ORIGINAL ARTICLE

Assessing the Risk of Estragole Consumption From Natural Products in the Malaysian Market by Using the Margin of Exposure Approach

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ABSTRACT

Introduction: Estragole is a naturally-occurring phytochemical found in variety of herbs, and spices, commonly used as food ingredients and in various natural products too. Despite some studies suggesting that estragole has health benefits, animal studies have shown that estragole is a hepatotoxic and genotoxic carcinogen, mainly due to the formation of its metabolites, specifically 1'-hydroxyestragole. **Objective:** This study aims to perform health risk assessment of estragole in natural products from Malaysia by using the margin of exposure (MOE) approach. **Methods:** 30 samples of natural product were obtained from Malaysian market using targeted sampling strategy. All of the samples were extracted using methanol extraction and analyzed using Ultra Performance Liquid Chromatography (UPLC). The estimated daily intake (EDI) was calculated by using the quantified estragole concentrations and the recommended daily intake as stated on the product label. To assess the risk associated with estragole, the MOE approach was employed. **Results:** 9 out of 30 samples were found to contain estragole with levels ranging from 55.03 to 418.02 µg/g. The EDI values of estragole in these samples ranged from 0.99 - 9.44 µg/kg bw/day. Using the MOE approach, all positive samples were found to have MOE values less than 10,000 for long-term consumption, indicating high priority for risk management. However, for shorter-than-lifetime scenarios, the MOE values were above 10,000, indicating low priority for risk management. **Conclusion:** The presence of estragole in Malaysian natural products may raise health concern, especially when it is consumed daily over extended period.

Keywords: Natural product; Risk assessment; Estragole; Alkenylbenzenes; Margin of exposure

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INTRODUCTION

Plants play a crucial role in human nutrition and have medicinal properties. Throughout history, humans have used plants as remedies for various ailments (1). However, despite the rising demands of natural products and plant food supplements (PFS) consumers in the developing countries, studies on their efficacies, safety and potential harmful effects are lacking (2). Due to the general perception that herbal products and PFS are "natural" and "safe", they are becoming increasingly popular worldwide, especially among specific demographics such as children, women, and cancer patients. PFS are dietary supplements which contain plant and botanical extracts, commonly sold in the form of capsules or tablets and are designed to supplement a person's regular diet (3). Many consumers prefer these products due to their perceived ability to improve health and prevent disease, without causing any adverse effects (4). Nevertheless, caution must be exercised while consuming these products since the research on their effectiveness and safety is still limited.

The Malaysian market for dietary supplement has recorded significant growth from RM 2.07 billion (488 million USD) in 2014 to RM 3.1 billion (730 million USD) in 2019 (5). Herbal and traditional medicines account for approximately one-third of the industry, and 50% of Malaysians have used health supplements, with one in every four Malaysians taking them on regular basis, whilst some take them on

alternate days or once a week (6). Herbal treatments are commonly used among Malay women to address both general and gender-specific health concerns such as postpartum care concerns, menstrual abnormalities, and infertility, because they believe that natural products are safer for consumption (7). However, there is growing concern about the unauthorized sales of unregistered health supplements with the Health Ministry seizing RM 9.7 million in unlawful health and beauty products, sold through drop-shippers and e-commerce companies in August 2021 (8). Natural products are widely used in Malaysia due to their low cost, accessibility, and availability, whereby they can be purchased without a prescription at supermarkets, health food stores, pharmacies, and even online markets. People often choose natural products because they believe that "natural" implies "safe." Despite their purported health benefits, these products may contain potentially dangerous compounds such as alkenylbenzenes, which are genotoxic and carcinogenic (2).

Alkenylbenzenes are a group of naturally occurring phytochemicals found in various plants including star anise, anise, parsley, fennel, and sweet fennel. They include estragole, eugenol, safrole, myristicin, elemicin, trans-anethole, apiol, β-asarone (BA) and methyleugenol, and are known to be genotoxic and carcinogenic (4). Estragole, which is one of the alkenylbenzenes can be found in a variety of botanicals, including fennel, lemon balm, tarragon, star anise, anise, sweet basil and betel. A study had found estragole in 5 out of 10 PFS samples, whereby 4 out of 7 samples that tested positive contained more than one type of alkenylbenzenes, such as myristicin and apiol (9). The presence of estragole in natural products may pose health risk, as various studies have shown that it can generate DNA adducts and liver tumours. Animal studies demonstrated that alkenylbenzenes generate DNA adducts and liver tumours by producing reactive 10-sulfoxy metabolites (10). High dosages of estragole, methyleugenol and safrole were found to cause liver tumour in rats, with identical modes of action and tumour formation pathway (11). Since September 2008, the European Commission has banned foods containing pure compounds of estragole, methyleugenol and safrole because of their genotoxic and carcinogenic properties. However, there are currently no specific restrictions regarding the use of supplements containing alkenylbenzenes-producing plants. While it is mandatory for natural products to be registered with the Drug Control Authority (DCA) prior to release in the Malaysian market, estragole is not listed as a prohibited ingredient. As a result, estragole may be present in any natural products approved by Malaysia's National Pharmaceutical Regulatory Agency (NPRA), leading to exposure to its genotoxicity and carcinogenicity effects in consumers.

Humans are exposed to estragole through consumption of foods, drinks, herbal tea and herbal products. Extracts of fennel (*Foeniculum vulgare*) have been used traditionally in nursing babies for prevention of flatulence and stomach spasm (12). Estragole consists of a benzene ring with a methoxy group and a propenyl group, with chemical formula of $C_{10}H_{12}O$, and molecular weight of 148.20 g/mol. Generally, upon consumption, estragole is metabolised in the liver by the CYP1A2 enzymes to form several metabolites via three major metabolic pathways. In the first pathway, estragole undergoes O-demethylation to produce 4-allylphenol and detoxified. Secondly, the allyl side chain undergoes epoxidation to form estragole-2′,3′-epoxide that is metabolised rapidly and excreted. Thirdly, estragole undergoes 1′-hydroxylation to form 1′-hydroxyestragole which further oxidise to 1′-oxoestragole. 1′-hydroxyestragole also undergoes glucuronidation to form 1-O-glucuronide. It also conjugates with sulphate to form 1′-sulfooxyestragole via the SULT1A1 enzyme. These metabolites are highly reactive and capable of forming adducts with GSH and endogenous amines and also DNA. The Committee on Herbal Medicinal Products (HMPC) had concluded that the genotoxicity and carcinogenicity of estragole is similar to safrole (13). Figure 1 shows the structure of these chemicals.

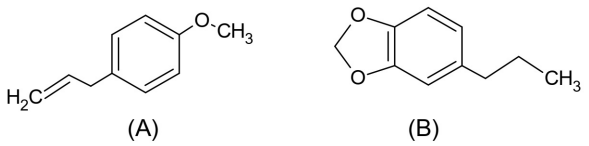


Figure 1 : Chemical structure of estragole (A) and safrole (B).

Hence, this study aims to evaluate the health risk of estragole present in Malaysian natural products, using the margin of exposure (MOE) approach. The World Health Organization (WHO) and the European Food Safety Authority (EFSA), support the use of MOE technique as a risk assessment tool for carcinogenic and genotoxic substances (14). The MOE refers to the ratio of estimated human exposure to the reference point based on dose-response data from experiments or epidemiological studies. The findings from this study can be utilized to inform risk management, allowing regulatory efforts to mitigate possible dangers associated with a compound's intake to be prioritized.

MATERIALS AND METHODS

Chemicals and reagents

4-Allylanisole (purity >98%) were purchased from Sigma-Aldrich (USA). Acetonitrile (ACN) and methanol (UPLC/MS grade) were obtained from Biosolve

(Valkenswaard, the Netherlands). Trifluoroacetic acid (TFA) (≥99.5%) were purchased from Fisher Scientific (United Kingdom). Nanopure water was obtained from Thermo Scientific water system.

Sample collection

A targeted sampling approach was employed, in which only natural products containing estragole-producing plants in the ingredient list were selected for analysis. The process started by performing a literature review to compile a list of estragole-producing plants. Estragole occurs naturally in the plant groups Apiaceae, Asteraceae, Magnoliaceae, Lamiaceae, and Piperaceae; for this study, products with *Foeniculum vulgare*, *Illicium verum*, *Pimpinella anisum*, *Piper betle*, *Melissa officinalis* and *Ocimum basilicum* in the ingredients list were selected for the analysis. A total of thirty samples were purchased from physical and online retailers. Detailed information of samples, such as the scientific and vernacular names of the botanical ingredients, dose recommended by manufacturer are summarized in Table I.

Extraction of estragole in natural products

Each sample was extracted using a modified methanol extraction procedure based on previous research (15). To improve the efficiency of extraction, all solid samples were ground to form fine particle or powder. A total of 10 ml of methanol was added to the sample, followed by 15 minutes ultra-sonification at room temperature. Next, the solution was centrifuged for 20 minutes at 20,000 rpm and supernatant was filtered using 0.22 µm syringe filter into a Ultra High Performance Liquid Chromatography (UPLC) vials, and stored at -80°C until analysis to ensure its integrity. The extraction and UPLC analysis were carried out in three independent experiments.

Quantification of estragole using UPLC

Detection and quantification of estragole were achieved based on the method described by Alajlouni et al. with minor modifications (3). The UPLC used was an Agilent UPLC-DAD system, comprising of a solvent manager, and photodiode array detector, and equipped with a Waters ACQUITY UPLC BEH Shield

Table I : List of the natural products samples used in the current study. The information was derived from the product labels.

Sample	Suspected ingredients	Vernacular names in English and Malay	Weight per unit (mg)	Recommendation for daily intake	Indication
S1	*Foeniculum vulgare*	Fennel/*Jintan hitam*	250	1 capsule, 2-3 daily after meal	For health and body strengthening
S2	*Foeniculum vulgare*	Fennel/*Jintan hitam*	250	1-2 capsules each time after food. 2 times daily	For health and body strengthening
S3	*Foeniculum vulgare*	Fennel/*Jintan hitam*	500	Chew 2 tablets each time, 3 times daily after meal	For general health
S4	*Foeniculum vulgare; Ocimum basilicum*	Fennel/*Jintan hitam;* Basil/*Selasih*	350	2 capsules, twice a day	For dyspepsia, flatulence, and indigestion
S5	*Foeniculum vulgare*	Fennel/*Jintan hitam*	600.57	1 tablet, once daily	To support eye health
S6	*Illicium verum*	Star anise/*Bunga lawang*	500	1 capsule after meal (morning and evening)	To relieve muscular pain, waist pain, joint pain, and mild constipation
S7	*Illicium verum*	Star anise/*Bunga lawang*	500	1 capsule after meal (morning and evening)	To regulate menstruation, relieve menstrual pain and vaginal discharge, constipation, and joint pain
S8	*Illicium verum*	Star anise/*Bunga lawang*	500	1 capsule after meal (morning and evening)	To regulate menstruation, relieve menstrual pain and vaginal discharge, constipation, and joints pain
S9	*Pimpinella anisum*	Anise/*Jintan manis*	1000	1-2 tablets, 3 times per day	To treat phlegm, coughs and sore throat
S10	*Pimpinella anisum*	Anise/*Jintan manis*	500	1 capsule, 2 times per day	To relieve weak limbs, smoothen blood circulation, and relieve mild pain in joints and muscles

S11	*Pimpinella anisum*	Anise/*Jintan manis*	250	2 capsules, 2 times per day	For men's health and energy, to relief waist pain, body weakness, joints pain, improve blood circulation, urination, and bowel movement
S12	*Pimpinella anisum*	Anise/*Jintan manis*	281.5	2 capsules, 2 times per day	To treat menstrual irregularities, headaches, increase blood circulation, and menstruation
S13	*Pimpinella anisum*	Anise/*Jintan manis*	300	2 capsules, 2 times per day	To relieve menstrual pain, mild itch, regulate menstruation
S14	*Pimpinella anisum, Piper betle*	Anise/*Jintan manis;* Betel/*Sirih*	500	4 capsules per day	To promote general well-being and strengthen body following child birth
S15	*Pimpinella anisum*	Anise/*Jintan manis*	400	2 capsules, 2 times per day	For general health
S16	*Pimpinella anisum*	Anise/*Jintan manis*	250	2 capsules, once per day	For menstruation and regulate blood circulation
S17	*Piper betle*	Betel/*Sirih*	350	2 capsules, 2 times per day	To regulate blood circulation, relief of cough, fever
S18	*Piper betle*	Betel/*Sirih*	570	3 capsules once daily after meal	To enhance menstrual flow, relieve menstrual pain, vaginal discharge, and flatulence
S19	*Foeniculum vulgare*	Fennel/*Jintan hitam*	500	2 capsules, 2 times per day	To improve menstrual flow for women after childbirth
S20	*Foeniculum vulgare*	Fennel/*Jintan hitam*	500	2 capsules, 2 times per day	For men's health and energy, relieving waist ache, body weakness, and joints pain
S21	*Foeniculum vulgare*	Fennel/*Jintan hitam*	560	1 capsule, 3 times daily after food	To enhance menstruation flow, relieve menstruation pain, vaginal discharge
S22	*Foeniculum vulgare*	Fennel/*Jintan hitam*	550	2 capsules, 2 times per day	To regulate menstruation
S23	*Piper betle*	Betel/*Sirih*	500	1-2 capsules, 3 times per day	For women's general health
S24	*Foeniculum vulgare*	Fennel/*Jintan hitam*	650	2 capsules, 2 times per day	To reduce pain and joints fatigue due to excessive uric acid
S25	*Melissa officinalis*	Lemon balm	400.2	1-2 capsule per day	For sleep difficulty
S26	*Piper betle*	Betel/*Sirih*	570	1 capsule, 3 times per day	To reduce excessive mucus in the feminine area
S27	*Foeniculum vulgare*	Fennel/*Jintan hitam*	500	2 capsules, 2 times per day	For women general health
S28	*Foeniculum vulgare; Illicium verum*	Fennel/*Jintan hitam;* Star anise/*Bunga lawang*	300	2 capsules per day	For health and strengthening of the body
S29	*Piper betle*	Betel/*Sirih*	250	2 capsules, 3 times per day	To reduce body heatiness
S30	*Foeniculum vulgare*	Fennel/*Jintan hitam*	2000	1 bottle (2g) each time	For stomach ache, mild diarrhoea, flatulence and vomiting

RP18 column (50 mm x 2.1 mm x 1.7 µm). 0.1% (v/v) ultra-pure water in TFA and 100% acetonitrile were used as mobile phase A and B, respectively. The flow rate was set to 0.6 mL/min. A gradient elution was performed as follows: 0.0 min 69% A/31% B, 5.0 min 69% A/31% B, 8.0 min 20% A/:80%. Temperature of column was maintained at 35.0 °C. Detection of estragole was performed using a photodiode array detector at 201 nm. A 3.5 µL of sample was injected into UPLC system.

An estragole calibration curve was constructed for determination of concentration of estragole in the natural product extracts. First of all, 10 mM stock solution of estragole was prepared in methanol and stored in a refrigerator at 4 °C until further use. The stock solution was further diluted in methanol to produce calibration solutions with concentrations of 25, 50, 100, 125, and 250 µM, in triplicates. Next, the calibration solutions were injected into the UPLC. The response (i.e., peak area) produced from each injection was recorded and plotted against concentration of estragole (uM) using Microsoft Excel software. The coefficient of determination (R2), slope and y-intercept were also recorded. The limit of detection (LOD) and limit of quantitation (LOQ) were determined according to the International Conference of Harmoni-sation Guidance for the Validation of Analytical Procedures: Text and Methodology (Q2) R1 (16). The LOD (Equation 1) and LOQ (Equation 2) were determined based on standard deviation of the response and slope, by using Microsoft Excel software, using the following equations:

$$\text{LOD} = \frac{(3.3 \times \text{residual standard deviation of a regression line})}{\text{Slope}}$$

Equation 1

$$\text{LOQ} = \frac{(10 \times \text{residual standard deviation of a regression line})}{\text{Slope}}$$

Equation 2

Calculation of estimated daily intake (EDI)

To determine the consumer's exposure to estragole in natural products, the EDI was calculated by using the concentration of estragole quantified in each sample, multiplied by the supplier's recommended daily intake and then divided by the average body weight of Malaysian adult, i.e. 66.7 kg (17). To simplify, the following equation was used to determine the EDI (ug/kg bw/day) for each sample:

$$\text{Estimated daily intake (EDI)} = \frac{W \times L}{BW}$$

Equation 3

"W" represents the recommended daily intake of PFS samples, in gram, based on the product label. "L" is the amount of estragole detected in the samples in µg/kg. "BW" is the body weight, which is set to 66.7 kg.

Calculation of the Margin of Exposure

The Margin of Exposure (MOE) approach is widely regarded as the most appropriate method for conducting a risk assessment of estragole, given its well-documented genotoxic and carcinogenic properties. This approach can be utilized for prioritisation of the risk management (18). MOE value is determined by dividing the BMDL10 by the EDI as displayed in Equation 4. Value of MOE of less than 10,000 indicates a high priority for risk management, while a MOE of more than 10,000 indicates low priority. To determine the MOE values for estragole consumed over shorter period of time, the Haber's rule was applied, i.e., k = C x T, where k is the constant toxic response for the chemical, C is the concentration or dose of chemical and T is the exposure duration in weeks (19). The application of Haber's rule combined with the MOE approach is important as it considers the risk of exposure when the exposure is shorter, i.e., only consumed during an illness or stressful event.

$$\text{Margin of exposure (MOE)} = \frac{\text{BMDL10}}{\text{EDI}}$$

Equation 4

"BMDL10" is the lower confidence limit of estragole for the benchmark dose that causes a 10% increase in tumour incidence. The BMDL10 value for estragole is 3300 µg/kg bw/day (4). "EDI" is the estimated daily intake of estragole by humans in µg/kg bw/day.

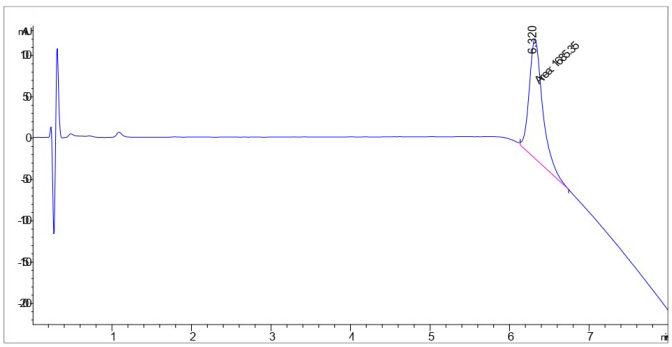
RESULTS

Construction of calibration curve and determination of LOD and LOQ

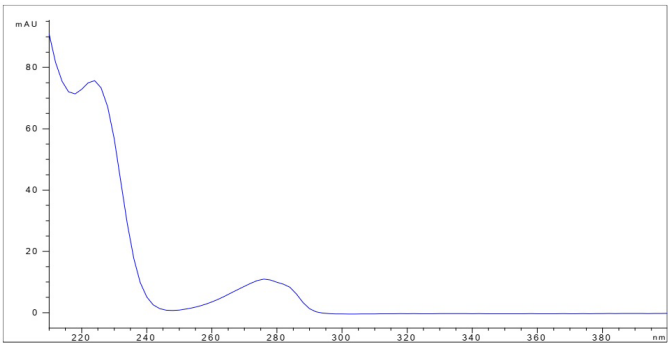
Figure 2 shows the chromatogram of estragole in 250 uM calibration solution and the corresponding UV spectrum. With the chromatographic parameters, estragole eluted between 6.305- 6.327 minutes. The response of the analyte peak area exhibits linearity within the range of 25 to 50 uM of estragole, following the equation of y = 6.653x + 35.286, and demonstrating a high coefficient of determination (R^2) of 0.0023. The LOD and LOQ were determined to be 50.6 µM and 153.4 µM respectively.

Levels of estragole in PFS

To allow risk assessment of estragole in PFS samples, the level of estragole in the samples of natural products were determined. Any sample showing peak at the retention time, and had similar UV spectrum as estragole standard is considered as positive for estragole. To calculate the concentration of estragole in PFS samples, the straight-line equation was used,



(A)



(B)

Figure 2 : (A) Chromatogram of estragole standard in methanol eluted under the chromatographic conditions; **(B)** UV-Spectra of estragole (λ = 201 nm).

and the value was adjusted according to the result for recovery test, i.e., 99.7% recovery from the previous study (15) mentioned in, 2.3 Extraction of estragole in natural products. The concentration of estragole recovered from the samples ranged from 55.03 µg/g to 418.02 µg/g, as shown in Table II.

EDI of estragole resulting from consumption of PFS

Based on the levels of estragole in PFS samples, the exposure to estragole, or EDI for a 66.7 kg person based on the recommended daily intake as indicated on the product's label was calculated, as presented in Table II. The EDI values obtained from positive PFS samples ranged from 0.99 - 9.44 µg/kg bw/day.

Risk assessment of consumption of PFS based on the MOE approach

The MOE values for the nine samples were obtained using Equation 4 and depicted in Figure 3. According to the EFSA-defined MOE approach, all MOE values are based on lifetime exposure which is 75 years. Based on the result obtained, all positive samples have MOE values less than 10 000. Among the nine samples with MOE values under 10,000, S18 had the lowest MOE values, followed by S24 and S22. Given that all the positive samples had MOEs of less than 10,000, it is clear that minimizing potential risks is necessary before taking these supplements over an extended period of time. Figure 4 depicts the MOE values that would result from shorter-than-lifetime consumption, i.e., assuming two- and six-weeks consumption. The results show that two- and six-weeks consumption of these PFS would not raise concern as their MOE values were more than 10,000.

DISCUSSION

In the present study, nine (9) out of thirty (30) PFS samples obtained from Malaysian market were found to contain estragole at various levels, from 55.0 to 418.0 µg/g. Many other studies have also found estragole and other types of alkenylbenzenes at various levels in different types of samples. For example, safrole (3.8 – 22.2 µg/g), estragole (13.3 – 23.9 µg/g), methyleugenol (15.5 – 128.6 µg/g), and myristicin (33.9 – 440.1 µg/g) were found in 92% of Indonesian jamu samples (20), all of the pesto sauce containing basil samples analyzed by Al-Malahmeh et al. were positive with alkenylbenzenes, namely apiol (3.4 µg/g), estragole (3.2 – 34.1 µg/g), methyleugenol (13.2 – 15.8 µg/g), and myristicin (3.6 – 99.3 ug/g) (11), 84.6% of PFS containing nutmeg samples contained

Table II : Summary of estragole presence in positive samples and Estimated Daily Intake (EDI).

Sample number	Recommended daily intake (g) of the PFS	Estragole concentration (ug/g)	EDI (µg/kg bw/day)
S13	1.2	55.03 ± 26.2	0.99
S16	0.5	418.02 ± 272.1	3.13
S18	1.71	368.20 ± 186.6	9.44
S19	2.0	61.47 ± 31.6	1.84
S20	2.0	90.82 ± 34.6	2.72
S22	2.2	229.35 ± 116.5	7.56
S23	1.5	290.85 ± 143.6	6.54
S24	2.6	233.60 ± 145.8	9.11
S26	1.71	164.72 ± 28.1	4.22

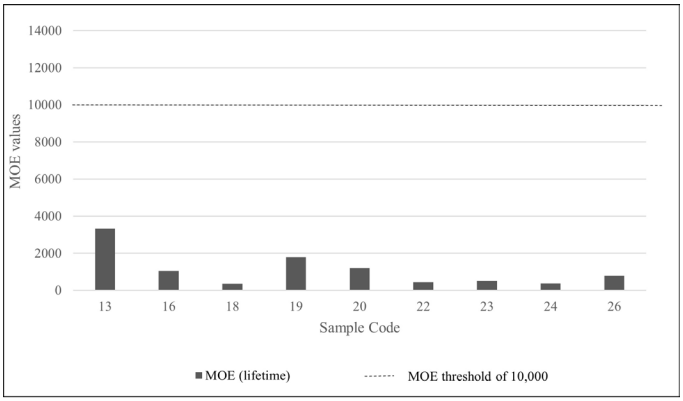


Figure 3 : MOE values for lifetime consumption of estragole from PFS.

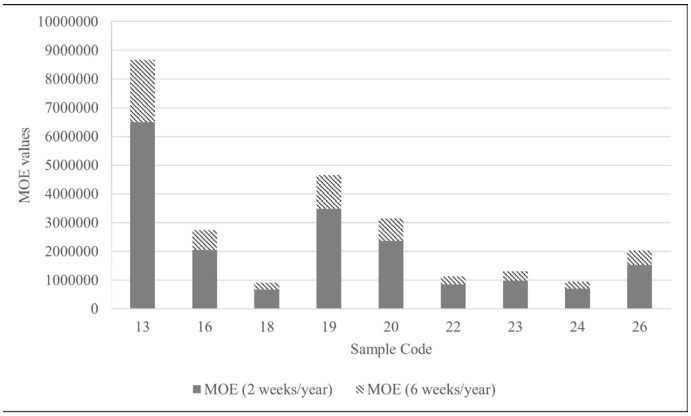


Figure 4 : MOE values assuming 2-weeks and 6-weeks consumption of estragole.

elemicin (59 – 9655 µg/g), methyleugenol (84 – 2814 µg/g), myristicin (13 – 101125 µg/g), and safrole (182 – 10494 µg/g) (3). Analysis on different types herbal teas and beverages also found various levels of alkenylbenzenes, such as in fennel tea (12) whereby all of the samples were positive with estragole (120 – 1029 µg/g), parsley and dill-based tea which were positive with apiol (18.5 – 667.9 µg/g), estragole (302.6 µg/g), methyleugenol (33.2 – 575.4 µg/g), and myristicin (64.6 – 1269.8 µg/g), and instant herbal beverages in Indonesia contained methyleugenol (2.6 – 443.7 µg/g) and eugenol (21.4 – 101.2 µg/g) (21).

The MOE values for the positive samples in this study varied according to the three exposure scenarios. When consumed as recommended by the manufacturer daily for 75 years, the MOE values were lower than 10,000 indicating potential risk to human health. On the other hand, shorter-than-lifetime consumption, i.e., two- and six- weeks consumption resulted in MOE much higher than 10,000. However, there are instances where the consumption of certain products may extend beyond the typical two to six-week timeframe. For example, herbal medicines intended for the treatment of cough and cold symptoms may be used repeatedly whenever an individual falls ill. Similarly, women may take PFS to treat menstrual symptoms and during postpartum which can extend beyond six weeks during lifetime. As a result, it is crucial to determine the safe duration of use to ensure that individuals can the benefit from these products while minimizing possible risks.

Genotoxicity and carcinogenicity of estragole is caused by the reactive metabolites that are produced in dose-dependent manner (13), therefore higher concentration of estragole will result in higher risk. The concentration of estragole in botanicals is influenced by various factors, including plant species geographical location, genetic factors, harvesting time, drying method and processing and manufacturing circumstances (9). This study revealed the presence of estragole in samples with concentration levels ranging from 0.99- 418.02 µg/g. Among the nine (9) estragole-positive samples, four samples contained suspected botanicals, *Foeniculum vulgare*, also known as fennel, which accounted for 27% of the total dietary intake of estragole (12). In another study, estragole was found in 37 of the 71 samples containing fennel (22). Eight (8) samples contained *Pimpinella anisum* or anise, also contain estragole naturally (13). Other samples contain other botanicals which are known to contain other types of alkenylbenzene. For example, *Piper betle* or betle leaf contains safrole (13,23), *Ocimum basilicum* or basil contains apiol, estragole, methyleugenol, and myristicin (11) and Illicium verum or star anise contains methyleugenol, and trans-anethole (24). Although *Melissa officinalis* does not produce genotoxic phytochemicals naturally, an extensive study have shown that Melissa tea contained hepatotoxic, mutagenic, and genotoxic pyrrolizidine alkaloids, probably due to contamination (25). However, the concentration of these compounds was not quantified in the current study, therefore the overall concentration of genotoxic and carcinogenic compounds, and EDI could be higher, and could result in lower MOE.

Although there are no human studies on estragole carcinogenicity, a study confirmed in their study that the major metabolites of estragole found in animals were also produced in humans, namely 1′-hydroxyestragole, p-allyphenol. 20% of the estragole dose administered was detectable in the urine samples in form of the two urinary metabolites, while the remaining 80% could be metabolized to others including 1′-oxoestragole, oxidation products of 1′-hydroxyestragole, and DNA, GSH or protein adducts (12). In fact, estragole undergoes 1′-hydroxylation very rapidly where the highest concentration of conjugated 1′-hydroxyestragole was detected in the urine samples after 1.5 hours of consumption. Furthermore, physiologically-based kinetic (PBK) model for

estragole for Caucasian and Chinese population has already been developed and validated (26,27). Both models predicted similar overall formation of 1′-hydroxyestragole which reaches peak plasma concentration at 0.5 to 2 hours. These models are considered adequate for prediction of estragole metabolism in humans, and can be extended for use in another ethnic group. Therefore, there is strong evidence that the metabolic activation and formation of DNA adducts of estragole demonstrated in rodents also occurs in humans.

Regulatory agencies, such as the European Commission (EU) and Australia's Therapeutic and Goods Administration (TGA), have established rules and limitations on the use of estragole in various of products due to the potential health risk associated with the compounds. TGA recognizes the acute toxicity of estragole in rats and potential health risk of its carcinogenic metabolites, 1′-hyroxyestragole, and thus restricts fennel oil and basil oil to contain less than 5% of estragole (28). Similarly, the European Union regulates estragole in food products, and recommends reductions in exposure and restrictions in use (29). The Committee on Herbal Medicinal Products (HMPC) under the European Medicines Agency (EMA) recommends using estragole less than 0.05 mg/person per day for adults, and 1.0 µg/kg bw for children (13). However, no such regulation or restriction for estragole in food products or natural products exist in Malaysia, putting Malaysians at risk of developing health issues.

The research conducted indicates that the Malaysian population is at risk of exposure to estragole through the consumption of natural products that are easily accessible, including through online platforms such as Lazada and Shopee. According to a recent survey, 22.4% of consumers purchase herbal products online, probably due to the convenience (7). In a recent survey, 71.89% of respondents had used herbal products in the previous year, which is consistent with recent research that evaluated the prevalence of use among university students in Klang Valley (30). Furthermore, the rise in the agricultural sector for medicinal plants, and licensing of natural products demonstrate the increasing demands for natural products. These factors, together with the lack of awareness on safety and risk associated with natural products, increases the potential of toxicity reactions, including hepatoxicity and carcinogenicity of estragole. The research findings suggests that lifelong exposure to estragole in some products had resulted in MOE values of less than 10,000, which is a high priority for risk management. However, short term consumption at two- and six-weeks in a lifetime resulted in MOE exceeding 10,000. Nevertheless, consideration needs to be given to the significant number of natural products marketed for general well-being or to alleviate symptoms of common illness that may occur multiple times in a lifetime.

In the future, it is important to investigate the impact of combined exposure from different types of alkenylbenzenes in natural products, such as methyleugenol, eugenol and safrole. This will allow thorough risk assessment to determine the overall effects on alkenylbenzenes on the health of Malaysians. Furthermore, the national regulatory agency should consider to implement limitations and restriction on the use of alkenylbenzene-containing plants in natural products.

CONCLUSION

In conclusion, estragole, a genotoxic and carcinogenic compound, can be found in natural products available on Malaysian market. Prolonged consumption of these products results in low MOE values, indicating priority in risk management. However, short-term exposure to estragole, i.e., two- and six- weeks to estragole from these products is not considered a high priority in risk management. Nonetheless it is important to exercise caution for some types of products, especially products indicated for women's health, which may be consumed more regularly.

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