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Polycyclic Aromatic Hydrocarbons (PAHs) Levels and Toxicity in Herbal Teas Marketed in Malaysia using QuEChERS and GC-FID

(Tahap dan Ketoksikan Hidrokarbon Polisiklik Aromatik (PAH) dalam Teh Herba di Pasaran Malaysia menggunakan QuEChERS dan GC-FID)

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ABSTRACT

In line with the growing health trend in Malaysia, more consumers drink herbal tea for medicinal benefits. However, herbal tea products could be contaminated with polycyclic aromatic hydrocarbons (PAHs) from various production sources. There is a little study focused on the detection of PAHs in herbal tea species distributed in Malaysia. This study was performed to investigate PAHs content and toxicity in selected commercial herbal teas in Malaysia. A total of seven different Malaysian herbal tea samples were extracted using QuEChERS extraction method and the contamination level of PAHs were evaluated using gas chromatography (GC) with a flame ionization detector (FID). The total content of 10 PAHs ($\sum_{10}$PAHs) in the herbal tea samples ranged from 2.53 to 9.39 µg/kg. Acenaphthene, fluorene, phenanthrene and anthracene were the most abundant compounds with 53% contribution of all PAHs content. All tested herbal teas species showed low toxic equivalency (TEQ) values ranging from 0.0027 to 0.1148. The least contaminated samples were *Strobilanthes crispus, Senna alata, Orthosiphon aristatus, Clinacanthus nutans,* and *Stevia rebaudiana*.

Keywords: Gas chromatography - flame ionization detector; herbal tea; polycyclic aromatic hydrocarbons; QuEChERS; toxic equivalency; toxic equivalent factors

ABSTRAK

Seiring dengan perkembangan amalan kesihatan yang semakin meningkat di Malaysia, lebih ramai pengguna meminum teh herba untuk manfaat perubatan. Walau bagaimanapun, produk teh herba boleh tercemar dengan hidrokarbon polisiklik aromatik (PAH) daripada pelbagai sumber. Kajian yang memfokuskan pada pengesanan PAHs dalam spesies teh herba yang diedarkan di Malaysia adalah sedikit. Penyelidikan ini dijalankan untuk mengkaji kandungan PAH dan ketoksikannya dalam teh herba komersial terpilih di Malaysia. Sebanyak tujuh sampel teh herba Malaysia yang berbeza telah diekstrak menggunakan kaedah pengekstrakan QuEChERS dan tahap pencemaran PAH telah dinilai menggunakan kromatografi gas (GC) dengan pengesan pengionan nyalaan (FID). Jumlah kandungan 10 PAH ($\sum$10PAH) dalam sampel teh herba ialah antara 2.53 hingga 9.39 µg/kg. Asenaftena, fluorena, fenantrena dan antracena adalah sebatian yang paling banyak dengan sumbangan 53% daripada semua kandungan PAH. Semua spesies teh herba yang diuji menunjukkan nilai kesetaraan toksik (TEQ) yang rendah antara 0.0027 hingga 0.1148. Sampel yang paling kurang tercemar ialah *Strobilanthes crispus* (pecah beling), *Senna alata* (gelenggang), *Orthosiphon aristatus* (misai kucing), *Clinacanthus nutans* (belalai gajah) dan *Stevia rebaudiana* (stevia).

Kata kunci: Faktor kesetaraan toksik; hidrokarbon polisiklik aromatik; kesetaraan toksik; kromatografi gas - pengesan pengionan nyalaan; QuEChERS; teh herba

Introduction

Herbal teas are popular dietary beverages made from medicinal plants that contain biologically active substances (Maria 2020). Some of the herbal teas are formulated with the combination of the tea plant (*Camelia sinensis*) with herbal plants such as jasmine (*Jasminum officinale*) and cinnamon (*Cinnamomum verum*). Jasmine tea is prepared by mixing the jasmine flower with green

tea (*Camelia sinensis*) leaves until their scent and flavors have been absorbed (Lin, Tu & Zhu 2005).

Consumers drink herbal tea mainly for its medicinal benefits such as antioxidant, anticarcinogenic, and antimutagenic (Fred-Ahmadu & Benson 2019). The screening on biological activities showed that *O. aristatus* has antioxidant properties (Vijayan et al. 2017). Studies showed that the ethanolic extract of *S. rebaudiana* has high antimicrobial and antioxidant properties (Ramya et al. 2014). In Malaysia, a study conducted using normal and streptozotocin-induced hyperglycemic rats showed that the aqueous extract of *S. crispus* tea has antihyperglycemic activities (Fadzelly, Asmah & Fauziah 2006).

Despite the potential health-promoting properties, aroma and flavor from herbal tea can be contaminated with undesirable compounds that might be dangerous to human health (Cacho et al. 2014). Among them, polycyclic aromatic hydrocarbons (PAHs) are among the most dangerous organic compounds due to carcinogenic and mutagenic potential for human health (Fred-Ahmadu & Benson 2019). PAHs are a group of contaminants produced by vehicle emission, industrial activities or industrial food processing (Mañana-López et al. 2021). PAHs chemical compounds contain only carbon and hydrogen atoms forming two or more fused aromatic rings (Lin, Tu & Zhu 2005). Light PAHs (e.g., naphthalene, fluorene, anthracene, acenaphthene, phenanthrene, fluoranthene, chrysene and benz[a]anthracene) compounds that consist of two to four rings are easy to vaporize compared to heavy PAHs (e.g., benzo[a]pyrene, benzo[b]fluoranthene and dibenzopyrene) compounds with five or more aromatic rings. Heavy PAHs have low volatility in the air, low solubility in water (Plaza-Bolaños et al. 2010) and are more toxic than light PAHs (Ciemniak et al. 2019).

The occurrence and toxicity of PAHs in green, black, and herbal teas commercialized in Nigeria showed that herbal teas have the highest carcinogenic and mutagenic potency factors than green and black teas (Fred-Ahmadu & Benson 2019). Among four species of herbal tea commercialized in Poland, linden tea showed the PAHs occurrence within the range of toxicological concerns (Ciemniak et al. 2019). The maximum limits for the sum of four PAHs (benzo[a]pyrene, benzo[a] anthracene, benzo[b]fluoranthene and chrysene) in dried herbs in accordance with Commission Regulation (EU) No. 2015/1933 of 27 October 2015 may not exceed 50 µg/kg. As for benzo[a]pyrene, the maximum limit in the dried herb is 10 µg/kg (Commission 2015). Based on the Agents Classified by the IARC (International for Research on Cancer Classification for Carcinogenicity) monographs (2016), benzo(a)pyrene was classified in group 1 as carcinogenic to humans. Each congener of PAHs exhibits a different level of toxicity and may be determined using toxic equivalent factors (TEF) (Ciemniak et al. 2019; Fred-Ahmadu & Benson 2019; Lin, Tu & Zhu 2005; Nisbet & LaGoy 1992). The total toxicity of these different congeners in each sample is expressed using toxic equivalency (TEQ) (Ciemniak et al. 2019; Nisbet & LaGoy 1992). PAHs content has been reported in various beverages from different countries. Only light PAHs with three to four rings were detected in 8 brands of Chinese tea products (Lin, Tu & Zhu 2005). Medicinal herbs and tea infusion in China were reported to contain 1.02 to 236.02 ng/g (Deng et al. 2021) and 0.21 to 0.62 µg/L (Zhou, Zhang & Wang 2021) of PAHs, respectively. PAHs content in tea infusion ranged from 52.9 to 2226.0 ng/L (Ciemniak et al. 2019) and 57 to 696 µg/kg (Roszko et al. 2018) were reported in Poland (Ciemniak et al. 2019) also found that herbal and fruit teas were the least polluted with PAHs compared to tea made from *C. sinensis* (green, red and white teas). Meanwhile in Chile, the PAHs content in tea infusion was ranged from 0.4 µg/L to 16.3 µg/L (Rivera-Vera et al. 2019). A study conducted in Spain reported that there is PAHs content in tea infusion (1.2 to 151.7 ng/L) and herbal beverages (11.5 ng/L) (Mañana-López et al. 2021). Herbal tea from a study conducted in Spain was reported to contain PAHs with a concentration of 0.65 ng/g (Lee et al. 2018).

The presence of essential oil in tea infusion was the factor that increased the PAHs solubility in water (Ciemniak et al. 2019). The content of PAHs compound in black tea increased after the drying process because the tea leaves absorbed smoke from the combustion of firewood (Lin & Zhu 2004). Green, herbal and black tea consumed in Nigeria were reported to contain PAHs (benzo[a]pyrene, benzo[a]anthracene, benzo[b] fluoranthene and chrysene) levels ranging from 1.28 to 44.57, 44.34 to 11.20 and 0.76 to 34.82 µg/kg, respectively (Fred-Ahmadu & Benson 2019). The detection of 16 PAHs in 10 medicinal plants from Syria was achieved using a reversed-phase HPLC coupled with the UV and fluorescence detector. Naphthalene and acenaphthylene were the most abundant compounds with more than 80% of all PAHs (Krajian & Odeh 2013). Different procedures of headspace sportive extraction were applied to analyze PAHs from black tea, green tea, lime flower, chamomile, and red tea. The authors

found a high PAHs level in the chamomile sample with a concentration of about 30 µg/L (Cacho et al. 2014). Long-term effects of PAHs exposure can cause cataracts, kidney and liver damage, breathing problems, and lung function abnormalities (Abdel-Shafy & Mansour 2016). A previous animal study conducted on mice reported that exposure to 16 PAHs mixture ranging from 0.5 to 1875 µg/kg resulted in neurotoxic oxidative effects such as DNA damage, spatial learning and memory abilities, and long-term potentiation (Kuang et al. 2022). In laboratory studies, the combination of light PAHs (1-methylanthracene and fluoranthene) and benzo[a] pyrene showed a co-carcinogenic effect in human lung epithelial cells (Bauer et al. 2022). Mice exposed to 5-500 ng/kg of phenanthrene (one of the most abundant PAHs in the environment) for a total of 210 days suffered from chronic kidney injury and fibrosis (Ruan et al. 2021).

There is little information regarding PAHs levels and toxicity in local herbal teas available in Malaysia. Among beverages, only coffee (Kasim et al. 2012; Loh et al. 2016, 2020), apple juice (Tan & Loh 2020), drinking water, mineral water, tea beverage, and tea infusion (*C. sinensis*) (Yih et al. 2020) were reported in Malaysia. Dispersive liquid-liquid microextraction (DLLME) technique combined with a high-performance liquid chromatography-fluorescence detector (HPLC-FLD) has been applied for extraction of three types of PAHs from green tea, chrysanthemum tea and coffee beverages (Loh et al. 2016). One of the PAHs (phenanthrene) was extracted using a headspace membrane-protected liquid-phase microextraction (HS-MP-LPME) combined with high-performance liquid chromatography-fluorescence detector (HPLC-FLD) in green tea and coffee beverage (Loh et al. 2020). A total of seven PAHs have been detected in drinking water, mineral water, tea beverage and tea infusion (Yih et al. 2020). The 10 most abundant PAHs in the environment selected from 16 Environmental Protection Agency (EPA) PAHs (Hamidi et al. 2016) were included in this study. Therefore, this study aimed to determine the 10 PAHs levels and toxicity in seven species of the commercialized herbal teas using the Quick, Easy, Cheap, Effective, Rugged and Safe (QuEChERS) sample preparation method followed by the Gas Chromatography separation and Flame Ionization Detection (GC-FID). The toxicity was assessed using the Toxic Equivalency Quotient (TEQ) (Ciemniak et al. 2019; Fred-Ahmadu & Benson 2019; Nisbet & LaGoy 1992). The QuEChERS extraction method developed by (Anastassiades et al. 2003) is fast, cost-effective, and environmentally friendly. It also generate small volume of waste (Mañana-López et al. 2021). The QuEChERS method was selected to reduce preparation time and minimize solvent consumption (Mañana-López et al. 2021; Zachara, Gałkowska & Juszczak 2018). QuEChERS has been widely used for extraction of PAHs in various food samples including fish (Ramalhosa et al. 2009), baby food (Petrarca & Godoy, 2018) and tea (Sadowska-Rociek, Surma & Cieślik 2014; Tfouni et al. 2018; Zachara, Gałkowska & Juszczak 2018).

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) and Strong Anion Exchange (SAX) sorbents were derived from Agilent Technologies, USA.

Samples Collection

Seven different herbal tea samples were purchased from local supermarkets in Malaysia: *J. officinale* (flower)*; S. crispus* (leaves)*; C. verum* (bark)*; S. rebaudia* (leaves)*; O. aristatus* (leaves and stem)*; C. nutans* (leaves) *and S. alata* (leaves). These seven herbal species were selected considering their common and traditional usage in Malaysia. For each herbal tea species, 3 different brands were obtained. The herbal tea samples were homogenized by crushing using a blender (Panasonic MX799S).

Determination of PAHs in Herbal Teas

The QuEChERS method was carried out using an optimized preparation method for PAHs determination in black, green, red and white tea adopted from Sadowska-Rociek, Surma and Cieślik (2014).

Samples Preparation

The homogenized herbal tea samples (2 g) were placed into a 50 mL polypropylene tube, and the hot water (10

mL) was added and cooled to room temperature (27 °C). 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 1 min at 10,500 rpm. A 6 mL aliquot of upper phase was transferred into the 15 mL polypropylene tube containing 0.15 g of Primary Secondary Amine (PSA), 0.15 g of Strong Anion Exchange (SAX) and 0.9 g of magnesium sulfate anhydrous. The mixture was vortexed for 1 min and then centrifuged for 5 min at 8000 rpm. Then, the upper phase (4 mL) 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 all extracted herbal tea 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 method quality control, *S. rebaudiana* was chosen as a spiked sample prepared by spiking herbal tea with the mixed PAH standard solution before being extracted using the same procedure used for herbal tea samples. Triplicate spiked samples were made and the percentage recovery was calculated.

TOXICITY OF PAHs

The total toxicity of all PAHs in each herbal tea species 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 below:

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

where TEQ is the total toxicity of different congeners of PAHs in each sample and *C*s is the concentration of PAHs congener. TEFs is 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 below:

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

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

The optimized QuEChERS-based extraction method (Sadowska-Rociek, Surma & Cieślik 2014) was applied as the analytical procedure to determine PAHs in herbal tea samples. In this study, herbal teas were selected from two categories according to tea processing mode; the first mode was the herbal tea with a combination of dried tea leaves (*C. sinensis*) and the second mode was 100% of herbal tea as stated on the product label. *J. officinale* (flower) and *C. verum* (bark) were produced from the first mode and another five herbal tea (*S. rebaudia* (leaves); *S. crispus* (leaves); *O. aristatus* (leaves and stem); *C. nutans* (leaves) and *S. alata* (leaves)) were produced from the second mode. In the first processing mode, tea leaves were used as a tea base (Xu et al. 2021). Table 1 reports the mean concentration of individual PAHs obtained from the studied herbal teas. The mean total of 10 PAHs ($\sum_{10}$PAHs) in herbal tea samples was varied between 2.53 and 9.39 µg/kg in *S. crispus* and *C.*

verum tea samples, respectively. These concentrations were considered as not harmful to consumers since carcinogenic PAHs compound (benzo[a]pyrene) was detected at a trace level. Acenaphthene, fluorene, phenanthrene, anthracene, fluoranthene, and pyrene were the most abundant compounds with 95% contribution of all PAHs content in this study. PAHs with five rings become less abundant with a content of 2 %. According to PAHs classified by International Agency for Research on Cancer (IARC), fluorene, phenanthrene, anthracene, fluoranthene, and pyrene have been unclassifiable as to carcinogenicity in humans (Group 3) (IARC, 2021). The PAHs compound classified as probably carcinogenic to humans (Group 2A) and possibly carcinogenic to humans (Group 2B) were found at a trace level in this study. No specific permissible concentration was stated by this agency, only substances and exposure circumstances have been classified in this list. Flouranthene was the highest PAH compound detected in all herbal tea species. Source analysis indicated that the high content of fluoranthene in all herbal tea species mainly came from the general uses of this compound. Fluoranthene is used as an intermediary in agricultural products such as synthetic fertilizers, hormones, and soil conditioning agents.

The recovery method was carried out by using *S. rebaudiana* tea sample. Figure 1 shows the GC-FID chromatogram of *S. rebaudiana* tea sample spiked with fluorene and acenaphthene at 10 µg/kg. The percentage recovery for acenaphthene and fluorene were 77% and 85%, respectively. The limit of detection (LOD) and limit of quantification (LOQ) were calculated as 3 and 10 times the standard deviation (SD), as shown in Table 2. LOD ranged from 0.08 to 0.17 µg/kg, while LOQ extended from 0.24 to 0.51 µg/kg.

The highest PAHs compound was found in *C. verum*, followed by *J. officinale* with approximately 30% and 16% contribution to the total of 10 PAHs concentration in the herbal tea samples, respectively. These two herbal teas were formulated with a combination of herbal tea and tea leaves (*C. sinensis*) as observed on the product labels. The drying and manufacturing process of tea leaves (*C. sinensis*) were most likely the main factors of PAHs contamination (Adisa et al. 2015).

Tukey HSD analysis results (Table 3) showed that total of 10 PAHs values in different brands of the same herbal tea species are insignificantly different from each other ($p > 0.05$). These insignificant differences might be due to no variation in the production process and environment contamination level among different brands and suppliers.

As shown in Table 4, Tukey's HSD test was applied to each herbal tea species to analyze which of them exhibits statistically significant differences. *C. verum* showed a statistically significant difference ($p < 0.01$) in a total of 10 PAHs levels between different herbal tea species evaluated. The high PAH content obtained in *C. verum* can be explained by the fact that *C. verum* was mixed with tea leaves during the production process. This is because tea leaves are commonly obtained from different locations of plantations and suppliers. This factor may expose tea leaves to different contamination sources such as chemical treatment of crops and post-harvest treatment.

Table 5 showed 10 PAHs content in herbal tea studied by different authors. Based on seven species of the selected herbs in this study, only jasmine tea was similar to herbal tea reported by other authors. The authors found a high level of 10 PAHs compound in jasmine tea with a total concentration of 1038.51 µg/kg (Lin, Tu & Zhu 2005). In this study, the mean total 10 PAHs concentration in jasmine tea was 4.83 µg/kg. Compared to the results presented in this study and by other authors, benzo[b]fluoranthene and benzo[a]pyrene content were the lowest with a total mean concentration of 1.14 µg/kg and 0.91 µg/kg, respectively. Roselle tea from Syria (Krajian & Odeh 2013) contains the lowest concentration of acenaphthene, anthracene, fluoranthene, and pyrene at concentrations of 2.10 µg/kg, 0.71 µg/kg, 2.89 µg/kg and 1.55 µg/kg, respectively. Jasmine tea from a study in China (Lin, Tu & Zhu 2005) has demonstrated the highest content of benz[a]anthracene, benzo[b]fluoranthene, chrysene, and benzo[a]pyrene with a total concentration of 151.91 µg/kg. The geographical factor affects the PAH content because the quality of soil and environmental exposure source (industrial activities, natural emissions from volcanoes and forest fires, and atmospheric deposition) (Abdel-Shafy & Mansour 2016) were different in the different countries.

The total toxicity of all PAHs in each herbal tea species is shown in Table 6. Each congener of PAHs exhibits a different level of toxicity and may be determined using toxic equivalent factors (TEF) (Ciemniak et al. 2019; Fred-Ahmadu & Benson 2019; Lin, Tu & Zhu 2005; Nisbet & LaGoy 1992). The toxicity expressed by TEQs depended mainly on the content of compounds belonging to the group of heavy PAHs. All tested herbal tea species showed the lower TEQ values due to PAHs congeners with a high TEF value such as benzo[a]pyrene, chrysene, benzo(b)fluoranthene, and

benz[a]anthracene were detected at trace levels of PAHs (below LOD). The TEQ values ranged from 0.0027 to 0.1148 in all herbal tea species. The total TEQ value for benzo[a]pyrene and four carcinogenic PAHs were 0.9100 and 1.1507, respectively. The values indicate that consuming herbal tea from the study product may not cause adverse health effect. The maximum limit for benzo[a]pyrene and sum of four PAHs (benzo[a] pyrene, chrysene, benzo(b)fluoranthene and benz[a] anthracene) were 10 µg/kg and 50 µg/kg, respectively. These maximum allowable limits were set for dried herbs by Commission Regulation (EU) 2015/1933 of 27 October 2015 (Commission 2015) and can be used as preliminary guidelines in herbal teas consumption (Joint & WHO Expert Committee on Food Additives). This result indicates that no risk of carcinogenicity can be expected from the consumption of tested herbal tea since TEQ values for carcinogenic PAHs (benzo[a] pyrene) were very low.

In this study, the most dangerous PAHs compound containing 4-5 rings (benzo[a]anthracene, benzo[b] fluoranthene, chrysene, benzo[a]pyrene) rings were detected at trace level (<LOD) in all herbal tea samples. Overall, the results of 10 PAHs compounds obtained in this study were lower than those reported by other authors. The results indicate that the selected Malaysian herbal tea products are less contaminated with PAHs compounds. This is primarily due to differences in the location of herbal tea plantations, processing methods, and herbal tea product ingredients.

TABLE 1. Results obtained in the analysis of herbal tea samples (µg/kg)

Compound	Brand	Mean of PAHs content in herbal tea species (µg/kg)										$\sum_{10}$PAHs
		Ace	Flu	Phe	Ant	Fluo	Pyr	BaA	Chr	BbF	BaP	
J. officinale	A	ND	ND	ND	1.330	1.960	1.700	ND	ND	ND	ND	4.990
	B	0.120	0.110	0.140	1.140	1.620	1.510	0.080	0.080	0.090	0.060	4.950
	C	0.150	0.140	0.130	1.250	1.440	1.140	0.070	0.080	0.070	0.070	4.540
S. crispus	A	ND	ND	ND	ND	1.220	1.480	ND	ND	ND	ND	2.700
	B	0.130	0.120	0.110	0.110	0.810	1.010	0.090	0.090	0.080	0.070	2.620
	C	0.140	0.150	0.140	0.120	0.710	0.880	0.080	0.070	0.090	0.070	2.530
C. verum	A	2.490	2.340	ND	2.240	1.210	1.110	ND	ND	ND	ND	9.390
	B	2.310	2.100	0.150	2.120	1.080	1.010	0.080	0.090	0.090	0.060	9.090
	C	2.340	2.080	0.130	2.140	1.010	0.960	0.080	0.090	0.080	0.070	8.980
S. rebaudiana	A	1.610	3.080	ND	ND	ND	ND	ND	ND	ND	ND	4.690
	B	1.240	2.590	0.150	0.100	0.110	0.100	0.090	0.090	0.090	0.070	4.630
	C	1.320	2.010	0.120	0.110	0.100	0.100	0.090	0.090	0.080	0.060	4.080
O. aristatus	A	ND	ND	ND	ND	1.980	1.400	ND	ND	ND	ND	3.380
	B	0.120	0.150	0.140	0.110	1.150	1.020	0.080	0.090	0.080	0.060	3.000
	C	0.110	0.130	0.110	0.120	1.180	1.050	0.090	0.070	0.090	0.070	3.020
C. nutans	A	0.660	ND	ND	ND	1.230	1.970	ND	ND	ND	ND	3.860
	B	0.360	0.140	0.150	0.110	1.070	1.150	0.080	0.090	0.070	0.060	3.280
	C	0.410	0.150	0.130	0.120	1.190	1.180	0.070	0.070	0.060	0.060	3.440
S. alata	A	ND	ND	3.350	ND	ND	ND	ND	ND	ND	ND	3.350
	B	0.160	0.150	2.010	0.110	0.110	0.100	0.090	0.090	0.080	0.070	2.970
	C	0.150	0.140	2.110	0.120	0.100	0.090	0.080	0.080	0.090	0.060	3.100

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

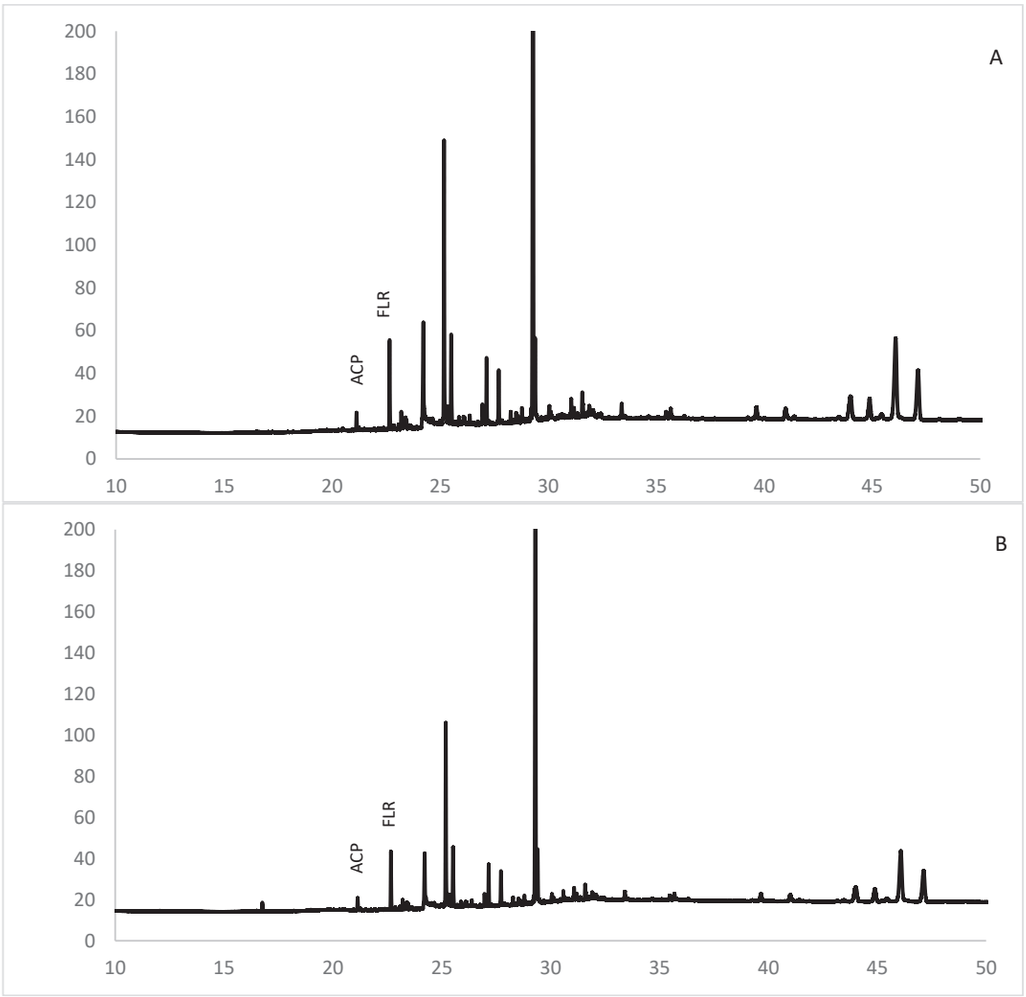

FIGURE 1. GC-FID chromatograms of (A) spiked with acenaphthene and fluorene at 10 μgkg^{-1} and (B) unspiked of *S. rebaudiana* tea sample. Column: Select PAH capillary column (30 m × 0.25 mm × 0.36 mm). Detection by flame ionization detector (FID). ACP: Acenaphthene; FLR: Fluorene

TABLE 2. Limit of detection (LOD) and quantification (LOQ), linearity of each PAHs

PAHs	Regression analysis	LOD (µg/kg)	LOQ (µg/kg)	R^2
Acenaphthene	y = 1.4791x + 0.0537	0.17	0.51	0.9999
Fluorene	y = 1.3216x + 0.2843	0.16	0.48	0.9999
Phenanthrene	y = 1.2917x + 0.2642	0.16	0.48	0.9999
Anthracene	y = 1.3217x + 0.1872	0.13	0.39	0.9999
Fluoranthene	y = 1.3217x + 0.1842	0.12	0.36	0.9999
Phyrene	y = 1.3217x + 0.0843	0.12	0.36	0.9999
Benz[a]anthracene	y = 1.3217x + 0.0989	0.10	0.30	0.9999
Chrysene	y = 1.3217x + 0.1041	0.10	0.30	0.9999
Benzo[b]fluoranthene	y = 1.3217x + 0.0841	0.10	0.30	0.9999
Benzo[a]pyrene	y = 1.3215x + 0.0857	0.08	0.24	0.9999

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

TABLE 3. Tukey HSD test for each brand in all herbal tea species

Herbal species	Treatments Pair for each brand	Tukey HSD Q statistic	Tukey HSD p-value	Tukey HSD Inference
	A vs B	0.0184	0.899995	insignificant
J. officinale	A vs C	0.2068	0.899995	insignificant
	B vs C	0.1884	0.899995	insignificant
	A vs B	0.06	0.899995	insignificant
S. crispus	A vs C	0.1875	0.899995	insignificant
	B vs C	0.1275	0.899995	insignificant
	A vs B	0.0947	0.899995	insignificant
C. verum	A vs C	0.1294	0.899995	insignificant
	B vs C	0.0347	0.899995	insignificant
	A vs B	0.0219	0.899995	insignificant
S. rebaudiana	A vs C	0.2227	0.899995	insignificant
	B vs C	0.2008	0.899995	insignificant
	A vs B	0.2213	0.899995	insignificant
O. aristatus	A vs C	0.2097	0.899995	insignificant
	B vs C	0.0116	0.899995	insignificant
	A vs B	0.3412	0.899995	insignificant
C. nutans	A vs C	0.2471	0.899995	insignificant
	B vs C	0.0941	0.899995	insignificant
	A vs B	0.1514	0.899995	insignificant
S. alata	A vs C	0.1315	0.899995	insignificant
	B vs C	0.0199	0.899995	insignificant

TABLE 4. Turkey HSD test comparison for each herbal tea species

Treatment Pair for each species	Tukey HSD Q statistic	Tukey HSD p-value	Tukey HSD Inference
J. officinale vs *S. crispus*	16.011	0.0010053	P<0.01
J. officinale vs *C. verum*	31.3458	0.0010053	p<0.01
J. officinale vs *S. rebaudiana*	2.6081	0.5387405	insignificant
J. officinale vs *O. aristatus*	12.2678	0.0010053	p<0.01
J. officinale vs *C. nutans*	9.4182	0.0010053	p<0.01
J. officinale vs *S. alata*	12.2195	0.0010053	p<0.01
S. crispus vs *C. verum*	47.3567	0.0010053	p<0.01
S. crispus vs *S. rebaudiana*	13.4028	0.0010053	p<0.01
S. crispus vs *O. aristatus*	3.7431	0.1837519	insignificant
S. crispus vs *C. nutans*	6.5928	0.005216	p<0.01
S. crispus vs *S. alata*	3.7914	0.1743301	insignificant
C. verum vs *S. rebaudiana*	33.9539	0.0010053	p<0.01
C. verum vs *O. aristatus*	43.6136	0.0010053	p<0.01
C. verum vs *C. nutans*	40.764	0.0010053	p<0.01
C. verum vs *S. alata*	43.5653	0.0010053	p<0.01
S. rebaudiana vs *O. aristatus*	9.6597	0.0010053	p<0.01
S. rebaudiana vs *C. nutans*	6.8101	0.0039563	p<0.01
S. rebaudiana vs *S. alata*	9.6114	0.0010053	p<0.01
O. aristatus vs *C. nutans*	2.8496	0.4490796	insignificant
O. aristatus vs *S. alata*	0.0483	0.8999947	insignificant
C. nutans vs *S. alata*	2.8013	0.4671641	insignificant

TABLE 5. Ten PAH contents in herbal samples reported in previous studies

Sampling place	PAHs content (μg/kg)									
	ACP	FLR	PHE	ANT	FLT	PYR	B[a]A	B[b]F	Chry	B[a]P
China (Fred-Ahmadu & Benson 2019)										
Natural liver flush tea	NT	NT	NT	NT	NT	NT	BDL	BDL	BDL	6.56
Tranquilizing and brain nourishing tea	NT	NT	NT	NT	NT	NT	4.24	BDL	6.89	11.07
Antihypertensive tea	NT	NT	NT	NT	NT	NT	1.20	BDL	2.53	8.48
Joint care tea	NT	NT	NT	NT	NT	NT	1.10	BDL	5.37	28.35
Kidney flush tea	NT	NT	NT	NT	NT	NT	BDL	BDL	BDL	4.03
Anticancer tea	NT	NT	NT	NT	NT	NT	BDL	BDL	BDL	8.40
China (Lin, Tu & Zhu 2005)										
Jasmine tea	177.00	53.00	231.00	56.30	196.00	173.00	67.30	54.00	45.9	28.10
Nigeria (Fred-Ahmadu & Benson 2019)										
Moringa herbal tea	NT	NT	NT	NT	NT	NT	BDL	BDL	BDL	0.76
India (Fred-Ahmadu & Benson 2019)										
Sahul slim herbal tea	NT	NT	NT	NT	NT	NT	10.22	1.97	20.21	NT
Syria (Krajian & Odeh 2013)										
Hollzhock	35.16	8.87	37.28	1.76	2.14	8.11	0.94	2.10	2.25	0.22
Chamomile	27.91	14.64	51.80	1.96	11.67	4.93	1.67	0.90	1.25	0.17
Roselle	2.10	5.40	18.01	0.71	2.89	1.55	0.27	0.17	0.78	0.08
Damask rose	19.50	9.67	20.36	0.76	3.23	4.49	0.34	0.65	1.16	0.41
Sage	5.13	17.93	61.56	3.52	141.97	30.74	12.93	1.37	2.54	0.78
Mellissa	10.27	14.90	33.58	2.07	17.97	6.25	0.45	0.29	0.64	0.19
Marjoram	40.90	13.62	37.75	2.47	8.92	6.60	1.38	1.77	7.87	0.41
Rosemary	40.61	8.80	32.99	1.48	51.60	67.90	10.43	1.17	12.24	0.68
Wild thyme	15.68	6.88	26.75	1.91	9.13	3.77	1.19	0.47	1.29	0.49
Mint	41.08	12.02	34.25	2.19	13.26	3.68	1.34	0.60	2.94	0.49

ACP = acenaphthene, FLR = fluorene, PHE = phenanthrene, ANT = anthracene, FLT = fluoranthene, PYR = pyrene, B[a]A = benzo[a]anthracene, B[b]F = benzo[b] fluoranthene, Chry = chrysene, B[a]P = benzo[a]pyrene, BDL = below detection limit, NT = not tested

TABLE 6. The toxic equivalency quotient (TEQ) calculated for each herbal tea species

	TEQs										$\sum_{10}$TEQs
	Ace	Flu	Phe	Ant	Fluo	Pyr	BaA	Chr	BbF	BaP	
TEFs	0.0010	0.0010	0.0010	0.0100	0.0010	0.0010	0.1000	0.0100	0.1000	1.0000	
J. officinale	0.0000	0.0000	0.0000	0.0133	0.0020	0.0017	0.0000	0.0000	0.0000	0.0000	0.0170
	0.0001	0.0001	0.0001	0.0114	0.0016	0.0015	0.0080	0.0008	0.0090	0.0600	0.0926
	0.0002	0.0001	0.0001	0.0125	0.0014	0.0011	0.0070	0.0008	0.0070	0.0700	0.1002
S. crispus	0.0000	0.0000	0.0000	0.0000	0.0012	0.0015	0.0000	0.0000	0.0000	0.0000	0.0027
	0.0001	0.0001	0.0001	0.0011	0.0008	0.0010	0.0090	0.0009	0.0080	0.0700	0.0911
	0.0001	0.0002	0.0001	0.0012	0.0007	0.0009	0.0080	0.0007	0.0090	0.0700	0.0909
C. verum	0.0025	0.0023	0.0000	0.0224	0.0012	0.0011	0.0000	0.0000	0.0000	0.0000	0.0295
	0.0023	0.0021	0.0002	0.0212	0.0011	0.0010	0.0080	0.0009	0.0090	0.0600	0.1058
	0.0023	0.0021	0.0001	0.0214	0.0010	0.0010	0.0080	0.0009	0.0080	0.0700	0.1148
S. rebaudiana	0.0016	0.0031	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0047
	0.0012	0.0026	0.0002	0.001	0.0001	0.0001	0.0090	0.0009	0.0090	0.0700	0.0941
	0.0013	0.002	0.0001	0.0011	0.0001	0.0001	0.0090	0.0009	0.0080	0.0600	0.0826
O. aristatus	0.0000	0.0000	0.0000	0.0000	0.0020	0.0014	0.0000	0.0000	0.0000	0.0000	0.0034
	0.0001	0.0002	0.0001	0.0011	0.0012	0.0010	0.0080	0.0009	0.0080	0.0600	0.0806
	0.0001	0.0001	0.0001	0.0012	0.0012	0.0011	0.0090	0.0007	0.0090	0.0700	0.0925
C. nutans	0.0007	0.0000	0.0000	0.0000	0.0012	0.002	0.0000	0.0000	0.0000	0.0000	0.0039
	0.0004	0.0001	0.0002	0.0011	0.0011	0.0012	0.008	0.0009	0.0070	0.0600	0.0800
	0.0004	0.0002	0.0001	0.0012	0.0012	0.0012	0.0070	0.0007	0.0060	0.0600	0.0780
S. alata	0.0000	0.0000	0.0034	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0034
	0.0002	0.0002	0.002	0.0011	0.0001	0.0001	0.0090	0.0009	0.0080	0.0700	0.0916
	0.0002	0.0001	0.0021	0.0012	0.0001	0.0001	0.0080	0.0008	0.0090	0.0600	0.0816

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

CONCLUSIONS

PAHs levels and toxicity in seven herbal tea samples widely consumed in Malaysia were successfully evaluated. The content of 10 PAHs in seven herbal tea products ranged from 2.53 µg/kg to 9.39 µg/kg. This finding indicates that the concentration of a total of 10 PAHs in tested herbal tea species was lower than in other studies cited in the literature. The TEQ values were

ranged from 0.0027 to 0.1148 in *S. crispus* and *C. verum*, respectively. There are no adverse health concerns from the consumption of tested herbal tea products. The herbal tea mixed with tea leaves (*C. sinensis*) contains a greater PAHs value than the single herbal tea. Therefore, it is recommended that consumers consider drinking herbal tea with less or no added ingredients that will contribute to PAH contamination.

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