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Polyaromatic Hydrocarbons (PAHs) present in aqueous ethanol, dichloromethane, and n-hexane extracts of Pumpkin (*Cucurbita pepo*) seed

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Abstract
The study evaluated the quantity of Polyaromatic hydrocarbon (PAH) present in aqueous ethanol (Aq. EtOH), dichloromethane (DCM), and n-hexane (n-hex) extracts of pumpkin (*Cucurbita pepo*) seed. Aliquots of dried extracts, gotten from the ground *C. pepo*, were subjected to Gas Chromatography Flame-Ionization Detector (GC-FID) analysis for quantitative identification of polycyclic aromatic hydrocarbons (PAHs) present in the samples. The procedure ensured that the analytical process had quality assurance and quality control. From the result, only naphthalene was present in all three samples (Aq. EtOH and DCM; 0.05ppm, n-hex.; 0.03ppm). Out of the eight (8) PAHs identified with potential carcinogenic effects by Environmental Protection Agency (EPA), only one, Fluorine, was present in only the n-hex extract (0.04ppm). From the result, DCM had more PAHs (5 PAHs) compared to n-hexane (4 PAHs) and Aq. EtOH (2 PAHs). The quantity of fluorine obtained from the extract is lower than the recommended daily intake in line with the European Union (EU) limits for fluorine, which is 0.05 ppm d^{-1} or mg kg^{-1} d^{-1}. Owing to the fact that only one chemical compound of public health importance (on the EPA priority list) was found, which was far below the standard daily intake limit by calculation, it is safe to suggest that the *C. pepo* seed used in this study may pose minimal or no risk to exposed organisms. This study is important in investigating extracts intended for experimental purposes, for contaminants and other inherent compounds that may be noxious to the biological system.

Keywords: GC-FID analysis, EU limits, EPA

Introduction
Polyaromatic hydrocarbons (PAHs) are compounds that are chemically formed from hydrogen and carbon, consisting of one or more benzene rings in various geometric configurations [1-4]. They can be found in the air, water and soil [5, 6]. PAHs are formed from pyrogenic process arising when fossil fuels and organic matter are incompletely combusted [7], and petrogenic processes from petrofilerous shale seepage and erosion [8]. Biologically or naturally, vegetative matter degradation and synthesis by certain plants and bacteria have been associated with PAHs formation [5, 9]. Artificially, human activities resulting in environmental pollution through petroleum spillages, waste treatment, industrialisation are related to PAHs input [7, 8].
According to findings, PAHs bio-accumulate in the tissues of living organisms [2, 5, 10]. Interestingly, scientific evidence suggests that PAHs may not be directly carcinogenic. However, when in contact with other compounds, they could become synergists, and their DNA binding properties determine their carcinogenicity [2, 11]. Some of the carcinogenic PAH compounds described by 'United States Environmental Protection Agency' (EPA) included "benz(a) anthracene, benzo(b) fluoranthene, benzo(k) fluoranthene, benzo(a) pyrene, chrysene, dibenz(ah) anthracene, and indeno (1,2,3-cd) pyrene" [5, 6, 12].
Scientifically, studies suggest that exposure to PAHs has low acute toxicity to humans; however, chronic exposure to PAHs using animal model studies found increased a rise in the cases of cancers associated with the liver, stomach, skin, lungs and bladder and also malignant tumours at injection sites [5, 13, 14]. Studies also reported hematopoietic and immune systems compromise that could induce neurologic, reproductive, and developmental effects [13, 15]. There are reports of dose-dependent non-carcinogenic effects of chronic exposure to PAHs [16], which could involve the dermatologic, gastrointestinal, pulmonary, and renal systems [17].
Studies report that PAHs are lipophilic and may accumulate in different cereals, grains, fruits, food crops, and vegetation [18-20], which are capable of indirect harm from consumption [21, 22]. Their presence in these food products makes diet the primary source of human exposure to these pollutants [28, 24]. [28, 25] found PAHs in a variety of plants and vegetables, respectively, with reports that leafy vegetables could act as an indicator of human exposure to PAHs [25].

The Pumpkin species regarded as *Cucurbita pepo* is an herbaceous plant that belongs to the Cucurbitaceae family [26, 27] and is commonly known as 'marrow' [28]. Studies have documented the beneficial potentials of *C. pepo* in tradomedicine for the management of several conditions [29-32]. However, studies on environmental contaminants present in pumpkin (*C. pepo*) seed remains scarce. This study therefore, evaluated the levels of Polyaromatic hydrocarbons (PAHs) in n-hexane, dichloromethane and aqueous ethanol seed extracts of *C. pepo.*

Materials and Methods

Collection of Plant material: The study obtained *C. pepo* (pumpkin) fruits from the popular Choba market (Latitude: 4°53'26"N and Longitude: 6°54'12"E), which is close to the University of Port Harcourt (Uniport), Obio/Akpor LGA, Rivers State, South-South, Nigeria. The fruit samples were identified and authenticated at the institute's Herbarium with the no. UPH/PSB/2021/071.

Preparation and Extraction Process: The preparation of the seed for extraction was done according to the method described by Fatope *et al.*, [33]. This involved air drying of seeds (exposed sacks in an aerated room for 2 weeks) and de-shelling and grinding into powdered form (using Vitamix E310 Explorian Blender). For extraction, fifty grams (50g) of the powdered plant materials were soaked in three separate conical flasks, containing 500ml of n-hexane, dichloromethane, and aqueous ethanol respectively, and kept for 24hrs in a shaker. The mixture was filtered, and the filtrate evaporated at room temperature.

GC - FID Analysis: The aqueous ethanol, dichloromethane, and n-hexane extracts were analyzed using Gas Chromatography (GC, Hewlett-Packard HP-5890 series II instrument with flame ionization detection (GC-FID).

Extraction Analysis

Liquid-liquid extraction (LLE) was used to extract the PAHs. To do this, 4 ml of the extract sample were mixed with 20 ml of dichloromethane (DCM). The former (extractant) was poured into the vials, sealed, and centrifuged for 20 seconds at 300 rpm. Using a pipette and pipette filter, the organic layer was separated and placed in an amber glass bottle that had undergone heat treatment and cleaning. To prepare the samples for the analyte to be eluted, silica gel fractionation was performed. After being concentrated by nitrogen blow-down, the eluted samples were put into a GC vial for examination.

GC - FID Analysis

For GC-FID analysis, Gas chromatography (GC) with a Flame-Ionization Detector (FID) was used to measure the amounts of each particular PAH. The GC retention times for each PAH in the analyte sample were used to determine the peaks. An HP 5890 series II Gas Chromatography Flame-Ionization Detector was utilized to analyse the extracted analyte according to Bayowa and Agbozu [34], with an HP-1 (Methyl Silicone Gum) Capillary column (Agilent, 30 m x 0.32 mm x 0.00025 mm film thickness) operating with Helium as carrier gas at 2µL per ml. The injection volume was 2µL, using a split less inlet mode. The GC temperature program was set according to an established method with slight modification. The oven was configured to 60 ºC and held for 1 minute, ramped to 320 ºC at the rate of 9 ºC/min, and held for 5 min. The injector and the FID were held at 275 ºC and 325 ºC, respectively. The solvent's (in the solution used in extraction and storage) peak was first noted in the GC-FID screen. The total PAHs were then calculated after detecting the spikes of the lightest to the heaviest chemicals in the PAHs and their corresponding concentrations. The GC-FID had a 40-minute retention period between injection and detection.

Quality Assurance and Quality Control

Since PAHs have been identified to be delicate to Ultraviolet light, the extracts were immediately placed in an amber bottle after preparation to avoid mutilation. The GC - FID was turned on for an hour prior to analysis in order to warm up the system and reduce the availability of undesirable chemicals inside the device. The GC's column was additionally cleaned and cleared by injecting blank organic solvent (DCM) into it four times: twice before the real analyte sample and twice after the analysis. These were carried out to guard against potential contamination of the examined samples with undesirable chemicals from earlier analyses. In order to prepare the instrument, sample Ionization Detectors (IDs) were inserted, and the analysis type (PAH) was chosen. Prior to and following every sampling event, each sampler was calibrated. For each PAH under investigation, calibration curves were created by plotting the GC output signal responses (represented by the peak area in the screen chromatogram, from lightest compound to heaviest) against the concentration of the analyte injected into the GC through an injection pot over a specific retention time (40 min in total).To guarantee the analytes' identification, validation ions and retention durations were also applied. The detection was 0.00 mg per gram.

For recovery analysis, to ensure an adequate quantification, the metric spike method was used. The compound was spiked into separately prepared aliquots of the MM5 train condensate samples before analysis. The spiked aliquots are then analysed, and the spike recovery is calculated. The recovery of these spikes (at ≥80%) provided an independent indicator of method accuracy and quantification relative to the sample matrix by assuming that the spiking compound has chemical characteristics that are identical to the PAH target compounds.

Results and Discussion

Results from the GC-FID analysis showed few PAH compounds in the Aq.EtOH, DCM, and n-hexane extracts (Table 1). The evidence of the presence of these compounds can be seen from the peaks associated with the retention time in the GC-FID chromatogram (Aq. EtOH, DCM and n-hex in Figs 1-3).

PAH compounds and their quantities found in Aq.EtOH, extracts were acenaphthylene 0.02 ppm (RT=5.285 mins) and naphthalene 0.05 ppm (3.875 mins), DCM were acenaphthene 0.02ppm (RT=5.336mins), acenaphthylene 0.01ppm (RT=5.316mins), anthracene 0.01ppm (RT=8.352mins), phenanthrene 0.010ppm (RT=8.352mins), naphthalene 0.050 (RT=4.000mins), and n-hex were anthracene 0.15ppm (RT=8.327 mins), fluorene 0.04ppm (RT=6.429), phenanthrene 0.04ppm (RT=8.426 mins), naphthalene 0.03ppm (RT=3.988 mins).

The result showed that only naphthalene was detected in all three samples (Aq. EtOH and DCM; 0.05ppm, n-hex.; 0.03ppm).

Out of the eight (8) PAHs identified with potential carcinogenic effects by EPA, only one (1), Fluorine was present in only one sample, which is; n-hex extract (0.04ppm).

Out of the 6 PAHs identified by the GC-FID, (Acenaphthene, acenaphthylene, anthracene, Fluorine, Phenanthrene, and naphthalene), DCM had more PAHs compounds (5) (acenaphthene 0.02ppm (RT=5.336mins), acenaphthylene 0.01ppm (RT=5.316mins), anthracene 0.01ppm (RT=8.352mins), phenanthrene 0.010ppm (RT=8.352mins), naphthalene 0.050 (RT=4.000mins), compared to n-hexane (4)(anthracene 0.15ppm (RT=8.327 mins), fluorine 0.04ppm (RT=6.429), phenanthrene 0.04ppm (RT=8.426 mins), naphthalene 0.03ppm (RT=3.988 mins) and Aq.EtOH (2) (acenaphthylene 0.02 ppm (RT=5.285 mins) and naphthalene 0.05 ppm (3.875 mins))

The quantity of fluorine obtained from the extract is lower than the recommended daily intake in agreement with the EU limits for fluorine, which is (0.05 ppm d−1 or mg kg −1 d−1) [36].

Table 1: Polyaromatic hydrocarbon levels in extract samples of C. pepo seed

Detected Compound	Aqueous Ethanol		Dichloromethane		n-hexane	
	Retention time	Quantity (ppm)	Retention time	Quantity (ppm)	Retention time	Quantity (ppm)
Acenaphthene	-	-	5.336	0.020	-	-
Acenaphthylene	5.285	0.020	5.316	0.010	-	-
Anthracene	-	-	8.352	0.010	8.327	0.150
Fluorene	-	-	-	-	6.429	0.040
Phenanthrene	-	-	8.352	0.010	8.426	0.040
Naphthalene	3.875	0.050	4.000	0.050	3.988	0.030

Note: '-'means not detected

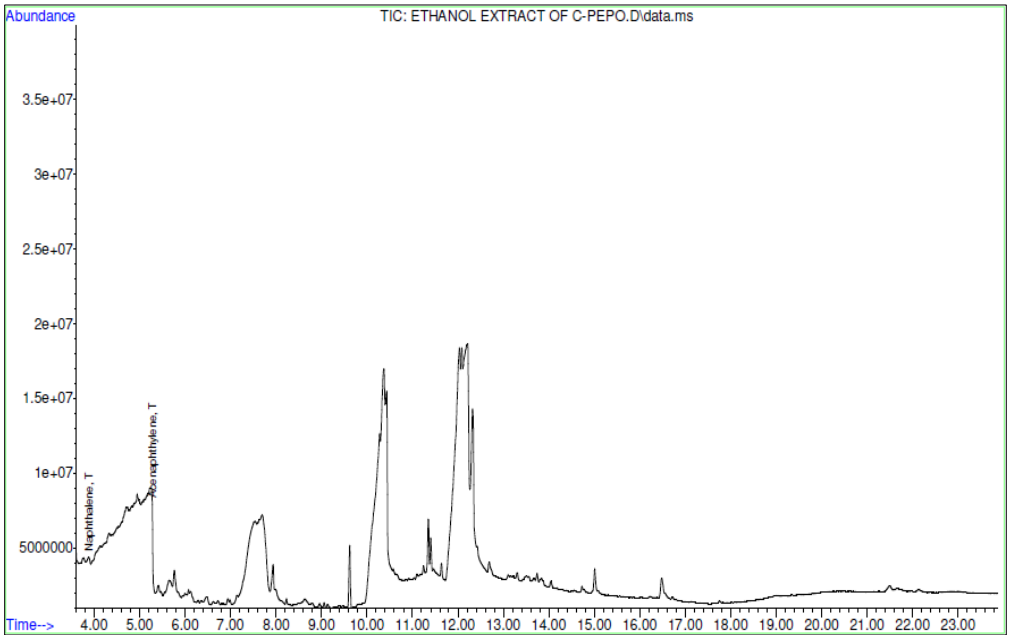

Fig 1: GC-FID chromatogram of aqueous ethanol extract of C. pepo seeds

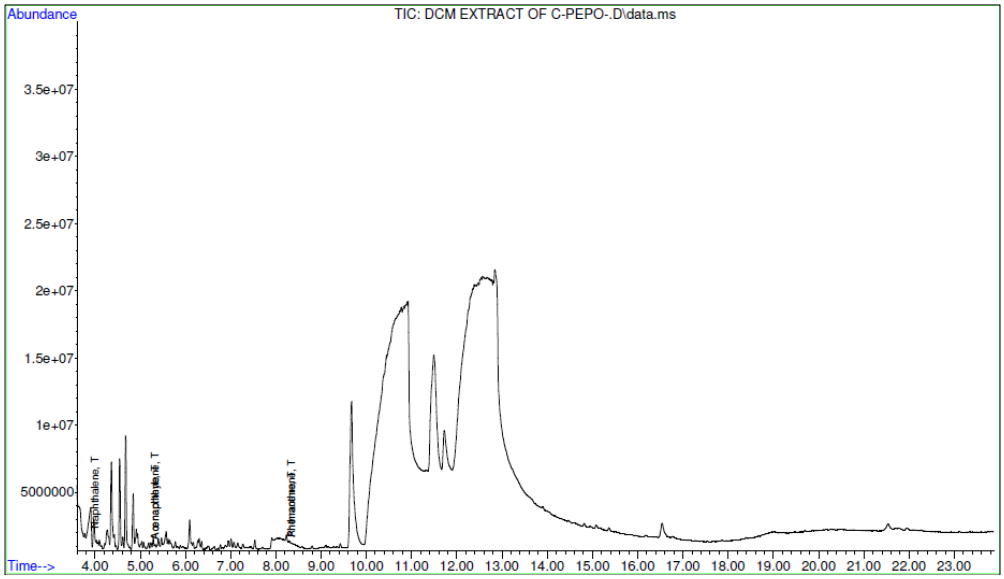

Fig 2: GC-FID chromatogram of dichloromethane extract of C. pepo seeds

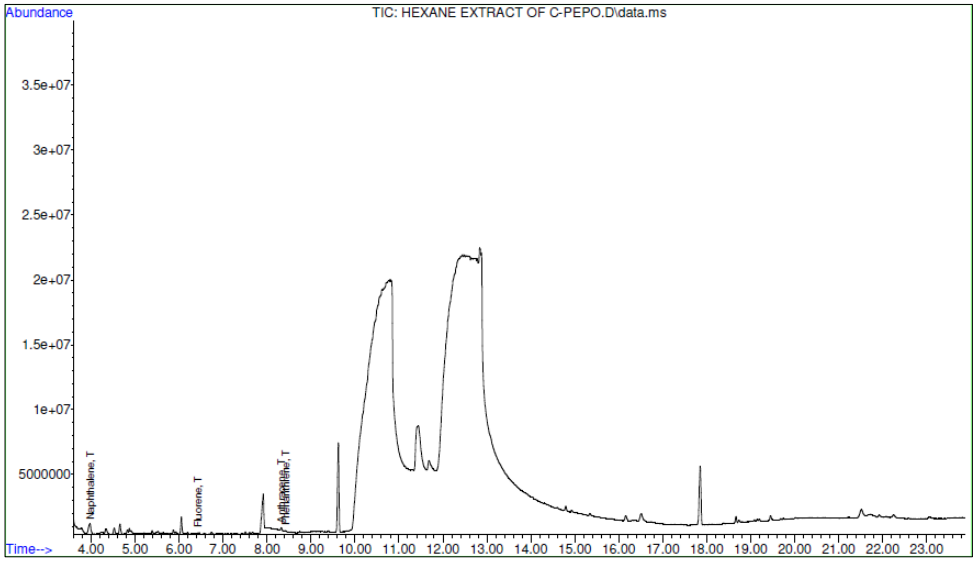

Fig 3: GC-FID chromatogram of n-hexane extract of C. pepo seeds

Conclusion

Fluorine (0.04ppm) was the only Polyaromatic hydrocarbon of significant interest (on the EPA priority list) identified in only the n-hexane extract. The detected level was far below the standard daily intake limit by calculation, thus may not pose any significant health risk to exposed organisms. This finding suggests that the *C. pepo* seed used in this study poses a minimal risk to organisms. This finding shows the importance of investigating for contaminants, in extracts and compounds intended for experimental purposes.

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