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Levels of Polycyclic Aromatic Hydrocarbons (PAHs) in Beers: Consumption and Public Health Concerns

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Authors' contributions

This work was carried out in collaboration among all authors. Author VNO designed and wrote the entire manuscript and sourced most of the data and literature and as well supervised authors UBU and RCA during laboratory work. Author UBU also provided some literature on PAHs while author RCA in addition assisted in the procurement of research materials and literature on beer. All authors read and approved the final manuscript.

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ABSTRACT

Aim: The aim is to investigate some physicochemical properties of beers and polycyclic aromatic hydrocarbons contaminants in beer brewed with isomerized hop extract and in comparison with beers brewed with extracts from four Nigerian potential hop substitutes.
Study Design: Beers were brewed using isomerized hop extract and extracts from four Nigerian bitter vegetables. Analyses of physicochemical properties of the beers and for the presence of 16 specific target PAHs were carried out using their respective standard methods.
Place and Duration of Study: Analysis of physicochemical properties of the beers was done at Nigerian Breweries PLC, Enugu while analysis for PAHs was conducted at Central Laboratory, Nigerian Institute for Oceanography and Marine Research, Lagos between July, 2018 and November, 2019.
Methodology: Physicochemical properties of the beers (alcohol content, bitterness level, pH, specific gravity, colour) were determined using their respective standard methods. Gas chromatography/mass spectrometry was used in analyzing for PAHs [naphthalene,

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acenaphthylene, acenaphthene, phenanthrene, anthracene, fluoranthene, pyrene, benzo(a)anthracene, chrysene, benzo(b)fluoranthene, benzo(k)fluoranthene, benzo(a)pyrene, dibenzo(a,h)anthracene, indeno(1,2,3-cd)pyrene and benzo(g,h,i)perylene]. Four isotopically labelled PAHs (acenaphthene-d_{10}, phenanthrene-d_{10}, chrysene-d_{12} and perylene-d_{12}) were used as internal standards.

Results: Alcohol content (%v//v) in the beer samples is A(5.20); B(4.28); C(4.40); D(4.43) and E(4.54), bitterness level in International Bitterness Units (IBU) is A(0.54); B(0.80); C(1.46); D(1.46) and E(0.08), pH is A(4.36); B(3.08); C(3.88); D(3.90) and E(3.87), specific gravity is A(10.06); B(10.00); C(10.00); D(10.06) and E(10.06), and beer colour is A(5.80); B(7.70); C(6.60); D(8.00) and E(7.40). All 16 EPA PAHs were not found in all the beer samples except pyrene which was detected in sample B at a concentration of 0.00402 mg/kg.

Conclusion: It is concluded that extracts from the four Nigerian bitter vegetables could be used as substitutes for isomerized hop extract and that consumption of beer produced using extract from *G. kola* poses great public health concerns.

Keywords: PAHs; isomerized hop extracts; extracts from Nigerian bitter vegetables; beer consumption; public health.

1. INTRODUCTION

Hops, the female flowers of the hop plant (*Humulus lupulus*) are grown in the temperate regions of the world, solely to meet the demands of the brewing industry [1]. The brewing value of the hop is found in hop resins and essential oils that are contained in the lupulun glands of the female hop cone. These contain bitter resins and ethereal oils which supply bittering and aroma components of beer [2]. Four Nigerian bitter vegetables had been reported as potential hop substitutes in beer brewing in our previous works [3,4].

The rate of beer consumption increases daily across the world and Nigeria is not an exception because of the country's favourable demographics with populous and vibrant youth and growing middle class, along with a growing, largely youth population with increased disposable incomes. The annual consumption rate of beer in Nigeria from 2008 to 2011 is shown in Table 1. Hence, the importation of hops to meet the demand of the brewing industries continues to constitute a significant proportion of the Nigerian economy.

Table 1. Annual beer consumption rate in Nigeria from 2008 to 2011

	Quantity consumed (mn hl)*
2008	115
2009	126
2010	151.2
2011	151.5

*Source: NIBREWNEWS (2012) [5]; *million hectoliter*

Our previous works sought to develop new sources of ingredients that could substitute hops from plant sources. We reported that the extracts from these four plants [*Garcinia kola* (bitter kola), *Azadirachta indica* (neem), *Vernonia amygdalina* (bitter leaf) and *Gongronema latifolium* (heckel)] could be used as suitable substitutes for isomerized hop extract in the Nigerian brewing industry. *Garcinia kola*, an angiospermae, belonging to the family *Guttiferae*, is known in commerce as bitter cola. On chewing, *G. kola* has a bitter astringent and resinous taste, somewhat resembling that of raw coffee, followed by a slight sweetness. *Azadirachta* is a genus of two species of trees in the Mahogany family, *Meliaceae*. Numerous species have been proposed for the genus but only two are currently recognized, *Azadirachta excelsa* and the more economically important tree, *Azadirachta indica* which is the only species in Nigeria [6]. *Vernonia amygdalina* is a shrub or small tree with petiolate leaf of about 6mm in diameter and elliptic shape. The leaves are green with a characteristic odour and a bitter taste [7]. *Gonogronema latifolium* is a climbing shrub of the family *Asclepiadeceae*. Water extracts of the powdered leaves of *G. latifolium* gave low bittering values, but extraction of the powdered leaves with organic solvents significantly increased analytical bitterness of levels comparable with hops [8].

Polycyclic aromatic hydrocarbons are bio-accumulating and bio-degradable through organism food chain [9]. They are components of most fossil fuels and are ubiquitous in the natural environment [10,11]. Stationary fuel sources are responsible for over 98% of polycyclic aromatic hydrocarbons emissions [11]. The study of polycyclic aromatic hydrocarbons is due mainly to their carcinogenic and widespread occurrences in environmental components;

including surface soils, most of the depositions after local and long-range transport which is supported by the presence of polycyclic aromatic hydrocarbons in soil of regions remote from any industrial activity [12,13]. Some examples of these compounds as proposed by WHO [14] are: naphthalene, 2- methylnaphthalene, acenaphthylene, acenaphthene, phenanthrene, anthracene, fluoranthene, pyrene, benzo(a)anthracene, chrysene, benzo(b)fluoranthene, benzo(k)fluoranthene, benzo(a)pyrene, dibenzo(a,h)anthracene, indeno(1,2,3-cd)pyrene and benzo(g,h,i)perylene.

PAHs belong to a group of over 100 hazardous substances of organic pollutants consisting of two or more fused-benzene aromatic rings [15]. Formation of PAHs is due to the incomplete combustion of organic matter through the condensation of ethylenic radicals in the gas phase to form the larger polycyclic compounds [16]. Those containing up to four benzene rings are known as light PAHs (l-PAHs), and those containing more than four benzene rings are known as heavy PAHs (h-PAHs). The h-PAHs are more stable and toxic than the l-PAHs [15]. According to Comprehensive Environmental Response, Compensation and Liability Act (CERCLA) list of hazardous substances, PAHs ranked 7th in the biennial ranking of chemicals deemed to pose the greatest possible risk to human health [17,18]. PAHs could be formed during processing of coal, crude oil and natural gas, incomplete combustion of coal, garbage and other organic substances [19], often as a result of pyrolitic processes especially the incomplete combustion of organic materials during industrial and other human activities [20]. They could also be found in cigarette smoke, exhaust from automobiles and machineries, asphalt, coal tar and creosote- treated wood product as well as from natural sources such as volcanoes. They are lipophilic, chemically stable [21] and can be found practically everywhere in soil, water, refuse dumpsite and food [22]. Their presence in food is of major interest as they could be found in spices, cereals, grains, flour bread, vegetables, fruits, meats, processed or pickled foods and even contaminated cow milk [23].

Analyses have been carried out on metals [24,25] in beers which may pose serious damages in the human system, if consumed but there may be little analysis on Polycyclic Aromatic Hydrocarbons(PAHs) in beers. PAHs have received considerable attention in recent years because several of them are known to be potential human carcinogens and have been implicated in various cancers [26-28]. They have also been implicated in numerous other toxicological manifestations such as reproductive toxicity, intra-uterine growth retardation, learning and intelligent quotient deficit, destruction of oocytes and inflammation of kidney cells [29,30].

It is important to note that beer consumption has not been so far implicated among the sources of PAHs but for the fact that cigarette smoke and foods were highly implicated and that alcoholic drinks can contain these carcinogenic chemicals through the charred insides of barrels, some ingredients such as caramel or the smoke released during the drying of germinated barley in beer or whisky [31], there is the urgent need to investigate levels of PAHs concentration in beers in order to ensure that they are free from these cancer causing compounds. The current work therefore is focused on evaluation of such physicochemical properties of beers as colour, bitterness level, alcohol content, pH, specific gravity, and the polycyclic aromatic hydrocarbons concentration in the beers.

2. MATERIALS AND METHODS

2.1 Extraction

Except isomerized hop extract that was purchased from Ritchies, England, United Kingdom, four fresh Nigerian bitter vegetables (*G. kola, A. indica*, *V. amygdalina* and *G. latifolium*) were procured, sorted and washed in tap water. They were air-dried for 10 min., after which they were transferred into an air drought oven, at a temperature of 57ºC for 24 h. The vegetables were allowed to cool at room temperature and were subsequently milled to powder. Five grams (5 g) of each of the vegetable was poured into four different beakers and 20 ml of methanol was added to each of the samples. The samples were transferred to a mechanical shaker which shook the mixtures vigorously and continuously until the mixture formed two layers. The extract was filtered, autoclaved and cooled at a temperature of 20ºC.

2.2 Brewing of Beers

The beers were brewed at Nigerian Breweries PLC, Ama, Enugu State, Nigeria. Three litres of star wort was obtained (star lager was used as control). Two hundred and fifty mililitre (250 ml) of wort was measured into five different conical flasks and 1 ml of isomerized hop extract was

added to the one of the flasks containing the wort as the control and labeled A. One mililitre (1 ml) each of the extracts from *Garcinia kola* (*G, kola*), *Azadirichta indica* (*A. indica*), *Vernonia amygdalina* (*V. amygdaina*) and *Gongronema latifolium* (*G. latifolium*) was added to each of the other four wort samples and labeled B, C, D and E respectively. Ten grams (10 g) of yeast was added to each mixture and was thoroughly stirred till all solids were dissolved. The mixtures were shaken at 3000 rpm for rapid fermentation for five days and were filtered after fermentation to obtain a bright beer. The bright beer was pasteurized at 57ºC to terminate the activities of the yeast. The beer samples were stored in a 20ºC refrigerator.

2.3 Determination of Physicochemical Properties

Alcohol content of the samples was determined by distillation method as described by Ceirwyn [32]. Bitterness was determined according to ASBC Beer 23A method [33]. pH was measured by Electrometric method using laboratory pH meter as described by Food Compliance Laboratory Unit of National Agency for Food and Drug Administration and Control (NAFDAC SOP Code: FC:06.5) [34]. A modified method used by De Clerk [35] was adopted in the determination of specific gravity of the beer samples. These methods had been described in our previous works [3,4,10]. European Brewing Convention (EBC) method 4.7.1 [36] was adopted in the determination of the colour of the samples using spectroscopic technique. The cuvette was filled with distilled water and the absorbance of the spectrophotometer was adjusted to 0.00. The cuvette was rinsed with brighter beer sample and was filled with the sample. The absorbance was read at 430 nm. The colour of the sample in EBC was calculated from the relation: Colour = 25Af where A is the absorbance at 430 nm in a 1ml cuvette and f is a dilution factor.

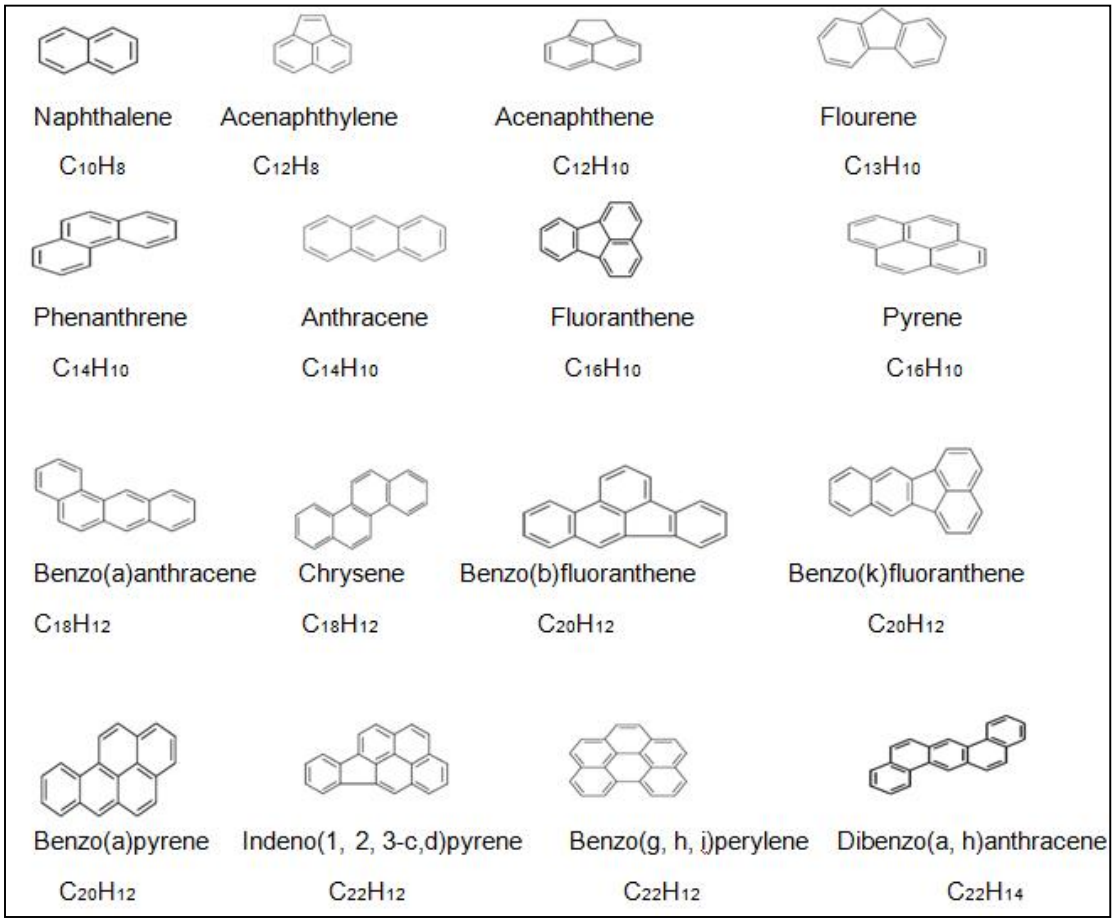

Fig. 1. Chemical structures and formulas of the 16 priority EPA PAHs

2.4 Determination of Polycyclic Aromatic Hydrocarbons

The beer samples, including the control were analysed for the presence of PAHs using the EPA 8100 method [37]. Extraction of hydrocarbons from the samples was done with a sonicator (Ultrasonic bath-Elmsonic S40H) in accordance with US SW – 846 Method 3550 [38]. Ten grams (10 g) each of the sample was extracted with 1:1 mixture of acetone and methylene chloride spiked with 1 ml of PAH internal standard and shaken thoroughly for proper mixing before placing in an ultrasonic bath. The 16 priority EPA PAHs determination was conducted at Central Laboratory, Nigerian Institute for Oceanography and Marine Research, Victoria Island, Lagos State, Nigeria using 7890A Agilent Gas chromatograph coupled with a HP-5 column (30 m × 0.32 mm × 0.25 µm). A splitless inlet mode, helium gas was used as the carrier gas, and nitrogen, the makeup gas. The ignition gases were hydrogen and compressed air. A 1 µm sample was injected into the Gas chromatograph under the following oven conditions; initial temperature: 60ºC held for 1 min, ramp rate 1: increased to 210ºC at 12ºC/min, ramp rate 2: increased to 320ºC at 8ºC/min, final temperature: 320ºC held for 5 min., total run time: 32.25 min. and detector temperature: 325ºC. Identification and quantification of individual PAHs was based on internal calibration standard containing known concentrations of the 16 EPA priority PAHs. Fig. 1 shows the 16 EPA priority PAHs with their chemical structures and formulas. The specificity of the 16 PAHs (EPA-16) sought for in the samples was confirmed by the presence of transition ions (quantifier and quantifier) as shown by their retention times which corresponded to those of their respective standards. The measured peak area ratio of precursor to quantifier ion were in close agreement with those of the standards. Results obtained were presented in mg/kg concentration per analyte.

3. RESULTS AND DISCUSSION

Table 2 shows the result of physicochemical properties of all the beer samples. Alcohol content ranged from 4.40 to 5.20 (% v/v) with Star lager beer (sample A) which is the control having the highest alcohol content. The results from the study carried out by Ifeanyi and Ihenatuoha [39] showed a disagreement with the present work; the former having lower alcoholic contents which might be as a result of low rate of fermentation in beers brewed by the former researcher.

The bitterness level in the samples and the control ranged between 0.54 and 1.46 IBU. Samples B and E have the same bitterness level while bitterness level in samples C and D is the same. The pH of the beers shows that sample A has the highest pH value of 4.36 while B has the lowest pH of 3.08. pH is an important factor in brewing quality beer. The pH levels during various stages of the brewing process affect extract potential, beer colour, hot-break formation, foam stability, hop oil extraction, hop bitterness and lauterability of the beer [40]. It is also an important consideration for beer quality during storage as a low pH inhibits bacterial growth. pH affects almost all the physical, chemical and biochemical reactions that occur within the brewing process. Brewers who understand the factors that affect pH and how to manage them during the brewing process will be able to consistently produce good beers. Although pH is clearly an important variable in the brewing process, it rarely requires a great deal of attention from the brewer [41]. It is evident from Table 2 that the specific gravity of all the beer samples are within the same range. These results are in agreement with that of Okafor [3] and other works [4,10,42,43]. From the result presented in Table 2, sample A has the least colour of 5.80 EBC while sample D has the highest colour of 8.00 EBC and the colour range compares favourably well. The colour of beer is largely determined by the melanoids and caramel present in the malt and adjuncts used but further caramelization occurs during wort boiling. Browning reaction occurs when malt is kilned and the amount of melanoidins depends upon the kilning temperature [44].

The results presented in Table 3 extracted from Figs. 2-6, GC-MS fingerprints (chromatograms of the beer samples) show that the retention times of naphthalene, acenaphthylene, acenaphthene, phenanthrene, anthracene, fluoranthene, benzo(a)anthracene, chrysene, benzo(b)fluoranthene, benzo(k)fluoranthene, benzo(a)pyrene, dibenzo(a,h)anthracene, indeno(1,2,3-cd)pyrene and benzo(g,h,i)perylene in all the samples are the same. A closer examination of Table 3 shows that the retention time of pyrene is the same in all the samples except in sample B which was 17.796 min. while it was 17.878 min. in samples A, C, D and E.

Table 2. Physicochemical properties of the beer samples

Sample	Physicochemical properties				
	Alcohol (% v/v)	Bitterness (IBU)	pH	Specific gravity	Colour
A	5.20	0.54	4.36	10.06	5.80
B	4.48	0.80	3.08	10.00	7.70
C	4.40	1.46	3.88	10.00	6.60
D	4.43	1.46	3.90	10.06	8.00
E	4.54	0.80	3.87	10.06	7.40

Table 3. Retention time of the beer samples

PAH	Sample retention time (min)				
	Sample A	Sample B	Sample C	Sample D	Sample E
Naphthalene	6.514	6.514	6.514	6.514	6.514
Acenaphthylene	9.242	9.242	9.242	9.242	9.242
Acenaphthene	9.565	9.565	9.565	9.565	9.565
Fiuorene	10.486	10.486	10.486	10.486	10.486
Phenanthrene	12.228	12.228	12.228	12.228	12.228
Anthracene	12.305	12.305	12.305	12.305	12.305
Fluoranthrene	14.450	14.450	14.450	14.450	14.450
Pyrene	14.878	14.796	14.878	14.878	14.878
Benzo[a]anthracene	17.539	17.539	17.539	17.539	17.539
Chrysene	17.638	17.638	17.638	17.638	17.638
Benzo[b]fluoranthrene	20.057	20.057	20.057	20.057	20.057
Benzo[k]fluoranthrene	20.115	20.115	20.115	20.115	20.115
Benzo[a]pyrene	20.779	20.779	20.779	20.779	20.779
Dibenzo[a, h]anthracene	23.183	23.183	23.183	23.183	23.183
Indeno[1, 2, 3-c, d]pyrene	23.247	23.247	23.247	23.247	23.247
Benzo[g, h, i]perylene	23.673	23.673	23.673	23.673	23.673

Results in Table 4 corroborated those in Table 3. Table 4 shows the absence of all the PAHs in all the samples except pyrene which was detected in only sample B (0.00402 mg//kg). The presence of pyrene in this sample is unexpected. The medicinal properties of *Garcinia kola* in African traditional medicine is well established [45-48]. So, it becomes a surprise and raises concern when pyrene, a carcinogen, was detected in a beer brewed with its extract. *G. kola* is a highly valued ingredient in African ethno-medicine because of its varied and numerous uses which are social and medicinal; thus making the plant an essential ingredient in folk medicine. Medicinal plants such as *G. kola* are found to be an important source of new chemical substances with potential therapeutic benefits [45]. *Garcinia kola* is regarded as a wonder plant because every part of the plant (bark, leaf, root, wood, seed) has been found to be of medicinal importance. The medicinal importance of bitter cola is based mainly on the phytochemical components of the plant. From its roots to its leaves, the plant is known to contain several phytochemicals noted for their medicinal importance [46]. *Garcinia kola* seed is believed to contain a wide spectrum of organic compounds such as flavonoids which confer on it some antimicrobial and antifungal actions against gram negative and gram positive micro-organisms. The biological activities of flavonoids include action against allergies, inflammation, free radicals and hepatoxins [47]. *Garcinia kola* seeds are also used in the treatment of diabetes, bronchitis and throat infections as well as treatment of liver disease and diarrhea [45,48]. Traditionally, the plant is used as a natural antimicrobial. Other medicinal properties of the plant include its usage in the treatment of skin infection in Liberia and Congo Democratic Republic. The powdered bark of the plant is applied to malignant tumors, cancers etc. The plant latex is taken internally for gonorrhea and externally to seal new wounds and prevent sepsis [49]. In Congo, a bark decoction is taken for female sterility and to ease child birth, the intake being daily till conception is certain and then at half quantity throughout the term. The bark is added to that of *Sarcocephalus latifolinus* which has a strong reputation as a strong anti-diuretic, in the treatment of urinary decongestion and chronic urethral discharge. In

Ivory Coast, a decoction of the bark is taken to induce the expulsion of a dead foetus, while the seed and the bark are taken for stomach pain [50]. In Sierra Leone, the roots and the bark are taken as a tonic for sexual dysfunction in men [51]. The bark is also added into palm wine to improve its potency. In Nigeria, a cold water extract of the roots and bark with salt are administered to cases of bronchial asthma or cough, or vomiting [46]. The medicinal properties of bitter cola can be classified under purgative, antiparasitic and antimicrobial.

However, the presence of pyrene in the beer sample may be explained from plantation point of view. It may be that the *G. kola* used in the current work was harvested from a refuse dumpsite. Refuse dumpsites had been reported as a candidate source of PAHs [52].

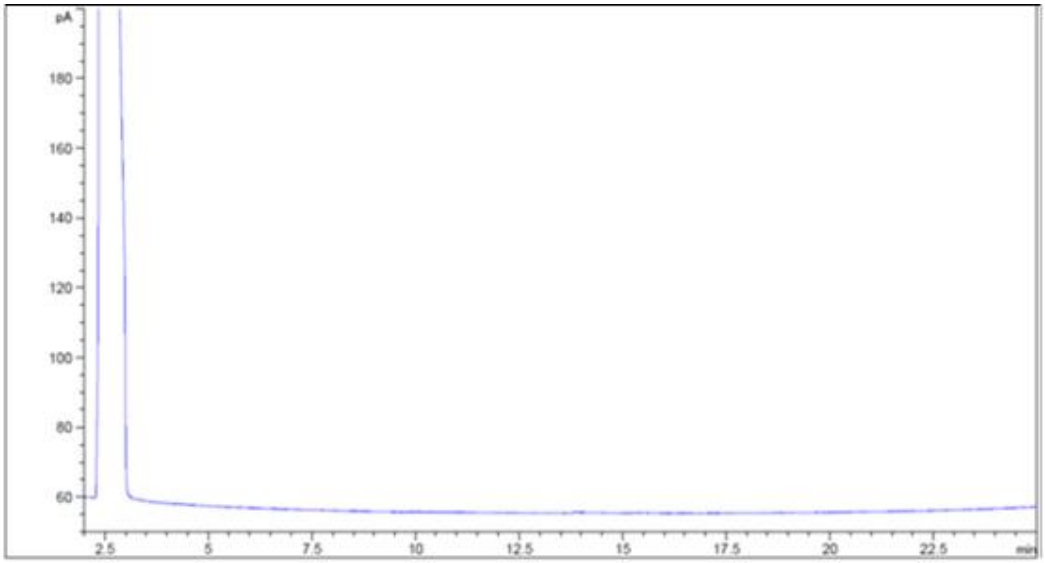

Fig. 2. Chromatogram of star lager beer (Sample A)

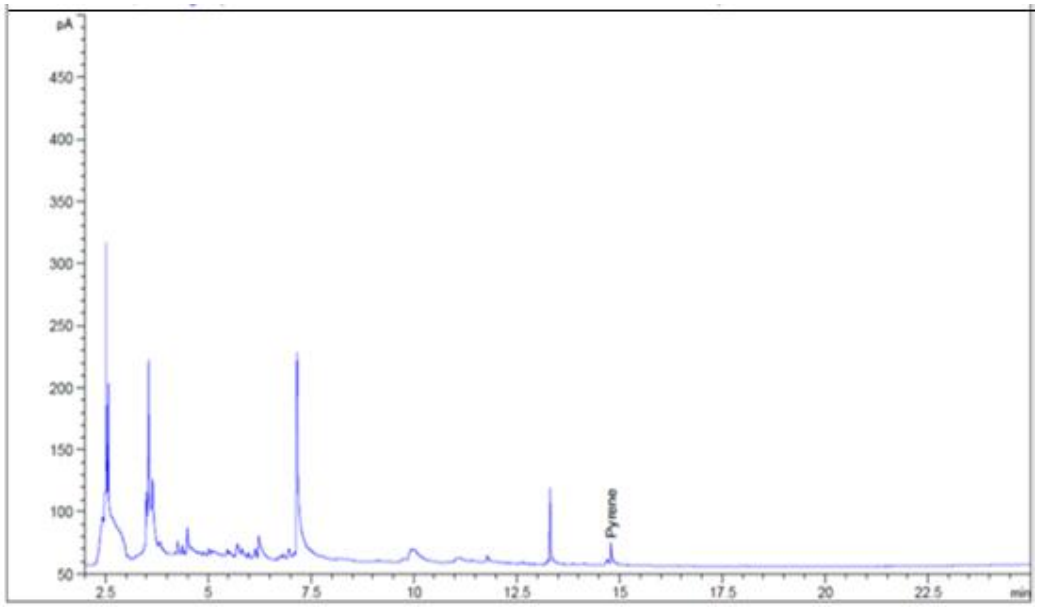

Fig. 3. Chromatogram of beer produced with *G. kola* extract (Sample B)

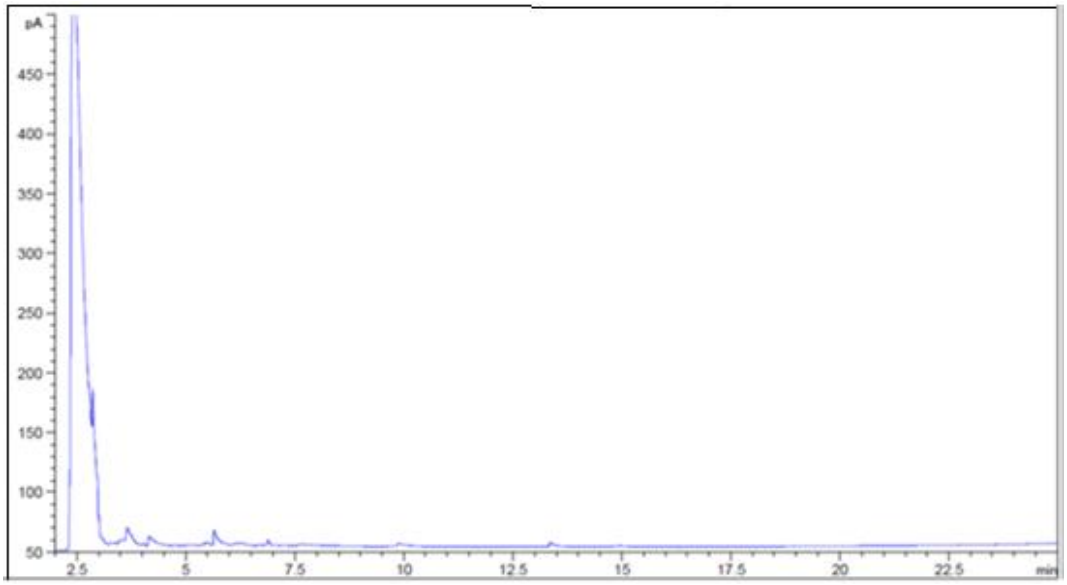

Fig. 4. Chromatogram of beer produced with *A. indica* extract (Sample C)

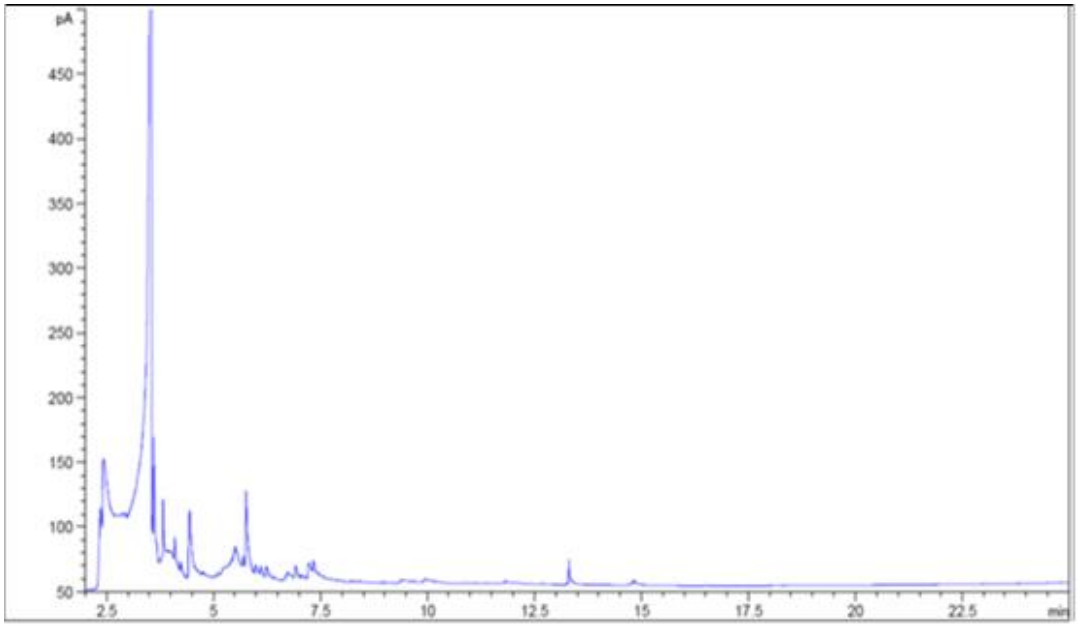

Fig. 5. Chromatogram of beer produced with *V. amygdalina* extract (Sample D)

Pyrene is a polycyclic aromatic hydrocarbon consisting of four fused benzene rings, resulting in a flat aromatic system. It is a colourless, crystal-like solid but sometimes looks yellowish. Benzo[a]pyrene, another PAH is synthesized from pyrene. Pyrene, being a polycyclic aromatic hydrocarbon compound has negative health effect and hence harmful showing that beers produced from the extract of *G. kola* is unsafe for consumption.

The presence of pyrene as recorded in this study should not be taken for granted since this compound irrespective of its concentration contributes greatly to carcinogenicity. Pyrene is implicated as carcinogen according to the US-EPA (California Environmental Protection Agency, 1994) [53]. Consequently, it is feared that the population of people consuming this product may be predisposed to high risk of cancer due to long term exposure and

consumption of the beer. The health effect of pyrene has been reviewed extensively [54-57]. These effects depend mainly on the extent of exposure, amount consumed, innate toxicity and exposure routes. Some studies have shown that pyrene can induce dioxin-like activity and weakened estrogenic responses [58-61]. The toxicity of pyrene has been extensively studied with well-established carcinogenic effects [62-64]. Its metabolites were said to be mutagenic and highly carcinogenic, and it is listed as a Group 1 carcinogen by the International Agency for Research on Cancer (IARC). The compound is one of the benzopyrenes formed by a benzene ring fused to pyrene, and is the result of incomplete combustion of organic matter at temperatures between 300ºC and 600ºC [65]. Pyrene toxicity results from its bioactivation to the ultimate toxic compound. Animal studies showed that mice that were fed with pyrene developed nephropathy, a kidney disease that decreases the weight of the kidney and increases that of the liver [66,67].

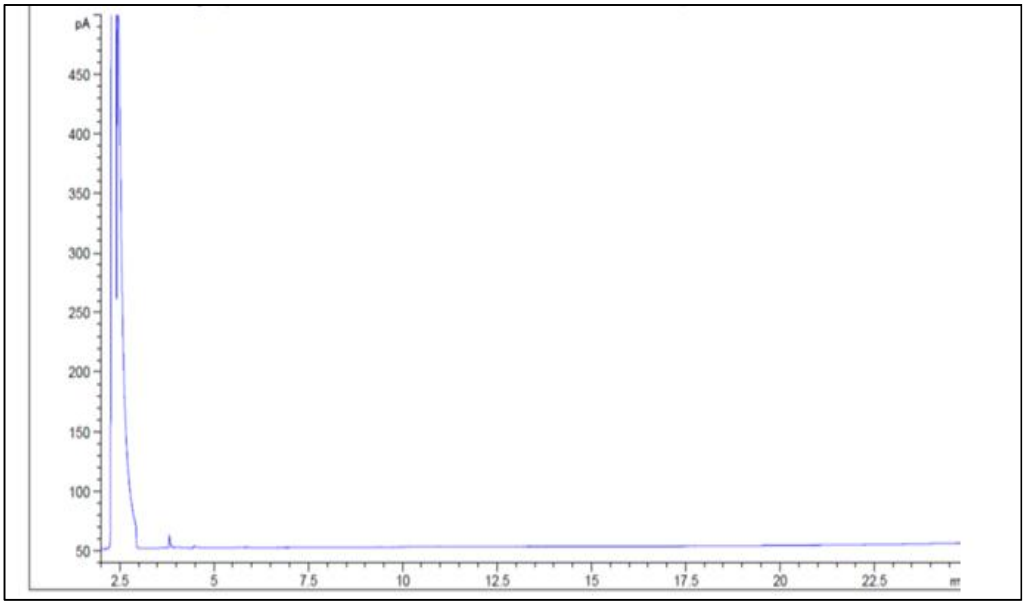

Fig. 6. Chromatogram of beer produced with *G. latifolium* extract (Sample E)

Table 4. Concentration of the 16 priority EPA PAHs in the samples

PAH	Concentration (mg//kg)				
	Sample A	Sample B	Sample C	Sample D	Sample E
Naphthalene	Nd	Nd	Nd	Nd	Nd
Acenaphthylene	Nd	Nd	Nd	Nd	Nd
Acenaphthene	Nd	Nd	Nd	Nd	Nd
Fiuorene	Nd	Nd	Nd	Nd	Nd
Phenanthrene	Nd	Nd	Nd	Nd	Nd
Anthracene	Nd	Nd	Nd	Nd	Nd
Fluoranthrene	Nd	Nd	Nd	Nd	Nd
Pyrene	Nd	0.00402	Nd	Nd	Nd
Benzo[a]anthracene	Nd	Nd	Nd	Nd	Nd
Chrysene	Nd	Nd	Nd	Nd	Nd
Benzo[b]fluoranthrene	Nd	Nd	Nd	Nd	Nd
Benzo[k]fluoranthrene	Nd	Nd	Nd	Nd	Nd
Benzo[a]pyrene	Nd	Nd	Nd	Nd	Nd
Dibenzo[a, h]anthracene	Nd	Nd	Nd	Nd	Nd
Indeno[1, 2, 3-c, d]pyrene	Nd	Nd	Nd	Nd	Nd
Benzo[g, h, i]perylene	Nd	Nd	Nd	Nd	Nd

Nd = Not detected

Properly speaking, pyrene is a pro-carcinogen, meaning that the mechanism of carcinogenesis of pyrene depends on enzymatic metabolism to the ultimate mutagen, pyrene diol epoxide. X-ray crystallographic and nuclear magnetic resonance (NMR) structure studies show that this binding distorts the DNA [68], including mutations by perturbing the double-helical DNA structure. This disrupts the normal process of copying DNA and induces mutations. This explains the occurrence of cancer after exposure [63]. Researches also indicated that pyrene diol epoxide specifically targets and destroys the protective gene thereby leading to cancer [69,70].

4. CONCLUSIONS AND RECOMMENDATION

It has been shown from this study that the extracts from the four Nigerian bitter vegetables could be used as potential substitutes for isomerized hop extract in the Nigerian Brewing industry. The study also revealed the absence of the 16 priority EPA PAHs in all the beer samples except in the beer produced using extract from *G. kola* where pyrene was detected which causes, on long term exposure cataracts, kidney and liver damages, jaundice, decreased immune function, breathing problems, asthma-like symptoms, lung function abnormalities, redness and skin inflammation, etc. We therefore recommend that the Nigerian brewery industry should not consider the substitution of isomerized hop extract with that from *G. kola* in beer brewing since the consumption of beers brewed with this extract can undermine public health of the consumers.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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