# Speculative Carcinogenicity Assessment of Polycyclic Aromatic Hydrocarbons Found in Some Anti-Diarrheal Herbal Drugs Sold in Ondo State of Nigeria

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**Abstract**

The conviction that herbal drugs have enormous health benefits has led to increase the rate of their consumption by Nigerians. The aim of this study was to assess the carcinogenic property of some popularly consumed anti-diarrheal herbal drugs via polycyclic aromatic hydrocarbons (PAHs) quantification. Three prevalent anti-diarrhea herbal drugs , Odunmo herbal drug *(Hibiscus rosa-sinensis* and *Bacopamonnieri),* Orogun herbal mixture (*Hibiscus sabdariffaI* and *Hedera helix)*, and Alora herbal syrup *(Aloe vera* and *Hibiscus sabdariffaI)* were bought for the purpose of this study and they were coded as samples A, B, and C, respectively. The ultrasonic extraction of the herbal drugs was carried out using standard procedures. The crude extracts obtained were purified using a chromatographic method. The concentrations of PAHs were quantified using gas chromatography flame ionization detector (GCFID). The diagnostic indices, group distribution, toxicity equivalence and exposure dosage were estimated. The cancer risk values were theoretically speculated based on concentrations of PAHs in the tested herbal drugs, associated with the published estimates of each concentration to cause cancer and the calculated exposure doses of the anti-diarrhea herbal drug samples were within three age groups (children, Preteen and adult). The highest concentration of total PAHs was observed in sample A (58.2815mg/kg) and the lowest concentration in sample B (44.1898mg/kg), but the concentration of total PAHs in sample C was 47.4169 mg/kg. The highest percentage of carcinogenic PAHs in the anti-diarrheal herbal drugs was found in sample C (48.66%) and the lowest in sample B (38.17%). The diagnostic indices confirmed a pyrogenic source of PAHs. Group distribution of PAHs showed that the herbal drugs are weakly carcinogenic due to high concentrations of low and moderate molecular weight PAHs. The cancer risk estimated for all the age groups where below the limit established by the United State Environmental Protection Agency (USEPA) for cancer (1 x 10$^{-6}$). This ascertained that the use of these herbal drugs cannot cause cancer. However, consumers of these herbal drugs should take necessary precautions as excessive intake can lead to dangerous health implications.

**Key words:** Polycyclic aromatic hydrocarbons, cancer risk estimation, anti-diarrheal herbal drugs, chromatography, toxicity.

## Introduction

Diarrhea disease is characterized with an increase in frequency of bowel, vomiting, presence of blood in stools, loss of appetite, loss of weight, and abdominal pains, while cancer remains a major public

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health problem worldwide [1, 2]. Diarrheal diseases has been rated as the second causative agent of death among children of five years and below, thereby accounting for the death of about 760,000 children annually [3, 4]. The world's highest prevalence and severity of diarrhea is recorded in the developing countries, especially in most African counties [5, 6]. In the year 2013, diarrhea disease was reported as the major public health problem in Ethiopia, causing high childhood mortality and morbidity [7,8]. The causes of diarrhea have been linked to bacterial infections with exposure to contaminated diet which could lead to infection that disrupts the intestinal absorptive and secretory functions of the body [9, 10]. Acute diarrhea is caused by parasitic infections and may persist for two days, while chronic diarrhea is connected with functional disorders comprising irritable bowel disease and Crohn's disease that can persist for a month [7]. Hyponatremia, malnutrition, dehydration, growth retardation, and low performance are impediments connected to diarrhea [8]. Oral rehydration therapy, supplements, antibiotics, adsorbents, and the use of prescriptions such as codeine and bismuth sub-salicylate have been used for the treatment of diarrhea [8]. Limitations such as abdominal discomfort, dry mouth, and vomiting are associated with the conventional therapies used for the treatment of diarrhea. The resistance of the causative agents to these commercial synthetic drugs is major challenges that need to be properly handled to eradicate diarrhea and its complications. Attempts to find better solutions to the aforementioned problems have led many researchers to investigate the anti-diarrheal activities of many natural products as an alternative treatment [9, 11]. About 80 % of the world population depends greatly on herbal medicines as remedy to the health challenges, among which millions of people in Africa [12, 13]. Recent studies confirmed that wide range of herbal drugs and medicinal plants have been widely adopted for the treatment of diarrhea and other ailments without any investigations of safety and therapeutic potentials [14, 15]. The use of various parts of medicinal plants in forming infusions, decoctions, and herbal drugs has remained an alternative approach for the treatment of diarrhea [16]. High safety profile, easy accessibility, and low cost are the major advantages associated with the use of herbal drugs as remedy to diarrheal disease [17]. Despite the advantages associated with the use of herbal drugs, toxicity and improper dosage remain a worrisome aspect [18, 19]. Side effects arisen from the contamination of herbal drugs with heavy metals and polycyclic aromatic hydrocarbons were reported by Akintelu *et al*. in 2018 [20]. The present study aimed at the carcinogenicity evaluation of randomly selected anti-diarrheal herbal drugs produced in Ondo State metropolis of Nigeria.

**Materials and methods**

Different prevalent anti-diarrheal herbal drugs produced locally in Irele Metropolis of Ondo State were bought from different stores. From the variety of anti-diarrheal herbal drugs, Odunmo herbal drug *(Hibiscus rosa-sinensis* and *Bacopamonnieri)*, Orogun herbal mixture (*Hibiscus sabdariffaI* and *Hedera helix)*, and Alora herbal syrup *(Aloe vera* and *Hibiscus sabdariffaI)* were selected and coded as samples A, B, and C, respectively. The selection was based on availability, high popularity among costumers, and efficiency against diarrheal infection. Five hundred milliliters (500 mL) of four bottles of each of the herbal drug samples were used for this study.

**Extraction of anti-diarrheal herbal drug sample**

The Akintelu *et al*. [20] method of extraction was modified. About 10 ml of the herbal drug sample was quantitatively measured with a measuring cylinder and transferred into a 50 ml clean beaker. Then, 4 ml of n-hexane was added. The beaker and its content were placed in an ultrasonic bath for 40 minutes to aid proper extraction. The extracting solvent was removed from the extract via concentration using a rotary evaporator. The crude extract obtained was kept in a cleaned sample bottle and stored in the refrigerator to prevent deterioration of the sample.

**The cleaning process of the extract**

Contaminants and other impurities extracted with PAHs in the extraction procedure were removed by column chromatography. The dry method of packing the column was used, in which a chromatographic column of $13 \times 200$ mm was packed with aluminum oxide and silica gel in a ratio of 1:3 as stationary phase. The obtained crude extract was quantitatively loaded on the packed column. Then, the elution of the extract was performed using the mobile phase that contains a mixture of acetone and hexane at different proportions. The eluent was collected drop wise into 100 ml test tubes. They were spotted on thin layer chromatographic plates and those with similar retardation factor values where bulked and properly air dried to remove the eluting solvents by direct exposure to the atmosphere at room temperature.

**The chromatographic conditions for GC-FID operation**

The obtained eluents were analyzed using GC-FID (Agilent 8860, Ukraine) to estimate the concentration of PAHs in the herbal drug samples. A triplicate analysis to each of the eluent was performed and recorded.

The analysis conditions included flow rate = 1.2 ml/min, column thickness = 1m, wavelength = 200 nm, temperature of column = 45°C, concentration of injected extract = 2 μl, mobile phase = helium and nitrogen gas, and method of elution = isocratic. Other operating set-up specifications were followed according to the operating instruction stated in the GC- FID manual.

**Calculation of the toxicity equivalent concentration of benzo (a) pyrene in the drug sample**

The carcinogenic effects of the long time usage of the herbal drugs were evaluated by calculating the baseline lifetime risk using the concentration of PAHs in the samples. The following equations were adopted from the study of Orisakwe *et al.* 2015 [21], in which the calculation of the concentration of benzo (a) pyrene toxicity equivalent was used [22, 23]:

$$TEQ = \Sigma(PAH_i \times TEF_i) \quad \dots \quad \text{Equation 1}$$

Where TEQ = toxicity equivalence, $PAH_i$ = concentration of carcinogenic PAHs in each sample, and $TEF_i$ = toxic equivalent factor (potency relative to benzo(a) pyrene).

USEPA TEF values were previously reported to be 0.1 for benzo[a] anthracene, 0.001 for chrysene, 0.1 for benzo[b] fluoranthene, 0.01 for benzo[k] fluoranthene, 1 for benzo[a] pyrene, 1 for dibenzo[a,h] anthracene, and 0.1 for indeno[1,2,3-c,d] pyrene [22].

**Cancer risk evaluation**

The following equations were applied to calculate cancer risk in the samples using the toxic equivalent obtained from the benzo(a)pyrene toxicity equivalent concentration in the herbal drugs via equation 1, according to Akintelu *et al.* [20].

$$Dose = \frac{(Concentration \times intake\ rate \times conversion\ factor \times exposure\ factor)}{Weight\ of\ the\ body} \dots Equation\ 2$$

Dose = estimated exposure dose in (mg/kg/day); Intake rate = volume of herbal drug sample which is 0.30 L per day; Body weight of average 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.

$$Cancer\ risk = \frac{(Exposure\ dose \times Number\ of\ years\ of\ sample\ usage \times CPF)}{User's\ life\ time} \dots\dots Equation\ 3$$

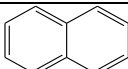CPF = cancer potency factor was given as (7.3); Years of sample intake = 30 years; User's life time = 55 years.
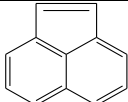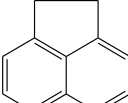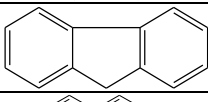
**Statistical analysis**
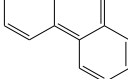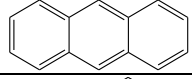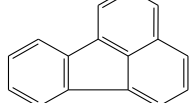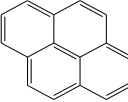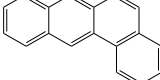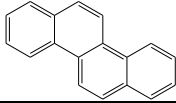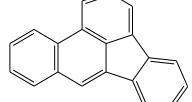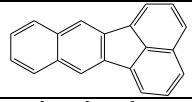
The means and standard deviations of the concentration of PAHs were determined with Microsoft excel package 2013.

**Results and discussion**

The concentrations of 16 individual PAHs found in the anti-diarrheal herbal drug samples are given in Table-1. Among the individual PAHs analyzed, pyrene had the lowest concentrati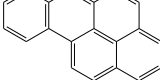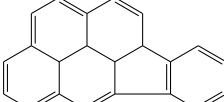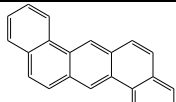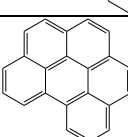on of 0.4567mg/kg in sample A, while benzo(g,h,i)perylene had the highest concentration of 9.2300 mg/kg. The highest concentration of total PAHs was observed in sample A (58.2815mg/kg) and the lowest was in sample B (44.1898mg/kg). The highest percentage of carcinogenic PAHs in the anti-diarrheal herbal drug samples was found in sample C (48.66%) and the lowest was found in sample B (38.17%). The existence of PAHs in the anti-diarrheal herbal drug samples might result from the deposition of PAHs in the soil in which the medicinal plants are grown. Another reason could be the combustion process during the preparation of the anti-diarrheal herbal drug samples [24].

**Table 1-**Concentrations of PAHs in the anti-diarrheal herbal drug samples

| PAHs | Structure | Concentrations of PAHs (mgkg$^{-1}$) | | |
|------|-----------|------|------|------|
| | | A | B | C |
| Naphthalene | | 6.1207±0.02 | 5.5431±0.03 | 5.8791±0.01 |

| | | | | |
|---|---|---|---|---|
| Acenaphthylene | | 4.3544±0.01 | 6.2470±0.00 | 4.2123±0.02 |
| Acenaphthene | | BDL | 5.9082±0.02 | BDL |
| Fluorene | | 7.8901±0.01 | BDL | BDL |
| Phanathrene | | 3.1234±0.03 | 1.2765±0.00 | 2.4290±0.03 |
| Anthracene | | 2.9832±0.04 | 1.4561±0.02 | 3.3457±0.01 |
| Fluoranthene | | 0.9578±0.04 | 1.4587±0.03 | 0.8723±0.01 |
| Pyrene | | 0.4567±0.03 | 2.0097±0.02 | 0.9711±0.01 |
| Benzo(a) anthracene | | 4.3298±0.01 | 3.3081±0.04 | 6.1009±0.02 |
| Chrysene | | 0. 8712±0.03 | 1.6134±0.03 | 1.2075±0.04 |
| Benzo(b) fluoranthene | | 3.0654±0.00 | 0.7651±0.02 | 2.9876±0.01 |
| Benzo(k) fluoranthene | | 7.8567±0.02 | BDL | 3.9871±0.03 |
| Benzo (a) pyrene | | 0.6721±0.03 | 0.8762±0.01 | 1.3091±0.04 |
| Indeno (1,2,3-cd) pyrene | | 3.1546±0.00 | 5.1547±0.01 | 4.2357±0.02 |
| Dibenzo(a,h) anthracene | | 3.2154±0.02 | 5.9112±0.03 | 3.2435±0.02 |
| Benzo(g,h,i) perylene | | 9.2300±0.01 | 6.1205±0.02 | 7.5083±0.01 |
| Total PAHs | | 58.2815 | 44.1898 | 47.4169 |
| Σ Carcinogenic PAHs | | 23.1652 | 17.6287 | 23.0714 |
| % Carcinogenic PAHs | | 39.75 | 38.17 | 48.66 |

Carcinogenic PAHs: Benzo(a)anthracene, benzo(b)fluoranthene, indeno(1,2,3-c,d)pyrene, benzo(k)fluoranthene, chrysene, benzo(a)pyrene and dibenzo(a,h)anthracene [25,26]BDL–below detection limit; means ±SD (n =3)

**Table 2-**Diagnostic indices of PAHs in the anti-diarrheal herbal drug samples

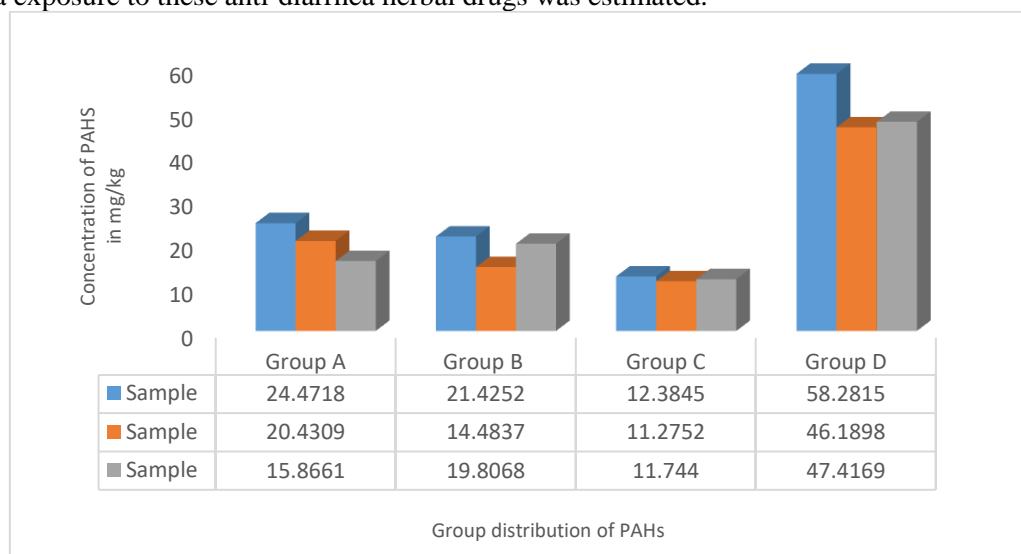| PAH ratio | Sample (mgkg⁻¹) | | | Value of ratio | Indication | Inference |
|---|---|---|---|---|---|---|
| | A | B | C | | | |
| $\frac{PHE}{ANT}$ | 1.05 | 0.88 | 0.73 | < 10 > 10 | Pyrogenic Petrogenic | Pyrogenic |
| $\frac{ANT}{(ANT+ PHE)}$ | 0.49 | 0.53 | 0.58 | < 0.1 > 0.1 | Petrogenic Pyrogenic | Pyrogenic |
| $\frac{FLT}{(FLT+ PYR)}$ | 0.68 | 0.42 | 0.90 | < 0.4 > 0.4 | Petrogenic Pyrogenic | Pyrogenic |

PHE = Phanathrene, ANT = Anthracene, PYR = Pyrene and FLT = Fluoranthene

The ratios obtained from the diagnostic indices were used to determine the sources of PAHs in the herbal drug samples, which could either be from pyrogenic or petrogenic sources. The concentration ratio of PHE/ANT in the samples analyzed was lower than 10, while that of ANT/ (ANT+ PHE) was higher than 0.1, and of FLT/ (FLT+ PYR) was higher than 0.4, as shown in Table-2. These results revealed that the PAHs found in these herbal drug samples are from pyrogenic sources. This finding is in agreement with previous reports by Dominguez *et al.* (2010), Tella *et al*. (2017), and Akintelu and Folorunso (2019) [27, 28, 29].

**Toxicity evaluation of the anti-diarrheal herbal drugs**

The distribution of PAHs in the anti-diarrhea herbal drug samples is shown in Figure-1. Low molecular weight PAHs (termed as group A in this study) have two and three fused rings in the chemical structure, medium molecular weight PAHs (group B) have four or five fused rings , while high molecular weight PAHs (group C) have more than five fused rings . Total PAHs in this study are termed group D. The order of group distribution of PAHs in the anti-diarrheal herbal drugs was as follows: group A > group B > group C for samples A and B, while the order in sample C was group B > group A > group C, as shown in Figure-1.

According to the findings of  Lijinsky (1991) [30], PAHs comprising four fused rings are regarded as weakly carcinogens, while those with five or six-fused rings are very potent carcinogens. This suggests that the herbal drug samples are weakly carcinogens because they have higher content of group A and group B than group C PAHs. However, the prolonged use of these drugs may lead to bioaccumulation that may cause cancer or affect the proper function of cells. Subsequently, the cancer risk via exposure to these anti-diarrhea herbal drugs was estimated.



| | Group A | Group B | Group C | Group D |
|---|---|---|---|---|
| ■ Sample | 24.4718 | 21.4252 | 12.3845 | 58.2815 |
| ■ Sample | 20.4309 | 14.4837 | 11.2752 | 46.1898 |
| ■ Sample | 15.8661 | 19.8068 | 11.744 | 47.4169 |

**Figure 1-**Group distribution of PAHs in the anti-diarrhea herbal drugs.

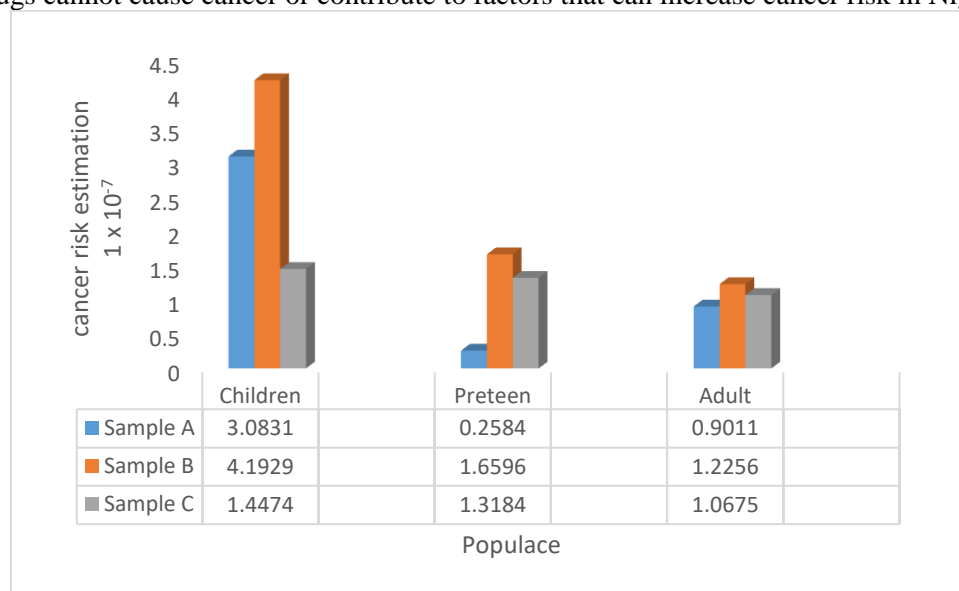**Cancer risk of exposure to selected anti-diarrheal herbal drug samples**

The toxicity equivalent concentration was used to calculate the cancer risk resulting from the exposure doses to the selected anti-diarrhea herbal drug samples. The concentrations were 5.7369, 7.8019, and 6.1967 $mgkg^{-1}$ in samples A, B, and C, respectively. The variation in the concentrations of total toxicity equivalence of the samples may be the result of differences in the concentration of carcinogenic PAH in the samples. The highest toxicity equivalence value observed in sample B might be due to the high concentrations of carcinogenic PAHs, while sample A, which has the lowest toxicity equivalence, possess low carcinogenic PAHs. The result of Benzo(a)Pyrene toxicity equivalent concentration in this study is higher than that obtained in the study of Orisakwe *et al.* (2015) [21].

The calculated exposure dose of herbal drug samples was adopted in estimating the cancer risk, as shown in Table-3.

**Table 3-**Calculated exposure doses in the anti-diarrhea herbal drug samples

| Age group | Calculated exposure dose in samples | | |
|:---:|:---:|:---:|:---:|
| | A | B | C |
| Children | $7.7430 \times 10^{-8}$ | $1.0530 \times 10^{-7}$ | $0.3635 \times 10^{-7}$ |
| Preteen | $0.0649 \times 10^{-7}$ | $0.4168 \times 10^{-7}$ | $0.3311 \times 10^{-7}$ |
| Adult | $0.2263 \times 10^{-7}$ | $0.3078 \times 10^{-7}$ | $0.2681 \times 10^{-7}$ |

The highest value of cancer risk calculated in the herbal drug samples was observed in sample B, with values of $4.1929 \times 10^{-7}$, $1.6596 \times 10^{-7}$ and $1.2256 \times 10^{-7}$ for children, preteen, and adult age groups, respectively. The lowest cancer risk calculated in this study for children was $1.4474 \times 10^{-7}$ and it was observed in sample C. However, sample A showed lowest cancer risk values of $0.2584 \times 10^{-7}$ and $0.9011 \times 10^{-7}$ for preteens and adults, respectively, as shown in Figure-2. However, the calculated values of cancer risk of all the samples were below the acceptable standard of $1 \times 10^{-6}$ set by the USEPA [31], as shown in Table-3. Cancer risk estimation of $1 \times 10^{-6}$ envisages the possibility of one additional cancer when a population of one million is exposed. This suggests that the intake of these herbal drugs cannot cause cancer or contribute to factors that can increase cancer risk in Nigeria.



| | Children | Preteen | Adult |
|:---|:---:|:---:|:---:|
| ■ Sample A | 3.0831 | 0.2584 | 0.9011 |
| ■ Sample B | 4.1929 | 1.6596 | 1.2256 |
| ■ Sample C | 1.4474 | 1.3184 | 1.0675 |

**Figure 2**-Cancer risk due to exposure to selected anti-diarrheal herbal drug samples.

**Conclusions**

The source diagnostic indices of the herbal drugs confirmed a pyrogenic source, which infers that the PAHs were incorporated into the samples via combustion. The carcinogenicity assessment of this study revealed that the use of these herbal drugs might not lead to cancer if taken at lower concentration and dosage. However, excessive exposure and accumulation might lead to serious health

risks. Subsequently, more accurate risk assessment of other herbal drugs should be encouraged to limit the potential health risks associated with locally made herbal drugs.

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