present study into the herbal drugs tested may have occurred during harvesting, transportation, post-harvest processing and production or manufacturing [5, 10, 25]. Herbal/medicinal plants normally carry bacteria and moulds often emanating from soil, manure application as well as individuals infected with pathogenic flora who may come into contact with these plants or the finished products during harvesting/collection, processing and production [10, 25]. Then and again, the proliferation of microbes in herbal drugs as stated early on can be triggered by the presence of moisture and from failure to regulate temperatures of liquid and finished herbal products [25].

Health Implications of Bacteriological Contaminants in Herbal Drugs

Of all the staphylococcal bacteria, *Staphylococcus aureus* has been cited as the most pathogenic. *Staphylococcus aureus* cause food poisoning and gastrointestinal illnesses through the production of toxins. Toxins produced by these bacteria are heat resistant. They have also been cited to cause skin infections, pneumonia, endocarditis and osteomyelitis [40].

Coliform bacteria are normally present in soil, vegetation and surface water. Most of these bacteria do not cause any disease. That notwithstanding, their presence in water or a water-based product is of a health concern as it indicates the presence of potential disease-causing organisms such as bacteria, viruses and protozoa [41].

Salmonella spp. commonly causes foodborne illness, sometimes called food poisoning. Many types of *Salmonella* bacteria exist. They can cause a range of illnesses, including typhoid fever and gastroenteritis. *Salmonella* infections may present symptoms including diarrhoea, stomach cramps, vomiting and fever [42].

V. CONCLUSION

Herbal drugs analysed in the current study all contained the heavy metals- Fe, Pb, Zn and Cu. Bacteriological analysis revealed the presence of microbial species including *Staphylococcus aureus*, coliforms and *Salmonella* in some of the herbal drugs tested. TVC counts suggest the presence of other microbial species other than the above-mentioned isolates in most of the tested samples. Levels of heavy metals recorded essentially pose no threat of heavy metal toxicity to consumers. That notwithstanding, these metals can bioaccumulate and bioconcentrate in human tissues and become injurious beyond certain thresholds. Herbal drugs found to contain microbes typically are unwholesome and pose a health threat to consumers taking into account the fact that these microbes can proliferate as a result of water activity since the tested herbal drugs were all water-based formulations. The presence of these microbes typically reflects poor practices along the harvesting and production chains. The findings of this study bring to bear the need for strict adherence to good manufacturing practices by herbal drugs producers to ensure the production of quality herbal drugs devoid of all forms of contaminants, a mandate that rests in the bosom of Ghana's Food and Drugs Authority. Thus, the Food and Drugs Authority must intensify monitoring and evaluation to ensure strict conformance of locally produced herbal drugs to agreed standards.

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CONFLICT OF INTEREST

The authors wish to declare no conflicts of interest.

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