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Polycyclic Aromatic Hydrocarbons (PAHs) in *Telfairia occidentalis* from two Markets in Ohafia Area, Abia State, Nigeria

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ABSTRACT
The presence and contents of polycyclic aromatic hydrocarbons (PAHs) were investigated in the leaves of *Telfairia occidentalis* obtained from Abia-Ohafia and Okagwe-Ohafia markets within Ohafia Local Government Area, Abia State, Nigeria. The samples from the two markets were divided into three portions. The first portion was not washed (UW), the second portion was washed with tap water (WTW) and the last portion was washed with an aqueous solution of sodium chloride (WSS) before drying, milling and extraction. Quantification of the PAHs was carried out using a Gas Chromatography/Mass Spectrometry (GC/MS) technique. Eighteen PAHs were analyzed and *T. occidentalis* samples from the Abia-Ohafia market gave a total PAHs content of 15.42, 6.43 and 3.47 mg kg^{-1} for UW, WTW and WSS, respectively, while the samples from the Okagwe-Ohafia market gave 19.06, 9.49 and 2.35 mg kg^{-1} for UW, WTW and WSS, respectively. The results obtained indicate that the content of PAHs in the samples decreased significantly when washed with tap water and further decreased when washed with the aqueous solution of sodium chloride. Also, the risk assessment of ΣPAH4 (the sum of benzo[*a*]pyrene, benzo[*a*]anthracene, benzo[*b*]fluoranthene and chrysene) in the samples indicated that there was no risk associated with the consumption of the vegetable samples.

Keywords: GC/MS, PAHs, *T. occidentalis*, Vegetables

INTRODUCTION
The consumption of vegetables by humans is as old as the humans themselves. Vegetables are eaten in various forms and are used in the preparation of different delicacies (Igwe *et al.*, 2020). One of the vegetables widely used in eastern Nigeria, and in so many other parts of the country, is *Telfairia occidentalis.* This is popularly known as 'Ugu' in Igbo language and commonly referred to as fluted pumpkin or fluted gourd (Igwe *et al.*, 2020).

T. occidentalis is a member of the family *Cucurbitaceae* and is indigenous to southern Nigeria. It is grown as a leafy vegetable for its edible seeds and leaves (Akoroda, 1990). The leaves of the plant are majorly used in the preparation of soups, yam porridge, sauce and stew, and also in herbal medicines. The seeds from the gourd of the plant are rich in protein and fat. The plant provides a cheap source of protein, vitamins, minerals and fat to the people of south eastern Nigeria (Igwe *et al.*, 2020; Akoroda, 1990).
Polycyclic aromatic hydrocarbons (PAHs) occur naturally in fossil fuels. They result from forest fires, burning coal, wood, oil, garbage, tobacco and cooking foodstuffs at high temperatures as well as from motor vehicle exhaust pipes (Iwegbue *et al.*, 2014). Humans are exposed to PAHs through breathing of air that had been contaminated with PAHs from these sources. In addition, consuming grilled or charred meats and foods and also eating foods upon which PAHs have been deposited may expose individuals to PAHs (Kipopoulou *et al.*,1999). These PAHs have been reported to be carcinogenic (Igwe *et al*., 2021).

This research probes the quantification of PAHs deposited on the leaves of *T. occidentalis* sold in two markets within Ohafia Local Government Area of Abia State, with respect to methods of washing before usage. It is commonplace to wash the vegetables before being used for the preparation of delicacies. While many people wash with tap water, others wash with a solution of common salt (sodium chloride) to ensure adequate removal of dirt and contaminants. The results of the aforementioned analyses are herein reported.

MATERIALS AND METHODS
Sample Collection
The leaves of *T. occidentalis* (Ugu) were bought from Abia-Ohafia and Okagwe-Ohafia markets in the month of December, 2021. The samples were identified and authenticated at the Taxonomy Section, Forestry Department, Michael

Okpara University of Agriculture, Umudike, Nigeria.

Sample Preparation

T. occidentalis from each of the two markets was divided into three portions of about 200 g per portion. The first portion was not washed prior to drying. The second portion was washed with ordinary tap water to remove dirt and impurities. The third portion was washed with 0.17 mol dm^{-3} of sodium chloride solution and finally rinsed with tap water. The various portions were sliced thinly with a clean kitchen knife and were oven-dried at 65℃ for 72 h. The dried samples were pulverized to fine and smooth particles with a wooden mortar and pestle and were stored in air-tight containers prior to analysis.

Extraction of Samples for PAHs Determination

In triplicates, l00 mg of each sample was weighed into clean extraction containers. 10 ml of the extraction solvent (dichloromethane) was added into each container and mixed thoroughly before being allowed to settle. The mixture was carefully filtered into clean solvent-rinsed extraction bottles, using filter paper fitted to Buchner funnels. The extracts were reduced to 2 ml volume and were cleaned up using column chromatography as described by Nwaichi *et al.* (2017). The cleaned-up eluents were then used for GC/MS analysis.

GC/MS Analysis

An Agilent 6890N gas chromatography equipped with an auto sampler connected to an Agilent mass spectrophotometric detector was used. 1 µl of sample extract was injected in the pulsed spitless mode onto a 30 m x 0.25 mm id DB 5MS coated fused silica column with a film thickness of 0.15 µm. Helium gas was used as a carrier gas and the column head pressure maintained at 20 psi to give a constant flow rate of 1 ml/mins. Other operating conditions were preset. The column temperature initially held at 55℃ for 0.4 mins, was increased to 200℃ at a rate of 25℃/mins, then to 280℃ at a rate of 8℃/mins and to final temperature of 300℃ at a rate of 25℃/mins, held for 2 mins. The identification time was based on retention time since each of the PAHs has its separate retention time in the column. The PAHs components with lower retention time were eluted before the ones with higher retention time. The column was calibrated with PAH standards supplied by the instrument manufacturer. A calibration curve was obtained by analyzing each of the standard PAHs solutions prepared on the GC/MS. The target PAH compound/internal standard peak heights were plotted against the PAH concentration to obtain a linear graph Y= mx + b, with an intercept (b) on the y-axis. All the samples were analyzed for eighteen PAH congeners: acenaphthene, acenaphthylene, anthracene, benzo[a]anthracene, benzo[b]fluoranthene, benzo[k]fluoranthene, benzo[g,h,i]perylene, benzo[a]pyrene, chrysene, dibeno[a,h]anthracene, fluoranthene, fluorene, indeno[1,2,3-cd]pyrene, 2-methylnaphthalene, naphthalene, phenanthrene, pyrene and 1,2,3-trimethylbenzene.

Risk Assessment of the PAHs

Risk assessment is the process that evaluates the potential health effects on humans from contaminant doses received through one or more exposure pathways (USEPA, 2007). Dietary and carcinogenic methods were used in this study to assess the potential risks posed by PAHs to human consumers of the vegetables.

Dietary Exposure Assessment

Daily dietary exposure level by the populace to PAH4 was calculated with the formula given in equation 1 according to Wu *et al*. (2016).

$$E = C \times IR \quad \text{..... (1)}$$

In equation 1, E= daily dietary ΣPAH4 exposure level (ng/day); C= PAH4 (the sum of benzo[a]pyrene, benz[a]anthracene, benzo[b]fluoranthene and chrysene) concentration in vegetables (ng/g); IR = daily ingestion rate vegetables which was estimated to be 65 g/day (Edogbo *et al*., 2020).

Cancer Risk Assessment

The Margin of Exposure (MOE) approach was used for the characterization of the risk posed to humans by exposure to PAHs which can cause cancer or damage to genetic materials. It is a ratio which for a specific population, assesses the dose at which a small but measurable negative effect is initially noticed and the level of exposure to the target substance (FSA, 2012).

$$MOE = \frac{BMDL_{10} \times BW}{E} \quad \text{..... (2)}$$

In equation 2, $BMDL_{10}$ (benchmark dose lower confidence limit 10 %) is an estimate of the lowest dose, which is 95 % certain to cause no more than a 10 % cancer incidence in animals. It was calculated as 0.34 mg/kg b.w./day for ΣPAH4 (EFSA, 2008). BW is the average body weight which is set as 60.7 kg for adults (Walpole *et al*., 2012). MOE ≥ 10,000 is assumed to be of low risk concern from a public health standpoint (Rozentale *et al*., 2015).

RESULTS AND DISCUSSION

The dry weight PAHs concentrations in the leaf sample of *T. occidentalis* obtained from the two market locations are shown in Tables 1 and 2.

Table 1: PAHs concentrations (mg kg^{-1}) in the *T. occidentalis* leaf samples obtained from Abia-Ohafia market

Contaminants	Unwashed	Washed with tap water	Washed with salt water
1,2,3-Trimethylbenzene	1.02±0.01	0.41±0.02	0.29±0.02
Naphthalene	1.19±0.01	0.46±0.04	0.24±0.01
2-Methylnaphthalene	0.98±0.05	0.43±0.02	0.20±0.03
Acenaphthylene	1.74±0.02	0.39±0.02	0.21±0.01
Acenaphthene	1.55±0.04	0.91±0.05	0.19±0.03
Fluorene	0.44±0.01	0.49±0.06	0.20±0.01
Anthracene	0.53±0.02	0.55±0.03	0.18±0.08
Phenanthrene	0.94±0.03	0.52±0.06	0.30±0.02
Fluoranthene	0.68±0.05	0.54±0.06	0.28±0.04
Pyrene	0.77±0.03	0.33±0.01	0.45±0.03
Benzo[a]anthracene	0.47±0.08	0.44±0.01	0.29±0.02
Chrysene	0.59±0.03	0.49±0.05	0.32±0.04
Benzo[b]fluoranthene	0.85±0.06	0.47±0.02	0.32±0.01
Benz0[k]fluoranthene	0.70±0.08	ND	ND
Benzo[a]pyrene	0.73±0.04	ND	ND
Diben[a,h]anthracene	0.57±0.01	ND	ND
Indeno[1,2,3-cd]pyrene	0.80±0.05	ND	ND
Benzo[g,h,i]perylene	0.87±0.02	ND	ND
∑PAH4	2.64	1.40	0.93
∑PAH18	15.42	6.43	3.47

Values are means± standard deviation of triplicate determinations. ND means not detected

Table 2: PAHs concentrations (mg kg^{-1}) in the *T. occidentalis* leaf samples obtained from Okagwe-Ohafia market

Contaminants	Unwashed	Washed with tap water	Washed with salt water
1,2,3-Trimethylbenzene	1.22±0.02	0.32±0.03	0.17±0.02
Naphthalene	3.18±0.01	0.77±0.01	0.06±0.001
2-Methylnaphthalene	2.21±0.01	0.92±0.01	0.15±0.01
Acenaphthylene	0.94±0.02	0.45±0.05	0.08±0.01
Acenaphthene	1.19±0.03	1.04±0.03	0.10±0.02
Fluorene	0.99±0.08	0.48±0.02	0.20±0.01
Anthracene	0.19±0.01	0.38±0.01	0.14±0.05
Phenanthrene	0.90±0.05	0.33±0.04	0.15±0.04
Fluoranthene	1.18±0.03	0.13±0.05	0.12±0.02
Pyrene	1.19±0.01	0.44±0.02	0.18±0.01
Benzo[a]anthracene	1.24±0.06	0.18±0.01	0.09±0.02
Chrysene	1.00±0.02	0.66±0.04	0.11±0.01
Benzo[b]fluoranthene	0.84±0.04	1.08±0.01	0.13±0.01
Benzo[k]fluoranthene	0.61±0.05	1.37±0.08	0.02±0.04
Benzo[a]pyrene	0.55±0.04	0.09±0.02	0.22±0.02
Diben[a,h]anthracene	0.82±0.02	0.44±0.03	0.15±0.03
Indeno[1,2,3-cd]pyrene	0.64±0.01	0.23±0.01	0.12±0.01
Benzo[g,h,i]perylene	0.17±0.03	0.18±0.07	0.16±0.03
∑PAH4	3.63	2.01	0.55
∑PAH18	19.06	9.49	2.35

Values are means± standard deviation of triplicate determinations

The PAHs concentrations (mg kg^{-1}) in *T. occidentalis* leaf samples from Abia-Ohafia market are shown in Table 1. The corresponding values for samples obtained from the Okagwe-Ohafia market are shown in Table 2. It is observed from the results that the total PAHs content of the analyzed vegetable (∑PAH18) from the two markets were highest in unwashed portions (15.42; 19.06 mg kg^{-1}), followed by the portions that were washed with tap water (6.43; 9.49 mg kg^{-1}). The third portions that were washed with aqueous solution of sodium chloride gave the least PAHs content (3.47; 2.35 mg kg^{-1}). Also, according to the ∑PAH4 which is the sum of benzo[*a*]pyrene, benzo[*a*]anthracene, benzo[*b*]fluoranthene and chrysene concentrations in the analyzed vegetable samples, the trends are (2.64; 3.63 mg kg^{-1}), (1.40; 2.01 mg kg^{-1}) and (0.93; 0.55 mg kg^{-1}) for the three portions in the

aforementioned order, respectively. The values reported by Igwe *et al.* (2020), for PAHs contents of *T. occidentalis* from some markets in Umuahia were higher compared to the values reported herein. From another study of PAHs contents in vegetables grown near markets within Aba metropolis, reported by Igwe *et al.* (2021), much higher values of PAHs were reported in vegetables grown around Ahiaohuru and Good-morning markets. These differences might be attributed to variable human activities and vehicular emissions within and around the harvest locations and handling methods prior and during transportation to the markets.

Benzo[a]pyrene and $\sum PAH4$ are used by different authorities as indicators or markers for the occurrence of PAHs in food (Igwe *et al*., 2021). However, the European Food Safety Agency has determined that $\sum PAH4$ is a better indicator than benzo[a]pyrene (FSAI, 2015). It is noteworthy that there are no regulatory limits for benzo[a]pyrene and total PAHs in vegetables (FSA, 2012).

Table 3: Risk assessment of ΣPAH4 in the vegetable sample

Risk Parameters	Abia-Ohafia Market			Okagwe-Ohafia Market		
	UW	WTW	WSS	UN	WTW	WSS
E (ng/day)	171.60	91.00	58.50	235.95	130.65	35.75
MOE	120268	226791	352786	87467	157964	57728

E = Dietary Exposure; MOE = Margin of Exposure, UW = Unwashed sample, WTW = Washed with tap water; WSS = Washed with salt solution

Table 3 shows the risk assessment for ΣPAH4 found in the vegetable sample. The daily dietary ΣPAH4 exposure level (ng/day) ranged from 35.75 to 235.95. Igwe *et al*. (2021) reported values of daily dietary exposure levels that ranged from 0.00 to 259.35 ng/day for *Talinum triangulare* and *Piper guineense* obtained from farms near markets within Aba metropolis. The use of the $BMDL_{10}$ value of 0.34 mg/kg bw/day set by EFSA (2008), a daily vegetable consumption of 65 g per person and an adult body weight of 60.7 kg resulted in MOE values higher than 10,000 in all the samples containing PAH4. Since MOE $\geq$ 10,000 is assumed to be of low risk concern from a public health standpoint (Rozentale *et al*., 2015), this implies that there are no health risks associated with the consumption of *T. occidentalis* obtained from Abia-Ohafia and Okagwe-Ohafia markets even under unwashed conditions.

CONCLUSION

This work probed the PAHs contents of *T. occidentalis* obtained from two markets in Ohafia Local Government Area, Abia State, Nigeria. Two culinary washing methods were adopted and the work revealed that washing of the vegetable with aqueous solution of common salt reduced the PAHs content more than ordinary washing with tap water. Considering the carcinogenic effect of PAHs, it is therefore, advisable to wash vegetables with common salt solution for optimal reduction of the PAHs content before consumption. This practice could be extended to fruits and other washable food items since they may have PAHs deposited on their surfaces. Although the risk assessment of the samples analyzed showed no potential risk associated with the consumption of the vegetable, a build-up could be avoided through proper washing with a solution of common salt.

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