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DETERMINATION OF As, Cd, Cr and Co IN SOME HERBAL MEDICINES SOLD IN THE MARKETS OF ZARIA USING NEUTRON ACTIVATION ANALYSIS

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ABSTRACT
The levels of elements with genotoxic potentials; As, Cd, Cr and Co in herbal medicines obtained from stem barks of Boswellia serrata, Prosopis africana, Anogeissus leiocarpus and Sclerocarya birrea were determined by instrumental neutron activation analysis and compared to WHO/EU (2007) permissible values in herbal products. The results of standard material IAEA 1515 (apple leaves) were not significantly different from the certificate values. Arsenic in the samples ranged from ND to 0.037 mg/kg. The maximum value (0.037mg/kg) was obtained in Boswellia serrata which was ten times and two times the value in Sclerocarya birrea and Anogeissus leiocarpus respectively. This maximum was below WHO/EU (2007) values. Arsenic was not detected in Prosopis africana herbal medicine. Cadmium in all the samples was below detection limit except in Anogeissus leiocarpus herbal medicine where the concentration was 0.0012mg/kg this was below WHO/EU (2007) values. Chromium in the samples ranged from ND to 0.06 mg/kg, the maximum was in Anogeissus leiocarpus herbal medicine; this concentration was slightly above EU (2007) values of 0.050 mg/kg but, below WHO (2007) value of 2.0 mg/kg. Cobalt was detected in all the samples, the concentrations ranged from 0.01mg/kg to 0.16 mg/kg with the highest concentration in Sclerocarya birrea herbal medicine, this concentration was about two and half times the concentration in Boswellia serrata . The Co concentration in Anogeissus leiocarpus herbal medicine was about five times lower than the concentration in Boswellia serrata. The concentrations of Co in the herbal medicines were in the order, Sclerocarya birrea > Boswellia serrata > Prosopis africana > Anogeissus leiocarpus. WHO/EU (2007) have not set Co permissible limits in food and drinking water. Although, most of the genotoxicants were below WHO/EU (2007) values none the less, these herbal medicines should be investigated further for other toxic and genotoxic compounds as a means of quality control because, genotoxicants have no threshold limit to cause cancer therefore, quality assurance measures should always be taken in the production and use of these substances.
Key words: Elements, genotoxic, herbal medicines, instrumental neutron activation analysis, Zaria.

INTRODUCTION

The World Health Organization (WHO) states that about 80% of the world population relies on herbs for their primary healthcare especially countries in Africa and Asia and that, those who take herbal remedies worldwide exceed those who take orthodox medicine by as much as two to three times (WHO, 1996). Due to the popularity of herbal remedies worldwide, scientists are taking a closer look at the safety and toxicity of these substances with regards to carcinogenic and genotoxic constituents in herbs. The renewed interest is because;herbal medicines play prominent roles in primary healthcare due to their acceptability in managing diseases in developing nations (Annan and Houghton, 2008). Much of the research on herbal medicines or medicinal preparations has focused on the phytochemical identification, quantification and the beneficial effects they exert on humans and by explaining the mechanism of how these substances inhibit cell injury and damage. Even though some researchers have dealt with toxicity in herbal medicines, but most of their work considers metal toxicity in general without being specific on genotoxic elements that may be present in herbal medicines (Sarkar *et al.,* 2008).

Genotoxic substances include heavy metals, PAHs and nitro-derived compounds. These substances could be carcinogenic through DNA damage. As a pointer to the carcinogenicity potential of constituents of herbal remedies, a multinational survey conducted in 2001 found that 35.90 % of cancer patients were either past or present users of complementary and alternative medicines which use herbs as ingredients in their preparations (Molassiotis *et al.,* 2001). Even though at least one third of new cancer cases seen every year are preventable through the control of tobacco and alcohol use, moderate diet and immunization against hepatitis B, the case of cancer keeps on growing (WHO, 1996). The incidences of cancer are increasing globally: the estimate of expected number of people to come down with cancer by 2020 is 30 million (Sloczynska *et al.,* 2014).

This study placed special interest on the toxic metals that may cause cancer through direct and indirect reaction with the genome; As Cd, Cr and Co were selected because they have been proven in the literature to be genotoxic (Fatai *et al*., 2013).

These elements may induce tumours that are benign or malignant and may increase the incidence of tumour formation or its malignancy (Valco *et al.,* 2005). The elements can change any of the multi-step processes of initiation, promotion, progression and metastasis in cancer formation and development (Mulware, 2013).The formation of complexes (DNA-adducts) by As, Cd, Cr, Co *in vivo* in the presence of DNA may lead to the eventual oxidation of DNA through reactive oxygen species thus, creating a mutation in which normal cells refuse to go through apoptosis (Valco *et al.,* 2005). Herbal medicines obtained from stem barks of *Boswellia serrata, Prosopis africana, Anogeissus leiocarpus* and *Sclerocarya birrea* medicinal plants were analysed for ofAs, Cd, Cr and Co by instrumental neutron activation analysis. The results from this study may serve as quality control measures that foster awareness of the safety or otherwise toxicity of genotoxicants in the selected herbal medicines.

MATERIALS AND METHODS

Sample Collection and Preparation

The ground stem bark samples of herbal medicines obtained from the medicinal plants *Boswellia serrata, Prosopis africana, Anogeissus leiocarpus* and *Sclerocarya birrea* were selected for analysis based on their popularity in markets of ZariaFigure (1).The selected herbal medicines were purchased from three markets in Zaria (Samaru, Sabon Gari and Zaria city) and their medicinal plants taken to the Herbarium in the Department of Botany, Faculty of Life Sciences Ahmadu Bello University for identification and confirmation.

Sample Preparation

Instrumental neutron activation analysis was carried out using NIRR-1(Nigeria Research Rector -1) at the Centre for Energy Research and Training, Ahmadu Bello University, Zaria. As a means of quality Control the certified reference material IAEA-1515(apple leaves)was run alongside the samples by weighing 200 mg of both samples and standard into polyethylene films and rabbit capsules that were cleaned by soaking in $1.1NHNO_3$ for 3 days and washed with de-ionized water. The rabbit capsules containing the samples and those containing standard reference material were sent to the reaction core pneumatically and after a pre-set time, they were received pneumatically through the Send/Receive station. For the irradiation regimes for the analysis of samples and standard, the short and the long schemes indicated in Table (1) were used (Jonah *et al.,* 2006).

Table1: Routine Irradiation and Measuring Regimes for NIRR-1 [Source: Jonah *et al.*2006]
Legend: Tr =irradiation time

Neutron flux/irradiation channel	Procedure	Tir	Td	Tc	Activation products
1×10^{11}n/cms/outer irradiation	S1	2min	2-15min	10min	^{28}Al,^{27}Mg,^{38}Cl,^{49}Ca,^{66}Cu,^{51}Ti^{52}V,^{116m}In
channel(B4,A2)	S2	2min	3-4h	10min	^{24}Na,^{42}K,^{165}Dy,^{56}Mn,^{152m}Eu,
5×10^{11}n/cms/inner irradiation	L1	6h	4-5d	30min	^{239}Np(U),^{72}Ga,^{122}Sb
channels(B1,B2, B3,L2 and A1)	L2	6h	10-15d	60min	^{46}Sc,^{141}Ce,^{60}Co,^{51}Cr,^{134}Cs,^{152}Eu,^{177}Lu,^{131}Ba,^{86}Rb,^{182}Tb,^{175}Yb,^{233}Pa(Th),^{65}Zn,^{59}Fe,^{181}Hf

Td= Decay Time
Tc= Counting Time

Safety Aspects of Instrumental Neutron Activation Analysis

For safety reasons, as soon as the samples arrived through the Send /Receive station, the Health Physicist immediately measured the dose rate of each irradiated sample. The rabbit capsules were accessed on lead castle that was kept below the Send / Receive station for the dose to be within permissible limits, after which the samples were taken out and the seal integrity was examined (IAEA-TECDOC-564, 1990),

Preparing Samples for Radioactive Assay and Counting

The activated samples plus standard reference material (SRM1515)and the resulting gamma ray energies and intensities were measured using a solid-state, high purity Germanium (HPGe) detector. The samples were wiped clean with wet tissue paper and mounted on flexi glass plate to measure the gamma activity with High Purity Germanium (HPGe) detector. The HPGe detector with a resolution of 1.9 KeV at 13332KeV of ^{60}Co was coupled to a multichannel analyser. When gamma –rays passed through the detector, they generate free electrons. The number of electrons (current) was related to the energy of the gamma ray. Given the differences in half-lives for various nuclides, there were optimum times to count an activated sample. Gamma ray spectra were accumulated in live time mode with dead time maintained at less than five percent; this we achieved by placing the sample at appropriate distance from the detector (IAEA-TECDOC-564, 1990).

The whole rector system consists of a horizontal dip-stick, high purity Germanium (HPGe) detector with a relative efficiency of 10% at 1332.5 KeV gamma ray line, MAESTRO emulation software compatible with the ADCAM ®Multi-Channel Analyser (MCA) card and the associated electronics modules all made by EG & ORTEC that is interfaced with a Personal Computer.

The efficiency curves of the detector system at near and far source detector geometries were determined by standard gamma –ray sources in the range of 59.5-2254KeV and extended to 4000KeV. Thedata processing and the gamma ray spectral peak areas were analysed using WINSPAN 2004 software (Liyu, 2004). The software requires that calibration factors be predetermined by a multi-element standard reference material for elements of interest using adopted irradiation and counting regimes (Jonah *et al.,* 2006).

RESULTS

Figure1shows the comparative percentages of the most popular herbal medicines obtained from the stem barks of *Anogeissus leiocarpus* of Combretaceae family, *Prosopis africana* of Mimosaceae family, *Boswellia serrata* of Burseraceae family and *Sclerocarya birrea* belonging to Anacardiaceae family as 29.06%, 29.04%, 23.26% and18.640% respectively.

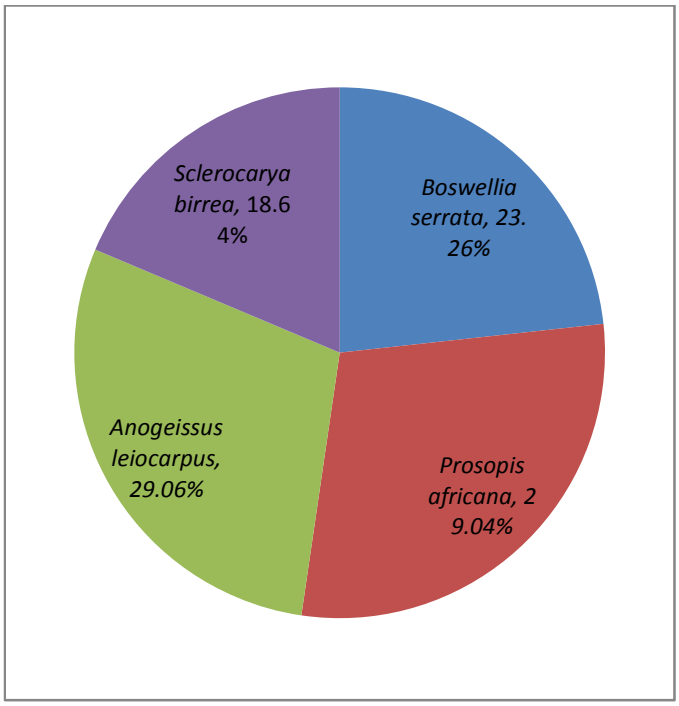

Figure 1: Comparative Popularity of Selected Samples in Markets of Zaria, Nigeria

Table 2 presents the information about the selected herbal medicines obtained from the stem barks of their corresponding medicinal plants. All the samples were presented as powdered samples composed of single plants. *Prosopis africana* and *Sclerocarya birrea* were prepared by pounding the stem barks obtained from the medicinal plants. *Boswellia serrata* and *Anogeissus leiocarpus* powdered stem barks of the herbal medicines were prepared for oral administration as decoctions and infusions respectively. All the samples were administered orally for treating piles and cancers, only *Prosopis africana* herbal medicine had in addition, topical application and filling routes for treating general body pains, and burns and the filling of cavities respectively. Table 3 presents the concentrations of As, Cd, Cr, Co in the selected crude herbal medicines purchased from the markets of Zaria. The concentration of the element As in all the samples ranged from ND to 0.037 mg/kg with the maximum value obtained in *Boswellia serrata* which was ten times and two and a half times the values in *Sclerocarya birrea* and *Anogeissus leiocarpus* respectively. The concentration of Cd in all the samples was below detection limit except in *Anogeissus leiocarpus* herbal medicine where the concentration was 0.0012mg/kg. This concentration is below WHO/EU (2007) values. The concentration of Cr in all the samples ranged from ND to 0.06 mg/kg. *Anogeissus leiocarpus* herbal medicine had the highest concentration of Cr at 0.06 mg/kg; this concentration is slightly above EU values of 0.050 mg/kg but below WHO (2007) value of 2.0 mg/kg. Chromium was not detected in *Prosopis africana* and in *Boswellia serrata* herbal medicines. Cobalt was detected in all the samples with concentrations ranging from 0.01mg/kg to 0.16 mg/kg. The concentration of Co in *Sclerocarya birrea* herbal medicine was about two and half times the concentration in *Boswellia serrata* and the concentration obtained in *Anogeissus leiocarpus* herbal medicine, this was about five times lower than the concentration in *Boswellia serrata*. The concentration of Co in the herbal medicines was in the order, *Sclerocarya birrea> Boswellia serrata > Prosopis africana > Anogeissus leiocarpus* (Table 3)

Name	Local name	Method of preparation	Local uses	Route of administration	Dose
Boswellia serrata	Indian frankincense	Decoction	Treating Piles and cancer	Oral	½ cup2x daily
Prosopis africana	African Mesquite	powder	Treating piles, general body pains, toothaches and burns	Oral for piles, topical for general body pains and burns and filling for toothaches	1 cup daily for piles, fill cavity 1x daily and apply topically once daily for pains
Anogeissus leiocarpus	African birch	pap or butter milk	Coughs/colds and malaria/fever	Oral	One cup daily
Sclerocarya birrea	Marula or cider tree	powder	Piles	Oral	Spoonful of powder with food once daily

Table 2 Information on Powdered Herbal Medicines Composed of Single Stem Barks of selected Medicinal Plants [Elisha *et al.,* 2016]

Table 3: Concentrations of Cr, As, Cd and Co in Herbal Medicines as Determined by INAA Compared to WHO (2007)/EU (2007)Guidelines Permissible Limits in Herbal Medicines

S/N	Sample	Cr (mg/kg)	As (mg/kg)	Cd (mg/kg)	Co (mg/kg)
1	*Boswellia serrata* herbal medicine	ND	0.037	ND	0.056
2	*Prosopis africana* herbal medicine	ND	ND	ND	0.045
3	*Anogeissus leiocarpus* herbal medicine	0.06	0.014	0.0012	0.01
4	*Sclerocarya birrea* herbal medicine	0.03	0.0037	ND	0.16
5	WHO values	2.0	5.0	0.3	NM
6	EU values	0.05	NM	0.005	NM
	IAEA Standard(1515)	0.3	0.038	0.013	0.09

NM=Not mentioned
ND =Not Detected

DISCUSSION

From our earlier work (Elisha *et al.,* 2016) the comparative percentages of the most popular herbal medicines obtained from the stem barks of medicinal plants showed that the most popular families from which these herbal medicines were obtained in Zaria were Combretaceae =Mimosaceae > Burseraceae> Anacardiaceae. Table 2 presents the information about the selected herbal medicines obtained from the stem barks of their corresponding medicinal plants. All the samples were presented in powdered form composed of single plants and were administered orally as decoctions and powders mixed with pap or applied topically in treating piles and cancer in agreement with Ampitan (2013).Only *Prosopis africana* herbal medicine had in addition, topical application for treating general body pains, and burns and for filling cavities.

Ajiboye *et al*. (2013) have revealed also that a combination of leaves and stem bark of *Prosopis africana* (Guill & Perr.) was used in treating rheumatism, skin diseases, and fever and used as eye wash.The herbal medicine with highest concentration of As(Table 3) was *Boswellia serrata* herbal medicine at 0.037mg/kg and this was belowWHO (2007)permissible values and EU (2007)has no mention of the values in herbal medicines. The concentration of As in *Boswellia serrata* was ten times the concentration in *Sclerocarya birrea* and about two and a half times the concentration in *Anogeissus leiocarpus*. The element As was not detected in *Prosopis africana* herbal medicine may be it was harvested from non-contaminated soil or good processing procedures were observed. Inorganic arsenic, arsenic (III) in particular, is very toxic and easily absorbed by all human vital systems, even to the point of crossing the placenta to cause foetal harm. Inorganic arsenic is carcinogenic to skin, lungs, liver and kidney (Nordberg *et al.,* 2007).Gebel (2001) has intimated that exposure to inorganic As leads to long-term toxicity especially to cancer and that As has the potential to cause tumours, it also has the potential to be genotoxic as shown by *in vitro* animal experimentation(Gebel*,* 2001). These animal experiments showed that As could induce chromosome aberrations and sister chromatid exchange without any point mutations in both humans and animals (Gebel*,* 2001).The mechanism of the genotoxic action is however not quite understood, although some researchers have indicated that As may act on DNA in an indirect way by inhibiting the DNA repair system or by interfering with DNA integrity by altering DNA methylation and phosphorylation of cell-cycle control proteins, which may lead to the resistance to cell apoptosis and eventually to cancer (Gebel, 2001).

The concentration of Cd in all the samples was below detection limit except in *Anogeissus leiocarpus* herbal medicine where the concentration was 0.0012mg/kg (Table3). This concentration is below WHO/EU (2007) values.Similar work done by Onwordi *et al.*(2015) had Cd levels above WHO (2007) permissible limit of 0.3mg/kg. This disparity seen between the present study and Onwordi *et al.* (2015) could be due to environmental influence or plant species. Cadmium compounds are carcinogenic in humans and weakly mutagenic in most assay systems (Filipic and Hei, 2004). Cadmium is genotoxic to Leydig cells (Yang *et al.,* 2003). The mechanism of Cd genotoxicity is by indirectly inducing oxidative stress in cells by inhibiting oxidative enzymes which may induce apoptosis in different cells (Yang*et al.,* 2003).

Chromium was not detected in *Prosopis africana* and in *Boswellia serrata* herbal medicines. The highest concentration was detected in *Anogeissus leiocarpus* at slightly higher value than WHO/EU (2007) permissible values in herbal medicines. From data available to the IARC (2006) it concluded that Cr (VI) compounds are carcinogenic being very active in cell transformation, mutagenicity and chromosomal damage with particular preference to phosphate backbone in DNA thus binding DNA to form DNA-adduct.

Cobalt was detected in all the samples with concentrations ranging from 0.01mg/kg to 0.16 mg/kg. The concentration of Co in *Sclerocarya birrea* herbal medicine was about two and half times the concentration in *Boswellia serrata* and the concentration obtained in *Anogeissus leiocarpus* herbal medicine, this was about five times lower than the concentration in *Boswellia serrata*. The concentration of Co in the herbal medicines was in the order, *Sclerocarya birrea*> *Boswellia serrata* > *Prosopis africana* >*Anogeissus leiocarpus*.Plants growing on contaminated soils may accumulate Co in the leaves, fruits and in the seeds (Ebere *et al.,* 2016). Cobalt is an essential element in human nutrition found as a component of vitamin B12, which aids in the formation of blood (Chengalvala *et al*., 1984). However, high concentrations of cobalt in the human system could be toxic causing vomiting and nausea also causing damage to vision, heart and the thyroid gland (Chengalvala et al., 1984). The International Agency for the Research on Cancer (IARC) has classified Co and its compounds as agents that are possibly carcinogenic to humans (IARC, 2006). The soluble salts interfere with cell proliferation by forming adducts with DNA. Cobalt salts could also inhibit the DNA repair system. Cobalt II ions in particular can cause the induction of chromosomal aberrations in plants. Cobalt-60 is radioactive and carcinogenic(www.epa.gov/radiation/radionuclide-basics-cobalt-60).

Animal and bacterial experimentations have shown that cobalt metal, alloys and compounds in form of injections and implants, do induce local and metastasing sarcomas in rats, rabbits and in mice (Jensen and Tuchsen, 1990). However, WHO/EU (2007) have not established any limits in foods and in drinking water for this metal.

Rehman *et al.* (2013) also investigated heavy metal contamination in selected herbal medicines in Pakistan and found that the Apocyaceae and Caryophyllaceae families had the highest Cd, Cr and Ni concentrations. In this study, only *Anogeissus leiocarpus* of the Combretaceae family contained all the selected metals and metalloid in which Cr exceeded the WHO (2007) permissible limits in herbal medicines.

CONCLUSION

The analysis of heavy metals with genotoxic potentials in some herbal medicines sold in the markets of Zaria has been done using instrumental neutron activation analysis.The comparative percentages of the most popular herbal medicines sold in the markets of Zaria was in the order; *Anogeissus leiocarpus*> *Prosopis africana*> *Boswellia serrata*> *Sclerocarya birre a*re presented by 29.06% > 29.04>23.25% >18.64%. respectively. These herbal medicines were powders obtained from single stem barks of the medicinal plants and were prepared as decoctions or powders mixed with food and administered orally for the treatment of piles and other human ailments. Genotoxic elements were detected in *Boswellia serrata, Prosopis africana* and *Sclerocarya birrea*herbal medicines at concentrations lower than WHO/EU (2007) permissible limits in herbal medicines except Cr in *Anogeissus leiocarpus* that exceeded the EU (2007) permissible limit of 0.05 mg/kg by 0.01mg/kg.

However, genotoxic compounds have no threshold limits in their ability to cause cancer; therefore the use of these samples should be investigated further for other toxic and genotoxic compounds.

RECOMMENDATIONS

From the results of this study it is recommended that the consumption of the aforementioned herbal medicines should always be monitored for toxic elements to avoid the accumulation of heavy metals in the body, which may lead to cancer. Further studies could be done to determine and standardize the total carcinogenic and genotoxic constituents in these herbal medicines.

Contributions of Autrhors

Edith Bolanle Agbaji helped in designing the experiment, critiqued and offeredcogent advice in structuring this work.

Abdulmumuni Abdulkadir Nuhu helped in designing the experiment and read through the work and offered meaningful suggestions that improved the quality of this work.

Stephen Eyije Abechi critiqued the work and offered helpful suggestions on how to prepare the work for final presentation.

Jamok Jacob Elisha helped in designing the work, carried out the experiment and did the write up and submitted the article.

Conflict of Interest

No conflict of interest declared.

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