UNIVERSITI PUTRA MALAYSIA

RISK ASSESSMENT OF PYRROLIZIDINE ALKALOID IN HERBAL SUPPLEMENT AND TEA USING MARGIN OF EXPOSURE APPROACH

AISHAH ZAHIRA BINTI ZAINUDIN

RISK ASSESSMENT OF PYRROLIZIDINE ALKALOID IN HERBAL SUPPLEMENT AND TEA USING MARGIN OF EXPOSURE APPROACH

BY

AISHAH ZAHIRA BINTI ZAINUDIN

Thesis submitted in fulfilment of the requirement for the degree of Bachelor of Science in Environmental and Occupational Health with Honours from the Faculty of Medicine and Health Sciences, Universiti Putra Malaysia

ACKNOWLEDGEMENTS

Alhamdulillah, all praises to Allah who give me the strengths, ability and His blessings for me to complete the thesis. Firstly, I would like to express my gratitude towards my parents and family for their continual supports in terms of moral and money for me to complete the journey. The completion of this thesis will not possible without the guidance and endless helps and motivation given during conducting this research from my supervisor, Dr Rozaini I also would like to give a thanks to my friends especially Atiqah my fypmates which always have my back during this journey.

ABSTRACT

RISK ASSESSMENT OF PYRROLIZIDINE ALKALOID IN HERBAL SUPPLEMENT AND TEA USING MARGIN OF EXPOSURE APPROACH

AISHAH ZAHIRA BINTI ZAINUDIN

Introduction: Herbal supplements and tea are frequently perceived as harmless, natural and effective for health benefit. However, pyrrolizidine alkaloid (PA) which naturally present in plants may induced genotoxicity and carcinogenicity in human. **Objectives:** This study aimed to assess the risk of PA from herbal supplement and tea using Margin of Exposure (MOE) approach. **Methodology:** The secondary data related to the presence of PA in herbal supplements and teas and animal toxicological data from exposure to PA was collected from the literature. The estimated daily intake (EDI) of PA from the literature data was calculated for a 70 kg person with recommended daily intake of 200 mg of herbal supplement and 2 g of herbal tea. Animal data on exposure to PA causing liver cancer was collected and analyzed using Benchmark Dose Modelling (BMD) version 3.2 to derive the BMD Lower confidence limit of 10 ($BMDL_{10}$) as a point of departure. Risk assessment to assess the potential health risk was performed using MOE approach with the ratio of $BMDL_{10}$ and EDI for lifetime exposure to PAs**. Results and Discussion**: A total number of 404 herbal supplements and teas contaminated with PA was collected from literature. The PA was found in the herbal supplements and teas ranging from 0.09 to 235376.00 µg/kg. The EDI of PA based on exposure to these samples were ranging from 0.000004 to 10.08 The BMDL10 calculated value was ranging from 176.76 µg/kg bw/day to 272.95 µg/kg bw/day. The calculated MOEs were ranging from 27.05 to 159220833.30 for herbal supplements and 3569.88 to 955325.00 for herbal teas. Out of 385 herbal supplements, 42 samples (10.09%) were below the MOE of 10,000 while out of 19 herbal teas, 4 samples (21.05%) were below the MOE. **Conclusion:** As conclusion, it shows that most of samples of herbal supplement and herbal teas are presents with PA with an MOE values of less than 10,000 indicating the urge for necessary risk management action to be taken.

Keywords: Risk Assessment, Pyrrolizidine Alkaloid, Margin of Exposure , herbal supplement, herbal tea

ABSTRAK

PENILAIAN RISIKO PYRROLIZIDINE ALKALOID DALAM SUPLEMEN HERBA DAN THE MENGGINAKAN KAEDAH 'MARGIN OF EXPOSURE'

AISHAH ZAHIRA BINTI ZAINUDIN

Pengenalan: Produk herba selalunya dianggap tidak berbahaya, semula jadi dan berkesan dengan penggunaannya namun pyrrolizidine alkaloid (PA) yang secara semula jadi terdapat dalam tumbuhan boleh menyebabkan genotoksisiti dan kekarsinogenan pada manusia. **Objektif:** Kajian ini bertujuan untuk menilai risiko PA daripada suplemen herba dan teh menggunakan kaedah Margin of Exposure (MOE). **Metodologi:** Data sekunder yang berkaitan dengan kehadiran PA dalam suplemen herba dan teh dan data toksikologi haiwan daripada pendedahan kepada PA dikumpulkan daripada literature. Anggaran pengambilan harian (EDI) PA daripada data literatur telah dikira untuk 70 kg orang dengan disyorkan pengambilan harian 200 mg suplemen herba dan 2 g teh herba. Data haiwan mengenai pendedahan kepada PA yang menyebabkan kanser hati dikumpul dan dianalisis menggunakan Benchmark Dose (BMD) versi 3.2 untuk memperoleh had keyakinan BMD Rendah sebanyak 10 ($BMDL_{10}$) sebagai titik berlepas. Penilaian risiko untuk menilai potensi risiko kesihatan dilakukan menggunakan kaedah MOE dengan nisbah $BMDL_{10}$ dan EDI untuk pendedahan seumur hidup kepada PA. **Keputusan dan Perbincangan:** Sejumlah 404 suplemen herba dan teh yang tercemar dengan PA telah dikumpulkan daripada literatur. PA ditemui dalam suplemen herba dan teh antara 0.09 hingga 235376.00 µg/kg. EDI PA berdasarkan pendedahan kepada sampel ini adalah antara 0.000004 hingga 10.08 Nilai pengiraan BMDL10 adalah antara 176.76 µg/kg bb/hari hingga 272.95 µg/kg bb/hari. MOE yang dikira adalah antara 27.05 hingga 159220833.30 untuk suplemen herba dan 3569.88 hingga 955325.00 untuk teh herba. Daripada 385 suplemen herba, 42 sampel (10.09%) berada di bawah MOE iaitu 10,000 manakala daripada 19 teh herba, 4 sampel (21.05%) berada di bawah MOE. **Kesimpulan:** Kesimpulannya, ia menunjukkan bahawa kebanyakan sampel suplemen herba dan teh hadir dengan PA dengan nilai MOE kurang daripada 10,000 menunjukkan keinginan untuk tindakan pengurusan risiko yang perlu diambil.

Kata kunci: Penilaian Risiko, Pyrrolizidine Alkaloid, '*Margin of Exposure'*, supplemen herba, teh herba

TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

LIST OF ABBREVATIONS

PA	Pyrrolizidine Alkaloid
BMD	Benchmark Dose Model
MOE	Margin of Exposure
POD	Point of Departure
EDI	Estimated Daily Intake
EFSA	European Food Safety Authority
BMDL	Benchmark Dose Level
IARC	International Agency for Research on Cancer

CHAPTER 1

INTRODUCTION

1.1 Research Background

For the past few years, the use of herbal medicine and supplements has surged, whereby more than 80% of people globally depend on them for some aspect of primary healthcare (Ekor, 2014). Herbal products are frequently perceived as harmless, natural, and effective by their users (Griffin et al., 2014). However, PAs (Figure 1.1) that present naturally may induce potential adverse effects to human (Allgaier & Franz, 2015). There are approximately more than 500 PAs and its N-oxides reported in over 6000 plant species based on chemotaxonomic considerations (Dusemund et al., 2018), which includes plants from families of Asteraceae, Boraginaceae, Symphytum and Fabaceae (JECFA, 2008). PAs have been detected in many herbal plants (Allgaier & Franz, 2015), which have been utilize medicinally (Steinhoff, 2019). For example, Comfrey (*Symphytum officinale*) are herbal teas which originally from China and claimed to be able to treat arthritis, bronchitis, allergies and diarrhea (Chen et al., 2019).

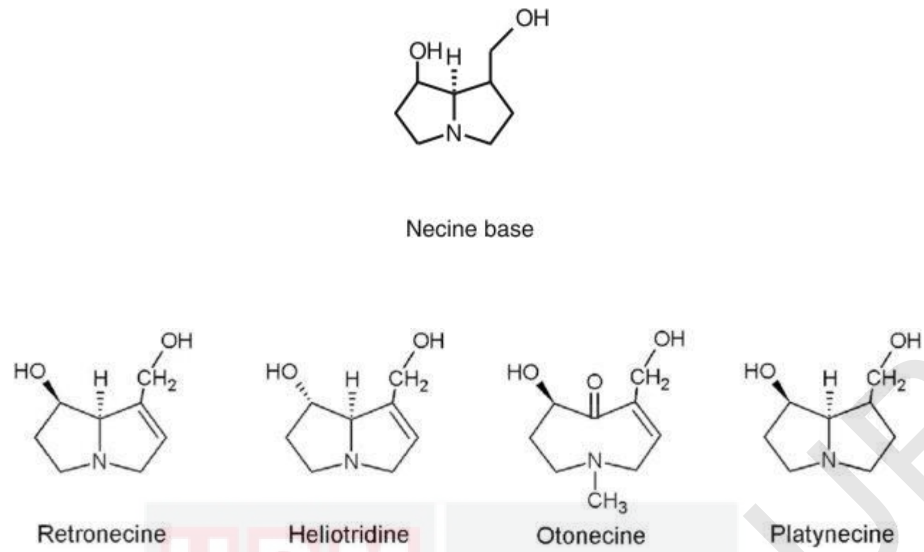

Figure 1.1: Common chemical structure of PA based on necine base

Pyrrolizidine alkaloids (PAs) are known as compounds that occur naturally which are generated from a variety of plants (Chen et al., 2017). There are approximately more than 500 pyrrolizidine alkaloids and its N-oxides reported in over 6000 plant species based on chemotaxonomic considerations (Dusemund et al., 2018). PAs can be divided into necine bases and necic acids (He et al., 2021). For necine bases, PAs can be further categorized into four different group which include the retronecine type, otonecine type, heliotridine type and platynecine type (He et al., 2021).The retronecine and otonecine types are known as the unsaturated PAs due to the presence of 1,2 double bonds in the necine base while platynecine is classified as the saturated PAs (Zheng et al., 2021). The 1,2-unsaturated PAs which include the lasiocarpine, monocrotaline and riddelliine compounds are claimed to be genotoxic and have been categorized as being possibly carcinogenic to humans (category 2B) by the International Agency for Research on Cancer (IARC) (Suparmi et al., 2020).

Humans can be exposed to the PA through the consumption of PA-based food, which is often generated from the plant (Bodi et al., 2014). The liver and lung are the specific targets of PA's short-term toxicity, whereas acute exposure of PAs may induce hepatic venoocclusive disease (HVOD), which causes serious liver damage and eventually death in some case (Knutsen et al., 2017; Suparmi et al., 2020).

1.2 Problem statement

Herbal and traditional remedies, a component of complementary medicine, have received much interest in both advanced and developing countries (Ismail et al., 2020), which function to cure minor and severe illnesses (Jantan, 2006). According to Ismail et al. (2020), numerous herbs are thought to effectively treat illnesses and enhance health status. However, as herbal and traditional remedies become more common, it has caused concern related to its detrimental side effects, which may outweigh the possible benefits of the products due to insufficient scientific evidence (Ismail et al., 2020). Nonetheless, PA which is abundant in the plant, has been observed to be present in herbal medicine (Schrenk et al., 2020). Moreover, PA-plant and PA-based products taken as herbal treatments, supplementation, veggies also have been shown to have considerable amounts of PA due to unintentional contamination with PA-plant or grains affected by the PA-plant (Beuerle et al., 2011).

There are several species of plants known to generate PAs that have been identified such as Borago, Echium, Eupatorium, Lithospermum, Petasitis, Pulmonaria, Senecio, Symphytum and Tussilago species. In addition, the plant species of Echium, Borago, Symphytum, Petasitis, and Tussilago also has been prescribe as a medications for the purpose of maintaining health (Mulder et al., 2015). The level of PA in herbal products have also been discussed and identified previously. For example, a total of 169 herbal teas available in German was analyzed using LC–MS/MS analysis. Based on the analysis, all of the herbal teas were presence with PA and tea mixtures shows the highest percentage (Schulz et al., 2015). Other than that, herbals teas that were brought in Swiss market also has been identified with PAs where 24 out 70 samples analyzed were presence with PA (Mathon et al., 2014). Aside of herbal teas, PA may also presence in herbal supplement or medicine. A study from Mulder et al. (2015), analyzed a total of 30 supplements which made of dry plant from PA-based plant. Based on the result, 29 out of 30 supplements were presence with PA.

PAs are natural phytotoxins, which may induce genotoxicity and carcinogenicity in the liver when metabolically activated (Zheng et al., 2021). PA is mainly compromised with necine base and necic acid. However, the majority of toxic PA can be identified from the necine base with double bond at C1 and C2 (Fu et al., 2002). The 1,2-unsaturated PA are known to be hepatotoxic and genotoxic carcinogen which may pose a possible threat towards human health (Chen et al., 2017) due to its ability to induce metabolic activation and form reactive pyrroles (Knutsen et al., 2017). Other than that, individual PA have different metabolic activation patterns, resulting in significant differences in hazardous potency among PA (He et al., 2021). According to International Agency for Research on Cancer (IARC), they identified lasiocarpine, monocrotaline, and riddelliine as possible human carcinogen (category 2B) based on available evidence but some other PAs tested were not classifiable (category 3) due to lack of evidence (JECFA, 2008).

Additionally, the toxicity exerts by each PAs are influenced by several factors which include the liver metabolites and its physical characteristic (Wiedenfeld & Edgar, 2011). Furthermore, the consumption of PA- based plants for medical reasons is possible to cause a threat to health (Allgaier & Franz, 2015) whereas the high quantity of PA consumption may lead to severe hepatic damage, and daily intake of a small dosage of PA for an extended period can cause severe liver problems (Schrenk et al., 2020).

The 1,2-unsaturated PA mainly affects the liver and the lung based on the human poisoning cases reported, which is commonly caused by PA-based herbal products and contamination of agricultural crop (Beuerle et al., 2011). In addition, more than 10,000 cases of poisoning linked with the use of PA-containing plants, are proven to be fatal (Ma et al., 2018; Seremet et al., 2018).Another study by Neuman & Steenkamp (2009) also claimed that PA-poisoning in human is linked with the development of veno-occlusive liver disease (VOD) which later causes cirrhosis and, eventually, death in different countries. For instance, there is a reported of a young woman who developed VOD after consuming cough medicine prepared made form a plant called *S. tephrosioides.*

1.3 Study Justification

Based on the previous studies, there are many studies have been conducted worldwide regarding the presences of PA in herbal product. However, there are still many that are not aware of the presence of PA in herbal supplement and tea thus, the finding from this study may be able to support the current evidence about the presence of pyrrolizidine alkaloid in the herbal products. For example, PA-derived plant species such as *Tussilago farfara* are common among European and Chinese user as herbal remedies to cure respiratory health issues (Adamczak et al., 2013).

In addition, according to Beuerle et al. (2011), it shows that exposure to PA primarily affects the liver and lungs based on experimental animal data. Additionally, based on the latest herbal supplement data, PA may be a third category of botanical compounds to be concerned. Long-term exposure may potentially lead to liver cirrhosis and pulmonary arterial hypertension (Dusemund et al., 2018). Hence, the finding of this study may be able to provide information on the toxicity potential of PAs upon exposure to humans.

Other than that, the hazardous potency of various PAs has been received less attention despite the increase of awareness related to their toxicity (He et al., 2021) hence, the finding of this study will be able to provide information on the hazardous potency between different types of PA. Also, this study may sum up the level of PA data in various types of herbal medicine all over the world, which is then being analyzed using the BMD method. Lastly, there is only a little toxicological information available on medicinal plants in Malaysia, particularly in terms of specific chemicals such as alkaloid and steroids (Jantan, 2006); therefore, the finding from this study may initiate further research in Malaysia related to PA in herbal medicine as there is no research regarding these issues have been discussed in Malaysia.

1.4 Research Question

1.What is the level of PA in the herbal supplement and tea?

2.What is the benchmark dose lower level of 10 ($BMDL_{10}$) of PA?

3.What is the acceptable estimated daily intake of PA for human to prevent from the occurrence of adverse effect?

4.Does the margin of exposure of pyrrolidine alkaloid in herbal supplement and tea are less than 10,000?

1.5 Research Objectives

1.5.1 General Objective

The aim of this study is to determine the risk assessment of PA in herbal supplement and tea using the Margin of Exposure approach.

1.5.2 Specific Objectives

1. To determine the level of PA in herbal supplement and tea.
2. To evaluate the benchmark dose lower level of 10 ($BMDL_{10}$) of pyrrolizidine alkaloid from animal toxicological data.
3. To determine the estimated daily intake of PA.
4. To assess the risk of PA from traditional herbal supplement and tea using the Margin of Exposure approach.

1.6 Hypothesis

The margin of exposure of PA in traditional medicine and herbal supplement are less than 10,000.

1.7 Conceptual Definition

1.7.1 Herbal Supplement

Herbal supplement is known as part of the dietary supplements which contain one or more herbal substances (Dietary and Herbal Supplement, 2020). An herbal supplement is a plant-based product which is only used internally and available in variety of forms which includes dried, chopped, powdered, capsule, or liquid.

1.7.2 Herbal Tea

Herbal tea is a drink extracted from the ingredients contained in a plant which served in both hot and cold conditions (Poswal et al., 2019). Moreover, a wide range of herbal teas also commercially available nowadays contained with majority of the herbal teas composed of one or combinations of ingredients with health purpose including a composition of many ingredients including different parts of botanical plants such as dried leaves, flowers and others (Ravikumar, 2007).

1.7.3 Pyrrolizidine Alkaloid

PAs are chemical components that present in plant that could induce hepatic damage in humans and animals (Griffin et al., 2014). It is commonly found in a plant from a family of Boraginaceae, Asteraceae, and Fabaceae (JECFA, 2008). There are two types of ester group in PAs which are the necine bases and necic acids where it can be assorted further into four different group based on its necine base including the retronecine type, otonecine type, heliotridine type and platynecine type PAs (He et al., 2021). Notably, the 1, 2-unsaturated PAs are hepatotoxic and genotoxic carcinogens, potentially harming human health (Chen et al., 2017).

1.7.4 Margin of Exposure (MOE)

Based on EFSA (On, 2005a), they suggested that the margin of exposure approach can be used to evaluate the risk of genotoxic and carcinogenic substances by dividing the reference point of Benchmark dose lower limit (BMDL), which is based on animal toxicological data with the estimated daily intake of humans.

1.8 Operational Definition

1.8.1 Herbal Supplement

Herbal and dietary supplements are widely utilized worldwide to cure the disease in which the products are available both in raw and commercial form (Bunchorntavakul & Reddy, 2012). In this study, herbal supplement that originally came from plant containing pyrrolizidine alkaloid will be considered. The data of herbal supplement containing pyrrolizidine alkaloid will be obtained from the previous study

1.8.2 Herbal Tea

Herbal teas are worldwide recognized drinks for health purposes, commonly in traditional remedies (Poswal et al., 2019). This study will consider herbal teas that originally came from plant-derived pyrrolizidine alkaloids. The data of herbal tea containing pyrrolizidine alkaloid will be obtained from the previous study.

1.8.3 Pyrrolizidine Alkaloid

In this study, data of herbal products containing pyrrolizidine alkaloid will be gather from various sources to derive the Estimated Daily Intake (EDI) based on the average amounts of pyrrolizidine alkaloid in herbal supplement and tea products, daily consumption of the samples, and the average body weight.

1.8.4 Margin of Exposure (MOE)

An MOE with a value less than 10,000 indicates that it may cause adverse effects on human health based on the animal toxicological data (On, 2005a).

1.9 Conceptual Framework

Based on Figure 1.2, it illustrates all of the variables that are included in this study. General population is involved in the study since the secondary data collected from variety of sources are used. The data collected is analyzed using BMD software, to generate the point of departure (POD) or $BMDL_{10}$ for the exposure of PAs in herbal products. The data is gathered from the article or resources worldwide which is relevant to the research topic.

The independent variable for this study is the risk assessment of PAs from herbal supplements and tea, which is analyzed using both animal toxicological data and the level of the contaminant data. The dependent variable for this study is the level of PAs and the Estimated Daily Intake (EDI) of PA. The cancer potential effect will also be included as the several 1,2-unsaturated PAs are claimed to be genotoxic and have been categorized as being possibly carcinogenic to humans (category 2B). To conclude, this study uses the MOE approach to conduct a risk assessment of PAs in traditional medicine and herbal supplement.

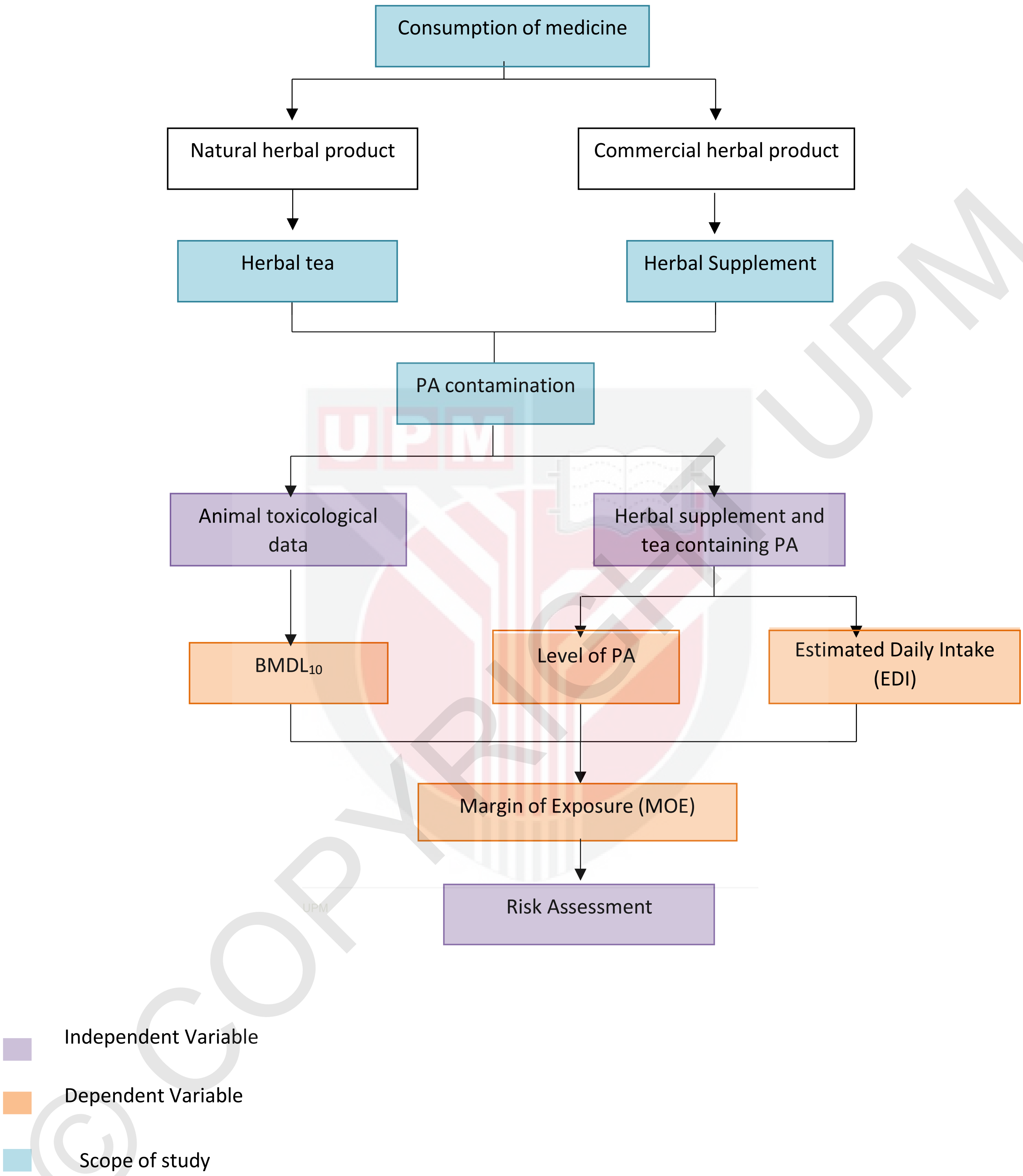

Figure 1.2: Conceptual Framework

CHAPTER 2

LITERATURE REVIEW

2.1 The use of herbal products

Nowadays, the herbal sector has been rapidly expanding all over the world (Ahmad et al., 2015). The herbs also have been known in history as a significant component of culture recognized for their dietary and medicinal benefits (Ahmad et al., 2015). According to WHO (2013), herbal medicines and the traditional medicinal approach have been acknowledged by many as one of the primary options in maintaining health.

Herbal products have become increasingly popular in Western countries over the last few decades whereas there are approximately 3,000 plant species marketed as herbal supplements in the United States, with up to 50,000 different products (Li et al., 2012). Moreover, herbal and dietary supplements are broadly applied all around the globe whereas the usage of herbal and dietary supplements in the United States has increased rapidly over the past few years in which 18.9% of people reported using them in 2004 (Bunchorntavakul & Reddy, 2012).

Additionally, the application of traditional medicine remains vital in Malaysia given the significant industrialization development (Ahmad et al., 2015). There is also increased demand and acceptability of alternative medicine and herbal supplements to treat disease among society's middle- and upper-income segments (Ahmad et al., 2015). Example of alternatives medicines uses in Malaysia includes the TCM, Ayurveda, Jamu and others (Ahmad et al., 2015).

2.2 Pyrrolizidine Alkaloid

Pyrrolizidine alkaloids (PAs) are naturally occurring toxins found in a wide range of plant species where it is also known as most commonly produced environmental contaminants which affect wildlife, poultry, and humans (Beuerle et al., 2011). PAs were discovered in over 6,000 plant species but only a few generally correspond to direct poisoning in humans and animals (Beuerle et al., 2011).

PAs are mainly found in the families of the *Asteraceae*, *Boraginaceae, and Leguminaceae* (Allgaier & Franz, 2015). Several PAs or N-oxides can be found in some plant species, and there are also some PAs that are expressed by several plant species (Beuerle et al., 2011). Aside from that, PA concentrations are higher in seeds and flower parts of plants and lower in leaves, stems, and roots.

PA consists of necine base, and necic acids (Gottschalk et al., 2015). The majority of PAs are heterocyclic compounds produced from necine bases (Fu et al., 2004). PA is classified into four types depending on the structure of the necine base: retronecine-type, heliotridine-type, otonecine-type, and platynecine-type (Gottschalk et al., 2015). Each group has its own representative structure in which for retronecine-type PAs includes riddelliine, retrosine, monocrotaline and symphytine while lasiocarpine and heliotrine are heliotridine-type PAs and otonecine type PAs structures are senkirkine and petasitenine (Chen et al., 2010)

The retronecine, heliotridine, and otonecine are known as unsaturated necine bases with a double bond at the C1 and C2 position in which their esters are considered as hazardous (Li et al., 2012). Majority of hepatotoxic PA are esters of both necine bases retronecine and heliotridine, which are diastereomers with differing C7 structures (Cheeke, 1988). It is also stated that the structure of PAs may affect their toxicity, for instance presence of 1,2-double bond and a branch in an esterified side chain may lead to hepatotoxicity (Cheeke, 1988). The 1,2-double bonded unsaturated necine is considered hazardous (Chen et al., 2010) where particularly known as hepatotoxic and genotoxic carcinogens, which may provide significant serious threat to humans (Chen et al., 2017).

The consumption of PA-derived plants is common worldwide for the purpose of health and to cure disease however has been diagnosed to also caused liver injuries among the consumer (Roeder & Wiedenfeld, 2009). Nevertheless, the reported cases of PA-poisoning due to consumption of herbal products were not only occur in developing but also developed country (Roeder & Wiedenfeld, 2009). The plant species which was frequently caused toxicities in humans are Heliotropium and Crotalaria in which it is common in weeds or legume crops (Beuerle et al., 2011) where the reported cases of toxicities were due to human subconsciously exposed to the food products contaminated with PA (Stickel & Seitz, 2000). Moreover, comfrey (*Symphytum spp.*) is one of the examples of plant consumed and used by human for the medicinal and health purposes such as gastritis and gastroduodenal ulcers but have been discovered to cause harmful effects to human.

However, despite its toxicities there are several examples of PA-derived plants that were used and consume for its health benefits. For examples, Coltsfoot or *Tussilago farfara* is PA-derived plant species which commonly used among European and Chinese herbal remedies to cure respiratory health issues (Adamczak et al., 2013). In addition, another Chinese herbal remedies which is common is called *Gynura japonica* with a health claimed of to aid the homeostasis during fatal injury (Adamczak et al., 2013).

2.3 Metabolic pathway and toxicity of PA

PA are known to be mutagenic and may cause carcinogenic effects on both humans and animals (Allgaier & Franz, 2015). According to Chen et al. (2010), several PA-based plant and particular PA chemicals has been identified to be carcinogenic in various tissues based on animal models. PA mutagenicity is strongly influenced by metabolic activity (Chen et al., 2010). PA must be metabolically activated just like other types of hazardous compounds before being able to cause toxic effects (Fu et al., 2004).

The 1,2-unsaturated PA can be absorbed via the gastrointestinal tract and then distributed throughout the body. Upon the absorption of PA, it will be delivered to the liver for the metabolism process (Beuerle et al., 2011). The liver is the main organ involved in the metabolism process of pyrrolizidine alkaloid (Fu et al., 2001). According to studies by Beuerle et al., three different metabolic pathways have been discovered for PAs (Figure 2.3). The first pathway involved the formation of necine base and necic acid through the hydrolysis of the ester linkages at C7 and C9. The second pathway is the N-oxidation of the necine bases to form N-oxides (Beuerle et al., 2011; Fu et al., 2004). The last metabolic pathway is the oxidative process, which includes two processes focusing on two different types of PA which are the retronecine- and heliotridine-type. The steps involved are the hydrolysis of necine base at the C3 or C8 position followed by a dehydration process to generate pyrrolic ester derivatives (Beuerle et al., 2011;Fu et al., 2004). Furthermore, based on the animal and human research data, cytochrome P450 is mainly responsible for inducing the metabolism of the 1,2-unsaturated PAs to generate the pyrrole esters (Beuerle et al., 2011).

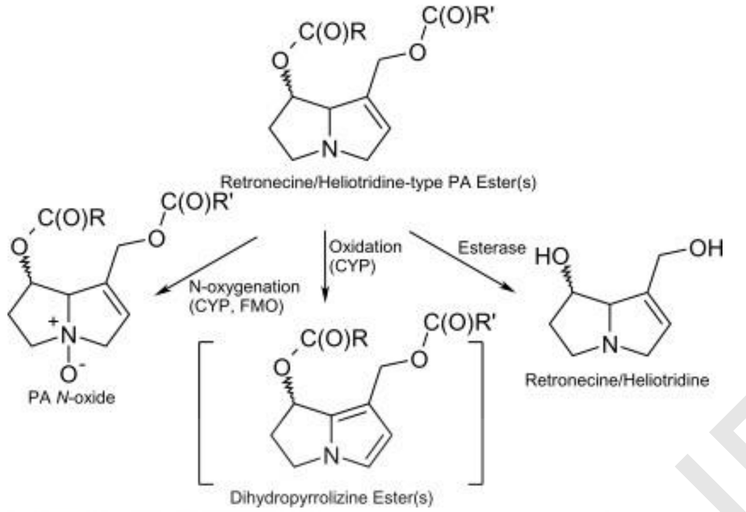

Figure 2.3: Metabolic pathway of PA

2.4 Presence of Pyrrolizidine Alkaloids in herbal products

Botanical products, including herbal teas and plant food supplements, are extensively being utilized worldwide but have been discovered to be contained with toxic PAs (Bodi et al., 2014). According to studies by Wiedenfeld & Edgar (2011), the traditional remedies have been proven beneficial with no adverse effects by experts in several Western countries. Yet, this situation thus results in a significant case of intoxication reported related to the intake of herbal remedies containing hazardous PAs. The presence of PA in herbal products has been widely assessed and investigated. In addition, according to Mulder et al. (2015), PAs were detected in a high percentage of plant-derived foods where 60 percent were coming from food supplements and more than 90 percent in teas.

Studies by Bodi et al. (2014) states that the probability of the existence of PA in herbal products is due to several factors, including the generated PA are from herbal remedies, the possibility of herbal products contaminated with similar PA-derived plant and a mixture exposure of herbal products with PA and contaminated PA-derive plant.

Previous assessment has been conducted by Bodi et al (2014), using a different dry herbal teas sample originally from German. It shows that most of the tea samples are presence with PA with rooibos teas has the highest level of PA presence. Other than that, based on a study by Chen et al. (2019), 6 out of 8 herbal medicines analyzed showed PA presence with the total concentration varied from 404 to 7883 μg/kg. In addition, they also analyzed 5 different types of herbal teas which extracted in two different form, intact and communised leaves. As a result, it shows that the total amount of PA extracted in intact leaves which is range from 30.7 to 845.4 g/L, is lower than the communited leaves values which are from 61.3 to 1120 g/L. Furthermore, another study by Mulder et al. (2015), which analyzed different types of different supplements, claimed that 60 percent of dietary supplement test samples were tested positives with PAs with lycopsamine-type dominating. Despite that, PAs were not found in supplements using oil-based extracts from both PA-based and non- PA-based plants.

2.5 Potential risk of Pyrrolizidine Alkaloid to human

Concerns have been raised regarding the impact of human health caused by exposure to pyrrolizidine alkaloid-containing plants that can be found worldwide (Fu et al., 2002). PAs toxic effects in humans can be seen based on the cases of intoxication due to the intake of PA-containing herbal products, epidemic cases, and death related to ingestion of PA-contaminated grain (Knutsen, Alexander, Barregård, et al., 2017).

Generally, the exposure to harmful PA and PA N-oxides among humans can happen via four ways which include as staple food contaminants, food, herbal teas and medicine and dietary supplement (Fu et al., 2001). Consumption of PA-based products may lead to the risk of having acute and chronic liver damage among the consumer (Chojkier, 2003).

The liver is known as the primary site of PA toxicity, as the bioactivation process mostly occurs in this organ (Moreira et al., 2018). PA-containing plant may lead to the development of hepatic veno-occlusive disease in humans which ought to be caused by PA intoxication (Chen et al., 2010). Other than that, it was proven that acute exposure of PAs in humans may result in hepatic veno-occlusive disease (HVOD), which causes severe liver damage and eventually death in exceptional situations (Suparmi et al., 2020). There are several clinical features that normally present, including nausea, enlarged liver, and bloody diarrhoea (Chen et al., 2010). However, certain individuals may only have occasional symptoms, with continual liver enlargement being the only sign of the illness (World Health Organization (WHO), 1989).

The toxicity of PA in humans can be observed based on the previous cases discussed in the literature following short-term consumption of PA (German Federal Institute for Risk Assessment, 2013). The poisoning cases involve a Mexican-American six months old girl and two months old boy who consume herbal tea known as *Senecio longilobus*. After the consumption, the girl later started to develop symptoms of ascites and pleural effusion, sinusoidal liver fibrosis and liver cirrhosis after half a year, while the boy started to develop a haematemesis, jaundice, disruption in central nervous systems and eventually die within a week (German Federal Institute for Risk Assessment, 2013). Furthermore, there have also been reports of a newborn baby developing VOD due to the consumption of breast milk from a mother that used to drink herbal tea containing PA during her pregnancy (Neuman & Steenkamp, 2009). Other than children, cases of PA toxicities in human adult also have been reported. For instance, a 49-year-old woman was reported to have VOD due to the consistent consumption of food supplement containing *Symphytum spp.* root for the purpose of health benefits (Ridker et al., 1985).

2.6 Laws and regulation related to herbal product

There has been growing collaborative efforts related to the herbal product's regulations worldwide (World Health Organization (WHO), 2013). The World Health Organization (WHO) has made significant efforts to integrate and standardize the herbal medicine regulations worldwide, whereas several guidelines have been issued to ensure its safety and quality which include the Quality Control Methods for Medicinal Plant Materials (1998), WHO Guidelines on Good Agricultural and Collection Practices (GACP) for Medicinal Plants (2003), and WHO Guidelines for Assessing Quality of Herbal Medicines with Reference to Contaminants and Residues (2007) (Ismail et al., 2020).

In addition, WHO also has introduced the WHO Traditional Medicine Strategy 2002– 2005, which mainly aims to achieve its four main goals. In response to the guidelines, there is an increase and consistent development of medicinal product regulation has been introduced by the Member of the States to support the safe usage of Traditional and Complementary products and practice (World Health Organization (WHO), 2013). Nevertheless, different countries have different varied approaches and legal frameworks that they shall comply related to the use of herbal and traditional medicine as pharmaceutical products (Ismail et al., 2020).

In Malaysia, different types of herbal medicine are governed by laws, including the Control of Drug and Cosmetic Regulations 1984, the Poison Act 1952, the Sale of Drug Act 1952, the Advertisement and Sale Act 1956, and the Protection of Wild Life Act 1972. (Ismail et al., 2020) Besides that, the Malaysian Health Ministry's Drug

Control Authority (DCA) is an essential authority in which all herbal and traditional medicine shall abide by all of their requirements (Ismail et al., 2020). In addition, the DCA is also responsible for ensuring that the level of heavy metals present in traditional medicine is within the permissible limit (Ismail et al., 2020). However, there are still no precise guidelines related to the use of herbal and natural ingredients (Ismail et al., 2020).

In European countries, the Medicines and Healthcare Products Regulatory Agency (MHRA) is responsible for overseeing the Traditional Herbal Medicines Registration Scheme (Ismail et al., 2020). Moreover, all applications for the registration of herbal medicine shall comply with the current requirement, whereas the applicant shall provide proof indicating that the herbal products have been traditionally used for medication purposes at least for 30 years (Ismail et al., 2020).

In Australia, the regulatory for complementary medicines is governed under the Therapeutic Goods Act 1989 (Ismail et al., 2020). Compared to other countries, their regulating approach is different, mainly focusing on a risk-based strategy with the support of a two-tiered structure (Ismail et al., 2020). The Therapeutic Goods Administration (TGA) is authority which responsible in ensuring that all of the therapeutic goods fulfil the needed requirements (Ismail et al., 2020).

The National Health Insurance is the healthcare system which responsible in covering nearly all of the Japan's population (Ismail et al., 2020). In Japan, herbal medicines are regulated similarly to the pharmaceutical products; for example, all manufactured Kampo medicines are covered by the Pharmaceutical Affairs Law and under the enforcement of Good Manufacturing Practice (Ismail et al., 2020).

2.7 Risk Assessment of Genotoxic and Carcinogenic Chemical

The risk assessment using margin of exposure (MOE) approach uses the reference point on the dose- response curve of a benchmark dose lower confidence limit (BMDL) obtained from animal toxicological data divided with estimated human intake (Benford, 2016). This approach has also been proposed by EFSA as a standardized method in assessing the risk of substances that contain carcinogenicity and genotoxicity characteristics (On, 2005b). The value of MOE demonstrates the concern on the chemical, but it does not quantify risk for a single chemical or compare the risk for various chemicals. The greater the MOE, the lower the potential threat posed by the substance under evaluation (Benford, 2016)

The MOE can be derived using a point of reference obtained through clinical or epidemiological research on the dose-response relationship (Benford et al., 2010). It is preferred to use the $BMDL_{10}$ as the reference point (On, 2005b). The $BMDL_{10}$ was based on the tumour endpoint chosen for the data analysis. Thus, different values are possibly used as the reference point when calculating the MOE value (Benford, 2016). There are several significant criteria that shall be take into account for carcinogenic mechanism of action in different tumour of the experimental animals such as sensitivity of different species, strains and sex, administration route and dose given in animals particularly in dietary studies (Benford, 2016).

The degree of the MOE values represents the level of risk (Benford et al., 2010). An MOE values of less than 10,000 suggest that there might be a risk to human health (Wang et al., 2021), while MOE values of 10,000 or more according to the animal toxicological data shall not risk human health and are not crucial for immediate risk management action (On, 2005a). In addition, EFSA also suggested to use the T25 if the gathered data is insufficient in determining the $BMDL_{10}$ for the risk assessment of both genotoxic and carcinogenic compounds (On, 2005a).

CHAPTER 3

METHODOLOGY

3.1 Research Design

This study used a secondary data to determine the relationship between the carcinogenicity of substances and its potential adverse effect. The secondary data used includes the animal toxicological data exposed to PA and level of PA that presence in herbal supplement and herbal tea worldwide. The animal data were used to calculate the $BMDL_{10}$ values and Estimated Daily Intake (EDI) values of the level PA in the samples. The MOE value was calculated based on the $BMDL_{10}$ and EDI values obtained from the literature.

3.2 Study location and population

This study research has no study location as there is no involvement of any human population for data collection. Other than that, this study also used the general population since all the data was gathered from varied resources worldwide.

3.3 Data collection criteria

Literature searches were conducted using Google Scholar Science Direct, Sage and Pubmed with the terms and keywords of "pyrrolizidine alkaloid", "herbal tea" and "herbal supplement". There was no time limit or country was set for the search of the literatures for the presences of PA in herbal supplement and teas. The literature search was collected from the year of 2014 to 2021 with a total of 5 eligible studies selected for both herbal supplements and tea. The selected data compromised of the PA which presence in herbal supplement for oral use which were obtained from the markets.

A total samples of 404 which includes 19 herbal teal and 385 herbal supplement were derived form 5 different studies (Chen et al., 2019; Griffin et al., 2014; Kaltner et al., 2020; Suparmi et al., 2020; Wang et al., 2021) which involve 6 different countries (China, Ireland, Indonesia, Netherland, Spain, German, Taiwan)

3.3.1 Inclusion criteria

Based on Gold et al. (1984), there are several standard criteria that have been recommended to be included in the animal study. The experimental data obtained shall met the standard criteria before being considered in the study.

Firstly, mammals were used in the experiment in which the period of exposure would be at least one-fourth of the species' standard lifetime. Next, the route of administration was thorough diet, water, gavage, inhalation, intravenous or intraperitoneal injection. Furthermore, the test agent shall be administered alone, instead of in combination with other chemicals and the exposure was chronic, with not over than 7 days between administration. Moreover, the research design shall include a control group and have at least 5 animals per group. Clear data on presence of dichotomous liver hemangiosarcoma must be proven in the animals. Other than that, the duration of the experiment, shall at least half the standard lifespan for that species.

Rat’s data set with dichotomous hemangiosarcoma endpoint has been chosen to be corrected. The collected dataset was corrected and adjusted using a method proposed by European Chemicals Agency, which is dose multiplied with [(w/104) (w2/104)] where w1 is week of dosing while w2 is the week of observation and 104 indicates the standard lifespan for rats.

3.4 Study Instrument

This study research was conducted by collecting data from a prior animal toxicological study which then be analyzed using a Benchmark Dose Modelling (BMD) version 3.2 to determine the MOE.

3.5 Estimated daily intake of PA

The EDI value was determined by the total PA concentration and its daily use. This method is used to estimate the possible risk exposure of PA to human due to the intake of herbal products. The EDI value of PA was calculated based on the assumption of 200 mg of herbal supplement (Chen et al., 2017) or 2 g per cup of tea, (German Federal Institute for Risk Assessment, 2013) for 70 kg person using the equation 3.5 (Chen et al., 2019):

$$EDI = \frac{\text{Total PA concentration x Daily use (µg/kg.bw/day)}}{\text{Body weight (70 kg)}}$$

Eq. 3.5

Where,

Total PA concentration = Level of PA in herbal products.

Body weight = Average body weight of 70 kg adult.

Daily use = Daily consumption assumption of 200 mg herbal supplement (Chen et al., 2017) or 2g per of tea (German Federal Institute for Risk Assessment, 2013).

3.6 Margin of Exposure (MOE)

The margin of exposure is a method proposed by EFSA (On, 2005) for determining the risk posed by genotoxic and carcinogenic substances. This method involves a calculation by dividing the $BMDL_{10}$ with EDI to generate value which indicates the necessity to take action (Benford et al., 2010). The MOE was determine based on the equation 3.6 (Suparmi et al., 2020):

$$MOE = \frac{BMDL_{10}}{\text{Estimated Daily Intake}} (\text{ng/kg.bw/day}) \quad \text{Eq 3.6}$$

CHAPTER 4

RESULTS

4.1 Literature search for herbal supplement and tea containing PAs

A total of 404 samples including 19 herbal tea samples and 385 herbal medicine samples was gathered from various online sources which involve 6 different countries (China, Ireland, Indonesia, Netherland, Spain, German, Taiwan) (Chen et al., 2019; Griffin et al., 2014; Kaltner et al., 2020; Suparmi et al., 2020; Wang et al., 2021). The summary data of herbal supplement and teas gathered was presented in Table 4.1.1 and 4.1.2.

Total PAs were found in herbal supplements were ranging from 0.09 ug/kg to 235376.00 ug/kg (Table 4.1.1) while in teas were ranging from 10.00 ug/kg to 1733.00 ug/kg (Table 4.1.2). The highest PAs were found in herbal supplement from Indonesia with PA level of 235376.00 ug/kg in which the level of PA in herbal supplement was 135-fold higher than in the herbal tea. Before deriving the MOE value, the EDI value of PA was calculated for a 70 kg person (On, 2005a) for assumptions on recommended daily intake of 200 mg for herbal supplement (Chen et al., 2017) and 2 g for one cup of tea (BfR, 2013) for the samples collected. The range of EDI value calculated for herbal supplements are within 0.000004 to 10.088 while for herbal teas, the EDI value is ranged from 0.0003 µg/kg bw/day to 0.0495 µg/kg bw/day. It was found that the EDI values are higher in herbal supplements than herbal tea.

Table 4.1.1: Product description of herbal supplements containing PAs including country of origin and the respective estimated daily intake. The remaining description for 375 samples is included in supplementary data (Appendix 3)

Herbal supplement (Name/species)	Country of origin	Product presentation	Total level of PA (µg/kg)	EDI (µg/kg bw/day)[a]	References
Sanxi Shangyao Pian	China	Tablet	4.2	0.0002	Chen et al., 2019
Feire Keli	China	Bag	4.00	0.0013	Chen et al., 2019
Arnebia euchroma	China	NA	25567.40	3.65	Wang et al., 2021
Tussilago farfara	China	NA	17600.20	2.51	Wang et al., 2021
Whitethorn dragées	German	Capsules	1.50	0.000009	Kaltner et al., 2017
Maca and hemp protei	German	Powder	0.70	0.0002	Kaltner et al., 2017
Bi Min Gan Wan	Ireland	Tablet	93.00	0.00002	Griffin et al., 2014
Xie Gan Wan	Ireland	Tablet	46.00	0.0001	Griffin et al., 2014
Lithospermum oriental	Indonesia	Radix	13.10	0.0003	Suparmi et al., 2020
Gynura pseudochina	Indonesia	folium	135.30	0.0270	Suparmi et al., 2020

[a]The estimated daily intake was estimated based on the consumption of 200 mg daily (Chen et al., 2017)

NA – Not available

Table 4.1.2: Product description of herbal teas containing PAs including country of origin and the respective estimated daily intake.

Herbal Tea (Name/species)	Country of origin	Product presentation	Total level of PA (µg/kg)	EDI (µg/kg bw/day)[a]	References
Coltsfoot (*Tussilago farfara)*	Netherland	Comminuted (leaves)	61.30	0.0017	Chen et al., 2019
Coltsfoot (*Tussilago farfara)*	Netherland	Intact (leaves)	30.70	0.0008	Chen et al., 2019
Comfrey *(Symphytum officinale)*	China	Comminuted (leaves)	415.00	0.011	Chen et al., 2019
Comfrey *(Symphytum officinale)*	China	Intact (leaves)	101.10	0.0028	Chen et al., 2019
Climbing groundsel *(Senecio scandens)*	China	Comminuted (leaves)	293.10	0.0083	Chen et al., 2019
Climbing groundsel *(Senecio scandens)*	China	Intact (leaves)	85.20	0.0024	Chen et al., 2019
Sunn hemp (Crotalaria juncea)	China	Comminuted (leaves)	192.80	0.0055	Chen et al., 2019
Sunn hemp (Crotalaria juncea)	China	Intact (leaves)	170.00	0.0048	Chen et al., 2019

Borage *(Borago officinalis)*	Spain	Comminuted (leaves)	1120.00	0.0320	Chen et al., 2019
Borage *(Borago officinalis)*	Spain	Intact (leaves)	845.40	0.0241	Chen et al., 2019
Expectorant	Ireland	Tea	29.00	0.0008	Griffin et al., 2014
Red bush	Ireland	Tea	140.00	0.0040	Griffin et al., 2014
C&H	Ireland	Tea	245.00	0.0070	Griffin et al., 2014
Ooolong	Ireland	Tea	51.00	0.00146	Griffin et al., 2014
Black tea	Ireland	Tea	19.00	0.00054	Griffin et al., 2014
Slimming aid	Ireland	Tea	107.00	0.00306	Griffin et al., 2014
Digestive aid	Ireland	Tea	1733.00	0.04951	Griffin et al., 2014
C&H	Ireland	Tea	10.00	0.00029	Griffin et al., 2014
C&S	Ireland	Tea	1438.00	0.04109	Griffin et al., 2014

[a]The estimated daily intake was estimated based on the consumption of 2g per cup of tea (German Federal Institute for Risk Assessment, 2013)

4.2 Benchmark dose modelling

Benchmark dose modelling (BMD) is a method for determining the point of departure (POD) or reference point for calculating margins of exposure (MOE) for genotoxic and carcinogenic substances (Haber et al., 2018; Hardy et al., 2017). The study used a carcinogenicity rat and mice experimental data to assess the risk of PA in human by assuming the risk shown from animal to human. There were 2 datasets obtained which is from NTP (2003) and NTP (1978) that met and fit the criteria to be run with the BMDS software. However, NTP (2003) present suitable animal data which recommended by EFSA (Knutsen, Alexander, Barregård, et al., 2017). The dataset from of animal study is as shown in Table 4.2.1. Based on the corrected dichotomous animal hepatic endpoint dataset by NTP (2003) and NTP (1978), there were two main information that shall be included in BMDS software which are the time adjusted dose (µg per kg body weight and day) and the tumour incidence. The data was analyzed using BMDS software version 3.2 developed by US Environmental Protection Agency (USEPA) to derived the BMD (benchmark dose) and BMDL (benchmark dose lower confidence limit). The software was set as a dichotomous model with extra risk of 95% confidence level.

Table 4.2: PA tumour incidences in female Fisher rat (NTP, 2003).

Rat	Type of diet	Concentration of PA in diet (µg/kg)	Duration of dosing (week)	Duration of observation (week)	Corrected dose (µg/kg bw/day)	Time adjusted dose[a] (µg/kg bw/day)	Tumour incidence
Female Fischer 344 rat	Riddelliine	0	105	105	0.00	0.00	0/50
		10	105	105	7.14	7.14	0/50
		33	105	105	23.57	23.57	0/50
		100	105	105	71.43	72.81	0/50
		330	105	105	235.71	235.71	3/50
		1000	97	97	714.29	621.37	38/50

[a]Time adjusted dose is calculation based on lifetime of rats which is 105 weeks (NTP, 2003).

4.3 $BMDL_{10}$ value from rat toxicological data

The point of departure (POD) of PA tumor incidence in female Fischer rats was shown in Table 4.3. The best fitted model was Multistage Degree 2 with p value of 0.005 and a ratio of BMD/$BMDL_{10}$ less than 10 (Abdullah et al., 2017). However, for this study, a range of $BMDL_{10}$ value of 176.76 μg/kg bw/day and 272.95 μg/kg bw/day from the analysis result of female rats by NTP (2003), was used for further analysis to determine the worst-case scenario that may occur. According to Chen et al. (2017), it is also recommended to use the lowest value of $BMDL_{10}$ for further assessment. The results from BMD analysis of PA tumour incidence in female Fisher rats (NTP, 2003) using BMDS software version3.2 are presented in Table 4.3.

Table 4.3: Results from BMD analysis of PA tumour incidence in female Fisher rats (NTP, 2003) using BMDS software version 3.2.

Model	Restriction	Number of parameters	Log-likelihood	*p*-value	Accepted[a]	BMD10 µg/kg bw/day	BMDL10 µg/kg bw/day
Dichotomous Hill	Unrestricted	3	-38.93	0.99873	Yes	271.68	217.02
Logistic	Unrestricted	3	-38.91	0.99977	Yes	270.03	215.99
Log-Logistic	Unrestricted	2	-39.94	0.83556	Yes	328.75	272.943
Log-probit	Unrestricted	2	-38.93	0.99990	Yes	271.68	217.02
Multistage Degree 5	Unrestricted	3	-38.90	0.99999	Yes	265.033	216.83
Multistage Degree 4	Unrestricted	6	-38.90	NA	No	NA	NA
Multistage Degree 3	Unrestricted	4	-38.90	0.99999	Yes	268.14	176.75
Multistage Degree 2	Unrestricted	4	-38.90	0.99999	Yes	275.92	210.69
Multistage Degree 1	Unrestricted	2	-38.902	**1**	Yes	262.14	212.52
Probit	Unrestricted	2	-57.09	<0.0001	No	NA	NA
Quantal Linear	Unrestricted	2	-39.33	0.96774	Yes	300.89	249.80
Weibull	Unrestricted	2	-57.09	<0.0001	No	NA	NA

[a]The criteria of acceptance is chosen based on $p > 0.05$ and BMD_{10} $BMDL_{10}$ ratio < 10 (Abdullah et al., 2017)

NA – Not available

4.4 Risk assessment of PA using MOE in herbal supplement and tea

The MOE approach was used to determine the risk caused by PA in herbal supplement and tea. Figure 4.4.1 represents the MOE from exposure to PA from herbal supplements while Figure 4.4.2 represents the MOE from exposure to PA from herbal tea based on their respective country with a lifetime exposure to PA. The calculated MOEs were ranging from 27.05 to 159220833.30 for herbal supplements and 3569.88 to 955325.00 for herbal teas. Out of 385 herbal supplements, 42 samples (10.09%) were below the MOE of 10,000 while out of 19 herbal teas, 4 samples (21.05%) were below the MOE of 10,000 which indicates necessity for risk management action.

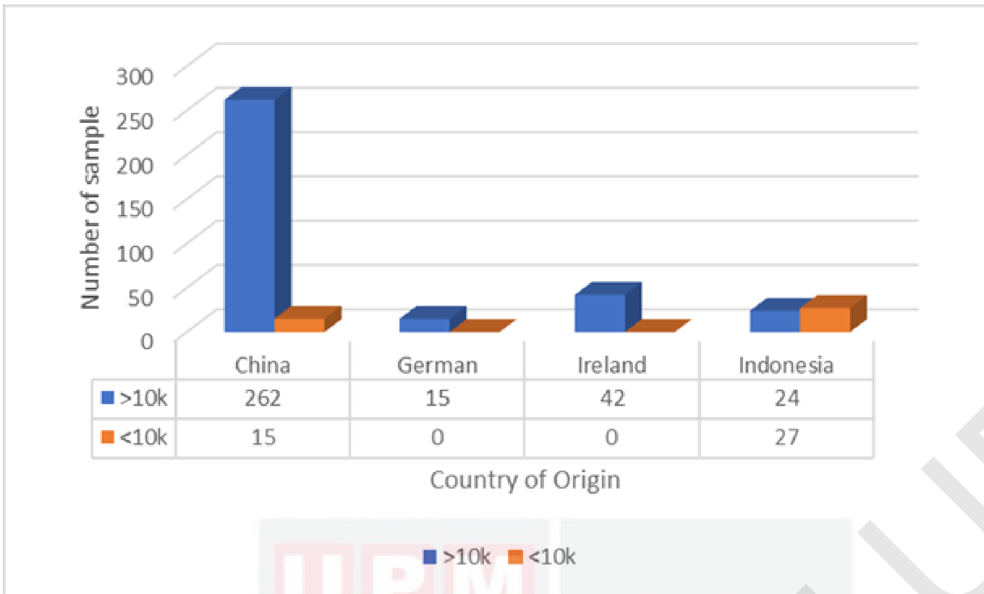

Figure 4.4.1: The calculated MOE for lifetime exposure to PA from herbal supplements with their respective country

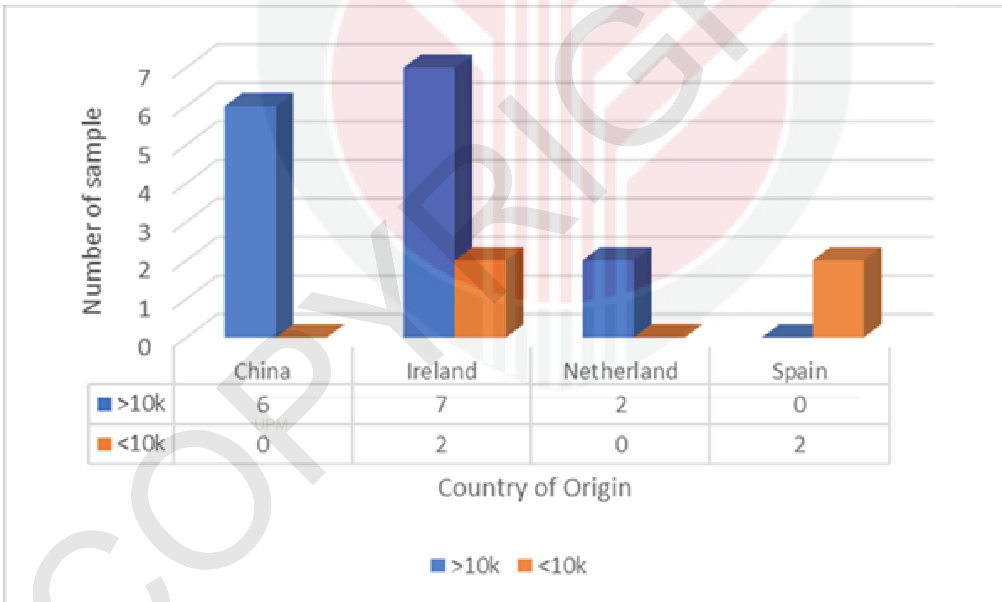

Figure 4.4.2: The calculated MOE for lifetime exposure to PA from herbal teas with their respective country

CHAPTER 5

DISCUSSION

5.1 Level of PA presence in herbal supplement and tea based on previous literature.

Based on the data collected, the level of PA in herbal supplement samples was up to 235376.00 µg/kg, while PA in herbal was up to 1733.00 µg/kg which compromises of 6 different countries (Chen et al., 2019; Griffin et al., 2014; Kaltner et al., 2020; Suparmi et al., 2020; Wang et al., 2021). This data revealed that the level of PA in herbal supplement was 135-fold higher than in the herbal tea. Other than herbal supplement and teas, PA can also be found in various foods including honey (EFSA, 2016; Knutsen et al., 2017; Merz & Schrenk, 2016; Schulz et al., 2015). There was also study stated about the probability of mix consumption of herbal teas with honey may lead to higher level of PA exposed to the consumer (Beuerle et al., 2011; Knutsen et al., 2017). Yet, the data related to the mix consumption of herbal products is less available leading to a challenge in the risk analysis (Chen et al., 2019).

In Malaysia, there are lacking in studies related to PA in food or herbal products but there are several plants that have been identified to contain PA with several species has been banned (Pauzi et al., 2022). However, there is no specific evidence shows that herbal plants with PA are still being consumed by the population in Malaysia for the purpose of health and other countries has also started to ban and introduce the limit for consumption of herbal products with PA including Germany, Austria, The Netherlands, Europe, Australia and USA (Ismail et al., 2020; Ma et al., 2018).

5.1 BMDL10 of animal toxicological data from the liver tumor formation endpoint

The Benchmark Dose method has been used in the study with a benchmark dose lower confidence limit of 10% ($BMDL_{10}$) which was recommended by EFSA (On, 2005b). A group of female rats has been chosen for this study and the dataset from the NTP (2003) was corrected before analyzed using BMD Software 3.2. Based on the result, the best fitted model was the $BMDL_{10}$ value from the Multistage Degree 1 with a value of 212.53 μg/kg bw/day. However, for this study it included a range of $BMDL_{10}$ from NTP (2003) with 176.76 μg/kg bw/day and 272.95 μg/kg bw/day with a 10% response to estimate the worst-case scenario that may occur upon human exposure. The data was chosen based on the criteria of p value > 0.05 and ratio of BMD/BMDL10 less than 10 as described by Abdullah et al. (2017). The value was slightly different from the one proposed by EFSA (Knutsen et al., 2017) which is 237 μg/kg bw/day. This may be due to different standardized value of average weight and daily food consumption for the calculation of corrected daily dose in which for this study, the corrected daily dose was calculated based on the standard value recommended by (Swirsky Gold et al., 1984).

The selection of dataset of female rats from NTP (2003) has been mentioned by EFSA (Knutsen et al., 2017) to be the most adequate dataset to be used for the point of departure (POD). Previously, there have been several oral experimental data involving various types of PAs, but only data on lasiocarpine and riddelliine are suitable for the BMD approach (Ning et al., 2019). However, after taking into account the differences in relative potency between the two chemicals, they began to propose the use of new POD value of 237 μg/kg bw per day of riddelliine for risk assessment of sum exposure of PAs (Knutsen et al., 2017; Ning et al., 2019) which have been used widely (Suparmi et al., 2020; Wang et al., 2021).

5.3 Risk assessment of pyrrolizidine alkaloid in herbal supplement and tea using the Margin of Exposure approach.

The EDI value derived from the herbal supplement samples are ranged from 0.000004 µg/kg bw/day to 10.008 µg/kg bw/day while for herbal teas are 0.0003 µg/kg bw/day to 0.0495 µg/kg bw/day using daily consumption assumptions of 200 mg PFS (Chen et al., 2017) or 2 g per cup of tea (BfR, 2013) for a 70 kg adult for general population. However, there are several countries which used its own general body weight of their respective country such 54 kg for Indonesia (Suparmi et al., 2020). Given that, this study involves samples collected from various countries, thus the 70 kg default weight recommended by EFSA (Beuerle et al., 2011) is being used.

Margin of exposure is an approach proposed by EFSA in determining the risk of substances with carcinogenicity and genotoxicity characteristics (On, 2005a) where a value of more or less of 10,000 for MOE value will indicates if the risk management are required or not. Based on the data obtained, the MOEs value were ranging from 27.05 to 159220833.30 for herbal supplements and 3569.88 to 955325.00 for herbal teas. Out of 385 herbal supplements, 42 samples (10.09%) were below the MOE of 10,000 while out of 19 herbal teas, 4 samples (21.05%) were below the MOE of 10,000 which indicates necessity for risk management action. However, according to Chen et al. (2017), the MOE values derived for the entire life may be unrealistic for the risk towards human as it was predicted basely on the daily consumption assumptions of supplement and teas throughout lifetime. It is possible that the consumption of herbal medicine and teas are for a shorter time or over per cup daily thus the MOE values will be higher implying there is no risk in the consumption. Considering that the consumption of herbal medicine is not too long, Harber's rule has been used to estimate the risk exposure for instance two weeks (Abdullah et al., 2017). All MOE values were more than 10,000, indicating no risk towards human health due to exposure to PA.

CHAPTER 6

CONCLUSION

In conclusion, it was found that 42 samples of herbal supplement and 4 samples of herbal teas containing PA that available in the market were below 10,000 of the MOE indicating the urge for risk management action to be taken. In addition, more research related to herbal products shall be conducted in Malaysia as there is no specific evidence showing that Malaysian are expose to PA from herbal supplement and teas.

For recommendation, further study is required to determine the accuracy of the finding obtained from this study and more research related to herbal products shall be conducted in Malaysia.There are several limitations that can be identified in the study. This study only used secondary data to estimate the exposure risk of the herbal product among humans thus further study is required to determine the real risk exposure of PAs to human. The data collected from previous studies only focussed on total level of PA instead of mean and maximum value in the herbal products. Other than that, there is high variability in the level of PA in different plant thus it is difficult to determine the exact value on level of PA in plants. Moreover, this study used recommended daily dosage intake from previous literature search instead of daily dosage provided by the buyer or manufacturer thus further study are required to determine the accuracy of the finding obtained from this study.

REFERRENCES

Abdullah, R., Diaz, L. N., Wesseling, S., & Rietjens, I. M. C. M. (2017). Risk assessment of plant food supplements and other herbal products containing aristolochic acids using the margin of exposure (MOE) approach. *Food Additives and Contaminants - Part A Chemistry, Analysis, Control, Exposure and Risk Assessment*, *34*(2), 135–144. https://doi.org/10.1080/19440049.2016.1266098

Adamczak, A., Opala, B., Gryszczyńska, A., & Buchwald, W. (2013). Content of pyrrolizidine alkaloids in the leaves of coltsfoot (Tussilago farfara L.) in Poland. *Acta Societatis Botanicorum Poloniae*, *82*(4), 289–293. https://doi.org/10.5586/asbp.2013.028

Ahmad, F., Azlan Shah Zaidi, M., Sulaiman, N., & Abdul Majid, A. (2015). Issues and Challenges in the Development of the Herbal Industry in Malaysia. *Prosiding Perkem*, *10*(September), 227–238. http://www.strategyr.com/MarketResearch/Infograhics_Images/MCP-1081/1081.jpg

Allgaier, C., & Franz, S. (2015). *Risk assessment on the use of herbal medicinal products containing pyrrolizidine alkaloids*. *73*, 494–500. https://doi.org/10.1016/j.yrtph.2015.09.024

Benford. (2016). The use of dose-response data in a margin of exposure approach to carcinogenic risk assessment for genotoxic chemicals in food. *Mutagenesis*, *31*(3), 329–331. https://doi.org/10.1093/mutage/gev064

Benford, D., Bolger, P. M., Carthew, P., Coulet, M., DiNovi, M., Leblanc, J. C., Renwick, A. G., Setzer, W., Schlatter, J., Smith, B., Slob, W., Williams, G., & Wildemann, T. (2010). Application of the Margin of Exposure (MOE) approach to substances in food that are genotoxic and carcinogenic. *Food and Chemical*

Toxicology, *48*(SUPPL. 1), S2–S24. https://doi.org/10.1016/j.fct.2009.11.003

Beuerle, T., Benford, D., & Cottrill, B. (2011). Scientific Opinion on Pyrrolizidine alkaloids in food and feed. *EFSA Journal*, *9*(11). https://doi.org/10.2903/j.efsa.2011.2406

Bodi, D., Ronczka, S., Gottschalk, C., Behr, N., Skibba, A., Wagner, M., Lahrssen-Wiederholt, M., Preiss-Weigert, A., & These, A. (2014). Determination of pyrrolizidine alkaloids in tea, herbal drugs and honey. *Food Additives and Contaminants - Part A Chemistry, Analysis, Control, Exposure and Risk Assessment*, *31*(11), 1886–1895. https://doi.org/10.1080/19440049.2014.964337

Bunchorntavakul, C., & Reddy, K. R. (2012). *Alimentary Pharmacology and Therapeutics Review article : herbal and dietary supplement hepatotoxicity*. *November*. https://doi.org/10.1111/apt.12109

Chen, Mulder, P. P. J., Louisse, J., Peijnenburg, A., Wesseling, S., & Rietjens, I. M. C. M. (2017). Risk assessment for pyrrolizidine alkaloids detected in (herbal) teas and plant food supplements. *Regulatory Toxicology and Pharmacology*, *86*, 292–302. https://doi.org/10.1016/j.yrtph.2017.03.019

Chen, Mulder, P. P. J., Peijnenburg, A., & Rietjens, I. M. C. M. (2019). Risk assessment of intake of pyrrolizidine alkaloids from herbal teas and medicines following realistic exposure scenarios. *Food and Chemical Toxicology*, *130*(May), 142–153. https://doi.org/10.1016/j.fct.2019.05.024

Chen, T., Mei, N., & Fu, P. P. (2010). Genotoxicity of pyrrolizidine alkaloids. *Journal of Applied Toxicology*, *30*(3), 183–196. https://doi.org/10.1002/jat.1504

Chojkier, M. (2003). Hepatic sinusoidal-obstruction syndrome: Toxicity of pyrrolizidine alkaloids. *Journal of Hepatology*, *39*(3), 437–446. https://doi.org/10.1016/S0168-8278(03)00231-9

Dusemund, B., Nowak, N., Sommerfeld, C., Lindtner, O., Schäfer, B., & Lampen, A. (2018). Risk assessment of pyrrolizidine alkaloids in food of plant and animal origin. *Food and Chemical Toxicology*, *115*(March), 63–72. https://doi.org/10.1016/j.fct.2018.03.005

EFSA. (2016). *Dietary exposure assessment to pyrrolizidine alkaloids in the European population European Food Safety Authority (EFSA). 14*(July). https://doi.org/10.2903/j.efsa.2016.4572

Ekor, M. (2014). The growing use of herbal medicines: Issues relating to adverse reactions and challenges in monitoring safety. *Frontiers in Neurology*, *4 JAN*(January), 1–10. https://doi.org/10.3389/fphar.2013.00177

Fu, P. P., Chou, M. W., Xia, Q., Yang, Y. C., Yan, J., Doerge, D. R., & Chan, P. C. (2001). Genotoxic pyrrolizidine alkaloids and pyrrolizidine alkaloid N-oxides - Mechanisms leading to DNA adduct formation and tumorigenicity. *Journal of Environmental Science and Health - Part C Environmental Carcinogenesis and Ecotoxicology Reviews*, *19*(2), 353–385. https://doi.org/10.1081/GNC-100107580

Fu, Peter P., Xia, Q., Lin, G., & Chou, M. W. (2004). Pyrrolizidine Alkaloids - Genotoxicity, Metabolism Enzymes, Metabolic Activation, and Mechanisms. *Drug Metabolism Reviews*, *36*(1), 1–55. https://doi.org/10.1081/DMR-120028426

Fu, Peter P, Yang, Y., Xia, Q., Chou, M. W., Cui, Y. Y., & Lin, G. (2002). *Pyrrolizidine Alkaloids -Tumorigenic Components in Chinese Herbal Pyrrolizidine Alkaloids - Tumorigenic Components in Chinese Herbal Medicines and Dietary Supplements*. *March 2015*. https://doi.org/10.38212/2224-6614.2743

German Federal Institute for Risk Assessment. (2013). *Pyrrolizidine alkaloids in herbal teas and teas* (Issue 018).

Gottschalk, C., Ronczka, S., Preiß-Weigert, A., Ostertag, J., Klaffke, H., Schafft, H., &

Lahrssen-Wiederholt, M. (2015). Pyrrolizidine alkaloids in natural and experimental grass silages and implications for feed safety. *Animal Feed Science and Technology*, *207*, 253–261. https://doi.org/10.1016/j.anifeedsci.2015.06.014

Griffin, C. T., Gosetto, F., Danaher, M., Sabatini, S., & Furey, A. (2014). Investigation of targeted pyrrolizidine alkaloids in traditional Chinese medicines and selected herbal teas sourced in Ireland using LC-ESI-MS/MS. *Food Additives and Contaminants - Part A Chemistry, Analysis, Control, Exposure and Risk Assessment*, *31*(5), 940–961. https://doi.org/10.1080/19440049.2014.900193

Haber, L. T., Dourson, M. L., Allen, B. C., Hertzberg, R. C., Parker, A., Vincent, M. J., Maier, A., & Boobis, A. R. (2018). Benchmark dose (BMD) modeling: current practice, issues, and challenges. *Critical Reviews in Toxicology*, *48*(5), 387–415. https://doi.org/10.1080/10408444.2018.1430121

Hardy, A., Benford, D., Halldorsson, T., Jeger, M. J., Knutsen, K. H., More, S., Mortensen, A., Naegeli, H., Noteborn, H., Ockleford, C., Ricci, A., Rychen, G., Silano, V., Solecki, R., Turck, D., Aerts, M., Bodin, L., Davis, A., Edler, L., … Schlatter, J. R. (2017). Update: use of the benchmark dose approach in risk assessment. *EFSA Journal*, *15*(1), 1–41. https://doi.org/10.2903/j.efsa.2017.4658

He, Y., Zhu, L., Ma, J., & Lin, G. (2021). Metabolism-mediated cytotoxicity and genotoxicity of pyrrolizidine alkaloids. *Archives of Toxicology*, *0123456789*. https://doi.org/10.1007/s00204-021-03060-w

Ismail, S. F., Azmi, I. M. A. G., Daud, M., Jalil, J. A., & Safuan, S. (2020). Regulatory Control of Herbal and Traditional Medicines in Malaysia: Issues and Concerns. *International Journal of Business and Society*, *21*(S1), 192.

Jantan, I. (2006). The Scientific Values of Malaysian Herbal Products. *Jurnal Sains*

Kesihatan Malaysia, *4*(1), 59–70.

JECFA. (2008). Safety evaluation of certain food additives and contaminants: Aflatoxins. In *Compendium of food additive specifications, WHO food additives series*.

Kaltner, F., Kukula, V., & Gottschalk, C. (2020). Screening of food supplements for toxic pyrrolizidine alkaloids. *Journal Fur Verbraucherschutz Und Lebensmittelsicherheit*, *15*(3), 237–243. https://doi.org/10.1007/s00003-020-01296-9

Knutsen, H. K., Alexander, J., Barreg, L., Ceccatelli, S., Cottrill, B., Dinovi, M., Edler, L., Grasl-kraupp, B., Hogstrand, C., Hoogenboom, L. R., Nebbia, C. S., Oswald, I. P., Petersen, A., Rose, M., Roudot, A., Schwerdtle, T., Vleminckx, C., Ruiz, A. G., Binaglia, M., & Wallace, H. (2017). *Risks for human health related to the presence of pyrrolizidine alkaloids in honey , tea , herbal infusions and food supplements*. *15*(June). https://doi.org/10.2903/j.efsa.2017.4908

Li, N., Xia, Q., Ruan, J., P. Fu, P., & Lin, G. (2012). Hepatotoxicity and Tumorigenicity Induced by Metabolic Activation of Pyrrolizidine Alkaloids in Herbs. *Current Drug Metabolism*, *12*(9), 823–834. https://doi.org/10.2174/138920011797470119

Ma, C., Liu, Y., Zhu, L., Ji, H., Song, X., Guo, H., & Yi, T. (2018). Determination and regulation of hepatotoxic pyrrolizidine alkaloids in food: A critical review of recent research. *Food and Chemical Toxicology*, *119*(May), 50–60. https://doi.org/10.1016/j.fct.2018.05.037

Ma, C., Liu, Y., Zhu, L., Ji, H., Song, X., Guo, H., & Yi, T. (2018b). Determination and regulation of hepatotoxic pyrrolizidine alkaloids in food: A critical review of recent research. *Food and Chemical Toxicology*, *119*(February), 50–60. https://doi.org/10.1016/j.fct.2018.05.037

Mathon, C., Edder, P., Bieri, S., & Christen, P. (2014). Survey of pyrrolizidine alkaloids in teas and herbal teas on the Swiss market using HPLC-MS/MS. *Analytical and Bioanalytical Chemistry*, 7345–7354. https://doi.org/10.1007/s00216-014-8142-8

Merz, K., & Schrenk, D. (2016). Interim relative potency factors for the toxicological risk assessment of pyrrolizidine alkaloids in food and herbal medicines. *Toxicology Letters*, *263*, 44–57. https://doi.org/10.1016/j.toxlet.2016.05.002

Moreira, R., Pereira, D. M., Valentão, P., & Andrade, P. B. (2018). Pyrrolizidine alkaloids: Chemistry, pharmacology, toxicology and food safety. *International Journal of Molecular Sciences*, *19*(6). https://doi.org/10.3390/ijms19061668

Mulder, P. P. J., López, P., These, A., Preiss-weigert, A., & Castellari, M. (2015). *Occurrence of Pyrrolizidine Alkaloids in food 1*. https://doi.org/10.2903/sp.efsa.2015.EN-859

Ning, J., Chen, L., & Rietjens, I. M. C. M. (2019). Role of toxicokinetics and alternative testing strategies in pyrrolizidine alkaloid toxicity and risk assessment; state-of-the-art and future perspectives. *Food and Chemical Toxicology*, *131*(June), 110572. https://doi.org/10.1016/j.fct.2019.110572

On, A. (2005a). Opinion of the Scientific Committee on a request from EFSA related to A Harmonised Approach for Risk Assessment of Substances Which are both Genotoxic and Carcinogenic. *EFSA Journal*, *3*(10), 1–31. https://doi.org/10.2903/j.efsa.2005.282

On, A. (2005b). *Opinion of the Scientific Committee on a request from EFSA related to A Harmonised Approach for Risk Assessment of Substances Which are both Genotoxic and Carcinogenic (Request No EFSA-Q-2004-020)*. 1–31.

Pauzi, N. A. M., Cheema, M. S., Ismail, A., Ghazali, A. R., & Abdullah, R. (2022). *Are Genotoxic and Carcinogenic Compounds Present in Malaysian Traditional*

Medicines and Herbal Supplements ? A Review Based on the Malaysian Herbal Monograph. *2022*(6), 207–222.

Poswal, F. S., Russell, G., Mackonochie, M., MacLennan, E., Adukwu, E. C., & Rolfe, V. (2019). Herbal Teas and their Health Benefits: A Scoping Review. *Plant Foods for Human Nutrition*, *74*(3), 266–276. https://doi.org/10.1007/s11130-019-00750-w

Ravikumar, C. (2007). *Review on Herbal Teas*. *6*(5), 236–238.

Ridker, P. M., Ohkuma, S., Mcdermott, W. V., Trey, C., & Huxtable, R. J. (1985). Hepatic Venocclusive Disease Associated With the Consumption of Pyrrolizidine Containing Dietary Supplements. *Gastroenterology*, *88*(4), 1050–1054. https://doi.org/10.1016/S0016-5085(85)80027-5

Roeder, E., & Wiedenfeld, H. (2009). Pyrrolizidine alkaloids in medicinal plants of Mongolia, Nepal and Tibet. *Pharmazie*, *64*(11), 699–716. https://doi.org/10.1691/ph.2009.9653

Schrenk, D., Gao, L., Lin, G., Mahony, C., Mulder, P. P. J., Peijnenburg, A., Pfuhler, S., Rietjens, I. M. C. M., Rutz, L., Steinhoff, B., & These, A. (2020). Pyrrolizidine alkaloids in food and phytomedicine: Occurrence, exposure, toxicity, mechanisms, and risk assessment - A review. *Food and Chemical Toxicology*, *136*(December 2019), 111107. https://doi.org/10.1016/j.fct.2019.111107

Schulz, M., Meins, J., Diemert, S., Zagermann-Muncke, P., Goebel, R., Schrenk, D., Schubert-Zsilavecz, M., & Abdel-Tawab, M. (2015). Detection of pyrrolizidine alkaloids in German licensed herbal medicinal teas. *Phytomedicine*, *22*(6), 648–656. https://doi.org/10.1016/j.phymed.2015.03.020

Seremet, O. C., Olaru, O. T., Gutu, C. M., Nitulescu, G. M., Ilie, M., Negres, S., Zbarcea, C. E., Purdel, C. N., Spandidos, D. A., Tsatsakis, A. M., Coleman, M. D., &

Margina, D. M. (2018). *Toxicity of plant extracts containing pyrrolizidine alkaloids using alternative invertebrate models*. 7757–7763. https://doi.org/10.3892/mmr.2018.8795

Steinhoff, B. (2019). Pyrrolizidine alkaloid contamination in herbal medicinal products: Limits and occurrence. *Food and Chemical Toxicology*, *130*(February), 262–266. https://doi.org/10.1016/j.fct.2019.05.026

Stickel, F., & Seitz, H. K. (2000). The efficacy and safety of comfrey. *Public Health Nutrition*, *3*(4 A), 501–508. https://doi.org/10.1017/S1368980000000586

Suparmi, S., Mulder, P. P. J., & Rietjens, I. M. C. M. (2020). Detection of pyrrolizidine alkaloids in jamu available on the Indonesian market and accompanying safety assessment for human consumption. *Food and Chemical Toxicology*, *138*(February), 111230. https://doi.org/10.1016/j.fct.2020.111230

Swirsky Gold, L., Sawyer, C. B., & Magaw, R. (1984). A carcinogenic potency database of the standardized results of animal bioassays. *Environmental Health Perspectives*, *VOL. 58*, 9–319. https://doi.org/10.2307/3429857

Wang, J., Zhang, M., Chen, L., Qiao, Y., Ma, S., Sun, D., Si, J., & Liao, Y. (2021). Determination of toxic pyrrolizidine alkaloids in traditional chinese herbal medicines by uplc-ms/ms and accompanying risk assessment for human health. *Molecules*, *26*(6). https://doi.org/10.3390/molecules26061648

Wiedenfeld, H., & Edgar, J. (2011). Toxicity of pyrrolizidine alkaloids to humans and ruminants. *Phytochemistry Reviews*, *10*(1), 137–151. https://doi.org/10.1007/s11101-010-9174-0

World Health Organization (WHO). (1989). *ALKALOIDS HEALTH AND*.

World Health Organization (WHO). (2013). WHO Traditional Medicine Strategy 2014-2023. *World Health Organization (WHO)*, 1–76. https://doi.org/2013

Zheng, P., Xu, Y., Ren, Z., Wang, Z., Wang, S., & Xiong, J. (2021). *Toxic Prediction of Pyrrolizidine Alkaloids and Structure- Dependent Induction of Apoptosis in HepaRG Cells*. *2021*.

SUPPLEMENTARY DATA

APPENDIX 1 – Corrected animal dataset

Type of diet	Gender	Concentration of PA (µg/kg)	Duration of dosing (week)	Duration of observation (week)	Corrected dose (µg per kg bw and day)	Time adjusted dose (µg per kg bw and day)	Tumour incidence	BMDL (µg/kg bw per day)	Route of administration	Referrences
riddelliine	M	0	72	72	0.00	0.00	0/50	-	Gavage	NTP, 2003
		1000	72	72	714.29	335.86	43/50			
	F	0	105	105	0.00	0.00	0/50	176.76 - 272.95		
		10	105	105	7.14	7.14	0/50			
		33	105	105	23.57	23.57	0/50			
		100	105	105	71.43	72.81	0/50			
		330	105	105	235.71	235.71	3/50			
		1000	97	97	714.29	621.37	38/50			
riddelliine	M	0	105	105	0.00	0.00	2/50	745.36 - 868.18		
		100	105	105	71.43	71.43	1/50			
		300	105	105	214.29	214.29	0/50			
		1000	105	105	714.29	714.29	2/50			
		3000	105	105	2142.86	2142.86	31/50			
	F	0	105	105	0.00	0.00	0/50	-		
		3000	105	105	2142.86	2142.86	1/50			
lasiocarpine	M	0	104	104	0	0.00	0/24	45.27 -	Gavage	NTP 1978

		7000	104	87	280	234.23	5/24	79.39		
		15000	104	74	600	426.92	11/24			
		30000	104	88	1200	1015.38	13/24			
	F	0	104	104	0	0.00	0/24	1.62		
		7000	104	84	350	282.69	8/24			
		15000	104	68	750	490.38	7/24			
		30000	104	69	1500	995.19	2/24			
lasiocarpine	M	0	55	59	0	0.00	0/10	-	Gavage	Rao & Reddy 1978
		50000	55	48	2000	488.17	9/20			

APPENDIX 2 – Results from BMD analysis of PA tumour incidence using BMDS software version 3.2

Model	Restriction	Number of paramaters	Risk Type	BMRF	Log-likelihood	*p*-value	Accepted	BMD (µg/kg bw per day)	BMDL (µg/kg bw per day)	BMD/BMDL
NTP 2003 - RAT (Female)										
Dichotomous Hill	Unrestricted	3	Extra Risk	0.1	-38.9301	0.9987377	Yes	271.68	217.03	1.25
Gamma	Unrestricted	3	Extra Risk	0.1	-38.9111	0.9997760	Yes	270.04	215.99	1.25
Logistic	Unrestricted	2	Extra Risk	0.1	-39.9445	0.8355663	Yes	328.75	272.95	1.20
Log-Logistic	Unrestricted	2	Extra Risk	0.1	-38.9301	0.9999001	Yes	271.68	217.03	1.25
Log-Probit	Unrestricted	3	Extra Risk	0.1	-38.9028	0.9999975	Yes	265.03	216.84	1.22
Multistage Degree 5	Unrestricted	6	Extra Risk	0.1	-38.9024	NA	No	268.40	124.38	2.16
Multistage Degree 4	Unrestricted	4	Extra Risk	0.1	-38.9024	0.9999996	Yes	268.14	176.76	1.52
Multistage Degree 3	Unrestricted	4	Extra Risk	0.1	-38.9024	0.9999996	Yes	275.92	210.69	1.31
Multistage Degree 2	Unrestricted	2	Extra Risk	0.1	-38.9024	1	Yes	262.14	212.53	1.23
Multistage Degree 1	Unrestricted	2	Extra Risk	0.1	-57.0990	<0.0001	No	88.43	68.70	1.29
Probit	Unrestricte	2	Extra	0.1	-39.3327	0.9677441	Yes	300.89	249.80	1.20

	d		Risk							
Quantal Linear	Unrestricte d	2	Extra Risk	0.1	-57.0990	<0.0001	No	88.43	68.70	1.29
Weibull	Unrestricte d	2	Extra Risk	0.1	-38.9693	0.9993916	Yes	281.21	217.31	1.29
NTP 2003 - MICE (MALE)										
Dichotomous Hill	Unrestricte d	4	Extra Risk	0.1	-56.31	0.1533717	Yes	1111.59	792.71	1.40
Gamma	Unrestricte d	3	Extra Risk	0.1	-56.31	0.3607410	Yes	1081.53	790.18	1.37
Logistic	Unrestricte d	2	Extra Risk	0.1	-57.16	0.2074609	Yes	1016.92	842.27	1.21
Log-Logistic	Unrestricte d	3	Extra Risk	0.1	-56.31	0.3608259	Yes	1111.45	792.73	1.40
Log-Probit	Unrestricte d	3	Extra Risk	0.1	-56.31	0.3604439	Yes	1037.19	771.85	1.34
Multistage Degree 4	Unrestricte d	5	Extra Risk	0.1	-55.02	NA	No	872.73	755.17	1.16
Multistage Degree 3	Unrestricte d	4	Extra Risk	0.1	-55.14	0.4517422	Yes	1037.79	783.59	1.32
Multistage Degree 2	Unrestricte d	3	Extra Risk	0.1	-55.03	0.8645479	Yes	1022.18	868.18	1.18
Multistage Degree 1	Unrestricte d	2	Extra Risk	0.1	-67.51	0.0001970	No	388.53	292.35	1.33
Probit	Unrestricte d	2	Extra Risk	0.1	-57.79	0.1012251	Yes	897.17	745.36	1.20
Quantal Linear	Unrestricte d	2	Extra Risk	0.1	-67.51	0.0001970	No	388.53	292.35	1.33
Weibull	Unrestricte	3	Extra	0.1	-56.32	0.3610487	Yes	1164.72	813.13	1.43

	d		Risk							
NTP 1978 (MALE)										
Dichotomous Hill	Unrestricted	4	Extra Risk	0.1	-45.39	NA	No	176.19	5.42	32.53
Gamma	Unrestricted	2	Extra Risk	0.1	-46.03	0.5225213	No	45.18	0.23	197.14
Logistic	Unrestricted	2	Extra Risk	0.1	-50.51	0.0199242	No	268.90	206.02	1.31
Log-Logistic	Unrestricted	3	Extra Risk	0.1	-45.93	0.2969752	No	69.40	0.40	174.28
Log-Probit	Unrestricted	2	Extra Risk	0.1	-45.91	0.5913678	No	80.42	0.76	105.90
Multistage Degree 3	Unrestricted	4	Extra Risk	0.1	-45.39	NA	No	149.85	37.81	3.96
Multistage Degree 2	Unrestricted	2	Extra Risk	0.1	-45.78	0.6764309	Yes	74.14	45.27	1.64
Multistage Degree 1	Unrestricted	2	Extra Risk	0.1	-46.47	0.3238210	Yes	106.94	79.39	1.35
Probit	Unrestricted	2	Extra Risk	0.1	-50.22	0.0230829	No	252.74	196.58	1.29
Quantal Linear	Unrestricted	1	Extra Risk	0.1	-46.47	0.5211715	Yes	106.94	79.39	1.35
Weibull	Unrestricted	3	Extra Risk	0.1	-46.01	0.2651588	No	50.49	0.09	589.17
NTP 1978 (FEMALE)										
Dichotomous Hill	Unrestricted	2	Extra Risk	0.1	-39.35	0.0918897	No	0	0	-
Gamma	Unrestricted	1	Extra Risk	0.1	-40.49	0.0721915	Yes	7.89	1.62	4.88

Logistic	Unrestricted	2	Extra Risk	0.1	-44.82	0.0015816	No	5483.09	535.84	-
Log-Logistic	Unrestricted	1	Extra Risk	0.1	-39.35	0.1890305	No	0	0	-
Log-Probit	Unrestricted	3	Extra Risk	0.1	-44.83	0.0003475	No	629163856.6	0	-
Multistage Degree 3	Unrestricted	4	Extra Risk	0.1	-36.65	NA	No	1025.38	533.38	1.92
Multistage Degree 2	Unrestricted	3	Extra Risk	0.1	-36.94	0.4426824	No	254768.64	0	-
Multistage Degree 1	Unrestricted	2	Extra Risk	0.1	-46.01	<0.0001	No	238.22	163.72	1.46
Probit	Unrestricted	2	Extra Risk	0.1	-44.82	0.0015726	No	5082.44	504.00	-
Quantal Linear	Unrestricted	2	Extra Risk	0.1	-44.80	0.0014274	No	2215.97	209.11	-
Weibull	Unrestricted	1	Extra Risk	0.1	-39.35	0.1890867	No	65535	0	-

APPENDIX 3 – Data on herbal supplements containing PAs including country of origin, estimated daily intake and MOE values.

No.	Herbal supplement (Name /species)	Country of origin	Product presentation	Total level of PA (µg/kg)	Estimated Daily Intake (µg/kg bw/day)[a]	Range of MOE[b]	References
1.	Sanxi Shangyao Pian	China	Tablet	4.20	0.0002700	654666.66 - 1010925.92	(Chen et al., 2019; Wang et al., 2021)
2.	Feire Keli	China	Bag	4.00	0.0013714	128887.5 - 199026.04	
3.	Runfei Zhisou Wan (coltsfoot)	China	Pill	6344.00	2.1750857	81.26 - 125.48	
4.	Qianbai Biyan Pian (Senecio scandens)	China	Tablet	7883.00	0.5946034	297.27 - 459.04	
5.	Qianxi Pian (Senecio scandens)	China	Tablet	403.90	0.0214644	8235.03 – 12716.40	
6.	Juhong Keli (coltsfoot)	China	Bag	1431.00	0.4497429	393.02 – 606.90	
7.	*Arnebia euchroma*	China	NA	25567.40	3.6524857	48.39 – 74.72	
8.	*Tussilago farfara*	China	NA	17600.20	2.5143143	70.301- 108.55	
9.	*Eupatorium fortunei*	China	NA	16943.60	2.4205143	73.02 – 112.76	
10.	*Eupatorium lindleyanum*	China	NA	1863.60	1.5973714	110.65 -170.87	
11.	*Senecio scandens*	China	NA	229.00	0.0981429	1801.04 - 2781.14	
12.	*Laggera pterodonta*	China	NA	198.50	0.0425357	4155.56 - 6416.96	

13.	*Artemisia scoparia*	China	NA	233.60	0.0333714	5296.74 - 8179.15	
14.	*Cassia angustifolia*	China	NA	220.60	0.0189086	9348.14 - 14435.25	
15.	*Euphorbia hirta*	China	NA	121.90	0.0156729	11278.09 - 17415.45	
16.	*Gynura japonica*	China	NA	531.00	0.0455143	3883.61 - 5997.018	
17.	*Scutellaria barbata*	China	NA	61.70	0.0264429	6684.602 - 10322.258	
18.	*Euphorbia humifusa*	China	NA	45.00	0.0128571	13748.00 - 21229.444	
19.	*Picria fel-terrae*	China	NA	58.20	0.0124714	14173.195 - 21886.025	
20.	*Cirsium japonicum*	China	NA	44.60	0.0095571	18495.067 - 28559.790	

21.	*Abrus cantoniensis*	China	NA	22.10	0.0094714	18662.443 - 272.95	
22.	*Equisetum hyemale*	China	NA	66.60	0.0085629	20642.642 - 31876.042	
23.	*Apocynum venetum*	China	NA	49.30	0.0084514	20914.807 - 32296.315	
24.	*Siphonostegia chinensis*	China	NA	51.80	0.0066600	26540.540 - 40983.483	
25.	*Achillea alpina*	China	NA	51.90	0.0333643	5297.880 - 8180.903	
26.	*Phryma leptostachya*	China	NA	45.70	0.0097929	18049.890 - 27872.355	
27.	Hedyotis diffusa	China	NA	7.24	0.0031029	56966.850 - 87967.311	
28.	Angelica sinensis	China	NA	1.06	0.0001514	1167283.019 - 1802500.00	

29.	Thlaspi arvense Linn	China	NA	14.14	0.0060600	29168.316 - 45041.254	
30.	Boehmeria nivea (L.) Gaudich	China	NA	0.50	0.0000710	2489577.465 - 3844366.197	
31.	Triticum aestivum L	China	NA	0.81	0.0001157	1527555.556 - 2358827.16	
32.	Zea mays L	China	NA	0.91	0.0001300	1359692.308 - 2099615.385	
33.	Benincasa hispida (Thunb.) Cogn.	China	NA	0.10	0.0000137	12888750.00 - 19902604.17	
34.	Pteris multifida Poir.	China	NA	0.96	0.0001371	1288875.00 - 1990260.417	
35.	Gynostemma pentaphyllum (Thunb.) Makino	China	NA	1.39	0.0001986	890158.273 - 1374568.345	

36.	Ramulus Euonymi	China	NA	0.24	0.0000337	5242881.356 - 8095974.576	
37.	Paederia scandens (Lour.) Merr	China	NA	0.23	0.0000331	5333275.862 - 8235560.345	
38.	Ilex pubescens Hook. et Arn.	China	NA	0.51	0.0000724	2440473.373 - 3768540.434	
39.	Pogostemon cablin (Blanco) Benth	China	NA	40.75	0.0058214	30363.680 - 46887.116	
40.	Eclipta prostrata L	China	NA	29.44	0.0050469	35023.777 - 54083.163	
41.	Taraxacum mongolicum Hand.-Mazz	China	NA	22.71	0.0048664	36322.324 - 56088.360	
42.	Erigeron breviscapus (Vant.) Hand.-Mazz	China	NA	20.86	0.0044700	39543.624 - 61062.639	

43.	Arctium lappa L	China	NA	25.42	0.0043577	40562.549 - 62636.047	
44.	Solidago decurrens Lour	China	NA	19.39	0.0041550	42541.516 - 65691.937	
45.	Desmodium styracifolium (Osb.) Merr.	China	NA	9.52	0.0040800	43323.529 - 66899.509	
46.	Imperata cylindrica Beauv. var. major (Nees) C. E. Hubb	China	NA	8.44	0.0036171	48867.298 - 75460.110	
47.	Clematis chinensis Osbeck.	China	NA	25.17	0.0035957	49158.522 - 75909.813	
48.	Dalbergia odorifera T. Chen	China	NA	16.24	0.0034800	50793.103 - 78433.908	
49.	Inula linariifolia Turcz.	China	NA	23.24	0.0033200	53240.963 - 82213.855	

50.	Vaccaria segetalis (Neck.) Garcke	China	NA	22.81	0.0032586	54244.629 - 83763.7001	
51.	Albizia julibrissin Durazz.	China	NA	15.62	0.0026777	66011.523 - 101933.952	
52.	Atractylodes lancea (Thunb.) DC	China	NA	20.71	0.0026627	66383.389 - 102508.181	
53.	Inula helenium L.	China	NA	18.12	0.0023297	75871.964 - 117160.289	
54.	Pinus tabulieformis Carr	China	NA	10.46	0.0022414	78860.420 - 121775.015	
55.	Mosla chinensis Maxim.	China	NA	14.75	0.0021071	83886.101 - 129535.593	
56.	Cirsium setosum (Willd.) MB.	China	NA	12.02	0.0020606	85782.029 - 132463.255	
57.	Aster tataricus L. f.	China	NA	13.89	0.0019843	89079.913 - 137555.795	

58.	Artemisia annua L.	China	NA	11.04	0.0018926	93396.739 - 144221.769	
59.	Siegesbeckia orientalis L	China	NA	10.95	0.0018771	94164.383 - 145407.153	
60.	Aucklandia lappa Decne.	China	NA	18.49	0.0015849	111530.557 - 172223.724	
61.	Smilax glabra Roxb	China	NA	1.76	0.0015086	117170.454 - 180932.765	
62.	Astragalus propinquus Schischkin	China	NA	3.42	0.0014657	120596.491 - 186223.196	
63.	Caesalpinia sappan L	China	NA	11.07	0.0014233	124191.508 - 191774.565	
64.	Rehmannia glutinosa Libosch.	China	NA	6.41	0.0013736	128686.427 - 198715.5486	

65.	Coix lacryma -jobi L. var. ma -yuen (Roman.) Stapf	China	NA	3.20	0.0013714	128887.5 - 199026.0417	
66.	Carthamus tinctorius L	China	NA	9.45	0.0013500	130933.3333 - 202185.1852	
67.	Ampelopsis japonica (Thunb.) Makino	China	NA	8.16	0.0011657	151632.3529 - 234148.2843	
68.	Glycine max (L.) Merr	China	NA	5.42	0.0011614	152191.8819 - 235012.3001	
69.	Echinops latifolius Tausch.	China	NA	8.10	0.0011571	152755.5556 - 235882.716	
70.	Inula japonica Thunb	China	NA	8.73	0.0011224	157479.9542 - 243178.0578	
71.	Trachelospermum jasminoides (Lindl.) Lem	China	NA	5.77	0.0009891	178700.1733 - 275945.985	

72.	Artemisia argyi Levl. et Vant	China	NA	7.53	0.0009681	182576.3612 - 281931.5331	
73.	Pulsatilla chinensis (Bge.) Regel	China	NA	4.36	0.0009343	189192.6606 - 292148.318	
74.	Xanthium sibiricum Patr	China	NA	6.53	0.0009329	189482.389 - 292595.7121	
75.	Hordeum vulgare L.	China	NA	4.28	0.0009171	192728.972 - 297609.0343	
76.	Sauropus spatulifolius Beille	China	NA	4.16	0.0008914	198288.4615 - 306193.9103	
77.	Phyllanthus emblica L	China	NA	6.45	0.0008293	213147.2868 - 329138.6736	
78.	Bupleurum chinense DC	China	NA	5.70	0.0008143	217073.6842 - 335201.7544	

79.	Bletilla striata (Thunb.) Reichb. f	China	NA	3.69	0.0007907	223544.7154 - 345194.2186	
80.	Fagopyrum dibotrys (D. Don) Ham	China	NA	1.13	0.0007264	243327.4336 - 375742.3795	
81.	Sophora japonica L	China	NA	4.88	0.0006971	253549.1803 - 391526.6393	
82..	Corydalis bungeana Turcz.	China	NA	3.13	0.0006707	263539.9361 - 406954.2066	
83	Adenophora stricta Miq.	China	NA	3.10	0.0006643	266090.3226 - 410892.4731	
84.	Carpesium abrotanoides L.	China	NA	5.09	0.0006544	270098.2318 - 417081.4233	
85.	Ghrysanthemum indicum L.	China	NA	2.95	0.0006321	279620.339 - 431785.3107	

86.	Albizia julibrissin Durazz	China	NA	4.24	0.0006057	291820.7547 – 450625.00	
87.	Vigna umbellata Ohwi et Ohash	China	NA	1.40	0.0006000	294600.00 - 454916.6667	
88.	Rosa rugosa Thunb.	China	NA	6.98	0.0005983	295444.1261 - 456220.1528	
89.	Epimedium koreanum Nakai	China	NA	3.83	0.0005471	323060.0522 - 498864.2298	
90.	Lycopodium japonicum Thunb.	China	NA	3.07	0.0005263	335863.1922 - 518634.6363	
91.	Canavalia gladiata (Jacq.) DC.	China	NA	3.75	0.0004821	366613.3333 - 566118.5185	
92.	Ophiopogon japonicus (L. f) Ker-GawL	China	NA	2.78	0.0004766	370899.2806 - 572736.8106	

93.	Allium macrostemon Bge	China	NA	3.26	0.0004657	379546.0123 - 586088.9571	
94.	Morus alba L.	China	NA	2.17	0.0004650	380129.0323 - 586989.2473	
95	Andrographis paniculata (Burm.f.) Nees	China	NA	3.35	0.0004307	410388.0597 - 633714.7595	
96.	Acanthoppanax gracilistylus W. W. Smith	China	NA	2.90	0.0004143	426662.069 - 658844.8276	
97.	Physalis alkekengi L. var. franchetii (Mast.) Makino	China	NA	3.19	0.0004101	430971.7868 - 665499.8258	
98	Rhaponticum uniflorum (L.) DC	China	NA	3.18	0.0004089	432327.044 - 667592.5926	
99.	Erodium stephanianum Willd	China	NA	1.88	0.0004029	438765.9574 - 677535.461	

100.	Polygonum aviculare L	China	NA	1.86	0.0003986	443483.871 - 684820.7885	
101.	Luffa cylindrica (L.) Roem.	China	NA	2.31	0.0003960	446363.6364 - 689267.6768	
102.	Sophora tonkinensis Gagnep	China	NA	4.54	0.0003891	454229.0749 - 701413.3627	
103.	Sarcandra glabra (Thunb.) Nakai	China	NA	0.89	0.0003814	463415.7303 - 715599.2509	
104.	Vitex trifolia L. var. simplicifolia Cham.	China	NA	2.53	0.0003614	489059.2885 - 755197.6285	
105.	Glycyrrhiza uralensis Fisch	China	NA	2.47	0.0003529	500939.2713 - 773542.5101	
106.	Paris polyphylla Smith var. chinensis (Franch.) Hara	China	NA	2.54	0.0003266	541259.8425 - 835804.8994	
107.	Gastrodia elata Bl.	China	NA	2.19	0.0003129	564986.3014 - 872442.9224	

108.	Homalomena occulta (Lour.) Schott	China	NA	2.16	0.0003086	572833.3333 - 884560.1852	
109.	Plantago asiatica L. & Plantago depressa Willd	China	NA	0.68	0.0002914	606529.4118 - 936593.1373	
110.	Paeonia lactiflora PalL	China	NA	1.68	0.0002880	613750 - 947743.0556	
111.	Glycine max (L.) Merr.	China	NA	0.67	0.0002871	615582.0896 - 950572.1393	
112.	Paeonia suffruticosa Andr	China	NA	1.64	0.0002811	628719.5122 - 970858.7398	
113.	Dolichos lablab L.	China	NA	1.26	0.0002700	654666.6667 - 1010925.926	
114.	Centipeda minima (L.) A.Br. et Aschers.	China	NA	2.05	0.0002636	670634.1463 - 1035582.656	

115.	Gentiana macrophylla Pall.	China	NA	1.67	0.0002386	740910.1796 - 1144101.796	
116.	Verbena Officinalis L	China	NA	1.67	0.0002386	740910.1796 - 1144101.796	
117.	Scrophularia ningpoensis Hemsl.	China	NA	1.10	0.0002357	749890.9091 - 1157969.697	
118.	Picrorhiza scrophulariiflora Pennell	China	NA	1.65	0.0002357	749890.9091 - 1157969.697	
119.	Akebia quinata (Thunb.) Decne	China	NA	1.80	0.0002314	763777.7778 - 1179413.58	
120.	Cuscuta australis R. Br.	China	NA	1.35	0.0002314	763777.7778 - 1179413.58	
121.	Gardenia jasminoides Ellis	China	NA	1.61	0.0002300	768521.7391 - 1186739.13	

122.	Acacia catechu (L. f.) Willd.	China	NA	5.22	0.0002237	790114.9425 - 1220083.014	
123.	Notopterygium inchum Ting ex H, T- Chang	China	NA	1.56	0.0002229	793153.8462 - 1224775.641	
124.	Kaempferia galanga L.	China	NA	1.66	0.0002134	828192.7711 - 1278882.195	
125.	Sophora flavescens Ai	China	NA	1.65	0.0002121	833212.1212 - 1286632.997	
126.	Setaria italica (L.) Beauv	China	NA	0.98	0.0002100	841714.2857 - 1299761.905	
127.	Astragalus membranaceus (Fisch.) Bge	China	NA	0.49	0.0002100	841714.2857 - 1299761.905	
128.	Lophatherum gracile Brongn	China	NA	1.41	0.0002014	877531.9149 - 1355070.922	

129.	Dendrobium nobile Lindl	China	NA	1.14	0.0001954	904473.6842 - 1396673.977	
130.	Eriocaulon buergerianum Koern	China	NA	1.36	0.0001943	909794.1176 - 1404889.706	
131.	Phragmites communis Trin.	China	NA	0.45	0.0001929	916533.3333 - 1415296.296	
132.	Spatholobus suberectus Dunn	China	NA	0.89	0.0001907	926831.4607 - 1431198.502	
133.	Santalum album L	China	NA	2.53	0.0001807	978118.5771 - 1510395.257	
134.	Cralaegus pinnatifida. Bge. var. major N.E.Br	China	NA	1.03	0.0001766	1001067.961 - 1545833.333	
135.	Lysimachia christinae Hance	China	NA	0.20	0.0001714	1031100 - 1592208.333	
136.	Ranunculus ternatus Thunb.	China	NA	0.39	0.0001671	1057538.462 - 1633034.188	
137.	Cichorium glandulosum Boiss.et Huet	China	NA	0.65	0.0001671	1057538.462 - 1633034.188	
138.	Polygonum multiflorum Thumb	China	NA	0.75	0.0001607	1099840 - 1698355.556	

139.	Stachyurus himalai -cus Hook.f.et Thoms	China	NA	1.85	0.0001586	1114702.703 - 1721306.306	
140.	Dimocarpus longan Lour.	China	NA	0.73	0.0001564	1129972.603 - 1744885.845	
141.	Pueraria lobata (Willd.) Ohwi	China	NA	0.69	0.0001479	1195478.261 - 1846038.647	
142.	Curcuma Longa L.	China	NA	1.03	0.0001471	1201281.553 - 1855000	
143	Selaginella tamariscina (Beauv.) Spring	China	NA	1.01	0.0001443	1225069.307 - 1891732.673	
144.	Ilex cornuta Lindl. ex Paxt.	China	NA	0.63	0.0001350	1309333.333 - 2021851.852	
145.	Paeonia lactiflora Pall	China	NA	0.60	0.0001286	1374800.00 - 2122944.444	
146.	Lobelia chinensis Lour	China	NA	0.59	0.0001264	1398101.695 - 2158926.554	
147.	Fraxinus rhynchophylla Hance	China	NA	0.72	0.0001234	1432083.333 - 2211400.463	
148.	Euphorbia pekinensis Rupr	China	NA	2.88	0.0001234	1432083.333 - 2211400.463	
149.	Atractylodes macrocephala Koidz.	China	NA	0.72	0.0001234	1432083.333 - 1502211400.463	
150.	Cassia obtusifolia L.	China	NA	0.56	0.0001200	1471513000 - 2274583.333	

151.	Piper kadsura (CHOisy) Ohwi	China	NA	0.70	0.0001200	1473000 - 2274583.333	
152.	Curcuma kwangsiensis S. G. Lee et C. F. Liang	China	NA	0.84	0.0001200	1473000.00 - 2274583.333	
153.	Lycium barbarum L.	China	NA	0.70	0.0001200	1473000.00 - 2274583.333	
154.	Sesamum indicum L	China	NA	0.56	0.0001200	1473000.00 - 2274583.333	
155.	Citrus medica L	China	NA	0.83	0.0001186	1490746.988 - 2301987.952	
156.	Glycine max (L.) Merr	China	NA	0.68	0.0001166	1516323.529 - 2341482.843	
157.	Salvia miltiorrhiza Bge	China	NA	0.54	0.0001157	1527555.556 - 2358827.16	
158.	Leonurus japonicus Houtt	China	NA	0.27	0.0001157	1527555.556 - 2358827.16	
159.	Polygonatum ordoratum (Mill.) Druce	China	NA	0.67	0.0001149	1538955.224 - 2376430.348	

160.	Aquilaria sinensis (Lour.) Gilg	China	NA	1.60	0.0001143	1546650.00 - 2388312.5	
161.	Cornus officinalis Sieb. et Zucc	China	NA	0.66	0.0001131	1562272.727 - 2412436.869	
162.	Angelica dahurica (Fisch. ex Hoffm.) Benth. et Hook. f	China	NA	0.79	0.0001129	1566227.848 - 2418544.304	
163.	Lonicera japonica Thunb.	China	NA	0.53	0.0001136	1556377.358 - 2403333.333	
164.	Nigella glandulifera Freyn et Sint.	China	NA	1.31	0.0001123	1574198.473 - 2430852.417	
165.	Codonopsis pilosula (Franch.) Nannf.	China	NA	0.26	0.0001114	1586307.692 - 2449551.282	
166.	Panax quinquefolium L	China	NA	1.29	0.0001106	1598604.651 - 2468540.052	

167.	Houttuynia cordata Thunb.	China	NA	0.31	0.0001107	1596541.935 - 2465354.839	
168.	Gleditsia sinensis Lam	China	NA	0.76	0.0001086	1628052.632 - 2514013.158	
169.	Prunus mume (Sieb.) Sieb. et Zucc	China	NA	1.50	0.0001071	1649760 - 2547533.333	
17.0	Citrus medica L. var. sarcodactylis Swingle	China	NA	0.74	0.0001057	1672054.054 - 2581959.459	
171.	Asparagus cochinchinensis (Lour.) Merr	China	NA	0.60	0.0001029	1718500.00 - 2653680.556	
172.	Zingiber officinale Rosc.	China	NA	0.80	0.0001029	1718500.00 - 2653680.556	
173.	Sanguisorba officinalis L.	China	NA	0.48	0.0001029	1718500.00 - 2653680.556	
174.	Isatis indigotica Fort.	China	NA	0.48	0.0001029	1718500 - 2653680.556	
175.	Mentha haplocalyx Briq	China	NA	1.16	0.0000994	1777758.621 - 2745186.782	

176.	Gremastra appendiculata (D.Don) Makino	China	NA	0.72	0.0000926	1909444.444 - 2948533.951	
177.	Gentiana manshuric Kitag	China	NA	1.06	0.0000909	1945471.698 - 3004166.667	
178.	Platycodon grandiflorum (Jacq.) A. DC	China	NA	0.62	0.0000886	1995677.419 - 3081693.548	
179.	Vladimiria souliei (Franch.) Ling	China	NA	0.66	0.0000849	2083030.303 - 3216582.492	
180.	Cynanchum atratum Bge.	China	NA	0.60	0.0000857	2062200 - 3184416.667	
181.	Cimicifuga heracleifolia Kom .	China	NA	0.59	0.0000843	2097152.542 - 3238389.831	
182.	Cynanchum stauntonii (Decne.) Schltr. ex Levi.	China	NA	0.59	0.0000843	2097152.542 - 3238389.831	
183.	Bambusa tuldoides Munro	China	NA	0.57	0.0000814	2170736.842 - 3352017.544	
184.	Dipsacus asper Wall. ex Henry	China	NA	0.37	0.0000793	2229405.405 - 3442612.613	
185.	Morinda officinalis How	China	NA	0.54	0.0000771	2291333.333 - 3538240.741	
186.	Lilium lancifolium Thunb	China	NA	0.45	0.0000771	2291333.333 - 3538240.741	

187.	Magnolia biondii Pamp.	China	NA	0.53	0.0000757	2334566.038 – 3605000.00	
188.	Cyathula officinalis Kuan	China	NA	0.53	0.0000757	2334566.038 - 3605000.00	
189.	Illicium verum Hook f.	China	NA	0.85	0.0000729	2426117.647 - 3746372.549	
190.	Oryza sativa L	China	NA	0.33	0.0000707	2499636.364 - 3859898.99	
191.	Rubus chingii Hu	China	NA	0.41	0.0000703	2514878.049 - 3883434.959	
192.	Prunus mume (Sieb.) Sieb. et Zucc.	China	NA	0.41	0.0000703	2514878.049 - 3883434.959	
193.	Alpinia katsumadai Hayata	China	NA	0.81	0.0000694	2545925.926 - 3931378.601	
194.	Liquidambar formosana Hance	China	NA	0.48	0.0000686	2577750 - 3980520.833	
195.	Alpinia officinarum Hance	China	NA	0.78	0.0000669	2643846.154 - 4082585.47	
196.	Rubia cordifolia L .	China	NA	0.46	0.0000657	2689826.087 - 4153586.957	
197.	Zingiber officinale Rosc	China	NA	0.46	0.0000657	2689826.087 - 4153586.957	
198.	Cinnamomum cassia Presl	China	NA	0.91	0.0000650	2719384.615 - 4199230.769	

199.	Achyranthes bidentata Bl	China	NA	0.36	0.0000617	2864166.667 - 4422800.926	
200.	Cynanchum paniculatum (Bge.) Kitag.	China	NA	0.36	0.0000617	2864166.667 - 4422800.926	
201.	Raphanus sativus L.	China	NA	0.36	0.0000617	2864166.667 - 4422800.926	
202.	Cyperus rotundus L	China	NA	0.42	0.0000600	2946000.00 - 4549166.667	
203.	Cnidium monnieri (L.) Cuss	China	NA	0.41	0.0000586	3017853.659 - 4660121.951	
204.	Asarum heterotropoides Fr. Schmidt var. mandshuricum (Maxim.) Kitag	China	NA	1.35	0.0000579	3055111.111 - 4717654.321	
205.	Panax notoginseng (Burk.) F. H. Chen	China	NA	0.44	0.0000566	3124545.455 - 4824873.737	
206.	Taxillus chinensis (DC.) Danser	China	NA	0.26	0.0000557	3172615.385 - 4899102.564	
207.	Potentilla chinensis Ser.	China	NA	0.26	0.0000557	3172615.385 - 4899102.564	
208.	Ziziphus jujuba Mill	China	NA	0.26	0.0000557	3172615.385 - 4899102.564	
209.	Broussonetia papyrifera (L.) Vent	China	NA	0.31	0.0000531	3326129.032 - 5136155.914	

210.	Tribulus terrestris L	China	NA	0.37	0.0000529	3344108.108 - 5163918.919	
211.	Citrus aurantium L.	China	NA	0.37	0.0000529	3344108.108 - 5163918.919	
212.	Curculigo orchioides Gaertn	China	NA	0.37	0.0000529	3344108.108 - 5163918.919	
213.	Panax ginseng C. A. Mey.	China	NA	0.40	0.0000514	3437000.00 - 5307361.111	
214.	Lygodium japonicum (Thunb.) Sw	China	NA	0.24	0.0000514	3437000.00 - 5307361.111	
215.	Sargentodoxa cuneata (Oliv.) Rehd. et Wils.	China	NA	0.24	0.0000514	3437000.00 - 5307361.111	
216.	Morus alba L.	China	NA	0.24	0.0000514	3437000.00 - 5307361.111	
217.	Eriobotrya japonica (Thunb.) Lindl.	China	NA	0.35	0.0000500	3535200.00 - 5459000.00	
218.	Dendrobium officinale Kimura et Migo	China	NA	0.28	0.0000480	3682500.00 - 5686458.333	
219.	Trichosanthes kirilowii Maxim	China	NA	0.22	0.0000471	3749454.545 - 5789848.485	
220.	Leonurus japonicus Houtt.	China	NA	0.33	0.0000471	3749454.545 - 5789848.485	
221.	Magnolia officinalis Rehd.et Wils	China	NA	0.36	0.0000463	3818888.889 - 5897067.901	

222.	Terminalia chebula Retz	China	NA	0.32	0.0000457	3866625.00 - 5970781.25	
223.	Celosia argentea L.	China	NA	0.21	0.0000450	3928000.00 - 6065555.556	
224.	Forsythia suspensa (Thunb.) Vahl	China	NA	0.21	0.0000450	3928000.00 - 6065555.556	
225.	Saussurea involucrata (Kar.et Kir.) Sch. -Bip	China	NA	0.52	0.0000446	3965769.231 - 6123878.205	
226.	Unacaria rhynchophylla (Miq.) Miq. ex Havil.	China	NA	0.25	0.0000429	4124400.00 - 6368833.333	
227.	Cibotium barometz (L.) J. Sm.	China	NA	0.23	0.0000394	4483043.478 - 6922644.928	
228.	Myristica fragrans Houtt	China	NA	0.28	0.0000400	4419000.00 - 6823750.00	
229.	Laminaria japonica Aresch	China	NA	0.23	0.0000394	4483043.478 - 6922644.928	
230.	Dioscorea nipponica Makino	China	NA	0.18	0.0000386	4582666.667 - 7076481.481	
231.	Nardostachys jatamansi DC	China	NA	0.44	0.0000377	4686818.182 - 7237310.606	
232.	Psoralea corylifolia L.	China	NA	0.26	0.0000371	4758923.077 - 7348653.846	
233.	Sinapis alba L.	China	NA	0.29	0.0000373	4740689.655 - 7320498.084	

234.	Eucommia ulmoides Oliv.	China	NA	0.25	0.0000357	4949280.00 – 7642600.00	
235.	Citrus reticulata Blanco	China	NA	0.25	0.0000357	4949280.00 – 7642600.00	
236.	Plantago asiatica L. & Plantago depressa Willd.	China	NA	0.16	0.0000343	5155500.00 - 7961041.667.00	
237.	Perilla frutescem (L.) Britt.	China	NA	0.24	0.0000343	5155500.00 - 7961041.667.00	
238.	Dianthus superbus L. & Dianthus chinensis L.	China	NA	0.16	0.0000343	5155500.00 - 7961041.667	
239.	Curcuma phaeocaulis Val	China	NA	0.26	0.0000334	5287692.308 - 8165170.94	
240.	Ephedra sinica Stapf .	China	NA	0.23	0.0000329	5379652.174 - 8307173.913	
241.	Ricinus communis L	China	NA	0.45	0.0000321	5499200.00 - 8491777.778	
242.	Clematis armandii Franch	China	NA	0.35	0.0000300	5892000.00 - 9098333.333	
243.	Astragalus complanatus R. Br.	China	NA	0.13	0.0000279	6345230.769 - 9798205.128	
244.	Siraitia grosvenorii (Swingle) C. Jeffrey ex A. M. Lu et Z. Y. Zhang	China	NA	0.13	0.0000279	6345230.769 - 9798205.128	

245.	Canarium album Raeusch.	China	NA	0.19	0.0000271	6512210.526 - 10056052.63	
246.	Stephania tetrandra S. Moore	China	NA	0.19	0.0000271	6512210.526 - 10056052.63	
247.	Cannabis sativa L	China	NA	0.12	0.0000257	6874000.00 - 10614722.22	
248.	Tinospora sagittata (Oliv.) Gagnep.	China	NA	0.19	0.0000244	7235789.474 - 11173391.81	
249.	Pyrola calliantha H. Andres	China	NA	0.11	0.0000236	7498909.091 - 11579696.97	
250.	Pueraria thomsonii Benth.	China	NA	0.11	0.0000236	7498909.091 - 11579696.97	
251.	Crocus sativus L	China	NA	0.54	0.0000231	7637777.778 - 11794135.8	
252.	Impatiens balsamina L.	China	NA	0.32	0.0000229	7733250.00 - 11941562.5	
253.	Schizonepeta tenuisfolia Briq.	China	NA	0.15	0.0000214	8248800.00 - 12737666.67	
254.	Trichosanthes kirilowii Maxim	China	NA	0.10	0.0000214	8248800.00 - 12737666.67	
255.	Schizonepeta tenuifolia Briq	China	NA	0.14	0.0000200	8838000.00 – 13647500.00	
256.	Morus alba L	China	NA	0.14	0.0000200	8838000.00 - 13647500.00	

257.	Gleditsia sinensis Lam.	China	NA	0.88	0.0000189	9373636.364 - 14474621.21	
258.	Rosa laevigata Michx.	China	NA	0.11	0.0000189	9373636.364 - 14474621.21	
259.	Sophora japonica L	China	NA	0.14	0.0000180	9820000.00 - 15163888.89	
260.	Typhonium giganteum Engl	China	NA	0.20	0.0000171	10311000.00 - 15922083.33	
261.	Cinnamomum cassia Presl	China	NA	0.12	0.0000171	10311000.00 - 15922083.33	
262.	Pyrrosia sheareri (Bak.)	China	NA	0.09	0.0000154	11456666.67 - 17691203.7	
263.	Cynomorium songaricum Rupr.	China	NA	0.11	0.0000157	11248363.64 - 17369545.45	
264.	Buddieja officinalis Maxim	China	NA	0.12	0.0000154	11456666.67 - 17691203.7	
265.	Amomurn kravanh Pierre ex Gagnep.	China	NA	0.17	0.0000146	12130588.24 - 18731862.75	
266.	Citrus grandis 'Tomentosa'	China	NA	0.17	0.0000146	12130588.24 - 18731862.75	
267.	Rhus chinensis Mill.	China	NA	0.16	0.0000137	12888750 - 19902604.17	
268.	Abutilon theophrasti Medic	China	NA	0.10	0.0000129	13748000.00 - 21229444.44	

269.	Trichosanthes kirilowii Maxim.	China	NA	0.09	0.0000129	13748000.00 - 21229444.44	
270.	Foeniculum vulgare Mill.	China	NA	0.14	0.0000120	14730000.00 - 22745833.33	
271.	Gleditsia sinensis Lam.	China	NA	0.49	0.0000105	16834285.71 - 25995238.1	
272.	Juncus effusus L.	China	NA	0.23	0.0000099	17932173.91 - 27690579.71	
273.	Melia toosendan Sieb. et Zucc	China	NA	0.10	0.0000086	20622000.00 - 31844166.67	
274.	Nelumbo nucifera Gaertn.	China	NA	0.12	0.0000086	20622000.00 - 31844166.67	
275.	Aconitum kusnezoffii Reichb.	China	NA	0.39	0.0000067	26438461.54 - 40825854.7	
276.	Oroxylum indicum (L.) Vent.	China	NA	0.10	0.0000043	41244000.00 - 63688333.33	
277.	Silybum mariamum (L.) Gaertrn	China	NA	5.50	NA	NA	
278.	Whitethorn dragées	German	Capsules	1.50	0.00001	20622000.00 - 31844166.67	(Kaltner et al., 2020)
279.	Maca and hemp protein	German	Powder	0.70	0.00020	883800.00 – 1364750.00	
280.	Smoothie balls	German	Pellet	0.40	0.00014	1237320.00 - 1910650.00	

281.	Red clover capsules	German	Capsules	5.10	0.00004	4043529.412 - 6243954.248	
282.	Green tea capsules	German	Capsules	0.10	0.00000	103110000.00 - 159220833.3	
283.	Artichoke capsules	German	Capsules	21.00	0.00069	256173.913 - 395579.710	
284.	Wheatgrass powder	German	Powder	0.30	0.00004	4582666.667 - 7076481.481	
285.	Nettle and herb tablets	German	Pill	50.10	0.00286	61742.51497 - 95341.816	
286.	Capsules with St. John's wort	German	Capsules	1.80	0.00001	17185000.00 - 26536805.56	
287.	Capsules with milk thistle	German	Capsules	3.20	0.00002	9666562.5 - 14926953.13	
288.	Herbadigestive tablets	German	Pill	105.10	0.00375	47091.15128 - 72717.411	
289.	Skin/hair/nails pellets	German	Pellet	33.10	0.00033	534018.1269 - 824622.356	
290.	Inner peace/relax pellets	German	Pellet	36.50	0.00083	211869.863 - 327166.095	
291.	Body balance preparation	German	Capsules	35.40	0.00035	499322.0339 - 771045.197	
292.	Stevia leaves	German	Leaves	8.60	0.00002	7193720.93 - 11108430.23	

293.	Bi Min Gan Wan	Ireland	Tablet	93.00	0.00083	665225.8065 - 1027231.183	(Griffin et al., 2014)
294.	Xie Gan Wan	Ireland	Tablet	46.00	0.00400	1344913.043 - 2076793.478	
295.	Zhuang Gu Guan Jie Wan	Ireland	Tablet	169.00	0.00700	366071.0059 - 565281.065	
296.	Wu Wei Xiao Du Yin	Ireland	Tablet	43.00	0.00146	1438744.186 - 2221686.047	
297	Han Shi Bi Wan	Ireland	Tablet	27.00	0.00054	2291333.333 - 3538240.741	
298.	Long Dan Xie Gan Wan	Ireland	Tablet	1918.00	0.00306	32255.47445 - 49808.394	
299.	Fu Ke Yang Rong Wan	Ireland	Tablet	14.00	0.04951	4419000.00 - 6823750.00	
300.	Jia Wei Xiao Yao Wan	Ireland	Tablet	29.00	0.00029	2133310.345 - 3294224.138	
301.	Ji Sheng Gui Yu Wan	Ireland	Tablet	31.00	0.04109	1995677.419 - 3081693.548	
302.	Dang Gui Wan	Ireland	Tablet	240.00	0.00027	257775.00 - 398052.0833	
303.	Ba Zhen Wan	Ireland	Tablet	644.00	0.00013	96065.21739 - 148342.3913	
304.	Baihe Gujin Wan	Ireland	Tablet	179.00	0.00048	345620.1117 - 533701.1173	

305.	Er Chen Wan	Ireland	Tablet	111.00	0.00012	557351.3514 - 860653.1532	
306.	Guan Jie Yan Wan	Ireland	Tablet	124.00	0.00008	498919.3548 - 770423.3871	
307.	Ba Zheng San	Ireland	Tablet	483.00	0.00548	128086.9565 - 197789.8551	
308.	Liu Wei Di Huang Wan	Ireland	Tablet	85.00	0.00004	727835.2941 - 1123911.765	
309.	Bai Zi Zi Xin Wan	Ireland	Tablet	112.00	0.00008	552375.00 - 852968.75	
310.	Tiang Wang Bu Xin Dan	Ireland	Tablet	74.00	0.00009	836027.027 - 1290979.73	
311.	"Purge Qi, Blood & Wei"	Ireland	Tablet	79.00	0.00069	783113.9241 - 1209272.152	
312.	"Strengthen Liver"	Ireland	Tincture	86.00	0.00184	719372.093 - 1110843.023	
313.	"Purge Qi"	Ireland	Tincture	140.00	0.00051	441900.00 - 682375.00	
314.	Kang Ning Wan	Ireland	Tablet	50.00	0.00032	1237320.00 - 1910650.00	
315.	Qing Re An Cang Wan	Ireland	Tablet	134.00	0.00035	461686.5672 - 712929.1045	
316.	Er Chen Wan	Ireland	Tablet	127.00	0.00138	487133.8583 - 752224.4094	

317.	Bai He Gu Jin Wan	Ireland	Tablet	59.00	0.00024	1048576.271 - 1619194.915	
318.	Fang Feng Xin Yi Wan	Ireland	Tablet	30.00	0.00032	2062200.00 - 3184416.667	
319.	Chuan Xiong Cha Tao Wan	Ireland	Tablet	190.00	0.00021	325610.5263 - 502802.6316	
320.	Er Chen Wan	Ireland	Tablet	599.00	0.00023	103282.1369 - 159486.6444	
321.	Ba Zhen Wan	Ireland	Tablet	65.00	0.00025	951784.6154 - 1469730.769	
322.	Xiao Yao San Pian	Ireland	Tablet	28.00	0.00040	2209500.00 - 3411875.00	
323.	Tan Wang Bu Xin Pian	Ireland	Tablet	49.00	0.00014	1262571.429 - 1949642.857	
324.	Qing Re An Cang Wan	Ireland	Tablet	169.00	0.00038	366071.0059 - 565281.0651	
325.	Xie Gan Wan	Ireland	Tablet	131.00	0.00036	472259.542 - 729255.7252	
326.	Pi Fu Zhi Yang Wan	Ireland	Tablet	152.00	0.00017	407013.1579 - 628503.2895	
327.	Qing Re An Cang Wan	Ireland	Tablet	432.00	0.00009	143208.3333 - 221140.0463	
328.	Wei Ling Xian	Ireland	Raw Herb	1722.00	0.00054	35926.82927 - 55477.64228	

329.	Chuang Xiong	Ireland	Raw Herb	3668.00	0.00171	16866.41221 - 26044.84733	
330.	Fang Feng	Ireland	Raw Herb	1434.00	0.00019	43142.25941 - 66619.59554	
331.	Ba Zhen Wan	Ireland	Tablet	16.00	0.00008	3866625.00 - 5970781.25	
332.	Suan Zhao Ren Tang	Ireland	Tablet	30.00	0.00014	2062200.00 - 3184416.667	
333.	Xiao Yao	Ireland	Tablet	52.00	0.00048	1189730.769 - 1837163.462	
334.	Yu Ping Feng San	Ireland	Tablet	13.00	0.00037	4758923.077 - 7348653.846	
335.	*Lithospermum orientale*	Indonesia	*radix*	13.1	0.00036	497115.307 - 767637.605	(Suparmi et al., 2020)
336.	*Gynura pseudochina*	Indonesia	*folium*	135.3	0.02706	6532.150- 10086.844	
337.	*Gynura procumbens*	Indonesia	*rhizoma*	31.8	0.00154	114439.511 - 176715.686	
338.	*Gynura segetum)*	Indonesia	*folium extract*	35066	0.55104	320.776 - 495.338	
339.	*Gynura sp.*	Indonesia	*folium*	17435	1.39480	126.727 - 195.691	
340.	*Gynura procumbens*	Indonesia	*folium extract*	73.4	0.00304	58128.347 - 89760.875	
341.	*Gynura procumbens*	Indonesia	*folium extract*	70055	0.80063	220.776 - 340.919	
342.	*Gynura procumbens*	Indonesia	*folium extract*	74837	3.10039	57.012 - 88.037	
343.	*Gyanura segetum*	Indonesia	*folium*	39632	1.69851	104.06 - 160.69	

344.	*Gyanura segetum*	Indonesia	*folium*	12.3	0.00065	271878.708 - 419830.8064	
345.	*Gynura segetum*	Indonesia	*folium extract*	105099	3.60339	49.05 - 75.74	
346.	*Gyanura procumbens*	Indonesia	*extract*	65.7	0.00282	62776.2557 - 96938.1024	
347.	*Tussilago farfara*	Indonesia	*flos farfarae*	131.1	0.08428	2097.330282 - 3238.6642	
348.	*Gyanura procumbens*	Indonesia	*bulbus extract*	21516	0.15369	1150.1394 - 1776.0271	
349.	*Gyanura procumbens*	Indonesia	*rhizoma + folium*	33.2	0.00052	338806.1336 - 523179.08	
350.	*Gyanura segetum*	Indonesia	*rhizoma*	235376	10.08754	17.522- 27.058	
351.	*Adenostemma lavenia*	Indonesia	*folium*	29.5	0.01264	13981.0169 - 21589.2655	
352.	*Gynura divaricata*	Indonesia	*folium extract*	453	0.01294	13656.9536 - 21088.8521	
353.	*Gynura segetum*	Indonesia	*folium extract*	276.6	0.01146	15425.2374 - 23819.4080	
354.	*Symphytum officinale*	Indonesia	*folium*	270.2	0.02316	7632.1243 - 11785.4058	
355.	*Gyanura procumbens*	Indonesia	*folium (extract)*	113.4	0.00227	77936.50794 - 120348.3245	
356.	*Gyanura procumbens*	Indonesia	*extract*	51425	0.73464	240.6067 - 371.5410	
357.	*Gyanura segetum*	Indonesia	*folium*	63877	0.82128	215.22 - 332.34	

358.	*Gyanura procumbens*	Indonesia	*folium*	1265	0.02169	8150.98 - 12586.62	
359.	*Gyanura procumbens*	Indonesia	*herba extract*	12173	0.24346	726.033 - 1121.128	
360.	*Gyanura segetum*	Indonesia	*folium extract*	933.9	0.05070	3486.567 - 5383.902	
361.	*Gyanura segetum*	Indonesia	*folium*	65763	0.65763	268.783 - 415.051	
362.	*Gyanura procumbens*	Indonesia	*folium*	205.9	0.00529	33385.138 - 51552.803	
363.	*Gyanura procumbens*	Indonesia	*bulbus extract*	42.5	0.00091	194089.4118 - 299709.8039	
364.	*Gyanura segetum*	Indonesia	*folium extract*	104842	5.09233	34.711 - 53.600	
365.	*Ageratum conyzoides*	Indonesia	*folium*	146977	1.04984	168.369 - 259.993	
366.	*Gyanura procumbens*	Indonesia	*folium (extract)*	357.2	0.01174	15060.616 - 23256.365	
367.	*Gyanura segetum*	Indonesia	*folium*	106712	6.40272	27.607 - 42.630	
368.	*Symphytum officinale*	Indonesia	*radix*	15.1	0.00004	4097086.093 - 6326655.629	
369.	*Zingiber officinale Rosc,Foeniculum vulgare Mill.,Myristica fragrans Houtt.*	Indonesia	*rhizoma, fruit seed*	127.8	0.02556	6915.492 - 10678.794	
370.	*Foeniculum vulgare Mill., Zingiber officinale Rosc, Myristica fragrans Houtt.*	Indonesia	*fruit, rhizome, seed*	313.7	0.06274	2817.341 - 4350.494	

371.	*Myristica fragrans Hout., Foeniculum vulgare Mill.,Piper betle L.*	Indonesia	*seed, fruit, folium*	40.6	0.01218	14512.315 - 22409.688	
372.	*Piper betle L. , Foeniculum vulgare Mill.*	Indonesia	*folium, fruit*	144.8	0.02896	6103.591 - 9425.069	
373.	*Eucalyptus alba Reinw,Foeniculum vulgare Mill., Zingiber officinale Rosc., Cinnamomum burmannii Blume*	Indonesia	*fruit, fruit, rhizome,bark*	253.2	0.05064	3490.521 - 5390.007	
374.	*Foeniculum vulgare Mill., Zingiber officinale Rosc, Cinnamomum burmannii Blume*	Indonesia	*fruit, rhizome, bark*	60.2	0.00602	29362.126 - 45340.531	
375.	*Eucalyptus alba Reinw., Zingiber officinale Rosc., Foeniculum vulgare Mill.*	Indonesia	*fruit, rhizome, seed*	149.8	0.02996	5899.866 - 9110.480	
376.	*Myristica fragrans Houtt., Foeniculum vulgare*	Indonesia	*seed, fruit*	436.8	0.06240	2832.692 - 4374.198	

377.	*Foeniculum vulgare Mill.*	Indonesia	*bark, fruit*	102.8	0.01469	12036.186 - 18586.089	
378.	*Myristica fragrans Houtt.*	Indonesia	*fruit*	35.7	0.00714	24756.302 - 38228.291	
379.	*Myristica fragrans Houtt.*	Indonesia	*seed*	26.2	0.00449	39354.961 - 60771.310	
380.	*Foeniculum vulgare Mill.*	Indonesia	*fruit*	49.5	0.00120	147037.433 - 227052.881	
381.	*Foeniculum vulgare Mill.*	Indonesia	*fruit*	125.2	0.00376	47060.702 - 72670.394	
382.	*Foeniculum vulgare Mill., Piper betle L., Thymus vulgaris L.*	Indonesia	*fruit, folium, folium*	86.7	0.01239	14271.280 - 22037.485	
383	*Foeniculum vulgare Mill., Nigella sativa L.*	Indonesia	*fruit, seed*	5.9	0.00118	149796.610 - 231313.559	
384	*Foeniculum vulgare Mill., Zingiber officinale Rosc*	Indonesia	*fruit, rhizome*	70.1	0.01402	12607.703 - 19468.616	
385	*Aristolochia debile*	Indonesia	*folium*	3421	0.17594	1004.676 - 1551.406	

[a]The estimated daily intake was estimated based on the consumption of 200 mg daily (Chen et al., 2017).

[b]The MOE value was calculated based on a range value of BMDL10 for PA induced liver tumour formation (176.76 µg/kg bw/day and 272.95 µg/kg bw/day) derived from the BMDS software 3.2 version.

NA – Not available

APPENDIX 4 – Data on herbal teas containing PAs including country of origin, estimated daily intake and MOE values

No.	**Herbal supplement (Name /species)**	**Country of origin**	**Product presentation**	**Total level of PA (µg/kg)**	**Estimated Daily Intake (µg/kg bw/day)[a]**	**Range of MOE[b]**	**References**
1.	Comfrey *(Symphytum officinale)*	China	Comminuted (leaves)	415.00	0.01185	14907.46 - 23019.87	(Chen et al., 2019)
2.	Comfrey *(Symphytum officinale)*	China	Intact (leaves)	101.10	0.00288	61192.87 - 94493.07	
3.	Climbing groundsel *(Senecio scandens)*	China	Comminuted (leaves)	293.10	0.00837	21107.47 - 32593.82	
4.	Climbing groundsel *(Senecio scandens)*	China	Intact (leaves)	85.20	0.00243	72612.67 - 112127.34	
5.	Sunn hemp (Crotalaria juncea)	China	Comminuted (leaves)	192.80	0.00550	32088.17 - 49550.05	
6.	Sunn hemp (Crotalaria juncea)	China	Intact (leaves)	170.00	0.00485	36391.76 - 56195.58	
7.	Coltsfoot (*Tussilago farfara)*	Netherland	Comminuted (leaves)	61.30	0.00175	100923.32 - 155844.20	

8.	Coltsfoot (*Tussilago farfara)*	Netherland	Intact (leaves)	30.70	0.00087	201517.91 - 311180.78	
9.	Borage *(Borago officinalis)*	Spain	Comminuted (leaves)	1120.00	0.032	5523.75 - 8529.68	
10.	Borage *(Borago officinalis)*	Spain	Intact (leaves)	845.40	0.02415	7317.95 - 11300.27	
11.	Expectorant	Ireland	Tea	29.00	0.00082	213331.03 - 329422.41	(Griffin et al., 2014)
12.	Red bush	Ireland	Tea	140.00	0.00400	44190.00 - 68237.50	
13.	C&H	Ireland	Tea	245.00	0.00700	25251.42 - 38992.85	
14.	Oolong	Ireland	Tea	51.00	0.00145	121305.88 - 187318.62	
15.	Black tea	Ireland	Tea	19.00	0.00054	325610.52 - 502802.63	
16.	Slimming aid	Ireland	Tea	107.00	0.00305	57818.69 - 89282.71	
17.	Digestive aid	Ireland	Tea	1733.00	0.04951	3569.87 - 5512.55	

18.	C&H	Ireland	Tea	10.00	0.00028	618660.00 - 955325.00	
19.	C&S	Ireland	Tea	1438.00	0.04108	4302.22 - 6643.42	

[a]The estimated daily intake was estimated based on the consumption of 2 g per cup of tea (German Federal Institute for Risk Assessment, 2013)

[b]The MOE value was calculated based on a range value of BMDL10 for PA induced liver tumour formation (176.76 µg/kg bw/day and 272.95 µg/kg bw/day) derived from the BMDS software 3.2 version.

NA – Not available