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Health risks evaluation of mycotoxins in plant-based supplements marketed in Malaysia

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Mycotoxins are toxic secondary metabolites produced by fungi, pose significant health risks when present in plant-based supplements (PBS), necessitating thorough risk assessment to ensure consumer safety. This study evaluates the health risks associated with mycotoxins, specifically aflatoxins (AFB_1, AFB_2) and ochratoxin A (OTA), in PBS sold in Malaysia. Contamination levels of AFB_1, AFB_2, and OTA were quantified in 14 PBS samples using Liquid Chromatography-Mass Spectrometry. All samples tested positive for AFB_2, while 28.57% and 42.86% tested positive for AFB_1 and OTA, respectively with some levels exceeding the regulatory limits set by the Malaysian Food Act 1983 and European regulations. The estimated daily intake of these mycotoxins was calculated based on the recommended daily intake of each supplement. To assess risk, Margin of Exposure (MOE) values were determined, showing that all AFB_2-positive samples had MOE values below the critical threshold of 10,000, indicating an urgent need for risk management. A quantitative cancer risk assessment also estimated the percentage of hepatocellular carcinoma and kidney cancer attributable to mycotoxin exposure. The findings emphasize the significant public health risks posed by mycotoxins, particularly in samples B2 and B10, where all three mycotoxins studied were present at concerning levels. This study highlights the urgent need for stricter regulations and better monitoring of mycotoxin levels in PBS to protect consumer's health.

Keywords Mycotoxin, Aflatoxin, Ochratoxin, Risk assessment, Supplement, Margin of exposure

Abbreviations

AFB_1	Aflatoxin B1
AFB_2	Aflatoxin B2
EDI	Estimated daily intake
EFSA	European food safety authority
HCC	Hepatocellular carcinoma
JECFA	Joint FAO/WHO expert committee on food additives
LB	Lower bound
MOE	Margin of exposure
OTA	Ochratoxin A
PBS	Plant-based supplements
UP	Upper bound

Mycotoxins, toxic secondary metabolites produced by various fungi, present a significant public health issue, particularly in regions like Malaysia, where plant-based supplements (PBS) are regularly consumed. These supplements, derived from natural sources, are susceptible to contamination by mycotoxins, such as aflatoxins, which have been associated with serious health effects, including hepatotoxicity and carcinogenicity[1,2]. As the

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use of herbal and natural supplements rises in Malaysia, concerns about the safety and quality of these products, particularly regarding mycotoxin contamination, have expanded[3]. Research has identified aflatoxins, particularly aflatoxin B_1 (AFB_1), a potent carcinogen, in many herbal products, posing substantial health risks to consumers who rely on these supplements[4]. In addition, the co-occurrence of multiple mycotoxins such as aflatoxin B2 (AFB_2) and ochratoxin A (OTA) in the product can lead to additive or synergistic toxic effects complicating the assessment of health risks associated with these supplements[5]. These risks are intensified by inadequate storage practices and lacking regulatory oversight, which contribute to fungal growth and contamination[2,6]. Strengthening regulatory frameworks and improving public education are essential steps toward ensuring the safety of PBS[7].

Upon ingestion, mycotoxins like AFB_1 are primarily metabolized in the liver, where they are converted into highly reactive intermediates. These intermediates form DNA adducts, such as aflatoxin-N7-guanine, which play a major role in promoting mutagenesis and carcinogenesis[8]. The genotoxicity of aflatoxins is widely documented, with studies showing that these toxins induce oxidative stress and DNA strand breakage, contributing to severe health outcomes, including liver cancer[9]. The interaction between aflatoxins and other environmental factors, such as hepatitis B virus infection, further elevates the risk of hepatocellular carcinoma (HCC). Understanding these interactions is essential to assessing the comprehensive risks associated with mycotoxin exposure[10].

Aflatoxins and OTA are among the most significant mycotoxins due to their widespread occurrence and pronounced toxicological effects. Studies reveal that these mycotoxins frequently co-occur in various food commodities, posing health risks. For instance, a study in Côte d'Ivoire highlighted a high prevalence of co-contamination, with multiple mycotoxins detected in foodstuffs, including OTA and AFB_1[11]. Furthermore, the interaction between these two toxins has been shown to increase their toxic effects. Research indicates that co-exposure to OTA and AFB_1 results in synergistic toxicity, amplifying cytotoxic and genotoxic impacts compared to individual exposures[12,13]. These effects are particularly concerning in vulnerable populations, such as children, where dietary exposure can lead to significant health risks[14,15].

In response to these public health concerns, regulatory agencies have set limits on mycotoxin levels in food products to minimize exposure. In Malaysia, the Ministry of Health regulates mycotoxins, with aflatoxin levels in certain food products set at 5 μg/kg[16]. Globally, organizations such as the Codex Alimentarius Commission and the European Commission have established stringent guidelines to control mycotoxin contamination, defining maximum residue limits for mycotoxins in various food items which are 5 μg/kg for AFB_1, 10 μg/kg for total aflatoxins, and 3 μg/kg for OTA[17,18]. However, challenges remain, particularly due to Malaysia's tropical climate, which promotes fungal growth and subsequent mycotoxin production. Inadequate storage and transportation conditions worsen contamination risks, highlighting the necessity for more stringent regulations and enhanced food safety measures[19].

Malaysia's tropical climate, marked by high humidity and temperatures, provides suitable conditions for the growth of mycotoxigenic fungi, such as *Aspergillus* and *Fusarium*[17]. This climatic condition along with inadequate post-harvest practices, raises the possibility of mycotoxin contamination in food products, including PBS. Research has shown that the occurrence of aflatoxins and other mycotoxins in agricultural products is significantly affected by these environmental factors[17]. The economic consequences of mycotoxin contamination in Malaysia are also considerable, leading to reduced agricultural productivity and increased healthcare costs from foodborne illnesses. With increasing temperatures due to climate change, the frequency and severity of mycotoxin contamination are forecasted to increase[20].

The public health burden of mycotoxin exposure in Malaysia is demonstrated by the rising incidence of liver cancer, which has been linked to dietary aflatoxin exposure. Studies indicate that AFB_1 is a significant contributor to liver cancer, particularly in areas with increased incidence of hepatitis B virus infection[21]. Malaysia is among the countries with the highest liver cancer incidence, with dietary aflatoxin exposure recognized as a major risk factor[22]. Beyond cancer, chronic exposure to mycotoxins has been associated with other health concerns, such as stunting and immune dysfunction[23]. The combined effects of exposure to multiple mycotoxins further worsen these health risks, highlighting the necessity for thorough risk assessments and targeted public health interventions[24].

The risk assessment of mycotoxins using Margin of Exposure (MOE) approach is essential for understanding their health impacts. Studies have highlighted alarming MOE values for aflatoxins; for example, in Indonesia, MOEs in maize and peanuts samples were generally below 10,000, signifying substantial health risks[25]. A systematic review further estimated that chronic aflatoxin exposure could contribute to 4.6% to 28.2% of global HCC cases, emphasizing the urgent need for effective risk management[26]. In Tanzania, recent studies revealed that aflatoxin-induced liver cancer poses a significant health burden, with MOE values pointing to serious concerns for those consuming contaminated food[27]. The MOE approach remains a critical tool for guiding regulatory actions and interventions to reduce mycotoxin-related risks[28].

Given the serious health risks associated with mycotoxin contamination in PBS, it is important to mitigate these risks through enhanced regulation, testing, and public education. Malaysia's current regulatory frameworks remain insufficient with limited monitoring and enforcement enabling contaminated products to reach consumers[29]. As the demand for natural supplements increases, so does the urgency for stricter safety standards. In Southeast Asia, where environmental conditions and agricultural practices lead to the high prevalence of mycotoxins, efforts to reduce contamination must prioritize improving storage practices, strengthening regulatory oversight, and raising consumer awareness about the hazards of contaminated supplements[17,30]. Hence, this study aims to assess the health hazard posed by PBS containing mycotoxins using MOE and cancer risk approaches.

Methods

Sample collection

The samples were collected from March to December 2023, focusing on commercially available herbs in Malaysia. A total of 14 samples were obtained from local drugstores and online marketplaces to capture the influence of Malaysia's varying climatic conditions on mycotoxin production. This approach provides a broader understanding of real-world contamination, and we accounted for variability during data analysis to ensure robust findings. In addition, these samples were chosen based on their inclusion in a prior cross-sectional survey that identified the most commonly used herbs in the region[31]. The selection criteria required the products to have been manufactured between 2022 and 2023 to reflect current market conditions. After collection, the samples were sealed in air-tight plastic bags, labelled, and stored at − 20 °C at the Institute of Bioscience, Universiti Putra Malaysia, until further analysis.

Chemicals and reagents

High-performance liquid chromatography (HPLC)-grade methanol and acetonitrile were obtained from R&M Chemicals (United Kingdom) for the extraction and testing of aflatoxins. Standards for AFB_1, AFB_2, and OTA mycotoxins were sourced from Sigma-Aldrich (United States). Reagents for the QuEChERS extraction method, including Magnesium Sulfate ($MgSO_4$) and acetonitrile, were purchased from R&M Chemicals (United Kingdom), while glacial acetic acid was acquired from Merck (Germany). These materials were essential for ensuring high purity and precision in the detection process.

Equipment and testing conditions

Chromatographic separation was performed using an Agilent G6125B LCMS system (Agilent, Santa Clara, USA) with a reversed-phase Supelco C18 (150 mm in length × 4.6 mm in internal diameter, 5 m particle size, Sigma Aldrich in Missouri, USA) with a quaternary pump, a vacuum degasser, an autosampler, and a thermostatic column oven kept at 35 °C. The extract was filtered through a 0.2 mm Nylon Syringe Filter (Corning, Massachusetts, USA) before analysis. The mobile phase consisted of nanopure water containing 0.1% formic acid (v/v)(solvent A) and acetonitrile containing 0.1% formic acid (v/v)(solvent B), applied with gradient elution. The elution starts at a concentration of 95% of solvent B and gradually reduces to 80% over a period of 1 min. Afterwards, it subsequently reduced to 75% during an interval of 1 min and remained at that level for an additional 2 min in isocratic mode. Then, solvent B was adjusted to 0% for a duration of 1 min and then restored at starting condition for an additional 1 min. The system operated at a flow rate of 0.3 mL/min, with an injection volume of 5 µL, and the column temperature was maintained at 40 °C.

Concentrations were determined using an Agilent tandem mass spectrometer, manufactured by Agilent in Santa Clara, USA, operating in electron spray ionization (ESI) positive ion mode. The quantification of the mass spectrometry (MS) system was operated in multiple reaction monitoring (MRM) mode. Details of MS parameters (declustering potential and collision energy), and retention time are described in Table 1.

QuEChERS extraction

The samples were homogenized and weighed to 2.5 g, then mixed with 7.5 mL of ultra-pure water. After homogenization with 10 mL of acetonitrile containing 1% acetic acid, the mixture was shaken for 30 minutes. The extraction process included purification with 4g anhydrous $MgSO_4$ and centrifugation at 1822 *g* for 10 min. The supernatant underwent solid-phase extraction using a dispersive solid-phase extraction (dSPE) tube, followed by shaking and centrifugation at 2200 *g* for 5 minutes. The extract was diluted, filtered, and stored in amber vials at − 20 °C until analysis by LC-MS.

Validation of the testing method

The method was validated by evaluating sensitivity through limits of detection (LOD) and quantification (LOQ), adhering to the criteria established by Afsah-Heji and co-workers[22]. Linearity was assessed by injecting a series of aflatoxin standards at varying concentrations into the LC–MS system and recording the corresponding responses. Recovery tests were performed by spiking samples with known concentrations of AFB_1, AFB_2, and OTA, followed by extraction and calculation of recovery rates. The method's linearity and precision were confirmed, with coefficient of determination (R^2) values exceeding 0.99[32].

Exposure evaluation

The estimated daily intake (EDI) of aflatoxins was calculated based on the concentration of mycotoxins detected in the samples and the average daily consumption rates of the products, according to data from the Malaysian population. The formula used was EDI = (Average concentration of mycotoxins × Average daily consumption)/Average body weight, with a body weight of 60 kg for the Malaysian population[33].

Mycotoxins	Ionization mode	Retention time (mins)	Parent ion (m/z)	Collision energy	Declustering potential
AFB_1	ESI+	6.47	313	55 V	30 V
AFB_2	ESI+	5.13	316		
OTA	ESI+	6.67	404		

Table 1. LCMS condition for AFB_1, AFB_2, and OTA.

Risk characterization

To assess the public health risk, both the Margin of Exposure (MOE) and cancer risk approaches were employed. The MOE was calculated by dividing the benchmark dose lower confidence limit ($BMDL_{10}$) by the EDI. For AFB_1 and AFB_2, the $BMDL_{10}$ is set at 0.4 µg/kg bw/day based on liver tumor incidences in rats[34] while $BMDL_{10}$ is set at 14.5 µg/kg bw/day for OTA based on kidney tumor incidences in rats[35]. An MOE value below 10,000 suggests a potential health concern as defined by the Scientific Committee of the European Food Safety Authority (EFSA) which include the inter-species and intra-species differences, the nature of the carcinogenic process, and the reference point on the dose–response curve factors[36].

Additionally, a quantitative risk assessment for liver cancer was conducted, incorporating the risk associated with HCC and kidney cancer attributable to the consumption of PBS was estimated based on the exposure, and the carcinogenic potency of AFB_1. For AFB_2 and OTA, the in vivo data are not sufficient to derive potency factors. Considering that AFB_1 is the major contributor to the exposure, the same potency as for AFB_1 was assumed for AFs and OTA[37]. The potency values for AFs/OTA estimated by Joint FAO/WHO Expert Committee on Food Additives (JECFA), which corresponded to 0.3 cancers/year/100,000 population/ng/kg body weight/day in HBsAg-positive individuals, and 0.01 cancers/year/100,000 population/ng/kg body weight/day in HBsAg-negative individuals, were adopted for this calculation. A HBsAG positivity rate of 5.24% was determined for the Malaysian population[38]. Therefore, the carcinogenic potency (P_{cancer}) was estimated $P_{cancer} = (0.3 \times 0.054) + (0.01 \times 0.9476) = 0.025$ cancers/year/100,000 individuals/ng AFB_1/kg bw/day.

The estimated risk of cancer in the adult population of Malaysia was calculated based on the total dietary exposure to AFs and OTA in PBS and the average potency of the population using the following equation: Cancer risk = Exposure × Average potency. The percentage of cancer attributable to dietary exposure to AFs and OTA in PBS was calculated as the ratio between the risk of the target population and the age-standardized incidence rate for HCC and kidney cancer per 100,000 population per year for both sexes[39,40].

Results

Collection of plant-based supplements for analysis

Table 2 shows the product description of the PBS analyzed in the present study. All the PBS were bought from physical drug stores and online shopping platforms. All the samples were originated from China and Malaysia but marketed in Malaysia.

Method validation

The LOD and LOQ for this investigation have been determined by evaluating the instrumental linearity. The calibration curves for each analyte may be seen in Supplementary Materials A. The correlation coefficients (R^2) values are consistently over 0.980 in all cases, specifically 0.997 for AFB_1, 0.999 for AFB_2, and 0.997 for OTA. The level of all mycotoxins detected were adjusted from a previous study recommended by a study conducted by Mbisana and co-workers[29] that matches with our present study. The percentage of recovery used was 90.10% for AFB_1, 92.07% for AFB_2, and 99.16% for OTA.

Liquid chromatography with mass spectrometry (LC–MS) analysis

The LC–MS analysis was performed to detect and quantify the presence of AFB_1, AFB_2, and OTA in the PBS. The quantification study revealed the presence of AFB_1, AFB_2, and OTA at 6.4, 5.1, and 6.6 min, respectively (Fig. 1).

Among the 14 samples tested using LC–MS, AFB_1 was detected in 4 samples (28.57%), AFB_2 was detected in all 14 samples (100%), and OTA was detected in 6 samples (42.86%) (Table 3). Both Sample B2 and Sample B10 from the PBS samples were found to have all three contaminants. Sample B2 contains 2.276 µg/kg of AFB_1, 233.073 µg/kg of AFB_2, and 30.565 µg/kg of OTA. The values for AFB_1, AFB_2, and OTA in sample B10 are 9.470 µg/kg, 187.389 µg/kg, and 14.946 µg/kg, respectively. Figure 1 also showed the chromatogram illustrating the co-existence of all three mycotoxins identified in sample B10.

Based on the list of PBS in Table 3, a noteworthy revelation emerged concerning the B2 and B10 PBS samples. These samples exhibited positivity for all tested mycotoxins contaminants, namely AFB_1, AFB_2, and OTA. The botanical ingredients identified in these positive samples encompass *Scutellaria Baicalensis Radix*, *Radix Angelicae*, *Fructus*, and *Semen Arecae*, unveiling a potential correlation between the presence of these botanical elements and the contamination profile. In addition, all samples that were detected positive with mycotoxins were exceeded the European regulatory limit of 10 µg/kg for total aflatoxins and 3 µg/kg for OTA except sample B4 for OTA exposure raises intriguing questions about potential sources and routes of contamination.

Estimated daily intake (EDI)

The average duplicate analysis was utilized to compute the EDI of AFB_1, AFB_2, and OTA. The EDIs were computed based on the quantities of AFB_1, AFB_2, and OTA detected in the PBS (Table 4), following the recommended daily intake by the supplier. The EDI of PBS varied from sample B11 contaminated with AFB_1, at a concentration of 0.000001 µg/kg bw/day, to sample B3 contaminated with AFB_2, at a concentration of 0.064 µg/kg bw/day.

Risk assessment of PBS containing mycotoxins

The risk assessment of PBS was calculated using MOE approach to determine whether the consumption of AFB_1, AFB_2, and OTA through PBS should be considered a high or low priority for risk management (Table 4). MOEs with values below 10,000 are considered a high priority for risk management. It was observed that samples B2, B9, and B10 included AFB_1, all PBS samples contained AFB_2, and samples B2, B3, B6, and B10 contained OTA. These positive PBS samples exhibited MOE values below 10,000, except for sample B11 (AFB_1), B1 (OTA), and B4 (OTA). Regarding AFB_2, it was observed that all PBS samples have values below 10,000 and are therefore prioritized for risk management.

Sample no	Product name	Product presentation	Dosage	Botanical ingredients	Country of origin	Function
B1	Teck Aun Chi-Kit Pills	Tablet	1 sachet, 4 times/day	*Rhizoma Atractylodis M, Oleum Menthae, Herba Menthae, Pericarpjum Citi R, Fructus Citri, Radix Glycyrizae, Radix Aucklandiae, Radix Angelicaed, Herba Asari, Fructus Chaenomelis, Poria, Semen Arecae, Herba Pogostemonis, Fructus Amomi*	Malaysia	Traditionally used for stomachache, minor diarrhoea, minor vomiting, indigestion, and motion sickness
B2	Fa Zhi Tan	Tablet	2 tablets, 4 times/day	*Olibanum, Scutellaria Baicalensis Radix, Sangulsorba Officinalis L Radix, Radix Et Rhizoma Rhei, Radix Angelicae Sinensis Extract, Ledebouriella Divaricata Radix, Fructus Aurantii Immaturus, Flos Sophorae, Arecae Semen, Angelicae Dahuricae Radix, Rhizoma Imperatae*	Malaysia	Traditionally used to relieve pain and itchiness to piles and for relief of mild constipation
B3	Chui Feng So Ho Wan	Tablet	2 tablets, 2 times/day	*Radix Glycyrrhiza uralensis, Fructus Amomum Villosum Lour, Rhizoma Ligusticum Chuanxiong, Radix Angelica Dahurica, Radix Ledebouriella Divaricata, Fructus Amomum Kravanh, Rhizoma Notopterygium Incisum, Honey, Lignum Santalum Album, Semen Areca Catechu, Herba Mentha Haplocalyx,* Excipient: *Menthol*	Malaysia	Traditionally used for symptomatic relief of stomach discomfort, flatulence, headache, mild vomiting, cough, and flu, dispels wind for women after childbirth
B4	Chuan Ann Tong Sian Ke Sen	Tablet	10 tablets, 2 times/day	NA	Malaysia	This medicine is traditionally used to relieve joint pain, smooth the menstrual cycle, for women after childbirth, for health, and energy
B5	Bao Ying Dan	Powder	2 bottles/day	*Calculus Bovis, Margarita Pteria Martensii, Rhizoma Acorus Tatarinowii, Succinum, Aisaema Cum Bile, Concretion Silicea Bambusa Textilis, Rhizoma Paris Polyphylla, Radix Saposhnikovia Divaricata, Rhizoma Pinellia Ternata, Bulbus Fritillaria Cirrhosa, Radix Scutellaria Baicalensis, Ramulus Uncaria Rhynchophylla, Radix Curcuma Wenyulin, Herba Mentha Haplocalyx, Rhizoma Gastrodia Elata, Periostracum Cicadae Cryptotympana Pustulata*	China	Traditionally used for phlegm, vomiting, crying at night, fever, cold, and cough
B6	Lotus Brand Ba Bao Zhu Po Plus Jing Feng San	Powder	1 spoon, 3 times/day	*Resina Succinum, Concha Cryptotympana Pustulata, Radix Glycyrrhiza Uralensis, Radix Trischosanthes Kirilowii, Caulis Polygoni Multiiflori Preparata*	Malaysia	Traditionally used for sleep difficulty
B7	Kwei Be Wan	Tablet	8 tablets, 3 times/day	*Radix Codonopsis, Poria, Semen Ziziphi Spinosae, Radix Angelicae Sinensis, Radix Aucklandiae, Rhizoma Atractylodis Macrocephalae, Arillus Longan, Radix Astragali,Fructus Jujubae*	China	Traditionally used for general health, improving appetite, and relief of fever
B8	Kapsul Herba Kempisa	Capsule	6 capsules/day	*Folium Cassia Alata, Folium Cassia Obtusifolia, Semen Nigella Sativa, Folium Cinnamomum Iners, Fructose*	Malaysia	Remedy for flatulence and blood wind. Treat constipation, deflate the stomach, and relieve urination. Suitable for those trying to get pregnant
B9	D' Senna Plus Capsule	Capsule	3 capsules/day	*Senna*	Malaysia	Helps treat constipation, relieve bowel movements, detox the body, get rid of dirt in the intestines, slim weight loss pro- gram, treat worm problems, treat high blood pressure, treat diabetes and high sugar
B10	Ubat Akar Teh Sejuk Cap Tan Ngan Lo	Leaves	1 sachet, 4 times/day	*Folium Camellia Sinensis, Cortex Schefflera Octophylla, Radix Bupleurum chinense, Radix Glycyrrhizae Uralensis, Cortex Ilex Rotunda, Radix Scutellariae Baicalensis, Semen Cleistocalyx Operculatus, Herba Progestemon Cablin, Herba Schizonepetae Tenuifolia, Radix Angelica Pubescens, Pericarpium Citri Reticulatae, Fructus Crataegi Pinnatifida, Radix Scrophularia Ningpoensis, Semen Arecae Catechu, Herba Menthae Haplocalyx*	Malaysia	Help in moderating health problems such as sore throats, flu, and lethargy
B11	Capsule Pegaga Bidan Liza	Capsule	2 capsules, 2 times/day	*Centella Asiatica*	Malaysia	Enhancing facial radiance removes dark spots on the face, shrinks the uterus, relieves body aches, and relieves headaches
B12	Jamu Nenek Kapsul Rapat Plus	Capsule	2 capsules, 2 times/day	*Hippocratea indica, Piper nigrum, Trachyspermum ammi, Quercus infectoria, Labisia pumillia lin*	Malaysia	Treats menstrua- tion and stabilises the menstrual cycle. Treating whiteness, bad smell, and itching in the intimate parts of women. Raises the complexion and stays young
B13	Jus Mengkudu Bermadu	Liquid	3 spoons, 1 time/day	*Phoenix dactylifera, Nigella sativa, Piper betle,Crocus sativus*	Malaysia	Helps to regulate the immune system, diabetes, cholesterol, and blood pressure
B14	Serai Mas Kurma Hidayah Plus Liquid	Liquid	2 spoons, 1 time/day	*Punica granatum, Zingiber officinale, Quercus infectoria. Elephantopus scaber, Plectranthus, Labisia pumila*	Malaysia	Helps to improve physical fitness and mental intelligence in addition to helping to recover various types of diseases that affect health. It is suitable for the whole family to practice obtaining an optimal level of health

Table 2. Product description of the PBS analyzed in the present study. NA Data not available/not provided by the supplier.

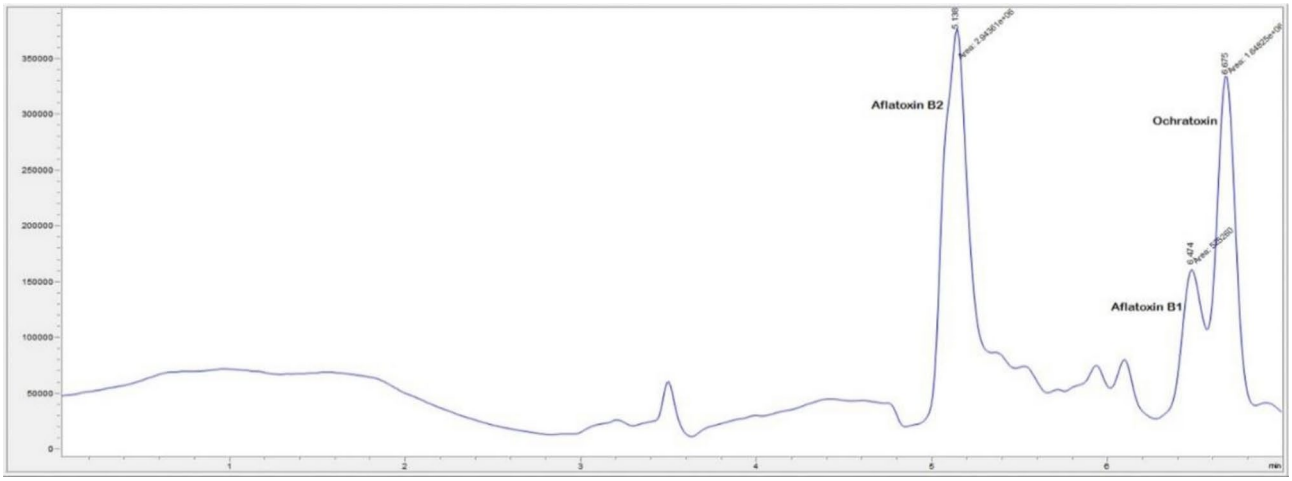

Fig. 1. Example of chromatogram of the co-existence of all contaminants studies which are AFB_1 (at 6.47 min), AFB_2 (at 5.13 min), and OTA (at 6.67 min) in sample B10.

Sample no	AFB_1 (µg/kg)	AFB_2 (µg/kg)	OTA (µg/kg)
B1	ND	277.611[b]	6.353[c]
B2	2.276[b]	233.073[b]	30.565[c]
B3	ND	238.381[b]	31.131[c]
B4	ND	26.373[b]	0.455
B5	ND	159.544[b]	ND
B6	ND	239.685[b]	61.898[c]
B7	ND	215.854[b]	ND
B8	ND	262.796[b]	ND
B9	66.377[a,b]	295.044[b]	ND
B10	9.470[a,b]	187.389[b]	14.946[c]
B11	0.087[b]	167.025[b]	ND
B12	ND	157.969[b]	ND
B13	ND	155.400[b]	ND
B14	ND	117.305[b]	ND

Table 3. Levels of mycotoxins detected in PBS samples. ND Not detected. [a]Exceeded the European regulatory limit of 5 µg/kg for AFB_1. [b]Exceeded the European regulatory limit of 10 µg/kg for total aflatoxins. [c]Exceeded the European regulatory limit of 3 µg/kg for OTA.

Apart from the qualitative MOE ratio, which was established by EFSA for carcinogenic and genotoxic substances, the characterization of risk results from oral exposure to AFs and OTA was evaluated using the quantitative cancer risk estimation proposed by the JECFA[42]. Table 5 shows the lowest (lower bound; LB) and highest (upper bound; UB) estimates of cancer risk and the percentage of HCC and kidney cancer caused by exposure to AFs and OTA in our PBS samples. The exposure of AFB_1 from the consumption of the PBS samples ranged from 0 to 1.11 ng/kg bw/day, while the exposure of AFB_2 ranged from 1.32 to 116.55 ng/kg bw/day. The exposure of OTA, which was detected in 6 samples (42.86%), ranged from 0 to 8.30 ng/kg body weight/day. The estimated cancer risk based on the potency estimate of 0.025 cancers/100,000 population/year per ng/kg bw/day for all mycotoxins tested ranged from 0 to 0.03 for AFB_1, 0.03 to 2.91 for AFB_2 and 0 to 0.21 cancers/100,000 population/year per ng/kg body weight/day for OTA. For all samples, the estimated percentage of HCC was between 0 and 0.44% due to AFB_1 exposure and between 0.52% and 46.25% due to AFB_2 exposure. According to estimates, up to 8.65% of kidney cancer cases could be attributed to OTA exposure.

Discussion

This study offers relevant insights into the occurrence of aflatoxins (AFB_1, AFB_2) and ochratoxin A (OTA) in PBS sold in Malaysia. Among the 14 PBS samples analyzed, all were contaminated with AFB_2, while over 28% and 42% were contaminated with AFB_1 and OTA, respectively. Samples B2 and B10 exhibited particularly had higher contamination, with AFB_1 concentrations of 2.276 µg/kg and 9.470 µg/kg. AFB_2 and OTA levels in these samples were also notably high. These results are consistent with previous studies documenting mycotoxin contamination in herbal supplements across Southeast Asia[3,43]. The presence of botanical ingredients like

Sample no	Estimated daily intake (µg/kg bw/day)			Margin of exposure		
	AFB_1	AFB_2	OTA	AFB_1	AFB_2	OTA
B1	ND	0.042	0.001	ND	9*	15 215
B2	0.001	0.062	0.008	659*	6*	1 779*
B3	ND	0.064	0.008	ND	6*	1 746*
B4	ND	0.001	0.000023	ND	303*	637,281
B5	ND	0.003	ND	ND	150*	ND
B6	ND	0.012	0.003	ND	33*	4685*
B7	ND	0.018	ND	ND	22*	ND
B8	ND	0.013	ND	ND	30*	ND
B9	0.001	0.005	ND	361*	81*	ND
B10	0.001	0.019	0.001	422*	21*	9 701*
B11	0.000001	0.003	ND	276,846	143*	ND
B12	ND	0.003	ND	ND	151*	ND
B13	ND	0.117	ND	ND	3*	ND
B14	ND	0.059	ND	ND	6*	ND

Table 4. Estimated Daily Intakes (EDI) and Margin of Exposure (MOE) of mycotoxins detected in PBS samples. ND Not detected. *MOE < 10,000.

Mycotoxins	Exposure (ng/kg bw/day)[a]		Estimated cancer risk (no. of cancers/100,000 population/year)[b]		% Cancer incidence	
	LB	UB	LB	UB	LB	UB
AFB_1	0	1.11	0	0.03	0*	0.44*
AFB_2	1.32	116.55	0.03	2.91	0.52*	46.25*
OTA	0	8.30	0	0.21	0**	8.65**

Table 5. The lowest and highest estimation of AFB_1, AFB_2, and OTA exposure, cancer risk, and percentage of cancer incidence in Malaysia. [a]Based on the mean bodyweight of the general adult population of 60 kg. [b]Calculated based on general adult population potency estimate of 0.025 cancers/100,000 population/year per ng/kg bw/day for AFs and OTA. *Based on the age-standardized incidence rate for liver cancer of 6.3/100,000 individuals/year[40]. **Based on the age-standardized incidence rate for kidney cancer of 2.4/100,000 individuals/year[39].

Scutellaria baicalensis and *Semen arecae* in the most contaminated samples suggests a possible link between specific plant species and increased mycotoxin contamination, emphasizing the need for further investigation into contamination mechanisms[44].

The EDI of mycotoxins from these PBS products indicates significant health risks, particularly for AFB_2, as all samples went beyond the MOE safety threshold of 10,000. Samples B2 and B10 posed the highest risk, with MOE values for AFB_2 as low as 6 and 21, respectively, highlighting the urgent need for risk management measures. These findings are consistent with studies that have reported elevated EDI levels of aflatoxins in dietary supplements, often exceeding the safety limits set by the EFSA[34,45]. Additionally, the co-occurrence of AFB_1, AFB_2, and OTA in these supplements raises about possible synergistic toxic effects, as indicated by prior research on mycotoxin mixtures[23].

The quantitative cancer risk assessment in this study revealed a significant risk of liver cancer linked to the consumption of contaminated PBS, especially those containing AFB_1 and AFB_2. The estimated dietary exposure to AFB_2 ranged from 1.32 to 116.55 ng/kg bw/day, contributing to an elevated risk of HCC, particularly in populations with frequent consumption of PBS[46]. These results are aligned with other studies that have identified increased cancer risks associated with aflatoxin exposure in vulnerable populations[47].

The findings of this study raise significant concerns regarding the health risks associated with consuming contaminated PBS, particularly for vulnerable groups such as children and immunocompromised individuals. Certain samples, such as B9, contained significant levels of mycotoxins, with AFB_1 reaching 66.377 µg/kg—far exceeding the regulatory limit of 5 µg/kg for total aflatoxins set by the Malaysian Food Act 1983[3]. Similar issues have been reported in other studies, which have documented elevated levels of mycotoxins in herbal supplements, pointing to a global concern that requires regulatory intervention[4,31].

The widespread AFB_2 contamination in Malaysian PBS, coupled with low MOE values and increased cancer risks, necessitates immediate regulatory intervention. The co-occurrence of multiple mycotoxins in some samples indicates that consumers may face greater health risks beyond those associated with individual toxins. Continued investigation is warranted to investigate the specific botanical and environmental factors leading to

contamination in these products, as well as the long-term health impacts of chronic mycotoxin exposure through dietary supplements[34,45]. Implementing robust regulatory measures and comprehensive product monitoring will be vital in mitigating the public health risks posed by mycotoxin contamination in plant-based supplements.

Ensuring the safety of PBS necessitates strict quality control measures across the entire supply chain, from cultivation to packaging. This study emphasizes the necessity of following good agricultural practices, maintaining proper storage conditions, and conducting regular mycotoxin testing. The findings are consistent with other research that points to the importance of robust regulatory frameworks to ensure the safety of dietary supplements[29]. Although regulatory frameworks exist in Malaysia, stronger enforcement is required to prevent contaminated products from reaching consumers.

This study has several limitations. First, the sample size of 14 PBS, while sufficient for preliminary evaluation, may not fully represent the diversity of products available in Malaysia, as it primarily focuses on more accessible, popular items. Expanding the sample to include a broader range of products and manufacturers would provide a more comprehensive assessment. Despite the limited sample size, the findings offer valuable preliminary data on mycotoxin contamination in PBS available in the Malaysian market. Second, the study only examined AFB_1, AFB_2, and OTA, excluding other mycotoxins such as fumonisins and zearalenone, which may have led to an underestimation of the total mycotoxin burden. Future research should include a wider range of mycotoxins to more comprehensive risk assessment. Additionally, the MOE and cancer risk approaches used for risk assessment do not account for cumulative exposure or interactions between mycotoxins, which may result in additive or synergistic effects. Future research should aim to address these complexities to provide a more comprehensive evaluation of combined mycotoxin health risks. Finally, the study assumed adherence to recommended dosages, though actual consumption patterns may vary, impacting the EDI and overall risk assessment. Future studies should incorporate consumer behaviors data to better represent potential exposures.

Conclusion

In conclusion, this study highlights the significant health risks associated with the presence of mycotoxins, specifically AFB_1, AFB_2, and OTA, in plant-based supplements sold in Malaysia. The detection of these toxic substances at levels exceeding regulatory limits points to the necessity of stricter quality control measures and stronger regulatory enforcement. The use of the EDI, MOE, and cancer risk approaches has provided important insights into the potential long-term health risks, particularly for vulnerable populations who regularly consume these supplements. Furthermore, the co-occurrence of multiple mycotoxins raises the cumulative risk, emphasizing the need for comprehensive testing for a wider range of contaminants in these products. Moving forward, it is essential that public health authorities and industry stakeholders collaborate to enhance the safety of plant-based supplements, ensuring compliance with safety standards and reducing consumer exposure to harmful mycotoxins.

Data availability

Raw data within the manuscript is provided in Supplementary Materials B.

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Author contributions

The authors' contributions were as follows—RA, NSK, and AHBA: designed the research; RA and NSK: performed the data analysis and interpretation, and wrote the initial draft of the manuscript; RA, MYA, AHBA: critically revised the manuscript; RA, NSK, SSA: conducted the research; RA and MYA: performed the LCMS analysis; RA: has primary responsibility for final content; and all authors: contributed to critically reviewing the manuscript, and read and approved the final manuscript.

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Declarations

Competing interests

The authors declare no competing interests.

Additional information

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