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POLYCYCLIC AROMATIC HYDROCARBONS (PAHS) IN THE LEAVES OF *PIPER GUINEENSE* FROM TWO MARKETS WITHIN UMUAHIA METROPOLIS

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ABSTRACT

Polycyclic aromatic hydrocarbons (PAHs) were probed in the leaves of *Piper guineense* obtained from Orieugba and Isigate markets within Umuahia metropolis in Umuahia North Local Government Area of Abia State. Prior to drying, milling and extraction procedures, *P. guineense* obtained from each of these markets was divided into three portions. The first portion was not washed (UW), the second portion was washed with ordinary tap water (WTW) while the third portion was washed with about 0.17 mol dm^{-3} of NaCl solution (WSS). Quantitation of the PAHs was done using Gas Chromatography/Mass Spectrometry (GC/MS) technique. Eighteen PAHs were analyzed and *P. guineense* samples from Orieugba market gave a total PAHs content of 30.71, 22.18 and 8.31 mg kg^{-1} for UW, WTW and WSS, respectively. The samples from Isigate market gave 18.27, 15.59 and 1.20 mg kg^{-1} for UW, WTW and WSS, respectively. This investigation showed that the level of PAHs in the leaf samples of *P. guineense* from the two markets decreased when washed with ordinary tap water but decreased more significantly when washed with the salt solution. In addition, the risk assessment of ΣPAH4 (the sum of benzo[*a*]pyrene, benz[*a*]anthracene, benzo[*b*]fluoranthene and chrysene) in the vegetable sample indicated that there was no risk associated with the consumption of the vegetable samples. However, it is pertinent to wash vegetables with a salt solution before proper rinsing as this would help to reduce the quantity of PAHs in them.

Keywords: PAHs; *P. guineense*; GC/MS; Vegetables; Leaf sample

INTRODUCTION

Vegetables are good sources of vital nutrients for man and animals. Most of them, though underexploited, are used in the preparation of different types of delicacies. They also possess medicinal values, hence are used as both food and medicine [1]. One of such plants is *Piper guineense*. *P. guineense* is a West African tropical plant that belongs to the family *Piperaceae* and grows in closed forests, forest edges and generally wet places in the forest [2]. It is commonly known as African black pepper and popularly called Uziza by the Igbos of southeast Nigeria and Iyre by the Yorubas of southwest [3]. The leaves and seeds are consumed widely as spice and used in the preparation of different dishes [4]. The plant parts such as roots, seeds, stem bark and leaves are used in traditional medicine [5].

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Polycyclic aromatic hydrocarbons (PAHs) are ubiquitous organic compounds that are chemically related, environmentally persistent with various structures and varied toxicity. They have toxic effects on organisms through various actions. They are mostly colorless, white, or pale yellow solids [6]. PAHs have been reported to be carcinogenic [3]. These substances emanate from motor vehicle exhaust pipes, forest fires and burning of wood, coal, oil, tobacco, garbage and cooking of foodstuffs at elevated temperatures [7]. Individuals may be exposed to PAHs by eating roasted foods and meats and by inhaling air contaminated with PAHs. Also, foodstuffs upon which PAHs have been deposited may unknowingly be consumed [8].

In the culinary art, there are variable practices which are dependent on culture, type of delicacy being prepared and choice. For instance, while some people wash vegetables with ordinary tap water before using them for cooking, others go as far as washing with a solution of common salt followed by rinsing. This present research, using *P. guineense* leaf samples from two markets, has adopted the two washing techniques and quantitated the level of PAHs in them in order to make available valid recommendations based on scientific evidence. Hence, we report herein the polycyclic aromatic hydrocarbons in the leaves of *Piper guineense* from two markets within Umuahia metropolis.

MATERIALS AND METHODS

Sample Collection

The leaves of *P. guineense* (Uziza) were bought from Orieugba and Isigate markets in the month of December, 2021. The samples were identified and authenticated at the Taxonomy Section of Forestry Department of Michael Okpara University of Agriculture, Umudike.

Sample Preparation

P. guineense 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 about 0.17 mol dm^{-3} of NaCl 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.

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Extraction of Samples for PAHs Determination

In triplicates, l0 g of each sample was weighed into clean extraction containers. 100 ml of extraction solvent (dichloromethane) was added into each and mixed thoroughly and allowed to settle. The mixture was carefully filtered into clean solvent-rinsed extraction bottles, using filter paper fitted to Buchner funnels. The extractions were concentrated to 2 ml and were cleaned up using column chromatography as described by Nwaichi *et al.* [9]. 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 microlitre 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 of 1 ml/min. Other operating conditions were preset. The column temperature initially held at 55℃ for 0.4 min, 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 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 PAHs

Risk assessment is the process that evaluates the potential health effects on humans from contaminant doses received through one or more exposure pathways [10]. Dietary and carcinogenic methods

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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 [11]

$$E = C \times IR$$

(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 [12].

Cancer Risk Assessment

The Margin of Exposure (MOE) approach was used for the characterization of the risk posed to human by exposure to PAHs which can cause cancer or damage genetic material. 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 [13].

$$MOE = \frac{BMDL_{10} \times BW}{E}$$

$BMDL_{10}$ (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 [14]. BW is the average body weight which is set as 60.7 kg for adults [15]. MOE ≥ 10,000 is assumed to be of low risk concern from a public health standpoint [16].

RESULTS AND DISCUSSION

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

Tables 1 and 2 show the PAHs concentrations (mg kg^{-1}) in *P. guineense* leaf samples from Orieugba and Isigate markets, respectively. From the available results, it is observed that the total PAHs (∑ PAH18) content of the leaf sample from the two markets were highest in unwashed portions (30.71; 18.27 mg kg^{-1}) followed by the portions washed with tap water (22.18; 15.59 mg kg^{-1}) while the third portions washed with aqueous solution of NaCl of about 0.17 mol dm^{-3} gave the least PAHs content (8.31; 1.20 mg kg^{-1}). In all the portions, leaf samples from Isigate market contained more PAHs than that from Orieugba market. This may be attributed to the different levels of exposure of these samples at their harvest locations as well as treatment received during storage and transportation to the markets.

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Table 1: PAHs concentrations (mg kg^{-1}) in *P. guineense* leaf samples from Orieugba market

Contaminants	UW	WTW	WSS
1,2,3-Trimethylbenzene	5.07±0.02	4.11±0.02	1.30±0.01
Naphthalene	4.65±0.03	3.01±0.03	1.31±0.02
2-Methylnaphthalene	1.66±0.04	ND	0.86±0.01
Acenaphthylene	1.59±0.01	ND	0.71±0.02
Acenaphthene	1.38±0.03	2.50±0.02	0.09±0.02
Fluorene	2.18 ±0.01	1.78±0.01	0.19±0.01
Anthracene	2.25±0.03	0.91±0.03	0.45±0.03
Phenanthrene	2.94±0.01	0.84±0.01	0.61±0.01
Fluoranthene	1.98±0.03	1.38±0.07	0.38±0.02
Pyrene	1.07±0.01	1.78±0.01	0.44±0.01
Benzo[a]anthracene	1.79±0.03	0.88±0.01	0.34±0.01
Chrysene	0.83±0.04	0.64±0.02	0.36±0.01
Benzo[b]fluoranthene	0.45±0.01	0.93±0.02	0.33±0.03
Benzo[k]fluoranthene	1.49±0.01	1.70±0.03	0.48±0.02
Benzo[a]pyrene	1.38±0.01	1.72±0.02	0.46±0.01
Diben[a,h]anthracene	ND	ND	ND
Indeno[1,2,3-cd]pyrene	ND	ND	ND
Benzo[g,h,i]perylene	ND	ND	ND
$\sum PAH4$	**4.45**	**4.17**	**1.49**
$\sum PAH18$	**30.71**	**22.18**	**8.31**

Values are means± standard deviation of triplicate determinations. ND means not detected
UW = Unwashed; WTW = Washed with tap water; WSS = Washed with salt solution

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Table 2: PAHs concentrations (mg kg^{-1}) in *P. guineense* leaf samples from Isigate market

Contaminants	UW	WTW	WSS
1,2,3-Trimethylbenzene	3.17±0.02	ND	0.10±0.01
Naphthalene	ND	ND	0.01±0.005
2-Methylnaphthalene	ND	2.47±0.05	0.01±0.006
Acenaphthylene	ND	1.47±0.04	0.01±0.001
Acenaphthene	ND	ND	0.01±0.002
Fluorene	ND	ND	0.14±0.06
Anthracene	1.58±0.01	ND	0.04±0.005
Phenanthrene	2.28±0.03	ND	0.11±0.02
Fluoranthene	1.84±0.01	9.96±0.08	0.14±0.008
Pyrene	1.05±0.05	ND	0.04±0.001
Benzo[a]anthracene	2.05±0.02	ND	0.08±0.005
Chrysene	1.14±0.01	0.50±0.02	0.03±0.007
Benzo[b]fluoranthene	3.18±0.02	0.31±0.01	0.05±0.001
Benzo[k]fluoranthene	0.54±0.02	0.39±0.03	0.04±0.004
Benzo[a]pyrene	1.44±0.05	0.49±0.01	0.06±0.009
Diben[a,h]anthracene	ND	ND	0.09±0.008
Indeno[1,2,3-cd]pyrene	ND	ND	0.05±0.001
Benzo[g,h,i]perylene	ND	ND	0.19±0.02
$\sum PAH4$	**7.81**	**1.30**	**0.22**
$\sum PAH18$	**18.27**	**15.59**	**1.20**

Values are means± standard deviation of triplicate determinations. ND means not detected
UW = Unwashed; WTW = Washed with tap water; WSS = Washed with salt solution

In the same vein, ∑ PAH4 which is the sum of benzo[*a*]pyrene, benz[*a*]anthracene, benzo[*b*]fluoranthene and chrysene concentrations in the analyzed vegetable samples followed the same trend i.e. (4.45; 7.81 mg kg^{-1}), (4.17; 1.30 mg kg^{-1}) and (1.49; 0.22 mg kg^{-1}) for the three portions stated earlier. Igwe *et al.* [3] reported values of 11.94, 7.17 and 0.27 mg kg^{-1} as the total PAHs content (∑ PAH18) in *Talinum*

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triangulare from Afule, Ahiaohuru and Ohanku markets (all within Aba metropolis) respectively. The values of total PAHs content ($\sum$PAH18) reported herein for UW and WTW are much higher. They also reported values of total PAHs content for *P. guineense* from Afule and Ohanku markets to be 5.70 and 9.03 mg kg^{-1} respectively. These values are lower than what is reported herein for UW and WTW.The values reported by Igwe *et al.* [1], for PAHs contents of *T. occidentalis* from Ubakala, Oriugba and Owerri-road markets in Umuahia were much lower compared to the values reported in this research. Benzo[a]pyrene and $\sum PAH4$ are used by different authorities as indicators or markers for the occurrence of PAHs in food. However, the European Food Safety Agency has determined that $\sum PAH4$ is a better indicator than benzo[a]pyrene [17]. It is noteworthy that there are no regulatory limits for benzo[a]pyrene and total PAHs in vegetables [13].

Table 3: Risk assessment of ΣPAH4 in the leaf sample of *P. guineense*

Risk Parameters	**Orieugba Market**			**Isigate Market**		
	UW	WTW	WSS	UN	WTW	WSS
E (ng/day)	289.25	271.05	96.85	507.65	84.50	14.30
MOE	71350	76141	213092	40654	244237	1443217

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 96.85 to 289.25 ng/day for the leaf sample from Orieugba market while that of Isigate market range from 14.30 to 507.65 ng/day. Igwe *et al*. [3] 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 [14], 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. This means that there are no health risks associated with the consumption of *P. guineense* obtained from Orieugba and Isigate markets within the experimental period.

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CONCLUSION

It has been shown in this research work that adequate washing of vegetables with a salt solution removes PAHs better than ordinary washing with tap water. Although there was no health risk found to be associated with the consumption of the analyzed vegetable sample, it is important to wash vegetables with a common salt solution. Also, obtaining *P. guineense* leaves for culinary use from Orieugba and Isigate markets are safer as they pose no health threats to the consuming population.

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