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

Akintelu Sunday Adewale1,2, Folorunso Femi Adekunle3, Folorunso Aderonke Similoluwa4*

1School of Chemistry and Chemical Engineering, Beijing Institute of Technology, Beijing, China
2Department of Pure and Applied Chemistry, Ladoke Akintola University of Technology, Ogbomoso, Nigeria
3Department of Anatomy, Osun State University, Osogbo, Nigeria
4Department of Chemistry, Louisiana State University, Louisiana, USA

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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), Orogu 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 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 47.4169mg/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
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 countries [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 sabdariffal and Hedera helix), and Alora herbal syrup (Aloe vera and Hibiscus sabdariffal) 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 × 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]:

\[
\text{TEQ} = \Sigma (\text{PAHi} \times \text{TEFi})
\]

Equation 1

Where TEQ = toxicity equivalence, PAHi = concentration of carcinogenic PAHs in each sample, and TEFI = 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].

\[
\text{Dose} = \frac{\text{Concentration} \times \text{intake rate} \times \text{conversion factor} \times \text{exposure factor}}{\text{Weight of the body}}
\]

\[
\text{CPF} = \frac{\text{Exposure dose} \times \text{Number of years of sample usage} \times \text{CPF}}{\text{User’s life time}}
\]

\[
\text{Cancer risk} = \frac{\text{Concentration} \times \text{total toxicity equivalent of benzo (a)pyrene}}{\text{Conversion factor}}
\]

\[
\text{CPF} = \text{cancer potency factor was given as (7.3)}; \text{Years of sample intake = 30 years}; \text{User’s life time = 55 years.}
\]

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 concentration of 0.4567 mg/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 (58.2815 mg/kg) and the lowest was in sample B (44.1898 mg/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].

| PAHs         | Structure | Concentrations of PAHs (mg kg⁻¹) | A       | B       | C       |
|--------------|-----------|---------------------------------|---------|---------|---------|
| Naphthalene  | ![Structure Image](image) | 6.1207±0.02                      | 5.5431±0.03 | 5.8791±0.01 |
| Compound                  | Mean ± SE 1 | Mean ± SE 2 | Mean ± SE 3 |
|---------------------------|-------------|-------------|-------------|
| 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       | Value of ratio | Indication       | Inference |
|-----------------|----------------|------------------|-----------|
|                | A   | B   | C   | < 10 | > 10 | Pyrogenic       | Pyrogenic |
| PHE/ANT         | 1.05| 0.88| 0.73|      |      | Petrogenic      | Pyrogenic |
| (ANT+ PHE)      | 0.49| 0.53| 0.58| < 0.1| > 0.1| Petrogenic      | Pyrogenic |
| FLT/ (FLT+ PYR) | 0.68| 0.42| 0.90| < 0.4| > 0.4| Petrogenic      | Pyrogenic |

PHE = Phenanthrene, 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.

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 mg kg\(^{-1}\) 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 x 10\(^{-8}\) | 1.0530 x 10\(^{-7}\) | 0.3635 x 10\(^{-7}\) |
| Preteen   | 0.0649 x 10\(^{-7}\) | 0.4168 x 10\(^{-7}\) | 0.3311 x 10\(^{-7}\) |
| Adult     | 0.2263 x 10\(^{-7}\) | 0.3078 x 10\(^{-7}\) | 0.2681 x 10\(^{-7}\) |

The highest value of cancer risk calculated in the herbal drug samples was observed in sample B, with values of 4.1929 x 10\(^{-7}\), 1.6596 x 10\(^{-7}\) and 1.2256 x 10\(^{-7}\) for children, preteen, and adult age groups, respectively. The lowest cancer risk calculated in this study for children was 1.4474 x 10\(^{-7}\) and it was observed in sample C. However, sample A showed lowest cancer risk values of 0.2584 x 10\(^{-7}\) and 0.9011 x 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 x 10\(^{-6}\) set by the USEPA [31], as shown in Table-3. Cancer risk estimation of 1 x 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.

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.

References
1. Raj, V., Rai, A., and Singh, M. 2017. Detection of bioflavonoids from methanol bark extracts of Acacia and their role as antidiarrhoeal agents. Journal of Analytical and Pharmaceutical Research, 5(4):1–7.
2. Shkkaer M. T., and Utba N. M. 2019. IL-18 Gene Polymorphisms Impacts on Its Serum Levels in Prostate Cancer Iraqi Patients. Iraqi Journal of Science, 60(6): 1188-1196.
3. CDC (Global Diarrhea Burden). 2012 [10 May 2019. Available from: http://www.cdc.gov/healthywater/global/diarrhea-burden.html.
4. WHO (World health organization). 2013. Diarrhoeal Disease. Available from: http://www.who.int/mediacentre/factsheets/fs330/en/#content. Accessed 10 may 2019.
5. Fischer, W.C.L., Perin, J., Aryee, M.J., Boschi-Pinto, C., and Black, R.E. 2012. Diarrhea incidence in low- and middle-income countries in 1990 and 2010: a systematic review. BMC Public Health, 12(1): 1-7
6. Fischer, W.C.L., Rudan, I., Liu, L., Nahir, H., Theodoratou, E., and Bhutta, Z.A. 2013. Global burden of childhood pneumonia and diarrhoea. Lancet, 381(7): 1405–1416.
7. Awoke, W., 2013 Prevalence of childhood illness and mothers’/caregivers’ care seeking behavior in Bahir Dar, Ethiopia: a descriptive community based cross sectional study. Open Journal of Preventive Medicine, 3(2): 155–9.
8. Mohammed, S., Tilahun, M., and Tamiru, D. 2013. Morbidity and associated factors of diarrheal diseases among under five children in Arba-Minch District, Southern Ethiopia. Science Journal of Public Health. 1(2): 102–6.
9. Suleiman, M.M., Balkisu, B.O., Ahmed, A., Mohammed, M., and Kamar-deen, T.B. 2017 A controlled study to investigate anti-diarrhea effect of the stem-bark fractions of Terminalia avicennioides in laboratory animal models. International Journal of Verteinary Science and Medicine 5: 14–22.
10. Lakshminarayana, M., Shivkumar, H., Rimaben, P., Bhargava, V.K. 2011. Antidiarrhoeal activity of leaf extract of MoringaOleifera in experimentally induced diarrhea in rats. International Journal Phytomedicine, 3: 68–74.
11. Amsalu, D., Ephrem, E., and Workineh, S. 2016. Evaluation of the anti-diarrheal activity of the leaf extract of Croton macrostachyus Hocsht. ex Del. (Euphorbiaceae) in mice model..BMC Complementary and Alternative Medicine 16(3): 1-11
12. Kim, H.S., 2005. Do not put too much value on conventional medicines. Journal of Ethnopharmacology, 100(1–2): 37–9.
13. WHO (World Health Organization). 2000. General guidelines for methodologies on research and evaluation of traditional medicine. WHO-Geneva.
14. Teklehaymanot, T., and Giday, M., 2007. Ethnobotanical study of medicinal plants used by people in Zegie Peninsula, Northwestern Ethiopia. Journal of Ethnobiology and Ethnomedicine. 12(3): 1-11
15. Hameed S.I., Al-Shahwany A.W., and Salih S. J., 2019. Investigation the potential role of some medicinal plants extracts in regulating serum lipid profile in female albino rats. Iraqi Journal of Science, 60(12): 2561-2571.
16. Afolanya, J.A., and Wintola, O.A. 2014, A survey of medicinal plants used in the treatment of dysentery in Amathole municipality, South Africa. Pakistan Journal of Botany 46(5): 1685–92.
17. Mahmood, H., Chaudhry, M.A., Masood, Z., Saeed, M.A., and Adnan, S.A. 2017. Mechanistic evaluation of the traditional uses of Nepetaruderalis in gastrointestinal and airway disorders. Pharmaceutical Biology, 55(1): 1017–21.
18. Buzescu, M. 2011 Advantages and disadvantages of complementary and alternative medicine in otitis media in children. Bulletin of the Transilvania University of Brașov, 4(2): 127- 132

1052
19. Salman A. D.A., and Abud-Rahman E.S., 2018. Bacteriological study Bacteriological study of Pseudomonas aeruginosa isolated from different infections and study antimicrobial activities of plant extract Solanum nigrum against it. *Iraqi Journal of Science*, 58(1): 2278-2284.

20. Akintelu, S.A., Abiola, B.E., Ajayi, S.O., and Olabemiwo, O.M. 2018. Quantification and Preliminary Estimation of Toxic Effects of Polycyclic Aromatic Hydrocarbon in Some Antimalarial Herbal Drugs in Southwest Nigeria. *Bulletin of Pharmaceutical Research*, 8(1): 1-6

21. Orisakwe, O.E., Mbagwu, H.O.C., Ukpa, P., and Udowellea, N.A. 2015. Survey of polycyclic aromatic hydrocarbons and lead in Chinese teas sold in Nigeria: Levels and health implications. *Roczinki Państwowego Zakładu Higieny journal*, 66(3): 225-232.

22. Orisakwe, O.E. 2014 The role of lead and cadmium in psychiatry. *North American Journal of Medical Sciences*, 6(8): 370-376.

23. Yoon, E., Park, K., Lee, H., Yang, J.H., and Lee, C. 2007. Estimation of excess cancer risk on time-weighted lifetime average daily intake of PAHs from food ingestion. *Human Ecological Risk Assessment*, 13: 669–680.

24. Ding, X., and Kammsky, L.S., 2003. Human extrahepatic cytoctromes P450, function in Xenobiatic in metabolism and tissue selective chemical toxicity in the respiratory and gastrointestinal tracts. *Annual Review of Pharmaceutical and Toxicology*, 43: 149-73.

25. ATSDR (Agency for Toxic Substances and Disease Registry) 1995. *Toxicological Profile for Polycyclic Aromatic Hydrocarbons (PAHs)*, U.S. Department of Health and Human Services, Public Health Service, Atlanta, GA.

26. EU (European Union), 2005. Commission Recommendation of 4 February 2005 on further investigation into the levels of polycyclic aromatic hydrocarbons in certain food, 2005 www.eurlex.europa.eu/LexUriServ/LexUriServ.do?uri=OJ:L:2005.034:0043:0045:EN.

27. Dominguez, C., Sarkar, S.K., Bhattachrya, A., Chatterjee, M., Bhattacharya, B.D., Jover, E., Albarges, J., Bayona, J.M., Alam, A.M., and Satpathy, K.K. 2010. Quantification and Source Identification of Polycyclic Aromatic Hydrocarbons in Core Sediments from Sundarban mangrove wetland, India. *Archives of Environmental Contamination and Toxicology*, 59(1): 49-61.

28. Akintelu, S.A., and Fololorunso, A.S., 2019. Cancer Risk Evaluation of Some Antidiabetic Herbal Drugs Sold in Irele Metropolis of Ondo State in Nigeria. *International Journal of Modern Chemistry*, 11(1): 82-93

29. Tella, A., Ajayi, S.O., Abiola, B.E., Adekunle, A.K., Akintelu, A.S., and Olabemiwo O.M. 2017. Assessment of the Levels of Polycyclic Aromatic Hydrocarbons in Wazo Market Topsoil, Ogbomoso,Nigeria. *Ewemen Journal of Analytical and Environmental Chemistry*, 3: 111-118.

30. Lijinsky, W., 1991. The formation and occurrence of polynuclear aromatic hydrocarbons associated with food. *Mutation Research*, 259: 251–61.

31. ATSDR (Agency for Toxic Substances Disease Registry) 2014. *Health Consultation*. EPA FACILITY ID: OHN000510571, U.S. Department of Health and Human Services, Fostoria, Wood County, Ohio, 2014.