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Evaluation of Polycyclic Aromatic Hydrocarbons (PAHs) in *Moringa oleifera* Leaves and Infusion

(Penilaian Hidrokarbon Aromatik Polisiklik (PAHs) pada Daun dan Infusi *Moringa oleifera*)

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ABSTRACT

Moringa oleifera infusion is a popular drink among herbal infusion consumers. Although *M. oleifera* is claimed to have medicinal value and promoted health benefits, it does not be simply considered safe if no specific study is conducted to determine Polycyclic aromatic hydrocarbons (PAHs) content in commercial *M. oleifera* herbal drink. Therefore, this study was conducted to investigate 10 PAHs content in *M. oleifera* dried leaves and in its infusion. The second objective was to assess the effect of mass-volume ratio on PAHs content in *M. oleifera* infusion. PAHs extraction procedure was performed using QuEChERS (Quick, Easy, Cheap, Effective, Rugged, and Safe) method. PAHs determination and quantification were performed using Gas Chromatography and Flame Ionization Detector (GC-FID). The mean of total 10 PAHs (∑10PAH) in *M. oleifera* dried leaves and infusion ranged from 1.06 µg/kg to 5.51 µg/kg and 0.62 µg/kg to 4.80 µg/kg, respectively. Four different mass-volume ratios were used in this study to determine PAHs content that could be extracted from *M. oleifera* dried leaves into an infusion; 2:250, 20:250, 10:100, and 10:50. The ratio of 2:250 showed the lowest PAHs content in comparison with other ratios. The PAHs content in *M. oleifera* dried leaves and infusion have statistically significantly different ($p > 0.01$) from each other. The toxic equivalency quotient (TEQ) value of *M. oleifera* dried leaves and infusion ranged from 0.01 to 0.09 and 0.00 to 0.07, respectively. PAHs content in *M. oleifera* dried leaves and infusions complied with the maximum limit set by Commission Regulation (EU) No. 2015/1933.

Keywords: Herbal infusion; *Moringa oleifera*; polycyclic aromatic hydrocarbons; QuEChERS; toxic equivalency

ABSTRAK

Infusi *Moringa oleifera* adalah minuman popular dalam kalangan penggemar infusi herba. Walaupun *M. oleifera* didakwa mempunyai nilai perubatan dan manfaat kesihatan, ia tidak sepatutnya dianggap selamat jika tiada kajian khusus dijalankan untuk menentukan kandungan hidrokarbon aromatik polisiklik (PAH) dalam minumaan herba *M. oleifera* komersial. Oleh itu, penyelidikan ini dijalankan untuk mengkaji kandungan 10 PAH dalam daun kering *M. oleifera* dan infusinya. Objektif kajian yang kedua adalah untuk menilai kesan nisbah jisim-isi padu ke atas kandungan PAH dalam infusi *M. oleifera*. Prosedur pengekstrakan PAH dilakukan menggunakan kaedah QuEChERS (Cepat, Mudah, Murah, Berkesan, Lasak dan Selamat). Penentuan dan kuantifikasi PAH dilakukan menggunakan Kromatografi Gas dan Pengesan Pengionan Nyalaan (GC-FID). Purata jumlah 10 PAH (∑10PAH) dalam daun kering dan infusi *M. oleifera* masing-masing dalam julat antara 1.06 µg/kg hingga 5.51 µg/kg dan 0.62 µg/kg hingga 4.80 µg/kg. Empat nisbah jisim isi padu berbeza telah digunakan dalam kajian ini untuk menentukan kandungan PAH yang boleh diekstrak daripada daun kering *M. oleifera* ke dalam infusinya; 2:250, 20:250, 10:100 dan 10:50. Nisbah 2:250 menunjukkan kandungan PAH yang paling rendah berbanding nisbah lain. Kandungan PAH dalam daun kering dan infusi herba mempunyai perbezaan yang ketara secara statistik ($p > 0.01$) antara satu sama lain. Nilai TEQ daun kering dan infusi herba masing-masing dalam julat antara 0.01 hingga 0.09 dan 0.00 hingga 0.07. Kandungan PAH dalam daun kering dan infusi *M. oleifera* mematuhi had maksimum yang ditetapkan oleh Peraturan Suruhanjaya (EU) No. 2015/1933.

Kata kunci: Hidrokarbon polisiklik aromatik; infusi; kesetaraan toksik; *M. oleifera*; QuEChERS

Introduction

Interest in natural products among different races of consumers greatly contributed to the increasing of herbal tea demand and consumption. Bioactive compounds in herbal tea promote health and nutritional benefits that attract consumers and make it become a popular drink (Maria 2020). Moringa tea is one of the popular herbal tea available in the market and is produced from *Moringa oleifera* dried leaves. *M. oleifera* belongs to the Moringaceae family and is native to India (Pandey et al. 2012).

M. oleifera was proven to have high antioxidant activity (Chauhan & Namdev 2022). A study conducted on rats showed that *M. oleifera* leaves infusion protected the layer of gastric mucosal from ulceration (Dalhoumi et al. 2022). *M. oleifera* dried leaves have been reported to contain nutritional values such as energy value, magnesium, potassium, calcium, carbohydrate, and protein (Ziani et al. 2019). Besides the usage of *M. oleifera* as herbal drink, *M. oleifera* leaves have also been used for food preservation due to their antimicrobial properties. Studies on food products such as smoked fish, hamburger, sausage, and chicken slices have shown beneficial effects including antifungal, sensory quality improvement, reduced fat levels, and lower microbial counts (Hodas, Zorzenon & Milani 2021). Phenolic compounds such as flavonoid, phenolic acid, quinone, xanthone, lignan, tannin, and coumarin were found in *M. oleifera* leaves. Biological activities associated with phenolic compounds included neuroprotective effects, antiepileptic, antidepressant, and hepatoprotective effects (Hassan et al. 2021).

Although herbal tea is referred to as a natural product that is considered beneficial for health, it might be contaminated by polycyclic aromatic hydrocarbons (PAHs) due to polluted soil, contaminated water, and a polluted environment during the plantation. Besides that, contamination of PAHs in herbal tea is also caused by industrial processing (Bratu et al. 2022). PAHs have many characteristics such as lipophilic (Maria 2020), potentially carcinogenic, and mutagenic (Fred-Ahmadu & Benson 2019; Iwegbue et al. 2016). Some studies have reported the PAHs content in dried leaves and herbal infusion from different herbal species including *Ilex paraguariensis* and *Camelia sinensis* (Panzl et al. 2022; Zachara, Gałkowska & Juszczak 2018). Herbal infusion is the aqueous beverage or drinks obtained after the dried herb in form of leaves, stem, fruit, or flower was brewed or soaked in hot water for a few minutes. Meanwhile, dried herbs of any part of the herbal plant species that undergo tea production processes such as drying and roasting can be used as herbal drink by infusing them in hot water. The occurrence of PAHs are not only in herbal drink but are also found in other food products such as coffee, oils, baby food, fish, and meat (Givechev, Tanev & Danalev 2021; Hamidi et al. 2016; Jahurul et al. 2013).

In dried leaves, the PAHs content mainly depends on the contamination during the manufacturing process and herbal plantation environment. This is different from herbal infusion where the content of PAHs depends on several parameters that determine the amount of PAHs extracted into the aqueous drinks during the infusion process. The parameters were the ratio of the mass of dried leaves to the volume of water and infusion time. Therefore, the main objective of this present study was to investigate the PAHs content in dried leaves and infusion of *M. oleifera*. The samples were analyzed both in dried form (dried leaves) and in liquid form (infusion). The second objective was to assess the effect of mass volume ratio on PAHs content in *M. oleifera* infusion. Consumers commonly infuse 2 gram of dried leaves in 250 mL water (Zachara, Gałkowska & Juszczak 2018). QuEChERS (Quick, Easy, Cheap, Effective, Rugged, and Safe) method was selected in the sample preparation of dried leaves and infusion of *M. oleifera.* This method was selected due to its ability to reduce the plant-interfering substances such as sugars, pigments, and organic acid in the PAHs extraction procedure (Zachara, Gałkowska & Juszczak 2018). Other benefits of QuEChERS were fast extraction, low cost, and minimal chemical consumption. The studied individual PAHs include acenaphthene, fluorene, phenanthrene, anthracene, fluoranthene, pyrene, benzo[a]pyrene, benz[a]anthracene, benzo[b]fluoranthene, chrysene, and benzo[a]pyrene. PAHs determination and quantification were performed using Gas Chromatography separation and Flame Ionization Detector (GC-FID). The toxicity of PAHs in dried leaves and infusion was further evaluated using Toxic Equivalency Quotient (TEQ) (Ciemniak et al. 2019; Fred-Ahmadu & Benson 2019; Nisbet & LaGoy 1992). Toxic equivalent factors (TEF) are significant in the determination of PAHs level and toxicity (Ciemniak et al. 2019; Fred-Ahmadu & Benson 2019; Lin, Tu & Zhu 2005; Nisbet & LaGoy 1992).

Materials and Methods

Standards and Chemicals

Analytical standards of acenaphthene (purity of 99.60%), fluorene (99.07%), anthracene (99.52%), phenanthrene (99.05%), fluoranthene (98.69%), pyrene (98.58%),

benz[a]anthracene (98.62%), benzo[b]fluoranthene (99.45%), benzo[a]pyrene (95.72%) and chrysene (99.44%) were purchased from Dr Ehrenstorfer, Germany. Acetonitrile was purchased from Supelco, Germany, and hexane was procured from QReC, New Zealand. Magnesium sulfate anhydrous and sodium chloride were purchased from Supelco, Denmark. Primary Secondary Amine (PSA) sorbents were derived from Agilent Technologies, USA.

SAMPLES COLLECTION

Ten different brands of *M. oleifera* tea samples were purchased from local supermarkets in Malaysia. This herbal species was selected considering its common and traditional consumption in Malaysia. For each brand, three sachets of samples with similar batches were obtained. The samples were available in tea sachets and in the form of dried leaves flakes. The *M. oleifera* tea samples were homogenized by crushing using a blender (Panasonic MX799S).

DETERMINATION OF PAHS IN *M. oleifera* HERBAL TEA

The samples preparation of *M. oleifera* dried leaves and it's infusion were adopted from method by Zachara, Gałkowska and Juszczak (2018).

SAMPLES PREPARATION OF *M. oleifera* DRIED LEAVES

The homogenized *M. oleifera* dried leaves (2 g) were placed into a 50 mL polypropylene tube. Next, acetonitrile (10 mL) was added, and the mixture was vortexed for 1 min. Sodium chloride (1 g) and magnesium sulfate anhydrous (4 g) were added, and the mixture was vortexed again for 1 min and then centrifuged for 15 min at 10,500 rpm. A 6 mL supernatant was transferred into the 15 mL polypropylene tube containing 0.15 g of Primary Secondary Amine (PSA), and 0.9 g of magnesium sulfate anhydrous. The mixture was vortexed for 1 min and then centrifuged for 15 min at 10,500 rpm. Then, 4 mL of supernatant was transferred into the 15 mL glass vial containing 4 mL of hexane and vortexed again for 1 min. Then, 3.5 mL of the upper phase was transferred into a glass vial (4 mL) and evaporated to dryness under a nitrogen gas stream. The residue was dissolved in hexane (1 mL) and filtered using a membrane filter. The filtered sample (1 µL) was then injected into the GC-FID for quantitative analysis. Triplicate determinations were made on each brand of *M. oleifera* dried leaves samples.

SAMPLES PREPARATION OF *M. oleifera* INFUSION

Four different mass-volume ratios of dried leaves and water were selected in this study. The mass-volume ratios were as follows: 2:250, 20:250, 10:100, and 10:50. The four different weights (2, 20, 10, and 10 g) of *M. oleifera* leaves were placed into a 100 mL conical flask, and the volume (250, 250, 100, and 50 mL) of hot boiled water (100 °C) was added to infuse and brew the tea for 15 min. The infusions were decanted and cooled to room temperature (27 °C). Next, acetonitrile (10 mL) was added to each infusion, and the mixture was vortexed for 1 min. Sodium chloride (1 g) and magnesium sulfate anhydrous (4 g) were added, and the mixture was vortexed again for 1 min and then centrifuged for 15 min at 10,500 rpm. A 6 mL supernatant was transferred into the 15 mL polypropylene tube containing 0.15 g of Primary Secondary Amine (PSA), and 0.9 g of magnesium sulfate anhydrous. The mixture was vortexed for 1 min and then centrifuged for 15 min at 10,500 rpm. Then, 4 mL of supernatant was transferred into the 15 mL glass vial containing 4 mL of hexane and vortexed again for 1 min. Then, 3.5 mL of the upper phase was transferred into a glass vial (4 mL) and evaporated to dryness under a nitrogen gas stream. The residue was dissolved in hexane (1 mL) and filtered using a membrane filter. The filtered sample (1 µL) was then injected into the GC-FID for quantitative analysis. Triplicate determinations were made on each mass-volume ratio of *M. oleifera* infusion samples.

GC-FID ANALYSIS

An Agilent 7890A Gas Chromatography system equipped with Flame Ionization Detector (GC-FID) was employed. Chemstation B.04.03 software was installed for quantitative data analysis. The gas chromatography separation was achieved on a Select PAH capillary column (30 m × 0.25 mm × 0.36 mm). The initial oven temperature was maintained at 100 °C, increased to 180 °C at 50°C min^{-1}, then ramped to 300 °C at 10 °C min^{-1}, and held for 20 min. The injector was maintained at 270 °C and 1 µL of the extract was injected in split mode (split ratio: 10:1). High purity helium (99.9999%) was employed as the carrier gas at a constant flow of 7.3 $mLmin^{-1}$ (Fred-Ahmadu & Benson 2019).

IDENTIFICATION AND QUANTIFICATION

Individual standard solutions, all prepared in hexane at five different points of concentration (2, 4, 6, 8, 10 µg/L) were analyzed using the flame ionization detector to establish a calibration curve.

QUALITY CONTROL

For analytical quality control method, a spiked sample was prepared by spiking dried leaves with the mixed PAH standard solution before being extracted using the same procedure used for dried leaves samples. Triplicate spiked samples were made.

TOXICITY OF PAHs

The total toxicity of all PAHs in dried leaves and infusion of *M. oleifera* is expressed as a toxic equivalency quotient (TEQ). The TEQ of PAHs was calculated using Equation (1) (Ciemniak et al. 2019; Nisbet & LaGoy 1992) as indicated herewith:

$$TEQ = \sum Cs \times TEFs \quad (1)$$

where TEQ is the total toxicity of different congeners of PAHs in each sample and *C*s is the concentration of PAHs congener. TEFs are the toxic equivalent factors for PAHs congener (Ciemniak et al. 2019; Fred-Ahmadu & Benson, 2019; Lin, Tu & Zhu 2005; Nisbet & LaGoy 1992).

STATISTICAL ANALYSIS

Data were analyzed using one-way Analysis of Variance (ANOVA) and Tukey's Honestly Significance Difference (HSD) test with 95% confidence using Statistica software (Statistica 5.5, Stat Soft Inc.). Tukey's HSD for each pair of mean was calculated using the formula as indicated herewith:

$$HSD = \frac{Mi - Mj}{\sqrt{MSw/N}}$$

where M_i-M_j is the difference between the pair of means. While, the MS_w is the mean square, and N is the number in the group or treatment (Nanda et al. 2021).

RESULTS AND DISCUSSION

PAHs CONTENT IN *M. oleifera* DRIED LEAVES

In this study, the determination of PAHs content was investigated in two stages; the first stage is determination in dried leaves, and the second is determination in infusion. The quality control method was carried out by using brand A sample. Figure 1 shows the GC-FID chromatogram of the brand A sample spiked with acenaphthene, fluorene, phenanthrene, and fluoranthene at 10 µg/kg. Table 1 shows the limit of detection (LOD) and limit of quantification (LOQ) calculated as 3 and 10 times the standard deviation (SD). LOD value ranged from 0.08 to 0.17 µg/kg, while LOQ value extended from 0.24 to 0.51 µg/kg.

Quantification of 10 PAHs ($\sum_{10}$PAHs) content in 10 different brands of *M. oleifera* tea samples were evaluated and the results obtained were presented in Table 2. The mean total of 10 PAHs ($\sum_{10}$PAHs) for each brand ranged from 1.06 µg/kg to 5.51 µg/kg. Flourene was the most PAH compound found in all samples followed by acenaphthene with percentages of 31% and 29%, respectively. The highest content of fluorene is indicated by the general uses of fluorene in agriculture as an herbicide and growth regulator. Acenaphthene is also used in the agriculture field as a pesticide. The contamination of these two PAHs compounds in dried leaves mainly came from surface soil. PAHs are easily bound with soil due to the small pore size of soil particles (Abdel-Shafy & Mansour 2016). Benzo(a)pyrene identified as carcinogenic to humans by International Agency for Research on Cancer (IARC) (IARC 2021) was found in the lowest percentage (1%). IARC also identified benz[a] anthracene, benzo[b]fluoranthene, and chrysene in group 2B in their classification list. Group 2B was categorized as possibly carcinogenic to humans. These three PAHs compounds were also found in low percentages (1-2%).

TABLE 1. Limit of detection (LOD) and quantification (LOQ), linearity of each PAHs

PAHs	Regression analysis	LOD (µg/kg)	LOQ (µg/kg)	R^2
Acenaphthene	y = 1.4781x + 0.0613	0.17	0.51	0.9999
Fluorene	y = 1.3221x + 0.2792	0.16	0.48	0.9999
Phenanthrene	y = 1.3176x + 0.1609	0.16	0.48	0.9999
Anthracene	y = 1.3216x + 0.1876	0.13	0.39	0.9999
Fluoranthene	y = 1.3217x + 0.1842	0.12	0.36	0.9999
Phyrene	y = 1.3264x + 0.0648	0.12	0.36	0.9999
Benz[a]anthracene	y = 1.3235x + 0.0989	0.10	0.30	0.9999
Chrysene	y = 1.3219x + 0.1032	0.10	0.30	0.9999
Benzo[b]fluoranthene	y = 1.3217x + 0.0840	0.10	0.30	0.9999
Benzo[a]pyrene	y = 1.3214x + 0.0863	0.08	0.24	0.9999

LOD = limit of detection, LOQ = limit of quantitation, R^2 = relative coefficient

TABLE 2. Results obtained in the analysis of *M. oleifera* dried tea samples (µg/kg)

Brands	Batch	PAHs content in *M. oleifera* dried tea (µg/kg)										$\sum_{10}$PAHs
		Ace	Flu	Phe	Ant	Fluo	Pyr	BaA	Chr	BbF	BaP	
A	1	0.300	0.250	0.190	0.150	0.170	ND	ND	ND	ND	ND	1.060
	2	0.320	0.220	0.190	0.150	0.170	ND	ND	ND	ND	ND	1.050
	3	0.300	0.250	0.190	0.150	0.170	ND	ND	ND	ND	ND	1.060
B	1	0.200	0.180	0.170	0.140	0.130	0.120	0.090	0.090	0.080	0.070	1.200
	2	0.190	0.170	0.160	0.140	0.130	0.120	0.090	0.090	0.080	0.070	1.240
	3	0.200	0.190	0.170	0.120	0.130	0.120	0.090	0.090	0.080	0.070	1.260
C	1	2.120	2.010	0.380	0.250	0.150	0.130	0.080	0.090	0.090	0.060	5.360
	2	2.100	2.000	0.380	0.260	0.150	0.130	0.080	0.090	0.090	0.060	5.340
	3	2.000	2.070	0.380	0.270	0.150	0.130	0.080	0.090	0.090	0.070	5.330
D	1	1.520	3.020	0.320	0.140	0.160	0.120	ND	0.090	0.070	0.070	5.510
	2	1.510	3.010	0.320	0.150	0.160	0.120	ND	0.090	0.090	0.070	5.52
	3	1.510	3.010	0.320	0.160	0.160	0.120	ND	0.090	0.080	0.060	5.51
E	1	0.170	0.190	0.180	0.150	0.180	0.120	0.080	0.070	0.070	0.070	1.28
	2	0.170	0.200	0.180	0.150	0.180	0.110	0.080	0.070	0.080	0.060	1.28
	3	0.150	0.210	0.180	0.150	0.180	0.130	0.080	0.070	0.090	0.070	1.31
F	1	0.700	0.180	0.160	0.150	0.150	0.120	0.080	0.080	0.070	0.070	1.76
	2	0.700	0.190	0.160	0.160	0.150	0.130	0.080	0.090	0.070	0.060	1.79
	3	0.650	0.170	0.160	0.170	0.150	0.110	0.080	0.070	0.070	0.060	1.69
G	1	0.170	0.200	0.230	0.140	0.140	0.140	0.070	0.070	0.080	.070	1.31
	2	0.170	0.220	0.230	0.140	0.140	0.140	0.070	0.090	0.080	0.070	1.35
	3	0.170	0.200	0.230	0.140	0.140	0.140	0.070	0.080	0.080	0.060	1.31
H	1	0.190	0.180	0.180	0.130	0.140	0.120	0.080	0.090	ND	ND	1.11
	2	0.190	0.190	0.180	0.130	0.140	0.120	0.080	0.090	ND	ND	1.12
	3	0.190	0.170	0.180	0.130	0.140	0.120	0.080	0.090	ND	ND	1.10
I	1	0.850	0.190	0.220	0.140	0.160	0.130	0.080	0.090	ND	ND	1.86
	2	0.800	0.190	0.220	0.140	0.160	0.130	0.080	0.090	ND	ND	1.81
	3	0.800	0.170	0.220	0.140	0.160	0.130	0.080	0.090	ND	ND	1.79
J	1	0.180	0.250	0.230	0.130	0.130	0.100	0.090	0.070	ND	ND	1.18
	2	0.190	0.240	0.210	0.110	0.130	0.100	0.090	0.090	ND	ND	1.16
	3	0.170	0.230	0.220	0.120	0.130	0.100	0.090	0.080	ND	ND	1.14

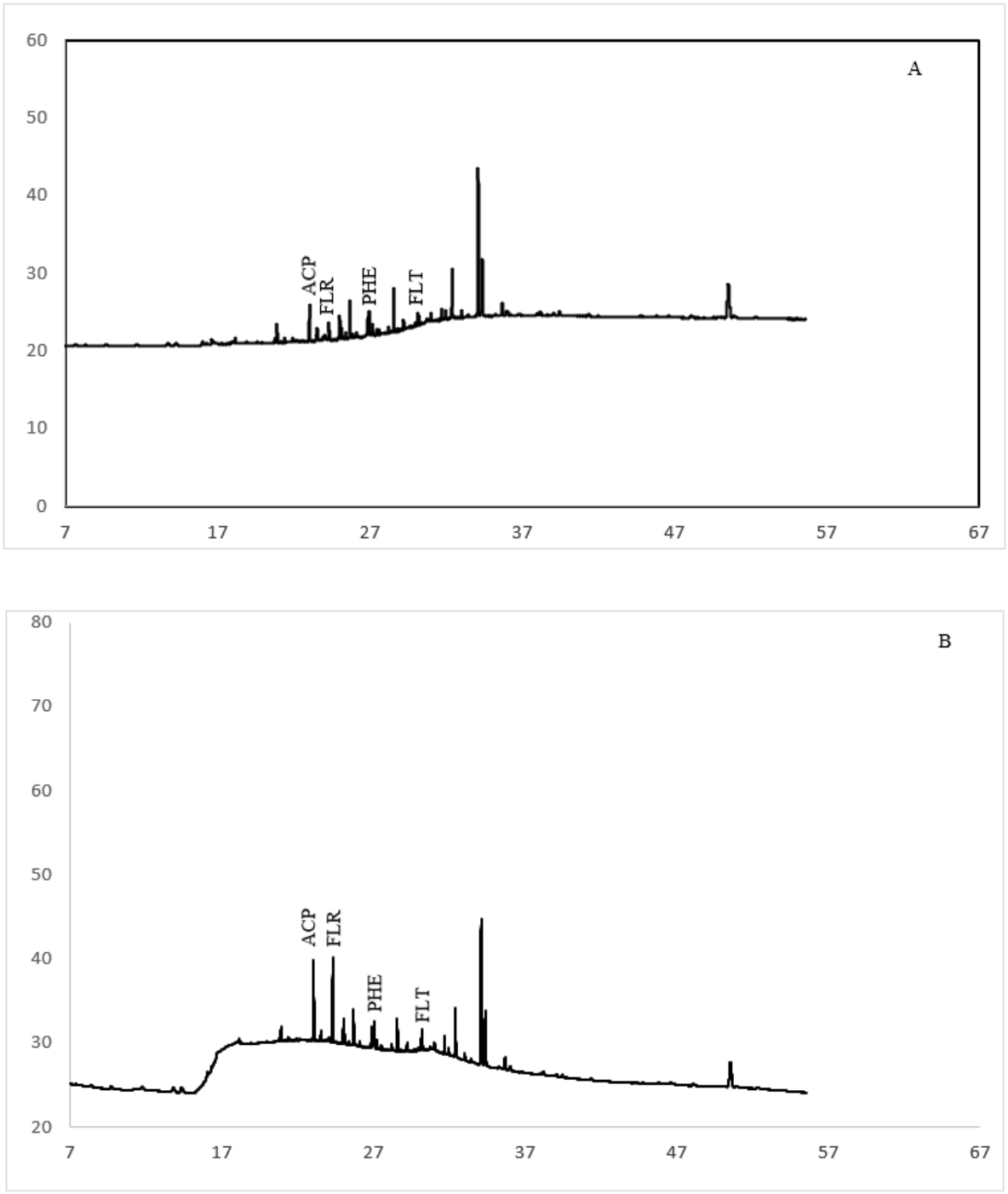

FIGURE 1. GC-FID chromatograms of (A) unspiked with acenaphthene, fluorene, phenanthrene, and fluoranthene at 10 µgkg^{-1}and (B) spiked of *M. oleifera* dried leaves sample (Brand A). Column: Select PAH capillary column (30 m × 0.25 mm × 0.36 mm). Detection by flame ionization detector (FID). ACP: Acenaphthene; FLR: Fluorene; PHE: Phenanthrene; FLT: Fluoranthene

PAHs CONTENT IN *M. oleifera* INFUSION AND THE EFFECT OF MASS-VOLUME RATIO ON PAHs CONTENT

The PAHs content in the infusion of different brands of commercial *M. oleifera* tea samples is shown in Table 3. The total of 10 PAHs (∑10PAHs) ranged from 0.62 µg/kg to 4.80 µg/kg. The individual PAHs content of infusion depends on the mass of dried leaves and the volume of water used in the brewing process. Four different mass-volume ratios were used in this study to determine PAHs content that could be extracted from dried leaves into an infusion. The ratio of 2:250 showed the lowest PAHs content in comparison with other ratios The PAHs content determined in infusion prepared from brewing 10 g of dried leaves in 50 mL of water was the highest in all tested brands. The results show that increasing the mass of dried tea to water will increase the PAHs content.

PAHs with high molecular weight (5 rings above) have low water solubility. This fact supports the finding that most PAHs with low molecular weight were determined in all infusion ratios. Essential oils help increase the solubility of PAHs in water. The essential oils also contribute to release the aroma of the infusion. Overall, this finding shows that the extraction of PAHs from dried leaves into infusion was facilitated by the mass-volume ratio. Another study has found that 70% of PAHs in infusion came from contaminated raw material (Ciemniak et al. 2019).

STATISTICAL ANALYSIS

Tukey HSD test (Table 4) showed that each batch in all brands of *M. oleifera* dried leaves is insignificantly different from each other ($p > 0.05$). These insignificant differences indicate that the PAHs exposure is similar for each batch. In comparison between each brand of *M. oleifera* dried leaves, most of the brands have significant differences ($p < 0.01$) as shown in Table 5. These significant differences might be caused by differences in the drying process, agricultural environment, and level of PAHs exposure.

Statistically significant differences ($p < 0.01$ and $p < 0.05$) between the content of 10 PAHs ($\sum_{10}$PAHs) were found for several brands in infusion (Table 6). These differences were due to several factors such as mass-volume ratios, the amount of essential oil, and the amount of high solubilities PAHs in water. Low molecular weight PAHs are more soluble in water than high molecular weight PAHs. PAHs have a lipophilic characteristic that needs compound such as essential oil to boost their solubility in water.

The 10 PAHs ($\sum_{10}$PAHs) content in *M. oleifera* dried leaves and infusion are statistically significantly different ($p > 0.01$) from each other as shown in Table 7. This is because the extraction of PAHs into infusion is affected by the amount of dried leaves and the volume of water. A large amount of dried leaves increases the PAHs content of the infusion. Based on this statistical analysis, consumption of herbal *M. oleifera* infusion has a low risk of exposure to PAHs because not all PAHs from dried leaves were extracted into the infusion.

TOXICITY OF PAHs IN *M. oleifera* DRIED LEAVES AND INFUSION

Toxic equivalency quotient (TEQ) was used in the assessment of PAHs toxicity in *M. oleifera* dried leaves (Table 8) and infusion (Table 9). TEQ represents the total toxicity of PAHs mixture in the samples. The TEQ value depends on the toxic equivalent factor (TEF). Samples that contain individual PAHs with high TEF values (benzo[a]pyrene, chrysene, benzo(b)fluoranthene, and benz[a]anthracene) will result in high toxicity levels. High molecular weight PAHs have high TEF values than low molecular weight PAHs (Ciemniak et al. 2019). Each individual PAHs compound has a different TEF value. TEQ was obtained by summing all the values after multiplying the concentration of individual PAHs with TEF. *M. oleifera* dried leaves have a higher TEQ value than *M. oleifera* infusions. The TEQ value of *M. oleifera* dried leaves ranged from 0.01 to 0.09. While in infusion, the TEQ value is in ranges of 0.00 to 0.07. At a ratio of 2:250, TEQ values of all commercial brands tested were the lowest compared to other ratios and ranged from 0.00 to 0.01. This is because of the ratio of 2:250 less facilitates the penetration of high molecular weight PAHs into the infusion. This finding indicates that consuming *M. oleifera* infusions with the brewing of 2 grams of dried leaves in 250 mL of water might not cause a toxic effect on humans since the TEQ value is very low. Based on the regulation, the maximum limit for benzo[a]pyrene and the sum of four PAHs (benzo[a] pyrene, benzo[a]anthracene, benzo[b]fluoranthene, and chrysene) in dried herbs were 10 µg/kg and 50 µg/kg, respectively (Ciemniak et al. 2019).

TABLE 3. Results obtained in the analysis of *M. oleifera* infusion samples (µg/kg)

Brands	Ratio	Batch	Mean of PAHs content in *M. oleifera* infusion (µg/kg)										$\sum_{10}$PAHs
			Ace	Flu	Phe	Ant	Fluo	Pyr	BaA	Chr	BbF	BaP	
A	2:250	1	0.150	0.140	.100	0.090	0.080	0.060	ND	ND	ND	ND	0.62
		2	0.150	0.140	0.100	0.090	.080	0.060	ND	ND	ND	ND	0.62
		3	0.150	0.140	0.100	0.090	0.080	0.060	ND	ND	ND	ND	0.62
	20:250	1	0.170	0.160	0.120	0.100	0.090	0.080	ND	ND	ND	ND	0.72
		2	0.170	0.160	0.130	0.100	0.090	0.080	ND	ND	ND	ND	0.73
		3	0.170	0.160	0.120	0.100	0.090	0.080	ND	ND	ND	ND	0.72
	10:100	1	0.190	0.170	0.130	0.110	0.100	0.090	ND	ND	ND	ND	0.79
		2	0.180	0.170	0.140	0.110	0.100	0.080	ND	ND	ND	ND	0.78
		3	0.170	0.170	0.140	0.110	0.100	0.090	ND	ND	ND	ND	0.78
	10:50	1	0.200	0.180	0.150	0.120	0.110	0.100	ND	ND	ND	ND	0.86
		2	0.190	0.170	0.150	0.120	0.110	0.110	ND	ND	ND	ND	0.85
		3	0.180	0.160	0.150	0.120	0.110	0.120	ND	ND	ND	ND	0.84
B	2:250	1	0.140	0.120	0.100	0.080	0.080	0.060	0.050	0.050	0.050	ND	0.73
		2	0.140	0.120	0.100	0.080	0.070	0.060	0.050	0.050	0.050	ND	0.72
		3	0.140	0.120	0.110	0.070	0.070	0.060	0.050	0.060	0.050	ND	0.73
	20:250	1	0.160	0.150	0.130	0.090	0.090	0.070	0.060	0.060	0.050	ND	0.86
		2	0.170	0.150	0.130	0.090	0.090	0.070	0.060	0.070	0.060	ND	0.89
		3	0.170	0.150	0.130	0.100	0.090	0.070	0.060	0.060	0.060	ND	0.89
	10:100	1	0.180	0.160	0.140	0.100	0.100	0.080	0.070	0.070	0.060	0.050	1.01
		2	0.170	0.160	0.130	0.100	0.100	0.080	0.070	0.070	0.060	0.050	0.99
		3	0.170	0.170	0.140	0.100	0.100	0.080	0.070	0.080	0.060	0.050	1.02
	10:50	1	0.180	0.170	0.150	0.110	0.110	0.110	0.080	0.080	0.070	0.060	1.12
		2	0.180	0.170	0.150	0.110	0.110	0.110	0.080	0.080	0.070	0.060	1.12
		3	0.180	0.170	0.160	0.110	0.110	0.110	0.080	0.080	0.070	0.060	1.13
C	2:250	1	0.480	0.410	0.150	0.120	0.080	0.080	ND	ND	0.050	ND	1.37
		2	0.480	0.410	0.150	0.120	0.080	0.080	ND	ND	0.050	ND	1.37
		3	0.470	0.410	0.150	0.120	0.080	0.080	ND	ND	0.050	ND	1.36
	20:250	1	1.000	0.970	0.200	0.180	0.100	0.090	0.050	0.050	0.060	ND	2.7
		2	1.010	0.980	0.200	0.180	0.100	0.090	0.050	0.050	0.060	ND	2.72
		3	1.020	0.980	0.200	0.180	0.100	0.090	0.050	0.050	0.060	ND	2.73
	10:100	1	1.830	1.440	0.250	0.190	0.110	0.100	0.060	0.060	0.070	ND	4.11
		2	1.840	1.450	0.250	0.190	0.110	0.100	0.060	0.060	0.070	ND	4.13
		3	1.840	1.450	0.250	0.190	0.110	0.100	0.060	0.060	0.070	ND	4.13
	10:50	1	1.980	1.780	0.300	0.200	0.120	0.110	0.070	0.070	0.080	0.050	4.76
		2	1.980	1.790	0.290	0.200	0.120	0.110	0.060	0.080	0.070	0.050	4.75
		3	1.970	1.780	0.300	0.210	0.120	0.110	0.070	0.080	0.070	0.050	4.76

Brands	Ratio	Batch	Mean of PAHs content in *M. oleifera* infusion (µg/kg)										$\sum_{10}$PAHs
			Ace	Flu	Phe	Ant	Fluo	Pyr	BaA	Chr	BbF	BaP	
D	2:250	1	0.490	0.470	0.100	0.090	0.110	ND	ND	ND	ND	ND	1.26
		2	0.490	0.470	0.100	0.090	0.110	ND	ND	ND	ND	ND	1.26
		3	0.490	0.470	0.100	0.090	0.110	ND	ND	ND	ND	ND	1.26
	20:250	1	0.980	1.010	0.150	0.100	0.120	0.090	ND	0.060	ND	ND	2.51
		2	0.980	1.000	0.150	0.100	0.120	0.090	ND	0.060	ND	ND	2.50
		3	0.980	1.010	0.150	0.100	0.120	0.090	ND	0.060	ND	ND	2.51
	10:100	1	1.130	1.950	0.250	0.120	0.130	0.100	ND	0.070	0.050	0.050	3.85
		2	1.130	1.950	0.250	0.120	0.130	0.100	ND	0.070	0.050	0.050	3.85
		3	1.130	1.950	0.250	0.120	0.130	0.100	ND	0.070	0.050	0.050	3.85
	10:50	1	1.450	2.490	0.280	0.130	0.140	0.110	ND	0.080	0.060	0.060	4.80
		2	1.450	2.480	0.290	0.130	0.140	0.110	ND	0.080	0.060	0.060	4.80
		3	1.440	2.500	0.290	0.130	0.140	0.110	ND	0.080	0.060	0.060	4.81
E	2:250	1	0.110	0.130	0.110	0.090	0.120	0.070	ND	ND	ND	ND	0.63
		2	0.110	0.130	0.110	0.100	0.120	0.070	ND	ND	ND	ND	0.64
		3	0.110	0.130	0.110	0.100	0.120	0.070	ND	ND	ND	ND	0.64
	20:250	1	0.120	0.150	0.130	0.120	0.140	0.080	0.050	ND	ND	ND	0.79
		2	0.120	0.150	0.130	0.120	0.140	0.080	0.050	ND	ND	ND	0.79
		3	0.120	0.150	0.130	0.120	0.140	0.080	0.050	ND	ND	ND	0.79
	10:100	1	0.130	0.170	0.150	0.130	0.150	0.090	0.060	0.050	0.050	ND	0.98
		2	0.130	0.170	0.150	0.130	0.150	0.090	0.060	0.050	0.050	ND	0.98
		3	0.130	0.170	0.150	0.130	0.150	0.090	0.060	0.050	0.050	ND	0.98
	10:50	1	0.140	0.180	0.170	0.140	0.170	0.100	0.070	0.060	0.060	0.050	1.14
		2	0.140	0.180	0.170	0.140	0.170	0.100	0.070	0.060	0.060	0.050	1.14
		3	0.140	0.180	0.170	0.140	0.170	0.100	0.070	0.060	0.060	0.050	1.14
F	2:250	1	0.160	0.130	0.120	0.110	0.090	0.050	ND	ND	ND	ND	0.66
		2	0.160	0.130	0.120	0.110	0.090	0.050	ND	ND	ND	ND	0.66
		3	0.160	0.130	0.120	0.110	0.090	0.050	ND	ND	ND	ND	0.66
	20:250	1	0.220	0.140	0.130	0.120	0.100	0.070	0.050	ND	ND	ND	0.83
		2	0.210	0.140	0.130	0.120	0.100	0.070	0.050	ND	ND	ND	0.82
		3	0.220	0.140	0.130	0.120	0.100	0.070	0.050	ND	ND	ND	0.83
	10:100	1	0.410	0.150	0.140	0.130	0.110	0.090	0.060	0.050	0.050	ND	1.19
		2	0.400	0.150	0.140	0.130	0.110	0.090	0.060	0.050	0.050	ND	1.18
		3	0.400	0.150	0.140	0.130	0.110	0.090	0.060	0.050	0.050	ND	1.18
	10:50	1	0.600	0.160	0.150	0.140	0.130	0.100	0.070	0.060	0.060	0.050	1.52
		2	0.600	0.160	0.150	0.140	0.130	0.100	0.070	0.060	0.060	0.050	1.52
		3	0.600	0.160	0.150	0.140	0.130	0.100	0.070	0.060	0.060	0.050	1.52

Brands	Ratio	Batch	Mean of PAHs content in *M. oleifera* infusion (µg/kg)										$\sum_{10}$PAHs
			Ace	Flu	Phe	Ant	Fluo	Pyr	BaA	Chr	BbF	BaP	
G	2:250	1	0.120	0.140	0.130	0.090	0.090	0.080	ND	ND	ND	ND	0.65
		2	0.120	0.140	0.130	0.090	0.090	0.080	ND	ND	ND	ND	0.65
		3	0.120	0.140	0.130	0.090	0.090	0.080	ND	ND	ND	ND	0.65
	20:250	1	0.130	0.160	0.170	0.100	0.100	0.090	ND	ND	0.050	ND	0.80
		2	0.130	0.160	0.170	0.100	0.100	0.090	ND	ND	0.050	ND	0.80
		3	0.130	0.160	0.170	0.100	0.100	0.090	ND	ND	0.050	ND	0.80
	10:100	1	0.150	0.170	0.180	0.110	0.110	0.100	0.050	0.050	0.060	0.050	1.03
		2	0.150	0.170	0.180	0.110	0.110	0.100	0.050	0.050	0.060	0.050	1.03
		3	0.150	0.170	0.180	0.110	0.110	0.100	0.050	0.050	0.060	0.050	1.03
	10:50	1	0.160	0.180	0.210	0.120	0.120	0.120	0.060	0.060	0.070	0.060	1.16
		2	0.160	0.180	0.210	0.120	0.120	0.120	0.060	0.060	0.070	0.060	1.16
		3	0.160	0.180	0.210	0.120	0.120	0.120	0.060	0.060	0.070	0.060	1.16
H	2:250	1	0.130	0.120	0.140	0.080	0.100	0.080	ND	0.050	ND	ND	0.70
		2	0.130	0.120	0.140	0.080	0.100	0.080	ND	0.050	ND	ND	0.70
		3	0.130	0.120	0.140	0.080	0.100	0.080	ND	0.050	ND	ND	0.70
	20:250	1	0.150	0.130	0.150	0.090	0.110	0.090	0.050	0.060	ND	ND	0.83
		2	0.150	0.130	0.150	0.090	0.110	0.090	0.050	0.060	ND	ND	0.83
		3	0.150	0.130	0.150	0.090	0.110	0.090	0.050	0.060	ND	ND	0.83
	10:100	1	0.170	0.140	0.160	0.110	0.120	0.100	0.060	0.070	ND	ND	0.93
		2	0.170	0.140	0.160	0.110	0.120	0.100	0.060	0.070	ND	ND	0.93
		3	0.170	0.140	0.160	0.110	0.120	0.100	0.060	0.070	ND	ND	0.93
	10:50	1	0.180	0.160	0.170	0.120	0.130	0.110	0.070	0.080	ND	ND	1.02
		2	0.180	0.160	0.170	0.120	0.130	0.110	0.070	0.080	ND	ND	1.02
		3	0.180	0.160	0.170	0.120	0.130	0.110	0.070	0.080	ND	ND	1.02
I	2:250	1	0.160	0.100	0.150	0.090	0.100	0.090	ND	0.050	ND	ND	0.74
		2	0.160	0.100	0.150	0.090	0.100	0.090	ND	0.050	ND	ND	0.74
		3	0.160	0.100	0.150	0.090	0.100	.090	ND	0.050	ND	ND	0.74
	20:250	1	0.340	0.140	0.170	0.110	0.130	0.100	0.050	0.060	ND	ND	1.10
		2	0.340	0.140	0.170	0.110	0.130	0.100	0.050	0.060	ND	ND	1.10
		3	0.340	0.140	0.170	0.110	0.130	0.100	0.050	0.060	ND	ND	1.10
	10:100	1	0.650	0.150	0.180	0.120	0.140	0.110	0.060	0.070	ND	ND	1.48
		2	0.650	0.150	0.180	0.120	0.140	0.110	0.060	0.070	ND	ND	1.48
		3	0.650	0.150	0.180	0.120	0.140	0.110	0.060	0.070	ND	ND	1.48
	10:50	1	0.750	0.160	0.200	0.130	0.150	0.120	0.070	0.080	ND	ND	1.66
		2	0.750	0.160	0.200	0.130	0.150	0.120	0.070	0.080	ND	ND	1.66
		3	0.750	0.160	0.200	0.130	0.150	0.120	0.070	0.080	ND	ND	1.66

Brands	Ratio	Batch	Mean of PAHs content in *M. oleifera* infusion (µg/kg)										$\sum_{10}$PAHs
			Ace	Flu	Phe	Ant	Fluo	Pyr	BaA	Chr	BbF	BaP	
J	2:250	1	0.100	0.140	0.140	0.080	0.080	0.050	0.050	ND	ND	ND	0.64
		2	0.100	0.140	0.140	0.080	0.080	0.050	0.050	ND	ND	ND	0.64
		3	0.100	0.140	0.140	0.080	0.080	0.050	0.050	ND	ND	ND	0.64
	20:250	1	0.130	0.170	0.160	0.090	0.090	0.070	0.060	0.050	ND	ND	0.82
		2	0.130	0.170	0.160	0.090	0.090	0.070	0.060	0.050	ND	ND	0.82
		3	0.130	0.170	0.160	0.090	0.090	0.070	0.060	0.050	ND	ND	0.82
	10:100	1	0.150	0.180	0.170	0.100	0.100	0.080	0.070	0.060	ND	ND	0.91
		2	0.150	0.180	0.170	0.100	0.100	0.080	0.070	0.060	ND	ND	0.91
		3	0.150	0.180	0.170	0.100	0.100	0.080	0.070	0.060	ND	ND	0.91
	10:50	1	0.160	0.210	0.200	0.110	0.120	0.090	0.080	0.070	ND	ND	1.04
		2	0.160	0.210	0.200	0.110	0.120	0.090	0.080	0.070	ND	ND	1.04
		3	0.160	0.210	0.200	0.110	0.120	0.090	0.080	0.070	ND	ND	1.04

ND = not detected, $\sum_{10}$PAHs = sum of 10 PAHs

TABLE 4. Tukey HSD test for each batch in all brands of *M. oleifera* dried leaves

Brands	Treatments Pair for each batch	Tukey HSD Q statistic	Tukey HSD p-value	Tukey HSD Inference
A	1 vs 2	0.0265	0.899995	insignificant
	1 vs 3	0.0000	0.899995	insignificant
	2 vs 3	0.0265	0.899995	insignificant
B	1 vs 2	0.2139	0.899995	insignificant
	1 vs 3	0.0713	0.899995	insignificant
	2 vs 3	0.1426	0.899995	insignificant
C	1 vs 2	0.0079	0.899995	insignificant
	1 vs 3	0.0118	0.899995	insignificant
	2 vs 3	0.0039	0.899995	insignificant
D	1 vs 2	0.0033	0.899995	insignificant
	1 vs 3	0.0000	0.899995	insignificant
	2 vs 3	0.0033	0.899995	insignificant
E	1 vs 2	0.0000	0.899995	insignificant
	1 vs 3	0.1822	0.899995	insignificant
	2 vs 3	0.1822	0.899995	insignificant

F	1 vs 2	0.0516	0.899995	insignificant
	1 vs 3	0.1203	0.899995	insignificant
	2 vs 3	0.1719	0.899995	insignificant
G	1 vs 2	0.2174	0.899995	insignificant
	1 vs 3	0.0000	0.899995	insignificant
	2 vs 3	0.2174	0.899995	insignificant
H	1 vs 2	0.0457	0.899995	insignificant
	1 vs 3	0.0457	0.899995	insignificant
	2 vs 3	0.0913	0.899995	insignificant
I	1 vs 2	0.0674	0.899995	insignificant
	1 vs 3	0.0944	0.899995	insignificant
	2 vs 3	0.0270	0.899995	insignificant
J	1 vs 2	0.0775	0.899995	insignificant
	1 vs 3	0.1550	0.899995	insignificant
	2 vs 3	0.0775	0.899995	insignificant

TABLE 5. Turkey HSD test comparison for each brand of *M. oleifera* dried leaves

Treatment Pair for each brand	Tukey HSD Q statistic	Tukey HSD p-value	Tukey HSD Inference
A vs B	12.0020	0.001005	p<0.01
A vs C	291.2200	0.001005	p<0.01
A vs D	302.7700	0.001005	p<0.01
A vs E	15.8520	0.001005	p<0.01
A vs F	46.8760	0.001005	p<0.01
A vs G	18.1160	0.001005	p<0.01
A vs H	3.6233	0.296135	insignificant
A vs I	51.8580	0.001005	p<0.01
A vs J	7.02010	0.002363	p<0.01
B vs C	279.2200	0.001005	p<0.01
B vs D	290.7700	0.001005	p<0.01
B vs E	3.8497	0.229616	insignificant
B vs F	34.8740	0.001005	p<0.01
B vs G	6.1143	0.009581	p<0.01
B vs H	8.3789	0.001005	p<0.01
B vs I	39.8560	0.001005	p<0.01
B vs J	4.9820	0.051898	insignificant
C vs D	11.5490	0.001005	p<0.01

C vs E	275.3700	0.001005	p<0.01
C vs F	244.3500	0.001005	p<0.01
C vs G	273.1100	0.001005	p<0.01
C vs H	287.6000	0.001005	p<0.01
C vs I	239.3600	0.001005	p<0.01
C vs J	284.2000	0.001005	p<0.01
D vs E	286.9200	0.001005	p<0.01
D vs F	255.8900	0.001005	p<0.01
D vs G	284.6500	0.001005	p<0.01
D vs H	299.1500	0.001005	p<0.01
D vs I	250.9100	0.001005	p<0.01
D vs J	295.7500	0.001005	p<0.01
E vs F	31.0240	0.001005	p<0.01
E vs G	2.2646	0.811954	insignificant
E vs H	12.2290	0.001005	p<0.01
E vs I	36.0060	0.001005	p<0.01
E vs J	8.8318	0.001005	p<0.01
F vs G	28.7600	0.001005	p<0.01
F vs H	43.2530	0.001005	p<0.01
F vs I	4.9820	0.051898	insignificant
F vs J	39.8560	0.001005	p<0.01
G vs H	14.4930	0.001005	p<0.01
G vs I	33.7420	0.001005	p<0.01
G vs J	11.0960	0.001005	p<0.01
H vs I	48.2350	0.001005	p<0.01
H vs J	3.3968	0.375736	insignificant
I vs J	44.8380	0.001005	p<0.01

TABLE 6. Turkey HSD test comparison for each brand of *M. oleifera* infusion

Treatments pair for each brand	Tukey HSD Q statistic	Tukey HSD p-value	Tukey HSD inference
A vs B	0.5139	0.8999947	insignificant
A vs C	6.9102	0.0011522	p<0.01
A vs D	6.5491	0.0022947	p<0.01
A vs E	0.3820	0.8999947	insignificant
A vs F	0.8403	0.8999947	insignificant
A vs G	0.4514	0.8999947	insignificant
A vs H	0.3403	0.8999947	insignificant
A vs I	1.3820	0.8999947	insignificant
A vs J	0.2917	0.8999947	insignificant

B vs C	6.3963	0.0030643	p<0.01
B vs D	6.0351	0.0060039	p<0.01
B vs E	0.1320	0.8999947	insignificant
B vs F	0.3264	0.8999947	insignificant
B vs G	0.0625	0.8999947	insignificant
B vs H	0.1736	0.8999947	insignificant
B vs I	0.8681	0.8999947	insignificant
B vs J	0.2222	0.8999947	insignificant
C vs D	0.3611	0.8999947	insignificant
C vs E	6.5282	0.0023878	p<0.01
C vs F	6.0699	0.0056297	p<0.01
C vs G	6.4588	0.0027208	p<0.01
C vs H	6.5699	0.0022051	p<0.01
C vs I	5.5282	0.0150495	p<0.05
C vs J	6.6185	0.0020113	p<0.01
D vs E	6.1671	0.0047001	p<0.01
D vs F	5.7087	0.0108957	p<0.05
D vs G	6.0976	0.0053487	p<0.01
D vs H	6.2088	0.0043499	p<0.01
D vs I	5.1670	0.0282392	p<0.05
D vs J	6.2574	0.0039721	p<0.01
E vs F	0.4584	0.8999947	insignificant
E vs G	0.0694	0.8999947	insignificant
E vs H	0.0417	0.8999947	insignificant
E vs I	1.0001	0.8999947	insignificant
E vs J	0.0903	0.8999947	insignificant
F vs G	0.3889	0.8999947	insignificant
F vs H	0.5000	0.8999947	insignificant
F vs I	0.5417	0.8999947	insignificant
F vs J	0.5486	0.8999947	insignificant
G vs H	0.1111	0.8999947	insignificant
G vs I	0.9306	0.8999947	insignificant
G vs J	0.1597	0.8999947	insignificant
H vs I	1.0417	0.8999947	insignificant
H vs J	0.0486	0.8999947	insignificant
I vs J	1.0904	0.8999947	insignificant

TABLE 7. Turkey HSD test comparison between *M. oleifera* dried leaves and infusion

Treatments pair	Tukey HSD Q statistic	Tukey HSD p-value	Tukey HSD inference
Dried leaves vs infusion	4.3445	0.0025	$p<0.01$

TABLE 8. The toxic equivalency quotient (TEQ) calculated for *M. oleifera* dried leaves infusions

Brands	Batch	TEQs										$\sum_{10}$TEQs
		Ace	Flu	Phe	Ant	Fluo	Pyr	BaA	Chr	BbF	BaP	
A	1	0.0003	0.0003	0.0002	0.0015	0.0002	0.0000	0.0000	0.000	0.0000	0.0000	0.0025
	2	0.0003	0.0002	0.0002	0.0015	0.0002	0.0000	0.0000	0.000	0.0000	0.0000	0.0024
	3	0.0003	0.0003	0.0002	0.0015	0.0002	0.0000	0.0000	0.000	0.0000	0.0000	0.0025
B	1	0.0002	0.0002	0.0002	0.0014	0.0001	0.0001	0.0090	0.0009	0.0080	0.0700	0.0901
	2	0.0002	0.0002	0.0002	0.0014	0.0001	0.0001	0.0090	0.0009	0.0080	0.0700	0.0901
	3	0.0002	0.0002	0.0002	0.0012	0.0001	0.0001	0.0090	0.0009	0.0080	0.0700	0.0899
C	1	0.0021	0.0020	0.0004	0.0025	0.0002	0.0001	0.0080	0.0009	0.0090	0.0600	0.0852
	2	0.0021	0.0020	0.0004	0.0026	0.0002	0.0001	0.0080	0.0009	0.0090	0.0600	0.0853
	3	0.0020	0.0021	0.0004	0.0027	0.0002	0.0001	0.0080	0.0009	0.0090	0.0700	0.0954
D	1	0.0015	0.0030	0.0003	0.0014	0.0002	0.0001	0.0000	0.0009	0.0070	0.0700	0.0844
	2	0.0015	0.0030	0.0003	0.0015	0.0002	0.0001	0.0000	0.0009	0.0090	0.0700	0.0865
	3	0.0015	0.0030	0.0003	0.0016	0.0002	0.0001	0.0000	0.0009	0.0080	0.0600	0.0756
E	1	0.0002	0.0002	0.0002	0.0015	0.0002	0.0001	0.0080	0.0007	0.0070	0.0700	0.0881
	2	0.0002	0.0002	0.0002	0.0015	0.0002	0.0001	0.0080	0.0007	0.0080	0.0600	0.0791
	3	0.0002	0.0002	0.0002	0.0015	0.0002	0.0001	0.0080	0.0007	0.0090	0.0700	0.0901
F	1	0.0007	0.0002	0.0002	0.0015	0.0002	0.0001	0.0080	0.0008	0.0070	0.0700	0.0887
	2	0.0007	0.0002	0.0002	0.0016	0.0002	0.0001	0.0080	0.0009	0.0070	0.0600	0.0789
	3	0.0007	0.0002	0.0002	0.0017	0.0002	0.0001	0.0080	0.0007	0.0070	0.0600	0.0788
G	1	0.0002	0.0002	0.0002	0.0014	0.0001	0.0001	0.0070	0.0007	0.0080	0.0700	0.0879
	2	0.0002	0.0002	0.0002	0.0014	0.0001	0.0001	0.0070	0.0009	0.0080	0.0700	0.0881
	3	0.0002	0.0002	0.0002	0.0014	0.0001	0.0001	0.0070	0.0008	0.0080	0.0600	0.0780
H	1	0.0002	0.0002	0.0002	0.0013	0.0001	0.0001	0.0080	0.0009	0.0000	0.0000	0.0110
	2	0.0002	0.0002	0.0002	0.0013	0.0001	0.0001	0.0080	0.0009	0.0000	0.0000	0.0110
	3	0.0002	0.0002	0.0002	0.0013	0.0001	0.0001	0.0080	0.0009	0.0000	0.0000	0.0110
I	1	0.0009	0.0002	0.0002	0.0014	0.0002	0.0001	0.0080	0.0009	0.0000	0.0000	0.0119
	2	0.0008	0.0002	0.0002	0.0014	0.0002	0.0001	0.0080	0.0009	0.0000	0.0000	0.0118
	3	0.0008	0.0002	0.0002	0.0014	0.0002	0.0001	0.0080	0.0009	0.0000	0.0000	0.0118
J	1	0.0002	0.0003	0.0002	0.0013	0.0001	0.0001	0.0090	0.0007	0.0000	0.0000	0.0119
	2	0.0002	0.0002	0.0002	0.0011	0.0001	0.0001	0.0090	0.0009	0.0000	0.0000	0.0118
	3	0.0002	0.0002	0.0002	0.0012	0.0001	0.0001	0.0090	0.0008	0.0000	0.0000	0.0118

TEFs = toxic equivalent factors for PAHs, TEQs = toxic equivalency quotient, $\sum_{10}$TEQ = total quotient of individual PAHs concentration in each herbal species (Ciemniak et al. 2019; Fred-Ahmadu & Benson 2019; Lin & Zhu 2004; Nisbet & LaGoy. 1992), ND = not detected

TABLE 9. The toxic equivalency quotient (TEQ) calculated for each brand of *M. oleifera*

BrandS	Ratio	TEQs										$\sum_{10}$TEQs
		Ace	Flu	Phe	Ant	Fluo	Pyr	BaA	Chr	BbF	BaP	
A	2:250	0.0002	0.0001	0.0001	0.0009	0.0001	0.0001	0.0000	0.0000	0.0000	0.0000	0.0015
	20:250	0.0002	0.0002	0.0001	0.0010	0.0001	0.0001	0.0000	0.0000	0.0000	0.0000	0.0017
	10:100	0.0002	0.0002	0.0001	0.0011	0.0001	0.0001	0.0000	0.0000	0.0000	0.0000	0.0018
	10:50	0.0002	0.0002	0.0002	0.0012	0.0001	0.0001	0.0000	0.0000	0.0000	0.0000	0.0020
B	2:250	0.0001	0.0001	0.0001	0.0008	0.0001	0.0001	0.0050	0.0005	0.0050	0.0000	0.0118
	20:250	0.0002	0.0002	0.0001	0.0010	0.0001	0.0001	0.0060	0.0006	0.0060	0.0000	0.0143
	10:100	0.0002	0.0002	0.0001	0.0010	0.0001	0.0001	0.0070	0.0007	0.0060	0.0500	0.0654
	10:50	0.0002	0.0002	0.0002	0.0011	0.0001	0.0001	0.0080	0.0008	0.0070	0.0600	0.0777
C	2:250	0.0005	0.0004	0.0002	0.0012	0.0001	0.0001	0.0000	0.0000	0.0050	0.0000	0.0075
	20:250	0.001	0.0010	0.0002	0.0018	0.0001	0.0001	0.0050	0.0005	0.0060	0.0000	0.0157
	10:100	0.0018	0.0014	0.0003	0.0019	0.0001	0.0001	0.0060	0.0006	0.0070	0.0000	0.0192
	10:50	0.0020	0.0018	0.0003	0.0021	0.0001	0.0001	0.0070	0.0008	0.0070	0.0500	0.0712
D	2:250	0.0005	0.0005	0.0001	0.0009	0.0001	0.0000	0.0000	0.0000	0.0000	0.0000	0.0021
	20:250	0.0010	0.0010	0.0002	0.0010	0.0001	0.0001	0.0000	0.0006	0.0000	0.0000	0.0040
	10:100	0.0011	0.0020	0.0003	0.0012	0.0001	0.0001	0.0000	0.0007	0.0050	0.0500	0.0605
	10:50	0.0015	0.0025	0.0003	0.0013	0.0001	0.0001	0.0000	0.0008	0.0060	0.0600	0.0726
E	2:250	0.0001	0.0001	0.0001	0.0009	0.0001	0.0001	0.0000	0.0000	0.0000	0.0000	0.0014
	20:250	0.0001	0.0002	0.0001	0.0012	0.0001	0.0001	0.0050	0.0000	0.0000	0.0000	0.0068
	10:100	0.0001	0.0002	0.0002	0.0013	0.0002	0.0001	0.0060	0.0005	0.0050	0.0000	0.0136
	10:50	0.0001	0.0002	0.0002	0.0014	0.0002	0.0001	0.0070	0.0006	0.0060	0.0500	0.0658
F	2:250	0.0002	0.0001	0.0001	0.0011	0.0001	0.0001	0.0000	0.0000	0.0000	0.0000	0.0017
	20:250	0.0002	0.0001	0.0001	0.0012	0.0001	0.0001	0.0050	0.0000	0.0000	0.0000	0.0068
	10:100	0.0004	0.0002	0.0001	0.0013	0.0001	0.0001	0.0060	0.0005	0.0050	0.0000	0.0137
	10:50	0.0006	0.0002	0.0002	0.0014	0.0002	0.0001	0.0070	0.0006	0.0060	0.0500	0.0663
G	2:250	0.0001	0.0001	0.0001	0.0009	0.0001	0.0001	0.0000	0.0000	0.0000	0.0000	0.0014
	20:250	0.0001	0.0002	0.0002	0.0010	0.0001	0.0001	0.0000	0.0000	0.0050	0.0000	0.0067
	10:100	0.0002	0.0002	0.0002	0.0011	0.0001	0.0001	0.0050	0.0005	0.0060	0.0500	0.0634
	10:50	0.0002	0.0002	0.0002	0.0012	0.0001	0.0001	0.0060	0.0006	0.0070	0.0600	0.0756
H	2:250	0.0001	0.0001	0.0001	0.0008	0.0001	0.0001	0.0000	0.0005	0.0000	0.0000	0.0018
	20:250	0.0002	0.0001	0.0002	0.0009	0.0001	0.0001	0.0050	0.0006	0.0000	0.0000	0.0072
	10:100	0.0002	0.0001	0.0002	0.0011	0.0001	0.0001	0.0060	0.0007	0.0000	0.0000	0.0085
	10:50	0.0002	0.0002	0.0002	0.0012	0.0001	0.0001	0.0070	0.0008	0.0000	0.0000	0.0098
I	2:250	0.0002	0.0001	0.0002	0.0009	0.0001	0.0001	0.0000	0.0005	0.0000	0.0000	0.0021
	20:250	0.0003	0.0001	0.0002	0.0011	0.0001	0.0001	0.0050	0.0006	0.0000	0.0000	0.0075
	10:100	0.0007	0.0002	0.0002	0.0012	0.0001	0.0001	0.0060	0.0007	0.0000	0.0000	0.0092
	10:50	0.0008	0.0001	0.0002	0.0013	0.0002	0.0001	0.0070	0.0008	0.0000	0.0000	0.0105
J	2:250	0.0001	0.0001	0.0001	0.0008	0.0001	0.0001	0.0050	0.0000	0.0000	0.0000	0.0063
	20:250	0.0001	0.0002	0.0002	0.0009	0.0001	0.0001	0.0060	0.0005	0.0000	0.0000	0.0081
	10:100	0.0002	0.0002	0.0002	0.0010	0.0001	0.0001	0.0070	0.0006	0.0000	0.0000	0.0094
	10:50	0.0002	0.0002	0.0002	0.0011	0.0001	0.0001	0.0080	0.0007	0.0000	0.0000	0.0106

TEFs = toxic equivalent factors for PAHs, TEQs = toxic equivalency quotient, $\sum_{10}$TEQ = total quotient of individual PAHs concentration in each herbal species (Ciemniak et al. 2019; Fred-Ahmadu & Benson 2019; Lin & Zhu 2004; Nisbet & LaGoy 1992), ND = not detected

Conclusions

The mean of a total of 10 PAHs ($\sum_{10}$PAHs) in *M. oleifera* dried leaves (5.51 µg/kg) is higher than in *M. oleifera* infusion (4.81 µg/kg). The maximum benzo[a] pyrene content in infusion (0.00-0.33 µg/kg) is lower than in dried leaves (0.38 µg/kg). The sum of four PAHs (benzo[a]pyrene, benzo[a]anthracene, benzo[b] fluoranthene, and chrysene) in infusion (0.15-0.29 µg/kg) was found lower than in dried leaves (2.26 µg/kg). PAHs content in *M. oleifera* dried leaves and infusions complied with the maximum limit set by Commission Regulation (EU) No. 2015/1933 (Commission 2015). The consumption of tested commercial *M. oleifera* tea is considered safe.

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