

Exploration of the Carcinogenic Properties of Some Antituberculosis Herbal Drugs Made in Nigeria from *Garcinia Kola* Plant's Parts

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Abstract: Tuberculosis is an antique disease that remains a major global health issue. *Garcinia kola* is a recognized medicinal plant with high medicinal values rendering it a prospective source of local herbs in undeveloped countries and a pharmaceuticals source in developed countries. This study was aimed at the assessment of the safety level of some antituberculosis herbal drugs made from *Garcinia kola* plant's part. The antituberculosis herbal drugs made from the Seed, leaves, bark and root of *Garcinia kola* were purchased and properly air dried. Ultrasonic extraction of the samples were done following standard procedure. The impurity in the herbal drug extracts were removed using column chromatography. The concentrations of PAHs in the purified herbal drugs extracts were determined with Gas Chromatography-Flame Ionization Detector (GC-FID). PAHs diagnostic ratios, group distribution and cancer risk estimation of PAHs were calculated from the concentration of PAHs. The highest concentration of total PAHs was detected in AHDR sample (22.434 mg/kg) and lowest concentration in AHDS sample (16.965mg/kg). Sample AHDS had highest percentage of carcinogenic PAHs (51.44%) while sample AHDB had lowest percentage (25.90%). Values obtained from the diagnostic indices confirmed that the source of PAHs were from pyrogenic sources. The estimated cancer risk via exposure to PAHs resulting from the use of these herbal drugs ranges from 2.0642 to 0.6034 x 10⁻⁷ and were below the USEPA set range (1 × 10⁻⁴ – 1 × 10⁻⁶). Nevertheless, to reduce health problem, excessive intake of these herbal drugs should be prevented as their biodegradation on accumulation are difficult.

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1. Introduction

Tuberculosis (TB) is a disease caused by a bacterial called Mycobacterium and its infection is one of the major disease that affects the lungs (Zhang et al 2016). Reports shows that tuberculosis is one of the most common infectious disease and it has been estimated that over two billion people or one-third of the world's population have been infected by *M. tuberculosis* (Sivakumar and Jayaraman 2011). More than eight million new cases of active TB disease are recorded yearly which result into two million deaths annually, thereby leading to a global epidemic (Dye 2006; WHO 2007). Multidrug-resistant tuberculosis (MDR-TB) is another important problem limiting the control of TB worldwide. The increase in rate of tuberculosis infections had been traced to increase in number of patients suffering from immuno deficiency virus (Shim and Jo 2013). Fever, night sweats, coughing up blood, chest pain, fatigue, breathing with difficulty and unintentional weight loss are the prominent symptoms of tuberculosis infection (MFMER 2019). The increase in multidrug resistant

strains of *M. tuberculosis* is connected to the inactive clinical nature of the disease which requires an extended period of treatment, approximately 6-9 months, as well as the abnormal microbiological attributes of the microorganism. Prolong therapy in cure of TB has led to poor patient compliance and often toxicity which are the cause of drug resistant (Zhang et al 2006). Chemotherapeutic cure for TB has led to discovery of several effective TB drugs such as rifampicin, isoniazid, pyrazinamide and ethambutol which are refer to as first line TB drugs, ciprofloxacin, levofloxacin, cycloserine and clofazimine second-line drugs while rifambutin, clarithromycin and linezolid are called third-line TB drugs (Eric et al 2016). Isoniazid, rifampin, pyrazinamide, ethambutol are the active commercially available synthetic drugs used as medication for treating tuberculosis while delamanid, sutezolid, PA-824, linezolid and bedaquiline are therapeutic drugs currently used for drug-resistant treatment of tuberculosis infection (MFMER 2019). The increase in multidrug resistant strains of *M. tuberculosis* has decreased the effectiveness of current

standard Tuberculosis treatment (Muthusamy et al 2013). The prevalence of multi drug resistant in conjunction with widespread drug resistant are major causes for finding novel anti-tuberculosis drugs. Inaccessibility and ineffectiveness of existing synthetic drugs to cure many microbial infections have led to the use of herbal drugs made from plants (Oyebamiji et al 2019; Akintelu et al 2019; Folorunso et al 2019a; Akintelu and Folorunso 2019; Folorunso et al 2019b).

Medicinal plants such as *Ranunculi Ternati Radix*, *Globularia alypum*, *Barlaria buxifolia*, *Ipomea turpethum*, *Withania somnifera*, *Humulus lupulus* as be used as remedy for tuberculosis infections especially among the citizen of developing countries of the world (Esmi et al 2012; Shivakumar et al 2012; Periyakaruppan et al 2012; Rahna et al 2013; Lin et al 2014). Among several medicinal plants utilized in Nigeria ethnomedicine for treatment of ailments, the antituberculosis activities of the seeds, bark, twigs, fruits, leaves and wood of *Garcinia kola* remain inestimable (Adesuyi et al 2012; Onyekwelu et al 2015; Kalu et al 2016). Despite the search for an alternative to cure tuberculosis infections the safety level of the alternatives must be study to prevent other health problems.

2. Material and Methods

All reagent used are of analytical grade.

Sample collection

The antituberculosis herbal drugs made from the Seed, leaves, bark and root of *Garcinia kola* were obtained. The antituberculosis herbal drugs were labelled AHDS for antituberculosis herbal drug made from the stem of *Garcinia kola*, AHDL for antituberculosis herbal drug made from the leaves AHDB for antituberculosis herbal drug made from the bark of *Garcinia kola* and AHDR for antituberculosis herbal drug made from the root of *Garcinia kola*. The antituberculosis herbal drug samples were properly air dried and stored in a neat labelled glass sample bottles.

Extraction of herbal drug samples

The extraction of PAHs from the herbal drug samples was done by weighing fifteen (15) grams of the herbal drug sample into a clean test tube. Five (5) mL of distilled n-hexane of analytical grade was added to the content in the test tube. The test tube was transferred into a beaker that is half filled with water and was kept on the ultrasonic bath for 30 minutes. The extract was filtered and the obtained filtrate was store in clean beaker. The residue in the test tube was returned into the beaker in the ultrasonic bath and five (5) mL of distilled acetone was added. The ultrasonic extraction of the residue also lasted for 30 minutes. The extract was filtered with fitter paper and the filtrate obtained was added to the filtrate in the beaker.

This procedure was repeated twice for each of the samples. The extracting solvent was removed from the filtrate via concentration on a rotary evaporator at 32^oC. The crude extract was stored in a cleaned sample bottle and placed in the refrigerator for further analysis.

Clean-up of the extract.

The impurity in the herbal drug extracts was removed by using column chromatograph approach. A column with a diameter of 1 cm was packed with two stationary phase namely; aluminum oxide (4 grams) and silica gel (12 grams). The crude extract was mixed with 2 grams of silica gel and was loaded on the packed column. Two solvents n-hexane and acetone were combined as mobile phase for eluting the extract. The eluent was collected into a beaker and air dried to remove the solvent used as mobile phase. This procedure was repeated for all the samples. The purified herbal drugs extracts were then analyzed with Gas Chromatography-Flame Ionization Detector (GC-FID) to determine the concentrations of PAHs in the herbal drug samples.

Chromatographic conditions for (GC-FID) Operation

The chromatographic conditions for the GCFID analysis of the purified extracts are; flow rate = 1.2 ml/min; wavelength = 200 nm, temperature of column = 45^oC, volume of extract injected = 2 µl; mobile phase = (helium and nitrogen gas); column thickness = 1m and an isocratic method of elution.

Evaluation of the Benzo (a) pyrene equivalent toxicity of the samples.

The estimation of the carcinogenic properties accompanying long time exposure to these herbal drugs were done by calculating the standard lifetime risk from the concentration of PAHs detected in the samples by modification of (Orisakwe 2014; Akintelu et al 2018) model.

Consequently upon this, the equivalent benzo (a) pyrene toxicity was calculated using the following equations.

$$TEQ = \Sigma(PAHi \times TEFi) \quad \rightarrow \text{Equation 1}$$

TEQ = Toxicity equivalence

PAHi = Concentration of carcinogenic PAHs in each sample

TEFi = Toxic equivalent factor (potency relative to benzo (a) pyrene)

The USEPA TEF values for benzo [a] anthracene, chrysene, benzo [b] fluoranthene, benzo [k] fluoranthene, benzo [a] pyrene, dibenzo [a, h] anthracene and indeno [1,2,3-c, d] pyrene are 0.1, 0.001, 0.1, 0.01, 1, 1, and 0.1 respectively (Larsen and Larsen 1998).

Daily Exposure

The Benzo (a) pyrene equivalent toxicity values were used as a parameter to evaluate the exposure

dose in order to determine the health risk linked with exposure to carcinogenic PAHs in the herbal drugs

samples by modification of the ATSDR equation (ATSDR 2005).

$$\text{Exposure dose} = \frac{(\text{Concentration} \times \text{intake rate} \times \text{conversion factor} \times \text{exposure factor})}{\text{Weight of the body}} \rightarrow \text{equation 2}$$

Dose = estimated exposure dose in (mg/kg/day);
Intake rate = volume of sample consumed which was 0.30L per day;
Body weight of consumer (children, preteens and adults) = 19 kg, 48 kg and 65 kg respectively
Conversion factor = 10^{-6}

Exposure factor = (6 times a week per year = 312/365)

Concentration = concentration of total toxicity equivalent of benzo (a) pyrene

Cancer risk evaluation

The lifetime cancer risk was calculate using equation 3

$$\text{Cancer risk estimation} = \frac{(\text{Exposure dose} \times \text{Number of year of sample usage} \times \text{CPF})}{\text{Average life time of user}} \rightarrow \text{equation 3}$$

CPF = cancer potency factor which is given as (7.3)

Years of sample intake = 30 years

User's life time = 55 years

3. Results

PAHs profiles in the selected anti-tuberculosis herbal drug samples

Concentration of PAHs in AHDS Sample

The average concentration of individual PAHs in the antituberculosis herbal drug made from the stem of *Garcinia kola* was in the range of 2.157 mg/kg (BkF) to 0.053 mg/kg (DhA) has showed in Figure 1a. The increasing order of the mean concentration of individual PAHs in this sample was given as DhA> NaP> PHE> BaP > ACP > ANT > FLT > BbF> ICP> PYR> ACY> BgP> FLR> CHR> BaA> BKF. The total concentration of PAHs in this herbal drug was 16.965 mg/kg. The percentage of carcinogenic PAHs in this drug was 51.44%.

Concentration of PAHs in sample AHDL Sample

The average concentration of individual PAHs in the antituberculosis herbal drug made from the leaves of *Garcinia kola* was in the range of 2.876 mg/kg (BkF) to 0.112 mg/kg (DhA) has shown in Figure 1b. The increasing order of individual PAHs in this sample AHBL was given as NAP> DhA> BgP>BaP> FLT> BbF > ACP > IcP > PyR> ACY> ANT > PHE> CHR> BaA> FLR> BkF. The total concentration of PAHs in the AHDL Sample was 20.59 mg/kg. The percentage of carcinogenic PAHs in AHDL Sample was 49.77%. The presence of high percentage of carcinogenic PAHs in this sample might have resulted from the deposition of gaseous PAHs bounded on growing leaves [23]. Thorough washing of plants leaves before usage may remove up to 50% of the total PAHs (Ding and Kamnsky 2003). It is therefore recommended for herbal drug producers to properly

rinse the surface of the leaves used for production of herbs and herbal drugs to reduce concentration of PAHs and possible other contaminants.

Concentration of PAHs in AHDB Sample

The concentration of individual PAHs in the antituberculosis herbal drug made from the bark of *Garcinia kola* was in the range of 2.98 mg/kg (FLR) to 0.079 mg/kg (ICP) has shown in Figure 1c. The increasing order of individual PAHs in this sample was given as ICP> BaA> NAP> BgP> BbF> BaP> BkF> DhA> PYR> CHR> FLT> ACY> ACP> PHE> ANT> FLR. The total concentration of PAHs in this herbal drug sample was 19.423 mg/kg and the percentage of carcinogenic PAHs was 25.90%.

Concentration of PAHs in AHDR Sample

The concentration of individual PAHs in the antituberculosis herbal drug made from the bark of *Garcinia kola* was in the range of 2.54 mg/kg (CHR) to 0.125 mg/kg (BkF) has shown in Figure 1d. The increasing order of individual PAHs in this sample was given as BkF> BgP> ICP>PYR> BaP> BaA> NAP> DhA> BbF> PHE> ACY> FLT> ACP> FLR> ANT> CHR. The total concentration of PAHs in sample AHDR was 19.423 mg/kg and the percentage of carcinogenic PAHs was 25.90%. Choi et al (2006) reported that exposure to high concentration of PAHs may lead to reduction in birth length. It is suggested that users of this herbal drug should take necessary measure to avoid excessive exposure through overdose or bioaccumulation.

In general, FLR had highest concentration of PAHs in sample AHDB (2.980 mg/kg) while IcP had the lowest concentration of PAHs in sample AHDB (0.079 mg/kg). The highest concentration of total PAHs among the studied samples was detected in sample AHDR (22.434 mg/kg) and lowest concentration of total PAHs was observed in sample AHDS (16.965mg/kg). Sample AHDS had the highest

percentage of carcinogenic PAHs (51.44%) while sample AHDB had the lowest percentage (25.90%). The variation in the percentage of carcinogenic PAHs resulted from the variation in the concentration of

carcinogenic PAHs in the samples studied. Previous study revealed that exposure to PAHs could produce reactive oxidative species which may induce oxidative stress (Valavanidis et al 2005; Valko et al 2005).

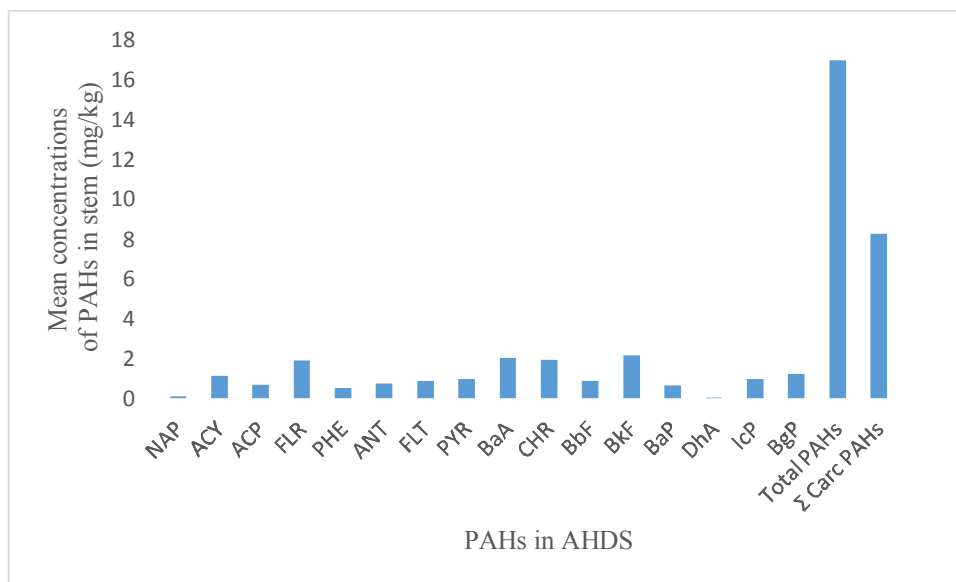


Fig 1a: Concentration of PAHs in AHDS sample

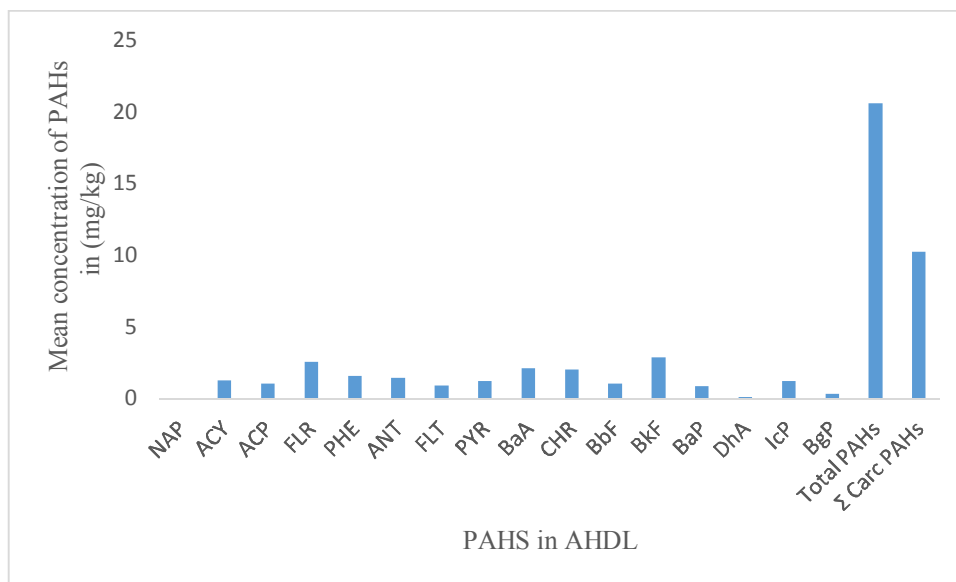


Fig 1b: Concentration of PAHs in AHDL sample

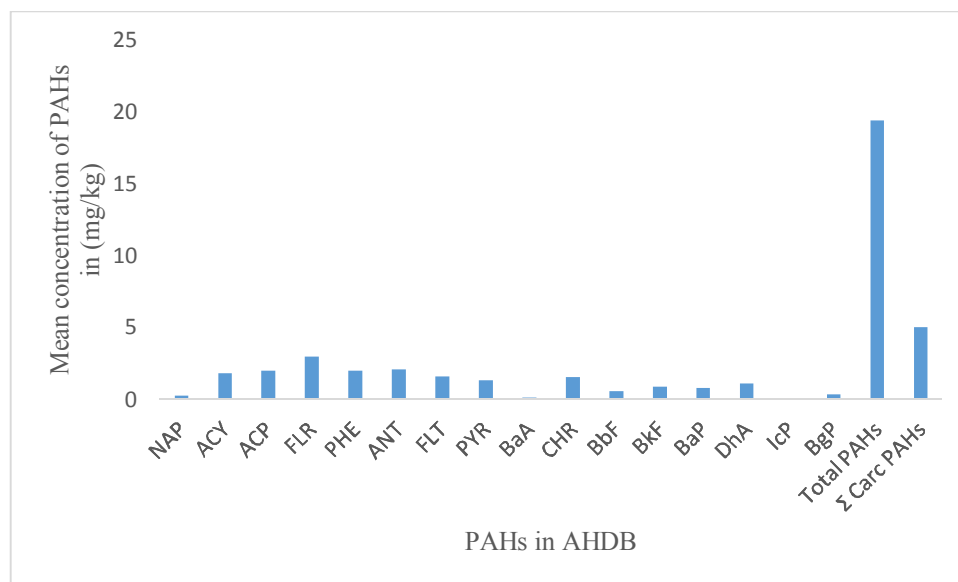


Fig 1c: Concentration of PAHs in AHDB sample

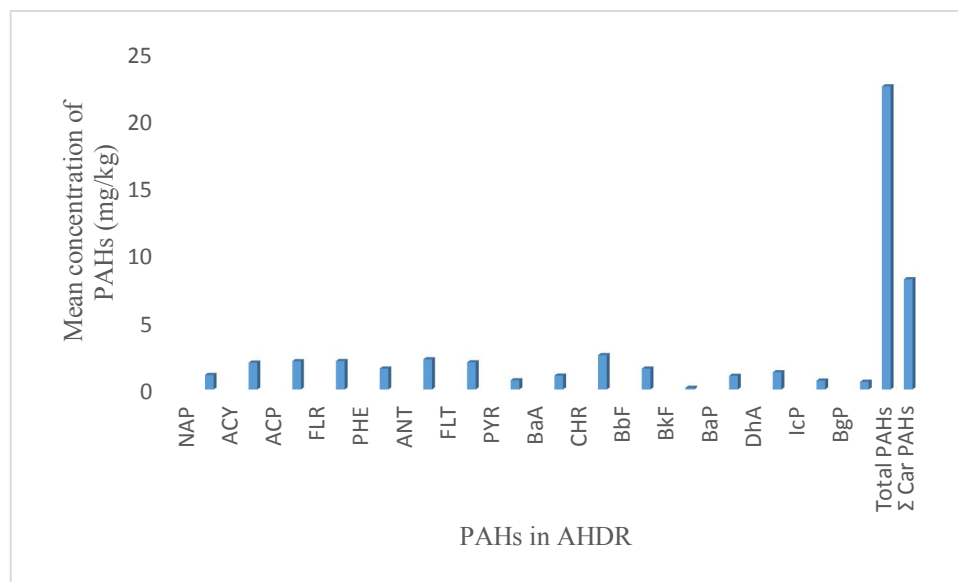


Fig 1d: Concentration of PAHs in AHDR sample

PAHs Diagnostic Ratios in the herbal drug samples

PAHs diagnostic indices of PAHs are extensively used to estimate the origins of PAHs found in samples (Liu et al 2005). PAHs diagnostic indices and ratios are accurate and dependable for the estimation of sources of PAHs (Guo et al 2010). The sources of PAHs in these herbal drug samples were examined by calculating several diagnostic ratios, and the results obtained are showed in Table 1. The PAHs ratio PHE/ANT calculated are 0.7, 1.1, 0.9 and 0.7 for samples AHDS, AHDL, AHDB and AHDR respectively. The values ratio of ANT/(ANT + PHE) were 0.6, 0.5, 0.5 and 0.6 for samples AHDS, AHDL, AHDB and AHDR respectively. The ratio of

PHE/ANT lesser than 10 and the ratio of ANT/(ANT + PHE) were greater than 0.1. These ratios are attributed to pyrogenic sources according to the findings of (Dominguez et al 2010). Thus the source of PAHs in the herbal drug samples were from pyrogenic source. This was further established by the result obtained from the calculation of the FLT/FLT + PYR ratios of 0.5, 0.4, 0.5 and 0.7 for samples AHDS, AHDL, AHDB and AHDR respectively which was also in the specified range for pyrogenic sources. These values obtained from the diagnostic indices confirmed that the source of PAHs were majorly from pyrogenic sources. The diagnostic indices of this study were in line with the study of (Tella et al 2017).

Table 1. Diagnostic indices of PAHs in the herbal drug samples.

PAH ratio	Sample (mgkg ⁻¹)				Value of ratio	Indication	Inference
	AHDS	AHDL	AHDB	AHDR			
<u>PHE</u> ANT	0.7	1.1	0.9	0.7	< 10 > 10	Pyrogenic Petrogenic	Pyrogenic
<u>ANT</u> (ANT+ PHE)	0.6	0.5	0.5	0.6	< 0.1 > 0.1	Petrogenic Pyrogenic	Pyrogenic
<u>FLT</u> (FLT+ PYR)	0.5	0.4	0.5	0.7	< 0.4 > 0.4	Petrogenic Pyrogenic	Pyrogenic

Preliminary toxicity of the antituberculosis herbal drugs

The concentrations of benzo [a] pyrene equivalent toxicity (TEQ) in the herbal drugs are showed in Table 2. The concentration of total benzo [a] pyrene equivalent toxicity ranges from 3.841 in sample AHDR to 2.449 in sample AHDB. The high

concentration of total benzo [a] pyrene equivalent toxicity in sample AHDR could be as a result of high concentration of total PAHs in the samples. The lowest benzo [a] pyrene equivalent toxicity observed in sample AHDB can be linked to few individual carcinogenic PAHs found in the sample.

Table 2. Toxicity Equivalent (TEQ) of benzo [a] pyrene concentration (mg/kg) in anti-cholera herbal drugs.

Sample	BaA	BbF	IcP	BkF	CHR	BaP	DhA	Total Bap TEQ
TEF	0.1	0.001	0.1	0.01	1	1	0.1	
AHDS	0.204	8.97 x10 ⁻⁴	0.097	21.5 x10 ⁻³	1.954	0.651	0.005	2.933
AHDL	0.211	0.10 x10 ⁻⁴	0.120	1.20 x10 ⁻³	2.043	0.865	0.011	2.752
AHDB	0.012	5.74 x10 ⁻⁴	0.008	8.52 x10 ⁻³	1.546	0.765	0.109	2.449
AHDR	0.103	0.15 x10 ⁻⁴	0.065	1.25 x10 ⁻³	2.540	1.005	0.127	3.841

Table 3 displays the exposure dose of carcinogenic PAHs in the herbal drug samples. Based on the result display in Table 3, the cancer risk

connected with exposure to these herbal drugs samples were calculated and are showed in Table 4.

Table 3: Exposure dose of carcinogenic PAHs in the herbal drugs sample.

Samples	Exposure dosage (1x10 ⁸)		
	Children	Preteen	Adult
AHDS	3.9587	1.5787	1.1571
AHDL	3.7144	1.4813	1.0857
AHDB	3.3054	1.3182	0.9662
AHDR	5.1842	2.0674	1.5154

The estimated cancer risk for these herbal drugs ranges from 2.0642 to 1.3161 x 10⁻⁷ in children, from 0.8232 to 0.5248 x 10⁻⁷ in preteen and from 0.6034 to 0.3847 x 10⁻⁷ in adult. Excess cancer risk is an expression of the quota of a population that may be infected by carcinogen throughout lifetime of exposure. Cancer risk estimation of 1 x 10⁻⁶ envisages

the possibility of one additional cancer occurrence when one million out of the population are exposed. The calculated cancer risk via exposure to PAHs resulting from the use of any of these herbal drugs were calculated to be below the set cancer risk range by USEPA (1 x 10⁻⁴ – 1 x 10⁻⁶) (ATSDR 2014).

Table 4: Cancer risk estimation in the herbal drugs samples.

Samples	Cancer risk estimation (1x10 ⁻⁷)		
	Children	Preteen	Adult
AHDS	3.9587	1.5787	1.1571
AHDL	3.7144	1.4813	1.0857
AHDB	3.3054	1.3182	0.9662
AHDR	5.1842	2.0674	1.5154

Group distribution of PAHs in the studied antituberculosis herbal drug samples

Low molecular weight (LMW) PAHs are PAHs with 2-3 fused ring structures, moderate molecular weight (MMW) PAHs are PAHs with 4 fused ring structures and high molecular weight (HMW) PAHs are PAHs with 5-6 fused ring structures. The increasing order of group distribution of PAHs in the studied antituberculosis herbal drugs was LMW PAHs > MMW PAHs > HMW PAHs for samples AHDL, AHDB and AHDR while the increasing order for sample AHDS was HMW PAHs > MMW PAHs > LMW PAHs as showed in Table 5. The higher concentrations of LMW PAHs in the samples might be due to slight rainfall during the harvest period of the medicinal plants which had helped in removing the particulate matters on the surface of the leaves and bark of the medicinal plants (Chun 2011). High

volatilization or dissolution of PAHs during extraction might also be responsible for the increase in concentration of LMW PAHs in these herbal drug samples because LMW PAHs has been reported to be relatively more volatile and evaporate rapidly at the high temperature (Tham et al 2008). HMWPAHs have been reported to possess greater hydrophobicity and less water solubility which are responsible for their low biodegradation and persistence in the environment where they exist (Wilcke 2000). In addition (Lijinsky 1991) reported that HMWPAHs are potent carcinogens. This suggest that exposure to all the herbal drugs may not be carcinogenic except in sample AHDS which had higher proportion of HMW PAHs. Nevertheless, to reduce health problem, excessive intake of these herbal drugs should be prevented as their biodegradation on accumulation are difficult.

Table 5: Group distribution of PAHs in the studied antituberculosis herbal drugs.

No of Rings	Group distribution of PAHs in Samples			
	AHDS	AHDL	AHDB	AHDR
LMWPAHs	5.126	7.918	11.054	11.029
MMWPAHs	5.871	6.249	4.583	6.250
HMWPAHs	5.968	6.423	3.708	5.155
TPAHs	16.965	20.29	19.345	22.434

Conclusion

The diagnostic indices of the herbal drugs revealed a pyrogenic source of PAHs. The toxicological investigation in this study revealed that the herbal drug samples may not cause health challenges based on the calculated cancer risk estimated values that are lower than the risk levels established by USEPA. Notwithstanding, caution should be taken as the group distribution of PAHs in sample AHDS confirmed high concentration of HMW PAHs which are less or non-biodegradable. However, excessive exposure to any of these antituberculosis herbal drugs should be avoided to prevent serious health jeopardy. In addition, it is crucial to set up monitoring agency for the assessment of herbal drugs produced in Nigeria before sale and use to reduce the toxicological effects.

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