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**Detection and risk assessments of multi-pesticides in 1771 cultivated herbal
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Guangwei Zhu, Jingyuan Song, Jianhe Wei, Cheng Peng, Han Zhang, and Wwei Zhang: sample collection. **ShumeiWang, and Lan Wu:** sample preparations. **Jianhui Li, JaneYang, and Zhengwei**

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Fitzgerald: solution strategy, reviewing and editing. **Jiaxing Liu:** reviewing and editing.

Detection and risk assessments of multi-pesticides in 1771 cultivated herbal medicines by LC/MS-MS and GC/MS-MS

Abstract

Focus on the safety of herbal medicines has mainly been directed towards the presence of intrinsic toxicity, as found in the cases of renal and hepatic dysfunction caused by aristolochic acids. However, contamination from extrinsic hazards may impart an even greater reduction in their safety and efficacy. This study reveals that pesticides were present in the majority (88%) of a comprehensive cross-section (n=1771) of herbal medicine samples. Alarming, more than half (59%) contained pesticides over the European Pharmacopoeia (EP) limit, and 43% of them contained 35 varieties of banned, extremely toxic pesticides, eight of which were detected at levels over 500 times higher than the default Maximum Residue Limit (MRL). DDTs, carbofuran, and mevinphos were confirmed as being among the most risk-inducing pesticides by three different risk assessment methods, reported to produce carcinogenic, genotoxic, reproductive, and developmental effects, in addition to carrying nephrotoxicity and hepatotoxicity. In light of these findings, and withstanding that extrinsic hazards can be controlled unlike intrinsic toxicity, the authors here strongly recommend the application of herbal medicine quality-control measures and solutions to safeguard against a neglected but certainly potentially serious health risk posed to the majority of the global population that consumes herbal medicines.

Key words

Herbal medicines; pesticide; extrinsic contamination; risk assessment; public health.

23

24 **1. Introduction**

25 Herbal medicines (HM) play both important medicinal and economic roles internationally, with 80% of
26 the world's population choosing traditional medicines to meet their healthcare needs (WHO, 2013), and
27 the global HM market reaching an estimated \$107 billion in 2017 (Sma, 2016). These figures are
28 expected to rise with the rapidly expanding globalization and commercialization of HM. Multiple
29 large-scale epidemiological studies claim that the higher occurrence of nephropathy found in Asia
30 compared to other continents is inherently due to more frequent exposure to HM in this region. (Yang et
31 al., 2014; Broe et al., 2012; Lord et al., 1999; Nortier et al., 2000; Debelle et al., 2008; Ng et al., 2017)
32 Attention to the cause of adverse effects from HM has focused on intrinsic toxicity, as seen in the
33 highly reported cases of hepatocellular carcinoma (Ng et al., 2017) and urothelial malignancies. (Broe
34 et al., 2012; Lord et al., 1999; Nortier et al., 2000; Debelle et al., 2008) However, the extrinsic aspects
35 of toxicity, pesticides in particular, are often neglected despite these being persistent, bioaccumulative,
36 and toxic. These extrinsic toxins are ranked highly amongst suspected carcinogens and endocrine
37 disruptors (Hajšlová et al., 2003), such as p,p'-DDT, aldicarb, and mevinphos. They possess a more
38 serious potential hazard than those of intrinsic nature.

39

40 Commercial cultivation of HM has gained ground within an economic climate of sharply rising
41 demand and associated profitability on a global scale (Singh et al., 2018). China is the world's largest
42 producer and supplier of the herbal industry, proving itself the epicenter of the trading of HMs,
43 with an annual production output of approximately over \$200 billion, which is expected to rise to
44 \$5 trillion by 2050 (Tripathy et al., 2015). The increasing demand requires more “intensive”

production of HMs leading to higher levels of development in techniques to optimize and maximize profits. The need to protect them from plagues and pests, increase yield and shelf life, reduce post-harvest and storage losses resulted in exponential use of pesticides over the last 50 years (Adusei-Mensah et al., 2018). Concerns were raised for the safety of HM application affecting its commercialization (Singh et al., 2018) regarding contamination with pesticides, microbial contaminants, heavy metals, aflatoxins, chemical toxins, mycotoxins, polycyclic aromatic hydrocarbons (PAHs) and fumigants (Pytlakowska et al., 2012). Particularly, pesticide residues in herbal products may accumulate due to agricultural practices, such as spraying, soil treatment, cultivation in contaminated soil, use of contaminated water sources. These contaminants can also accumulate during the cultivation, storage, and processing of herbs (Tripathy et al., 2015).

The increasing use and the abusive and disordered employment of pesticides will certainly bring a broad spectrum of adverse effects with risks and damages to public health, farm animals, economy, and the alteration of the local environment (Zuin et al., 2000). Highly hazardous pesticides, although banned or strictly controlled, are still heavily promoted and widely applied in HM cultivation, especially in developing nations (Tripathy et al., 2015). Exposure to these contaminants may have cumulative, additive, or synergistic effects on human health (Adusei-Mensah et al., 2018). These contaminants can potentially interfere with the endocrine system, causing endocrine and metabolic disruption, presenting immune-suppressive, carcinogenic, and teratogenic effects (Pytlakowska et al., 2012). They may cause irreversible organ toxicity, fetotoxicity, embryotoxicity, or immunotoxicological effects (Chou et al., 2019) and reproductive issues such as infertility and birth defects. They may also affect and damage the nervous system, causing neurodegenerative and

hepatorenal disorders (Garcia et al., 2012; Adams et al., 2018). Epidemiological studies have associated pesticide contamination with the occurrence of multiple server disorders including cancer, sarcomas, leukemia, non-Hodgkin lymphoma, and myeloma (Garcia et al., 2012). There have been various reports regarding the presence of pesticide contaminants in medicinal herbs. Pesticide residues have been detected in many HMs distributed in the market, especially some are categorized as banned, highly hazardous ones, or extremely hazardous ones by the WHO (Singh et al., 2018). In China, of the 313 samples tested, 94.57% contained pesticide residues (Chou et al., 2019). A study conducted by American researchers has shown that 42 different pesticides were detected in 108 HM samples (36.7%) (Zuin et al., 2000). In Portugal, 50% of the market samples contained organochlorine pesticide residues and 53.1% of samples were detected with pesticides (Silva et al., 2018). Banned pesticides Aldrin, dieldrin, and chlordane in Ghana were also identified in 50% of the studied herbal preparations (Adusei-Mensah et al., 2018). In India, a total of 40 samples of single crude herbs (Dashmool) were examined and the results indicated that 5% contain residue of DDT or its metabolites and presence of α -HCH and γ -HCH, and the main constituents of commercial HCH were detected in 97.5% samples (Bhanti et al., 2007). However, the sample numbers and pesticide categories were not sufficient enough to cover all the commonly prescribed in the clinic or widely adopted ones in dietary products. A comprehensive study focusing on commonly used HMs with a large sample number and sampling areas is in urgent need.

However, the current depth-of-coverage pesticide risk assessment in HMs still relies on the comparison of toxicity exposure ratios (Silva et al., 2018), which is always limited due to lack of

pesticide indicator or consumption data. To address these limitations and to grasp a general idea of the types and amount of pesticides applied in HMs, the adoption of banned pesticides, a better categorization of the top-ranking pesticides in HMs and the contributing factors of the risks, a multi-residue pesticide method has been developed for the determination of pesticides in HM. Furthermore, better insight into pesticide contamination over large-scale HM cultivation areas with large representative sample populations is necessary to fully evaluate HM safety on a global scale (Abou-Arab et al., 2001). A more comprehensive risk-assessment approach is needed to fully assess health damage from pesticides to provide a suitable foundation on which to build an effective and well-targeted quality-control strategy. The objectives of this study were to: summarize pesticide content observed in a large sample of cultivated HM samples and compare observed levels to corresponding standards; conduct a comprehensive risk assessment of pesticide contamination in HMs and classify the top risk-inducing pesticides and related HMs; and discuss resolutions to control the quality and efficacy of HMs for policymakers.

2. Methods

2.1 Quality assessment rationale

For detection rate of banned pesticides, we compiled a list of 35 banned pesticides according to collective regulations of the European Union (EU), United States (US) and China. Banned pesticides from EU were found in Regulation or Directives of EU of the European Parliament and of the Council (EC) (EC, 2004; EC, 2004; EC, 2003; EC, 2003; EC, 2003; EC, 2003.), these from the US were collected from the list of banned and severely restricted pesticides in Prior Informed Consent (PIC)

procedure drafted by the U.S. Environmental Protection Agency (EPA) (Buffington et al., 2018; Colorado State University, 2018; USEPA, 2003; Licensing and Regulatory Affairs, 2018), and these banned in China were obtained from the announcements of Ministry of Agriculture and Rural Affairs of China and Chinese Pharmacopoeia (CP, 2015 edition) (Ministry of Agriculture and Rural Affairs, 2018; National Pharmacopoeia Commission, 2015.).

2.2 Sample collection

Pesticide contamination mainly occurs through agricultural practices. Therefore, a survey of HM categories with major HM cultivation areas and a large artificial cultivation scale was conducted to determine which HM samples were representative for analysis. Here a relatively complete investigation is presented following the collection of all currently available HM samples from large-scale cultivation areas covering major HM markets in China, with a few samples from Southeast Asia, without regard to possible pollution sources. For each available HM category in each HM market, at least one HM sample from each cultivation area was collected. A total of 1771 HM samples from 503 major cultivation areas (Table S1.1, S1.2, appendix) were sampled for pesticide analysis, using either Liquid chromatography-tandem mass spectrometry (LC/MS-MS) (Shah et al., 2015) or Gas chromatography-tandem mass spectrometry (GC/MS-MS) (Cherta et al., 2013). A total of 136 pesticides (Table S2.1, appendix) with high toxicity and high detection rates were selected for detection.

2.3 Sample preparation and instrumentation

The sample preparation protocol applied was slightly modified from a previously published protocol by Waters Corporation (Waters Corporation, 2018). One gram of HM sample powder was weighed in a 50 mL centrifuge tube and then homogenized with 10 mL of water. The tube was shaken for 30 seconds using a hand-motion shaker (IKA VORTEX GENIUS 3, VG 3 S25). Subsequently, 10 mL of 1% glacial acetic acid in acetonitrile was added as an extraction solvent, followed by the addition of QuEChERS AOAC3 material (Waters DisQuE QuEChERS pouches, p/n 186006812). The tube was capped, vortexed for 1 min, and centrifuged (Eppendorf, 5920 R) at 4500 RPM for 5 minutes. One mL aliquot of the supernatant was removed and dried. The dried extract was then re-dissolved in 2 mL QuEChERS d-SPE tubule containing 50 mg Primary Secondary Amine (PSA), 150 mg anhydrous MgSO_4 , and 50 mg C18 followed by 30 seconds whirlpool and centrifugation (CK1060, Biotake Corporation) at 12 000 RPM for 3 minutes. In order to detect different kinds of pesticides, LC-MS/MS or GC-MS/MS was applied separately for different HM samples. Therefore 0.1 mL supernatant was diluted with 0.9 mL of water and filtered with a microporous membrane (0.22 μm) before LC-MS/MS analysis. While for GC-MS/MS analysis, 0.1 mL supernatant was transferred into a vial and diluted with 0.9 mL isooctane.

UPLC-MS/MS analysis was performed adopting Ultra Performance LC (UPLC) coupled to tandem quadrupole mass spectrometry (Xevo TQ-XS) with ACQUITY UPLC I-Class system. The chromatographic column (ACQUITY UPLC BEH C18 2.1×100 mm, 1.7 μm) was utilized for LC separation at 45 °C. The injector was operated with 5 μL volume, the flow rate was at 0.45 $\text{mL} \cdot \text{min}^{-1}$.

The mobile phase consisted of 10 mM ammonium acetate (pH=5) in water (A) and 10 mM ammonium acetate (pH=5) in methyl alcohol (B). The gradient elution program was as follows: 0-0.25 min, B, 2%; 0.25-12.25 min, B, 2%-99%; 12.25-13.00 min, B, 99%; 13.00-13.01 min, B, 99-2%; 13.01-17.00 min, B, 2%. The capillary voltage of MS was 3 KV with a source temperature of 150 °C and desolvation temperature of 500 °C. The desolvation was run at the speed of 1000 L·h⁻¹ while the cone at 150 L·h⁻¹.²¹ (Table S2.2, S2.4, appendix)

APGC-MS/MS analysis was employed by ESI/APGC+TQ-S platform using the Agilent 7890B system (Agilent 7890B, Palo Alto, CA, USA) coupled to a triple quadrupole (QqQ) mass spectrometer (Xevo TQ-S, Waters Corporation, Manchester, UK), operating in APCI mode. A fused silica DB-5MS capillary column (30 m × 0.25 mm i.d. × 0.25 µm film thickness) (J&W Scientific, Folsom, CA, USA) was used for GC separation. Helium was used as a carrier gas in constant flow mode (2 mL·min⁻¹). One microliter of the sample was injected. The GC inlet was operated in pulsed splitless mode and worked at 280 °C. The oven temperature was programmed as follows: initial temperature 70 °C held for 1 min, 15 °C·min⁻¹ to 150 °C and 10°C·min⁻¹ to 300 °C held for 3 min. A pulsed splitless injection was carried out using an initial pressure of 240 kPa, maintained for one min, and then changed to a constant flow of 2 mL·min⁻¹, which corresponded to a linear velocity of 52 cm·s⁻¹. In the MRM method, automatic dwell time (values ranging from 4 to 52 ms) was applied in order to obtain at least 12 points per peak. The interface temperature was set to 300 °C using N₂ as auxiliary gas at 250 L·h⁻¹, a makeup gas at 270 mL·min⁻¹ and cone gas at 170 L·h⁻¹. The APCI corona discharge pin was operated at 1.8 µA. Water was used as a modifier when working under proton-transfer conditions was placed in an uncapped vial, which was located within a holder placed in the source door. All data were analyzed by

Targetlynx (a module of MassLynx) and Quanpedia database software (Cherta et al., 2013) (Table S2.3, S2.5, appendix).

2.4 Quantitative methods

In our experiment, the mixture of Multi-residue Pesticide Standards for LC/MS-MS was bought from Waters® LC Multi-residue Pesticide Standards Kit, p/n 186007574 and for GC/MS-MS from Sigma Aldrich as reference substances. The $5 \mu\text{g}\cdot\text{mL}^{-1}$ multi-pesticide standards mixtures were prepared in acetonitrile and stored in a freezer at -20°C . The working standard mixtures were prepared by volume dilution of stock solutions in hexane to prepare matrix-matched calibrants and in acetone for sample fortification (Cherta et al., 2013). The detailed standard curves, R^2 , retention time, quantification trace, and ion trace of each pesticide were listed in the appendix (Table S2.1-S2.5, appendix). The methodologies of LC/MS-MS and GC/MS-MS were modified from previously validated studies by Shah et al. (Shah et al., 2015) and Cherta et al. (Cherta et al., 2013). With respect to LC/MS-MS, the linearity range and standard calibration curves were established by spiking the pesticide mixtures from $0.00125 \text{ mg}\cdot\text{kg}^{-1}$ to $0.32 \text{ mg}\cdot\text{kg}^{-1}$ respectively. Quantification was performed against a matrix-matched calibration curve. All of the incurred pesticide residues detected were identified from comparison with the retention time and ion ratio from reference standards within the tolerance limit specified by the European Commission (EC, 2016). As for GC/MS-MS, the selected reaction monitoring (SRM) method was modified from the study by Cherta et al. to evaluate linearity, precision, and selectivity (Cherta et al., 2013). Linearity was tested by injecting standards in hexane at eight concentrations, 0.1, 0.5, 1, 5, 10, 25, 50, and $100 \text{ ng}\cdot\text{mL}^{-1}$, considered acceptable as regression coefficients were over 0.99. Precision was estimated as repeatability in terms of relative standard deviation (RSD, %) ($n = 6$)

calculated for each fortification level. The selectivity forevaluating each of the three specific SRM transitions measured was satisfactory. For the identification of pesticides, criteria were set for the ratio of the response obtained for the transitions measured (EC, 2011).

2.5 Statistic analysis

The lowest linearity range of this experiment is $0.02 \text{ mg} \cdot \text{kg}^{-1}$. Only data equal to or above it were retained in data analysis (PAN, 2006). For detection rate and the over-limit ratio of 136 pesticides, the total number of samples detected with at least one pesticide, the number of samples detected of each pesticide, and the number of pesticides detected in single HM were calculated. In addition, to simplify comparisons HM was divided into five categories, according to the part of the plant used for medicinal properties: fructus & semen, radix & rhizoma, flos, folium & cortex, and herba & others. The numbers of pesticides in five medicinal properties of HM were aggregated by classes 1-5, 6-10, 11-15, 16-20, and >20, respectively, as well as detection frequency of six pesticide types.

The total number of samples detected with banned pesticides, the number of samples detected with each of the 35 pesticides, and the number of banned pesticides detected in a single sample were calculated.

To analyze the over-limit ratio of pesticide in HM, Maximum Residue Limits (MRLs) of pesticides is needed as a reference, yet the MRLs of 136 pesticides are not available in any existing regulation in any country. Therefore we calculated the MRLs of 136 pesticides in 182 HM according to the

European Pharmacopoeia (EP, 8th Edition) (Europe pharmacopoeia, 2014). If the pesticide limits are neither listed in EP nor Regulation (EC) No. 396/2005, its MRL shall be calculated using the following expression:

$$\text{MRL} = \frac{\text{ADI} \times \text{BW}}{\text{IR}_d \times 100} \quad (1)$$

Where ADI ($\text{mg} \cdot \text{kg}^{-1}$) is the acceptable daily intake of each pesticide. BW is the body weight 60 kg; IR_d refers to the daily dose of HM in kilograms and here we used maximal dose derived from CP 2015. Besides, for pesticides whose ADIs are not available, default MRL $0.01 \text{ mg} \cdot \text{kg}^{-1}$ was applied according to the regulation by EP. Additionally, banned pesticides identified in our experiment based on the stipulations of the EU, US, and China were defined as Not Detected (ND). (Table S3)

For pesticides whose ADIs are vacant but NOELs available, ADI was derived from NOEL by:

$$\text{ADI} = \frac{\text{NOEL}}{\text{safety factor}} \quad (2)$$

Where ADI is acceptable daily intake ($\text{mg} \cdot \text{kg}^{-1}$); NOEL is no-observed-effect level; safety factor is set as 1000 (USFDA, 2018; Vania, 2000).

Times of highest concentrations detected over the MRLs were calculated by concentration of pesticides detected minus MRL calculated, then divided by MRL:

$$\text{Times over MRL} = \frac{\text{C-Limit}}{\text{Limit}} \quad (3)$$

238

239 When calculating times of concentrations detected over the MRLs for ADI-vacant and banned
 240 pesticides, default MRL 0.01 mg·kg⁻¹ was adopted.

241

242 2.6 Three approaches of risk assessments

243 Dietary exposure assessment (Bhandari et al., 2019) was employed to classify acceptable and
 244 unacceptable risks of pesticides in HMs posed to health due to elevated background of dietary exposure
 245 and risk-ranking score (VRC, 2007) to categorize and prioritize pesticides and HMs by ranking.
 246 Additionally, for the first time, patient health impact was characterized to combine both cancerogenic
 247 and non-cancerogenic risks, and ultimately connected these risks of pesticides interpreted to disease
 248 probability in a lifetime (Fantke, 2012). Hazard Quotient (HQ) and Hazard Index (HI) were applied to
 249 quantify acute, chronic, and cumulative risks posed by pesticide residues in HM for dietary exposure
 250 assessment (Bhandari et al., 2019). The risk-ranking score (S) for pesticides and Risk Index (RI) for
 251 HM were obtained by multiplying three groups of individual scores which represent the toxicity of the
 252 pesticide, the intake of pesticides, and the level of detectable residues (VRC, 2007). Also, we quantified
 253 potential patient intake caused by pesticide residues in HMs and related cancer and non-cancer
 254 aggregated effect estimates based on methods developed for comparative impact assessment, and
 255 combined both into a set of comparative patient health impact scores (IS), expressed as
 256 disability-adjusted life years (DALYs) (Fantke, 2012).

257

258 Using this approach, the absence of dietary exposure of certain pesticides due to lack of Acceptable

Daily Intake (ADI) and Acute Reference Dose (ARfD) was compensated for by applying risk-ranking score and patient health impact characterization, which also aggregates both cancerous and non-cancerous effects. Therefore, comprehensive and detailed data on pesticide content in HM were obtained to support a robust interpretation of their impacts on health. Revealed here is a more critical cause of the adverse effects of HM.

2.6.1 Dietary Exposure Assessment

The Hazard Quotient (HQ) and Hazard Index (HI) were applied here to quantify adversity raised by pesticide residues in HM. The acute, chronic and cumulative risks were evaluated according to the following equations (Bhandari et al., 2019; Xiao et al., 2019; Zuo et al., 2019):

$$ESTI = \frac{Ef \times Ed \times IR_h \times C_h}{AT \times BW} \quad (4)$$

$$HQ_a = \frac{ESTI}{ARfD} \quad (5)$$

Where ESTI ($\text{mg} \cdot \text{kg}^{-1} \cdot \text{bw}^{-1}$) is the estimated short-term intake, Ef is exposure frequency, here the 95th percentile of annual consumption on HM was adopted which was set 90 days per year according to a questionnaire on HM consumption of 20917 people (Wang et al., 2017); Ed is the exposed days over a lifetime which is set as 20 years; IR_h (kg) here refers to 95th percentile of HM taken on a single day per person, which is set to 0.5 kg, C_h ($\text{mg} \cdot \text{kg}^{-1}$) is the highest residue level of pesticides; AT is the average

lifetime=365 days×70 years; BW is the mean body weight, set as 60 kg. The estimate of pesticide intake in HM was then compared to the Acute Reference Doses (ARfD) (mg.kg⁻¹) (Zuo et al., 2019).

$$EDI = \frac{Ef \times Ed \times IR_m \times C_m}{AT \times BW} \quad (6)$$

$$HQ_c = \frac{EDI}{ADI} \quad (7)$$

Where EDI (mg.kg⁻¹.bw⁻¹) is the estimated daily intake; IR_m (kg) here refers to the mean dosage of each HM which is 0.2 kg and C_m (mg.kg⁻¹) is the mean residue level of each pesticide in the single herb (Kumari et al., 2019).

HQ_as and HQ_cs of each pesticide in the same HM were summed up to give a cumulative hazard index of single HM as HI_a and HI_c (USEPA, 2002; EFSA, 2013):

$$HI = \sum_{i=1}^n HQ_i \quad (8)$$

An HQ of <1 or HI of <1 for any pesticide is often considered as acceptable; whereas an HQ or HI of >1 not. As the HQ or HI increases, the risk does as well. For HI_a>1 and HI_c>1 HM, the contribution of each pesticide was calculated by (HQ_a/HI_a)×100% and (HQ_c/HI_c)×100% to explore which pesticide contributes the most to HM posing risks (USEPA, 2002; EFSA, 2013).

2.6.2 Risk-ranking score

The risk-ranking score (S) is obtained by multiplying three groups of individual scores which represent the toxicity of the pesticide, the consumption of pesticides and the evidence of detected residues, calculated by the following equation (Nie et al., 2014; Li et al., 2018):

$$\text{Overall Risk-ranking Score } (S) = (A+B) \times (C+D+E) \times F \quad (9)$$

Where A refers to nature of the hazard, here refers to LD_{50} which determines the toxicity of pesticides (Table S4, appendix); B is the potency of the substance, here refers to ADI of pesticides; C is exposure 1, here refers to the maximal dosage of HM (CP 2015); D is exposure 2, which is calculated by Frequency=times of pesticide application/planting days. Considering the data regarding the pesticide application in HM is not available, and in most cases pesticide is applied relatively less frequently in HM than in crops, the D value was considered less than 2.5% which signifies 0 for all cases. E is exposure 3, here refers to the chance of exposure of HMs to the population. Since HM could be applied in medicinal, dietary, and tea occasions, herbs with only one function are set with 1 point, with two functions 2 points and three functions 3 points; F refers to evidence for detected residues. (Tables S2.1, appendix)

For overall risk-ranking score of each herb, Risk Index (RI) is calculated by summing up S of each pesticide in one single herb.

$$RI = \sum_{i=1}^n S \quad (10)$$

Where RI is the Risk Index of single HM, S is the risk-ranking score of each pesticide, n is the number of pesticides detected in one single herb. Sensitivity analyses were performed by estimating the contribution of A+B, C+D+E and F to the variance in the overall risk score by Spearman correlation coefficient analysis (Spss 17.0) (Li et al., 2018).

2.6.3 Patient health impact assessment

Patient health impact score (IS), expressed as disability-adjusted life year (DALY), was developed to evaluate health impact by multiplying IF, the human intake factor and EF, the human toxicological effect factor which reflects the change in lifetime disease probability due to change in lifetime intake of pesticides (Fantke et al., 2012; Frischknecht et al., 2016). DRF is the Human dose-response slope factor (incidence risk.kg a.i. intake⁻¹), which can be derived for cancer effects from human or extrapolated from animal TD₅₀ and for non-cancer effects from human ED₅₀ or extrapolated from animal ED₅₀, NO(A)EL, LO(A)EL or LD₅₀ by the following equations (Rosenbaum et al., 2011; Huijbregts et al., 2005). Only five TD₅₀s of carbaryl, methidathion, p,p'-DDD, p,p'-DDT, and piperonyl butoxide were available and have been certainly proven with cancerogenic effects. The lowest ED₅₀ was applied to derive the highest Impact Score (IS) to be conservative.

$$DRF_{\text{non-cancer}} = \frac{f_{ED\ 50}}{ED\ 50} \quad (11)$$

$$DRF_{\text{cancer}} = \frac{f_{TD\ 50}}{TD\ 50} \quad (12)$$

$$ED_{50 \text{ non-cancer}} = \frac{NOEL \times f_{NOEL} \times BW \times AT}{f_s \times f_{time} \times f \frac{mg}{kg}} \quad (13)$$

$$ED_{50 \text{ non-cancer}} = \frac{LOAEL \times f_{LOAEL} \times BW \times AT}{f_s \times f_{time} \times f \frac{mg}{kg}} \quad (14)$$

$$ED_{50 \text{ non-cancer}} = \frac{LD_{50}}{26} \quad (15)$$

$$ED_{50 \text{ cancer}} = \frac{TD_{50} \times BW \times AT}{f_s \times f \frac{mg}{kg}} \quad (16)$$

f_{ED50} or f_{TD50} = 0.5 is the human response level corresponding to ED_{50} or TD_{50} ; ED_{50} refers to the median effective dose of pesticide which can induce 50% effects on exposed subjects; TD_{50} is a numerical description of carcinogenic potency, which corresponds to the daily dose-rate (in $mg \cdot kg^{-1} \cdot bw^{-1} \cdot d^{-1}$) over a lifetime, to induce tumors in half of the test animals; NOEL refers to no-observed-effect level; f_{NOEL} = 9 is the NOEL-to- ED_{50} extrapolation factor (University of Hertfordshire, 2019); f_s corrects for interspecies differences between the studied animal and humans. f_s = 4.1 for rat, f_s = 7.3 for mouse, f_s = 1.5 for dog, f_s = 2.4 for rabbit and f_s = 1.9 for monkey (USEPA, 2002); BW = 60 kg person denotes average body weight; AT = 365 days × 70 years is the average human lifetime; f_{time} is the extrapolation factor correcting for differences between exposure duration of the study and chronic exposure. f_{time} = 5 for (sub-)acute exposure and f_{time} = 2 for (sub-)chronic exposure. The same principle applies to other animals. If the exposure duration is not mentioned at all, the acute test was assumed to be conservative; $f \frac{mg}{kg}$ = $10^6 \text{ mg} \cdot \text{kg}^{-1}$ corrects for mg per kg; LOAEL refers to the Lowest dose at which there was an observed toxic or adverse effect; f_{LOAEL} = 2.25 is the LOEL-to- ED_{50} extrapolation factor (Huijbregts et al., 2005); LD_{50} is lethal dose 50, a dose that proves lethal to 50% of a given population; Both $EF_{\text{non-cancer}}$

with ED_{50} and EF_{cancer} with TD_{50} were calculated. If more than one parameter exists per chemical (NOEL or LOAEL or LD_{50}), lowest converted human lifetime ED_{50} was applied;

From DRF and the estimated residue-related patient intake, we derived impact scores based on measured residues of pesticide active ingredients (a.i.) in different consumed herbs:

$$IS_{i,h,d} = IR_{i,h} \times C_i \times PF_{i,h} \times \sum_e (DRF_{i,e} \times SF_e) \times f \frac{kg}{mg} \times f \frac{\mu DALY}{DALY} \quad (17)$$

$$IS_{i,h} = IR_{i,h} \times Ef \times Ed \times C_i \times PF_{i,h} \times \sum_e (DRF_{i,e} \times SF_e) \times f \frac{kg}{mg} \times f \frac{\mu DALY}{DALY} \quad (18)$$

$$IS_h = \sum_i IS_{i,h} = IR_{i,h} \times Ef \times Ed \times \sum_i (C_i \times PF_{i,h} \times \sum_e (DRF_{i,e} \times SF_e)) \times f \frac{kg}{mg} \times f \frac{\mu DALY}{DALY} \quad (19)$$

Where $IS_{i,h,d}$ ($\mu DALY \cdot person^{-1} \cdot d^{-1}$) refers to IS per person per day for single herb-pesticide pair; $IS_{i,h}$ ($\mu DALY \cdot person^{-1}$) is IS per person for a single herb-pesticide pair over lifetime and IS_h ($\mu DALY \cdot person^{-1}$) is IS per person for a single herb over lifetime; $IR_{i,h}$ ($kg \cdot person^{-1} \cdot d^{-1}$) is the individual daily intake dose of single herb, here we adopted the maximal dose from CP 2015 to be conservative; C ($mg \cdot kg^{-1}$) refers to the measured residual concentration of pesticide active ingredient i in HM, we adopted maximal and mean concentration of each pesticide detected in single HM; Ef refers to the number of days of individual daily intake of each herb every year, here we assume an individual takes HM 90 days each year; Ed is the exposed days over lifetime, set as 20 years here (Wang et al., 2017); $PF_{i,h}$ is food processing factor ($mg_{a.i. \text{ in processed herb}} \cdot kg^{-1}_{\text{processed herb}}$ per $mg_{a.i. \text{ in harvested herb}} \cdot kg^{-1}_{\text{harvested herb}}$), considering no official data is available, we assume $PF=1$; SF_e refers to severity factor ($DALY \cdot incidence^{-1}$) derived from general statistics about disease severity for cancer and non-cancer effects with $11.5 DALY \cdot incidence^{-1}$ for cancer and $2.7 DALY \cdot incidence^{-1}$ for non-cancer (Huijbregts et al., 2005); f is the conversion factors adjusting for kg per mg (10^{-6}) and for $\mu DALY$ per DALY (10^6),

where $1 \mu\text{DALY} = 31.56$ seconds or 0.526 minutes of lost healthy life per person over lifetime. Both $\text{DRF}_{\text{non-cancer}}$ and $\text{DRF}_{\text{cancer}}$ were derived and summed per pesticide to obtain $\text{IS}_{\text{i,h}}$ per HM-pesticide and IS_{h} per HM, both of which were calculated in three categories with maximal, mean and minimal concentrations of each pesticide detected.

2.7 Data sources

The indicators of pesticides including CAS number, pesticide type, substance group, f_s , f_{time} , TD_{50} ($\text{mg} \cdot \text{kg}^{-1} \cdot \text{day}^{-1}$), ADI ($\text{mg} \cdot \text{kg}^{-1}$), ARfD ($\text{mg} \cdot \text{kg}^{-1}$), NOEL ($\text{mg} \cdot \text{kg}^{-1} \cdot \text{d}^{-1}$), NOAEL ($\text{mg} \cdot \text{kg}^{-1} \cdot \text{bw}^{-1} \cdot \text{d}^{-1}$), LOAEL ($\text{mg} \cdot \text{kg}^{-1} \cdot \text{d}^{-1}$) and LD_{50} ($\text{mg} \cdot \text{kg}^{-1}$) were obtained from pesticide databases including Pesticide Properties Database (PPDB) (University of Hertfordshire, 2019), EU Pesticide Database (EC, 2016), Pesticide Action Network Pesticide Database (PAN) (PAN, 2018), Chemical book (Beijing Xilinbuke Network Technology Co., Ltd, 2016), Cancer Potency Database (CPDB) (The Carcinogenic Potency Project, 2007), Toxicology Data Network from U.S. National Library of Medicine (TOXNET IRIS) (TOXNET, 2019), and Foodmate (Foodmate, 2012). For indicators not available, national documents including *The WHO Recommended Classification of Pesticides by Hazard And Guidelines to Classification 2009* (WHO, 2009), *Acceptable Daily Intakes (ADI) for Agricultural and Veterinary Chemicals Used in Food Producing Crops or Animals (Australia)* (Australia Pesticides and Veterinary Medicines Authority, 2017), *National Food Safety Standard-Maximum Residue Limits for Pesticides in Food (China)* (CFDA, 2016), *Pesticide MRLs in Agricultural Commodities (South Korea)* were referred to (Ministry of Food and Drug Safety, 2019). If data neither available in the databases nor the documents, articles containing relative parameters were applied. All data were collected based on

the listed sequence of references above.

The classification of pesticides by hazard was categorized according to WHO Class which defines pesticides into four groups: Ia (extremely hazardous, $LD_{50} < 5$), Ib (highly hazardous $5 < LD_{50} < 50$), II (moderately hazardous, $50 < LD_{50} < 2000$) and III (slightly hazardous with LD_{50} Over 2000) (WHO, 2009). For exposure time, one-time admission or 1 week is considered acute, 2-6 weeks exposure is subacute, 7-28 weeks exposure is subchronic and 1-2 years exposure is considered chronic (Vermeire, 2001; Fantke, 2011). HM was classified into five categories according to which part of herbal plants were applied for medicinal use. For information regarding five medicinal properties and dosage of HM, *Chinese Pharmacopoeia (CP, 2015 edition)* was referred to. For information on HM not available in CP, the *Encyclopedia of Chinese Herbal Medicine* was considered (Nanjing University of Chinese Medicine, 2006). For NOEL only available with unit ppm diet, according to *Food additives Guidelines for the preparation of toxicological working papers for the Joint FAO/WHO Expert Committee on Food Additives (2000)*, it is converted to $mg \cdot kg^{-1} \cdot bw^{-1}$ per day (rat 0.05, rabbit 0.025, and dog 0.03) (FAO/WHO, 2000).

Figures were plotted using OriginPro 2018C (64-bit) SR1b 9.5.1.195 (One Roundhouse Plaza OriginLab Corporation Northampton, MA 01060 USA) and R language [no IDE (integrated development environment), R from the linux terminal R version 3.5.1. (2018-007-02)-- "Feather Spray" Copyright (C) 2018, the R Function for Statistic Computing Platform: x86_64-pc-linux-gnu (64-bit)] (The R Core Team, 2019).

3. Results

3.1 Detection of 136 pesticides in 1771 HM samples

Totally 98 pesticides in 1000 HM samples were detected by LC/MS-MS and 44 in 771 samples by GC/MS-MS, therefore a total of 136 pesticides (6 in common) were determined in 1771 samples with concentrations ranging from 0.02 mg·kg⁻¹ to 20.00 mg·kg⁻¹ (linearity range). In total, 136 pesticides, including 35 banned, five extremely toxic, and 20 highly toxic, were detected in 1771 HM samples by either LC/MS-MS or GC/MS-MS (Table S2.1, S2.2, appendix).

3.1.1 Detection rate of pesticides in herbal medicines

Totally 88.03% (1559) samples were detected with at least one pesticide in 1771 samples (Fig.1). The detection rate of each pesticide ranges from 0.10% to 36.70% in the total of 98 pesticides detected by LC-MS and from 0.13% to 44.62% in 44 pesticides by GC-MS. Bifenthrin was detected in 44.62% samples, followed by diphenylamine (42.41%), and metolachlor (41.50%). Additionally, 51.38% of the total samples were detected with three or more pesticides, 24.45% with five or more pesticides, and 69.79% with one to five different pesticides. Notably, five HMs were detected with over 21 different kinds of pesticides. Chrysanthemi flos was detected with 37 different kinds of pesticides, followed by Crataegi fructus (29), and Alpiniae oxyphyllae fructus (27). 48.56% of the pesticide residue contamination was located in the radix & rhizoma of HMs, followed by fructus & semen (29.83%), and folium & cortex (8.98%). Insecticides (45.42%) and fungicides (33.69%) are most frequently detected pesticides (Table S 5,6,7, appendix).

Fig.1 Detection rate of 136 pesticides in 1771 samples by LC/MS-MS and GC/MS-MS

(A) Total detection rate of overall pesticides by LC/MS-MS, GC/MS-MS and LC/MS-MS & GC/MS-MS. (B) Detection rate of five medicinal properties in different number of pesticides by LC/MS-MS. (C) Detection rate of five medicinal properties in different number of pesticides by GC/MS-MS. (D) Detection rate of five medicinal properties in different number of pesticides by LC/MS-MS & GC/MS-MS. (E) Detection rate of five medicinal properties by LC/MS-MS, GC/MS-MS and LC/MS-MS & GC/MS-MS. (F) Detection rate of six categories of pesticides in five medicinal properties by LC/MS-MS. (G) Detection rate of six categories of pesticides in five medicinal properties by GC/MS-MS. (H) Detection rate of six categories of pesticides in five medicinal properties by LC/MS-MS & GC/MS-MS. (I) Detection rate of top ten pesticides by LC/MS-MS. (J) Detection rate of top ten pesticides by GC/MS-MS. (K) Number of pesticides detected in top ten HMs by LC/MS-MS. (L) Number of pesticides detected in top ten HMs by GC/MS-MS.

3.1.2 Detection rate of banned pesticides

The detection rate of banned pesticides in 1000 samples by LC/MS-MS was 57.70% and 23.87% in 771 samples by GC/MS-MS, so the total detection rate of banned pesticides was 42.97% in 1771 samples (Fig.2). The detection rate of 21 banned pesticides by LC/MS-MS ranged from 0.10% to 24.20%, and 15 banned pesticides by GC/MS-MS ranged from 0.13% to 13.10%. Mevinphos (I+II) was the banned pesticide with the highest detection rate (24.20%), followed by carbendazim (16.20%),

aldicarb (15.00%), and total DDT (13.10%). 149 samples were detected with 3 or more banned pesticides. 47.44% of the samples detected with banned pesticides were found in radix & rhizoma and 30.62% in fructus & semen, 8.67% in folium & cortex, 7.88% in herba & others, and 5.39% in flos. Crataegi Fructus were detected with ten different kinds of banned pesticides, followed by Herba agastaches (9), and Dendrobii caulis (8) (Table S5, S6, appendix).

Fig.2 Detection rate of banned pesticide by LC/MS-MS, GC/MS-MS and LC/MS-MS & GC/MS-MS

(A) Total detection rate of banned pesticides by LC/MS-MS, GC/MS-MS and LC/MS-MS & GC/MS-MS. (B) Detection rate of top ten banned pesticides by LC/MS-MS. (C) Detection rate of top ten banned pesticides by GC/MS-MS. (D) HMs with over seven banned pesticides detected by LC/MS-MS. (E) HMs with over three banned pesticide detected by GC/MS-MS. (F) HMs with over six banned pesticide detected by LC/MS-MS & GC/MS-MS (F) Proportion of five medicinal properties detected with banned pesticides by LC/MS-MS. (H) Proportion of five medicine properties detected with banned pesticides by GC/MS-MS. (I) Proportion of five medicine properties detected with banned pesticides by LC/MS-MS & GC/MS-MS.

3.1.3 Over-limit ratio of pesticides in herbal medicines

474 Based on the equation from the EP (8th Edition), all MRLs were set up for 136 pesticides in 182 HMs
475 (Table S3, appendix). Among which, 35 banned pesticides in EU, US, and China were set Not Detected
476 (ND), eight pesticides in lack of ADI were set as 0.01 mg·kg⁻¹, and the MRLs ranged from 0.0005
477 mg·kg⁻¹ (Dicrotophos) to 3120.00 mg·kg⁻¹ (Chlorantraniliprole). Except for NDs and default MRLs
478 (0.01 mg·kg⁻¹), 14 MRLs (0.08%) were below 0.001 mg·kg⁻¹, 181 were between 0.01 mg·kg⁻¹ and
479 0.001 mg·kg⁻¹, 1083 between 0.01 mg·kg⁻¹ and 0.10 mg·kg⁻¹, 6047 between 0.10 mg·kg⁻¹ and 1.00
480 mg·kg⁻¹, 8399 between 1.00 mg·kg⁻¹ and 10.00 mg·kg⁻¹, 1467 between 10.00 mg·kg⁻¹ and 100.00
481 mg·kg⁻¹, 96 between 100.00 mg·kg⁻¹ and 1000.00 mg·kg⁻¹, and 3 MRLs were above 1000.00 mg·kg⁻¹.
482 Comparing the concentrations of each pesticide detected in individual herb with MRLs calculated
483 according to EP, 1045 out of 1771 (59.01%) samples were detected with concentration above the
484 threshold and the total over-limit ratio has reached 59.70% by LC/MS-MS and 58.11% by GC/MS-MS
485 (Fig.3).

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487 **Fig.3: Over-limit ratio of samples by LC/MS-MS, GC/MS -MS and LC/MS -MS & GC/MS -MS**

488 (A) Over-limit ratio of samples by LC/MS-MS, GC/MS-MS and LC/MS-MS & GC/MS-MS. (B) HMs detected with over seven

489 kinds of over-limit pesticides. (C) Over-limit ratio of top ten pesticide by LC/MS-MS. (D) Over-limit ratio of top ten pesticide by

490 GC/MS-MS. (E) HMs with over eight over-limit pesticides detected by LC/MS-MS. (F) HMs with over five over-limit pesticides

491 detected by GC/MS-MS. (G) Times over the limit of top ten pesticides by LC/MS-MS. (H) Times over the limit of top ten

492 pesticides by GC/MS-MS.

The concentrations of 49 pesticides have exceeded the EP standard by LC/MS-MS with over-limit ratios from 0.10% to 24.20% and 36 by GC/MS-MS from 0.13% to 19.33%. The highest over-limit ratio was 24.20% by mevinphos (I+II), followed by propetamphos (19.33%), carbendazim (16.20%), aldicarb (15.00%), and total DDT (13.10%). Fifteen over-limit pesticides were detected in *Crataegi fructus* and ten in *Pogostemonis herba*. Totally 170 samples were detected with four to 15 pesticides exceeding the EP limit. Comparing detected residues with MRLs calculated according to EP, eight banned pesticides were detected with residues more than 500 times above the EP limit. Isocarbofos was 1997.0 times exceeding the EP limit, followed by carbendazim (1731.0), and total DDT (1521.0). (Table S5, 6, appendix).

3.2 Risk assessments of pesticide contents in herbal medicines

3.2.1 Dietary Exposure Assessment

The Hazard Quotient (HQ) and Hazard Index (HI) were applied here to quantify adversity raised by pesticide residues in herbal medicines. Since 76 out of 136 pesticides are in lack of ARfD values, so only 60 pesticides participate in the acute risk assessment (Table S2.1, appendix). 72.95% of HQ_a was below 0.01, 22.98% ranged from 0.01 to 0.1, 3.64% from 0.1 to 1.0 and only 0.43% HQ_a was above one (Fig.4). Pesticides carbofuran showed unacceptable risk in four HMs including *Crataegi fructus* ($HQ_a=10.80$), *Zanthoxyli pericarpium* ($HQ_a=10.49$), *Lycii fructus* ($HQ_a=1.61$), and *Corydalis bungeanae herba* ($HQ_a=1.15$), as well as pesticide Mevinphos I in *Paridis rhizoma* ($HQ_a=1.08$) and *Myristicae semen* ($HQ_a=1.03$), Methomyl in *Rhei radix et rhizome* ($HQ_a=1.30$). The HQ_a s of other pesticides detected are all below one, thus considered as acceptable. Eight pesticides didn't take part in the chronic risk assessment for their ADIs were not available. 75.20% HQ_c was below 0.01, 18.20% ranged from 0.01 to 0.1, 6.41% ranged from 0.1 to 1.0, and 0.20% was above one thus considered as

unacceptable risk. The HQ_cs of carbofuran in *Crataegi fructus* and *Zanthoxyli pericarpium* were 4.32 and 1.52, respectively, of mevinphos I in *Paridis rhizome*, *Myristicae semen*, and *Cnidii fructus* were 2.83, 2.72, and 2.26, respectively.

We calculated both acute cumulative risk and chronic cumulative risk for 182 HMs with 60 pesticides for acute risk and 128 for chronic risk with data available. 4.40% HI_as was below 0.01; 61.54% ranged from 0.01 to 0.1; 28.57% is from 0.1 to 1.0, and 5.49% was above one which considered as unacceptable. Totally ten HMs were presented with above-one HI_as, and *Crataegi fructus* ranked the top (HI_a=12.09), followed by *Zanthoxyli pericarpium* (HI_a=11.54), *Lycii fructus* (HI_a=1.86), *Corydalis bungeanae herba* (HI_a=1.48), *Chrysanthemi flos* (HI_a=1.43), *Myristicae semen* (HI_a=1.42), *Rhei Radix et Rhizoma* (HI_a=1.41), *Ganoderma* (HI_a=1.15), *Paridis rhizome* (HI_a=1.14), and *Cnidii fructus* (HI_a=1.07). While for chronic cumulative risk assessment, 1.65% HI_cs was below 0.01, 17.03% ranged from 0.01 to 0.1, 68.68% ranged from 0.1 to 1.0, and 12.64% was above one and considered unacceptable. The HI_cs of a total of 23 HMs were above one, with *Crataegi fructus* (HI_c=6.62) ranking the top, followed by *Myristicae semen* (HI_c=3.51), and *Zanthoxyli pericarpium* (HI_c=3.38).

Mevinphos I contributed 72.98% in the total HI_a of *Cnidii fructus*, 72.41% in *Myristicae semen*, and 94.41% in *Paridis rhizome*, while carbofuran contributed 77.95% in *Corydalis bungeanae herba*, 89.35% in *Crataegi fructus*, 86.84% in *Lycii fructus*, and 90.89% in *Zanthoxyli pericarpium*. Carbendazim contributed 44.35% in *Ganoderma*, Chlorpyrifos contributed 47.69% in *Chrysanthemi flos*, and Methomyl contributed 92.14% in *Rhei radix et rhizoma*. As per HI_cs, coumaphos, isocarbofos, mevinphos I, carbofuran, propetamphos, methidathion, and chlorpyrifos were the chief pesticides made

the major contributions in $HI_c > 1$ HMs. Conclusively, HMs *Crataegi fructus* and *Zanthoxyli*
pericarpium were most risk-inducing with highest HQ_a , HQ_c , HI_a , and HI_c , while pesticides carbofuran
 (banned) and mevinphos I (banned) are posing unacceptable risk based on dietary exposure assessment.
 Additionally, HMs applied with fructus & semen part showed the highest risks. (Fig.4, 5, Table 1,2).

Fig.4 Dietary Exposure Assessment of pesticide residues in herbal medicines

(A) Violin plot and proportion of acute hazard quotient (HQ_a). (B) Violin plot and proportion of chronic hazard quotient (HQ_c). (C) Violin plot and proportion of acute hazard index (HI_a). (D) Violin plot and proportion of chronic hazard index (HI_c). (E) $HQ_a > 1$ pesticides and HM. (F) $HQ_c > 1$ pesticides and HM. (G) Contribution of pesticides in acute risk assessment of $HI_a > 1$ HM (only pesticides with contribution > 10% were included). (H) Contribution of pesticides in chronic risk assessment of $HI_c > 1$ HM (only pesticides with contribution > 10% were included). Chr is short for Chrysanthemi flos, Cni for Cnidii fructus, Cor for Corydalis bungeanae herba, Cra for Crataegi fructus, Gan for Ganoderma, Lyc for Lycii fructus, Myr for Myristicae semen, Par for Paradisi rhizoma, Rhe for Rhei radix et rhizoma, Zan for Zanthoxyli pericarpium, Ali for Alismatis rhizoma, Alp for Alpiniae oxyphyllae fructus, Ane for Anemarrhenae rhizoma, Atr for Atractylodis macrocephalae rhizoma, Chu for Chuanxiong rhizoma, Cit for Citri reticulatae pericarpium, Cur for Curcumae longae rhizoma, Epi for Epimedii folium, Eri for Eriobotryae folium, Far for Farfarae flos, Her for Herba sea radix amaranthi viridis, Lig for Ligustri lucidi fructus, Lon for Longan arillus, Peu for Peucedani turgeniifolii herba, and Sap for Saposhnikoviae radix.

3.2.2 Risk-ranking Score

The risk-ranking scores (S) of 136 pesticides detected in 182 HMs all participated in the analysis, as well as the Risk Index (RI) of all 182 HMs, which supplemented the dietary exposure assessment due to lack of ADIs and ARfDs. As per pesticide, 2.07% was calculated with S over 100, 14.12% from 50 to 100, 51.27% from 10 to 50, and 32.53% were below 10 (Fig.S1). Carbofuran-3-hydroxy and leptophos were with the highest S (140) and considered highly risky when detected in Crataegi fructus, Lycii fructus, Lonicerae japonicae flos, and Taraxaci herba. As per RI, 25 herbs (13.74 %) were calculated with RI below 200, 64 (35.16%) ranging from 200 to 500, 79 (43.41%) from 500 to 1000, and 14 (7.69%) were over 1000. Crataegi fructus ranked the top with a RI of 1925, followed by

Dendrobii caulis (1315) and Saposhnikoviae radix (1144) Additionally, the level of detection ($p=0.783$) contributed the most to the risk-ranking, followed by the toxicity of pesticide ($p=0.691$), and the least relevant was HM consumption ($p=0.370$) according to Spearman correlation coefficients. For HMs with RI over 1000, we calculated the proportion of S of each pesticide contributed to its RI, the result is in accordance with the contributions of pesticides in HMs derived through HQ_a/HI_a and HQ_c/HI_c . (Table 1, 2, Fig.S1, S3, S5 appendix).

3.2.3 Patient health impact assessment

Same as the risk-ranking score above, with three approaches of ED_{50} calculations, all pesticides detected were included in patient health impact assessment. With minimal concentrations detected of each pesticide in each HM, the $IS_{i,h,d}$ ranged from $3.51E-07$ to $1.52 \mu\text{DALY}\cdot\text{person}^{-1}\cdot\text{d}^{-1}$; with mean concentrations, it ranged from $6.11E-07$ to $1.58 \mu\text{DALY}\cdot\text{person}^{-1}\cdot\text{d}^{-1}$; with maximal concentrations, it ranged from $6.11E-07$ to $2.05 \mu\text{DALY}\cdot\text{person}^{-1}\cdot\text{d}^{-1}$. The average value of all min $IS_{i,h,d}$ was $0.01 \mu\text{DALY}\cdot\text{person}^{-1}\cdot\text{d}^{-1}$ and the median value was $8.02E-04 \mu\text{DALY}\cdot\text{person}^{-1}\cdot\text{d}^{-1}$. For mean $IS_{i,h,d}$, the average and median were 0.01 and $0.001 \mu\text{DALY}\cdot\text{person}^{-1}\cdot\text{d}^{-1}$, respectively, while for maximal $IS_{i,h,d}$, 0.02 and $0.001 \mu\text{DALY}\cdot\text{person}^{-1}\cdot\text{d}^{-1}$. As per $IS_{i,h}$, the minimal values ranged from 0.0006 to $2729.59 \mu\text{DALY}\cdot\text{person}^{-1}$, the mean from 0.001 to $2837.91 \mu\text{DALY}\cdot\text{person}^{-1}$, and the maximal from 0.001 to $3682.78 \mu\text{DALY}\cdot\text{person}^{-1}$. The average values of minimal, mean, and maximal $IS_{i,h}$ were 19.06 , 23.00 , and $28.19 \mu\text{DALY}\cdot\text{person}^{-1}$, respectively. Their median values were 1.44 , 2.22 , and $2.62 \mu\text{DALY}\cdot\text{person}^{-1}$. The ranges of minimal, mean, and maximal IS_h were from 1.93 to $3945.40 \mu\text{DALY}\cdot\text{person}^{-1}$, from 1.97 to $3972.07 \mu\text{DALY}\cdot\text{person}^{-1}$, and from 2.00 to $4287.78 \mu\text{DALY}\cdot\text{person}^{-1}$. Their averages values were 378.87 , 457.19 , and $560.34 \mu\text{DALY}\cdot\text{person}^{-1}$, and median values were

210.22, 269.63, and 326.58 $\mu\text{DALY}\cdot\text{person}^{-1}$. With min and mean IS_h , Coicis semen ranked the top (min $\text{IS}_h=3945.40 \mu\text{DALY}\cdot\text{person}^{-1}$, mean $\text{IS}_h=3972.07 \mu\text{DALY}\cdot\text{person}^{-1}$. While with max IS_h , Chuanmingshinis violacei presented with the highest IS_h ($4287.78 \mu\text{DALY}\cdot\text{person}^{-1}$). Pesticide o,p'-DDT in Coicis semen also ranked the top in min $\text{IS}_{i,h}$ ($2729.58 \mu\text{DALY}\cdot\text{person}^{-1}$), yet in Radix chuanmingshinis violacei ranked the top in both mean and max $\text{IS}_{i,h}$ (mean $\text{IS}_{i,h}=2837.91 \mu\text{DALY}\cdot\text{person}^{-1}$, max $\text{IS}_{i,h}=3682.78 \mu\text{DALY}\cdot\text{person}^{-1}$). (Table 1, 2, Fig.S2, S4, S5, appendix).

4. Discussion

In total, 136 pesticides including 35 banned, five extremely toxic, and 20 highly toxic were detected in 1771 HM samples by either LC/MS-MS or GC/MS-MS. Summarizing results from both LC/MS-MS and GC/MS-MS experiments, at least 88.03% (1559) samples were present with pesticide residues and 59.01% (1045) samples with over-limit pesticides levels. Furthermore, 35 banned pesticides were detected in at least 42.97% samples (761), of which mevinphos and total dichlorobiphenyl trichloroethane (DDT) accounted for 24.20% (242 of 1000 samples by LC/MS-MS) and 13.10% (101 of 771 samples by GC/MS-MS), respectively (Fig.1, 2, 3). Additionally, eight banned pesticides appeared at concentration levels over 500 times higher than the EP limit (Table 1). Chrysanthemi flos were detected with 37 pesticides (eight over-limit and seven banned), followed by Crataegi fructus (29 pesticides) and Alpiniae oxyphyllae fructus (27 pesticides) (Table 2). Our findings indicated pesticides were most widely detected in radix & rhizoma (48.62%, $n=1559$) and the least in flos (5.77%, $n=1559$) (Table S7.1, appendix). Pesticides presenting the highest risk belonged to the organophosphorus insecticide group (Fig.5). Insecticides and fungicides were those most frequently detected (45.42% and 33.69%, respectively, $n=6387$) (Table S7.2, appendix).

608

609 As per risk assessments, pesticide carbofuran in *Crataegi fructus*, *Lycii fructus*, *Zanthoxyli pericarpium*,
 610 and *Corydalis bungeanae* Herba, pesticide mevinphos in HM *Myristicae semen*, *Cnidii fructus* and
 611 *Paridis rhizoma*, Methomyl in *Rhei radix et rhizoma*, and isocarbofos in *Ganoderma* and *Epimedii*
 612 *folium* posed unacceptable acute or chronic risk ($HQ_a > 1$ or $HQ_c > 1$). Additionally, 25 HM ($HI_a > 1$ or
 613 $HI_c > 1$) posed unacceptable cumulative risk according to dietary exposure assessment (Table 1, 2, Fig.4,
 614 appendix). Carbofuran-3-hydroxy and leptophos appeared with the highest risk-ranking score ($S=140$),
 615 while *Crataegi fructus* occupied the top in Risk Index ($RI=1925$) (Table 1, 2, Fig.S1, S4, S5, appendix),
 616 whereas pesticide o,p'-DDT and HM *Radix chuanmingshinis violacei* and *Coicis semen* posed the most
 617 severe risk according to patient health impact approach (Table 1, 2, Fig.S2, S4, S5, appendix).
 618 Mevinphos, carbofuran, DDTs, leptophos, and methomyl are all banned pesticides and highly toxic
 619 (Fig.5). *Crataegi fructus* ranked high in three assessments and it belongs to the fructus & semen
 620 category (Table 2), thus considered the most risk-inducing HM in need of special surveillance and
 621 monitoring.

622 **Table 1: Summary of pesticides ranking the top in detection and risk assessments**

Pesticide	Times appeared	Detection rate	Over-limit ratio	Times of highest concentration over S		Mean (μ DALY.person ⁻¹)	IS _{i,h}
				the EP limit	HQ _a HQ _c		
o,p'-DDT*	17	8.4306%	8.4306%	1521.0000	80 ..	0.1909	2837.9061

Mevinphos I*	10	21.6000%	21.6000%	549.0000	120	1.0763	2.8274	633.6051
o,p'-DDD*	10	5.8366%	5.8366%	943.0000	60	..	0.2217	1155.3816
Carbofuran*	6	2.4000%	2.4000%	275.0000	100	10.8023	4.3209	298.9317
Aldicarb*	5	15.0000%	15.0000%	61.0000	120	0.1213	0.0438	1155.3816
Isocarbofos*	5	10.4000%	10.4000%	1997.0000	100	..	1.4153	444.0290
p,p'-DDT*	4	0.1297%	0.1297%	409.0000	64	..	0.0963	952.4230
Carbendazim*	3	16.2000%	16.2000%	1731.0000	40	0.5084	0.2034	625.3503
Carbofuran-3-hydroxy*	3	1.3000%	1.3000%	117.2000	140	..	..	61.5434
Bifenthrin	2	44.6174%	11.6732%	18.1200	45	0.2806	0.1551	137.8599
Methomyl*	2	0.4000%	0.4000%	550.8000	100	1.2958	0.5183	174.3136
Propetamphos	2	19.4553%	19.3256%	78.9167	60	..	0.6661	314.1194
Temephos*	2	11.3000%	11.3000%	5.0000	40	..	0.0001	1.4442

623

624 Arranged in descending order by times appeared in both top detection and risk assessment lists. Pesticides marked with * are

625 banned pesticides (ND). .. means no data obtained due to lack of ADI or ARfD.

626 We selected pesticides appeared over once in the top ten detection list by respectively summarizing the top ten detected, banned

627 and over-limit pesticides as well as top ten regarding the maximal times over the limit comparing to MRLs derived by EP

628 equation. We also collected those with $HQ_a > 1$, and $HQ_c > 1$ pesticides in dietary exposure assessment, $S=140$ in risk-ranking score,

629 and those ranking top 10 in minimal, mean and maximal $IS_{i,h}$ (only mean $IS_{i,h}$ shown here) in patient health impact assessment.

630 Totally 13 pesticides were summarized in the table above. Of the 13 pesticides, 11 are banned, 11 are insecticides, one is

631 fungicide, and one is metabolite. Four of them belong to organophosphorus groups and another four are carbamates. Two of them

632 are extremely toxic and four are highly toxic. o,p'-DDT appeared 17 times in the top ranking pesticides which indicated that it is

the pesticide posing the most widely and serious risk in herbal medicines, followed by Mevinphos I (10 times) and o,p'-DDD (10 times).

Table 2: Summary of herbal medicines ranking the top in detection and risk assessments

Herbal medicine	Times appeared	Number detected pesticides	Number banned pesticides	Number over-limit pesticides	S	RI	HI _a	HI _c	Mean IS _h (μ DALY.person ⁻¹)
Crataegi fructus	12	29	10	15	140	1925	12.0903	6.6217	1817.9194
Myristicae semen	7	16	7	8	72	849	1.4161	3.5063	1299.8473
Acanthopanax senticosi radix et rhizoma seu caulis	6	19	7	9	96	856	0.0506	0.9490	2809.1422
Chrysanthemi flos	6	37	7	8	96	1096	1.4329	2.2635	942.1945
Paridis rhizoma	6	7	2	3	48	242	1.1401	3.0070	1642.6345
Pogostemonis herba	6	20	7	10	96	1044	0.3673	0.6485	698.0653
Zanthoxyli pericarpium	5	18	6	8	84	993	11.5404	3.3763	728.3205
Anemarrhenae rhizoma	4	11	3	4	120	1070	0.0947	1.1800	1564.7528
Cnidii fructus	4	16	5	9	72	1011	1.0672	3.2433	1012.6341
Ganoderma	4	6	2	2	120	905	1.1463	3.1432	1294.7426
Herba sea radix amaranthi viridis	4	11	5	6	72	627	0.0596	1.2770	1793.1331
Alpiniae oxyphyllae fructus	3	27	5	8	72	897	0.3331	1.2865	532.6463

Coicis semen	3	8	2	5	120	910	0.0709	0.4822	3972.0719
Corydalis bungeanae herba	3	12	4	6	84	621	1.4762	1.5270	932.4413
Cynanchi paniculati radix et rhizoma	3	19	7	8	96	728	0.1060	0.7369	377.2584
Dendrobii caulis	3	16	8	8	120	1315	0.0510	0.7085	429.6968
Herba agastaches	3	21	9	9	96	776	0.2731	0.7643	260.3077
Lycii fructus	3	9	3	4	140	825	1.8569	0.7606	414.0162
Pruni semen	3	9	2	3	72	492	0.1632	0.5377	2046.1625
Radix aucklandiae	3	8	1	5	32	254	0.8530	0.5679	2081.1195
Radix chuanmingshinis violacei	3	7	3	4	96	624	0.0666	0.2505	3439.8703
Pinelliae rhizoma	2	4	1	2	64	256	0.0828	0.1028	1465.5672
Chuanxiong rhizoma	2	20	4	7	96	952	0.4741	1.4029	629.7785
Citri reticulatae pericarpium	2	20	5	6	96	756	0.9027	1.3135	333.3653
Epimedii folium	2	16	5	7	96	704	0.0458	1.3005	359.1246
Eriobotryae folium	2	13	4	8	112	720	0.3838	1.0552	483.3975
Rhei radix et rhizoma	2	13	4	6	120	1015	1.4064	0.8296	707.8251
Saposhnikoviae radix	2	11	3	5	96	1144	0.2038	2.5268	899.4202
Taraxaci herba	2	13	5	6	140	1035	0.0327	0.0681	255.5016

636

637 Arranged in descending order by times appeared in both top detection and risk assessment lists.

638 We selected HM appeared over once in the top ten detection list by respectively summarizing the top ten HM with number of

639 detected, banned and over-limit pesticides, as well as $HI_a > 1$ and $HI_c > 1$ HM in dietary exposure assessment, top ten with highest

RI in risk-ranking score assessment, and those ranking top ten in minimal, mean and maximal IS_h (only mean IS_h shown here) in patient health impact assessment. Those appeared over once were included, and totally 29 HM were summarized here. Considering HM detected with banned, under-limit and above-limit pesticides, *Chrysanthemi flos* was present with 37 pesticide (seven banned and eight over-limit), followed by *Crataegi fructus* and *Alpiniae oxyphyllae fructus*. As for medicinal properties, 11 belong to radix & rhizoma, nine are of fructus & semen category, six belong to herba & others, two folium & cortex, and one belongs to flos. *Crataegi fructus* appeared 12 times in the indicators of three risk approaches, seemingly the most risky herbal medicine due to pesticide contamination, followed by *Chrysanthemi flos*, *Pogostemonis herba*, *Acanthopanax senticosi radix et rhizoma seu caulis*, *Myristicae semen*, and *Paridis rhizoma*.

These detected pesticides are likely to exhibit fetotoxic, embryotoxic, as well as immunotoxic effects, potentially causing irreversible organ damage and reproductive issues (The Carcinogenic Potency Project, 2007; Beyond Pesticides, 2018), such as infertility or birth defects. They can potentially interfere with the endocrinium, causing endocrine and metabolic disruption (Beyond Pesticides, 2018). They may also damage the nervous system, causing neurodegenerative and hepatorenal disorders (Sahoo et al., 2010) (Fig.5). Widely used as insecticides, DDTs are chemically stable, possessing a half-life of degradation (DT_{50}) as long as 30 years, capable of inducing carcinogenic effects (Vašíčková et al., 2019). Other frequently detected pesticides are likely to impart multisystem damage to the human body (Fig.5). Organophosphorus pesticides, such as azinphos-ethyl, chlorpyrifos, famphur, mevinphos, omethoate, and propetamphos, are cholinesterase inhibitors which potentially induce Central Nervous System (CNS) symptoms including restlessness and depression of the respiratory or cardiovascular system (Han et al., 2017). Meanwhile, aldicarb, DDTs, and parathion methyl are qualified as Persistent Organic Pollutants (POPs), notable in their reputation for persistent toxicity to

humans and animals, and ability to be transported around the globe through atmospheric vectors (Chen et al., 2015). Heretofore unknown synergistic effects due to the combined effect of mixing the pesticides found in this study could result in multisystem dysfunctions yet to be discovered.

Fig.5: Heatmap of Impact Scores (mean $IS_{i,h}$) of top seven risk-inducing (banned) pesticides in 11 HM and their possible connections with health impacts

Collecting $HQ_a > 1$ and $HQ_c > 1$ pesticides, and top ten pesticides with highest risk-ranking score ($S=140$) and Impact Score (mean $IS_{i,h}$), ten pesticides were obtained. Since p,p'-DDT, leptophos, and methomyl were not detected in the 11 HM included thus not shown here, totally seven banned pesticides were summarized above. Collecting $HQ_a > 1$ and $HQ_c > 1$ HM, and top ten HM with highest risk index (RI) and Impact Score (mean $IS_{i,h}$), 11 risk-inducing herbal medicines appeared over once thus included here. The possible health impacts of seven pesticides were derived from Pesticide Properties Data Base (PPDB) (University of Hertfordshire., 2019), European Food Safety Authority (EFSA) (EFSA, 2019), United States Environmental Protection Agency (USEPA)⁴⁷, Agency for Toxic Substances and Disease Registry (ATSDR) (Agency for Toxic Substances and Disease Registry, 2011), Toxicology Data Network from U.S. National Library of Medicine (TOXNET) (TOXNET, 2019), and Beyond Pesticides

(Beyond Pesticides, 2018). Data showing positive, possible, mixed and ambiguous results are considered a possible connection, while no data and negative results considered no connection.

In general, we propose four reasons why safety problems emerge from herbal medicinal products. First and most importantly, failure of surveillance and monitoring coupled with poor quality controls (Sahoo et al., 2010), this has resulted in non-standard and disproportionate use of pesticides and fertilizers and consequently contamination. This is in addition to adulteration, substitution, misidentification, incorrect processing, and inappropriate labeling and advertising (Garcia et al., 2012). Secondly, pesticide residues may not always be directly derived from their application but from accumulated residues in soil, wastewater irrigation, and aerial deposition (Vašíčková et al., 2019). Both fructus and radix may assimilate pesticides from the soil through their roots (Han et al., 2017), which may be an explanation of why most pesticides were present in the radix and rhizome (Garcia et al., 2012). Thirdly, relatively safe pesticides with low toxicity and obvious effectiveness are neither sufficiently available nor well regulated, while highly and extremely toxic pesticides are easily accessible and inexpensive. Lastly, education and training institutions relating to safety, pesticide selection and, application for farmers is scarce.

Unlike intrinsic toxicity, contamination from extrinsic hazardous elements in HM can be regulated and controlled. Here we express a recommendation that regulatory efforts should be based on well-defined specifications that clearly standardize and classify HM cultivation, thus, ensuring better uniformity of quality, safety, and efficacy. A Chinese solution is herein proposed for regulating and establishing a universal standard for the production of HM. It includes four parts (Fig.6): (1) Good Agriculture

Practice (GAP) & Good Agricultural and Collection Practice (GACP) during cultivation, collection, and post-harvest operations for medicinal plants shall be implemented to ensure the quality of HM. Standardized and regulated practices including ecological planting, wild nurturing, and bionic cultivation should be developed within formalized cultivation areas; (2) An appropriate unified standard with enforcement criteria regarding hazardous elements in HM must be established for HM production to control their safety and quality (Silva et al., 2019); (3) A legal framework regarding authentication and quality assessment, which incorporates three levels of certification, could be granted according to different levels of hazardous element contents such as pollution-free, green, and organic; (4) A comprehensive prediction, surveillance, and invigilation system should be created to predict, track, and check adverse events throughout the whole HM supply chain including shipping, importing, exporting, and maintenance of plant materials (Chen et al., 2015). An electronic database showcasing each step of HM production and its profile shall be constructed (Eisenberg et al., 2011). Therefore special focus on control and monitoring of risk-inducing pesticides including carbofuran, mevinphos, methomyl, isocarbofos, leptophos, and o,p'-DDT, as well as HMs *Crataegi fructus*, *Lycii fructus*, *Zanthoxyli pericarpium*, *Corydalis bungeanae Herba*, *Myristicae semen*, *Cnidii fructus*, *Paridis rhizoma*, *Rhei radix et rhizoma*, *Radix chuanmingshinis violacei*, and *Coicis semen* shall be needed, whose MRLs and dosages applied in clinics shall be refined based on our findings. (Table 1, 2)

Fig.6 Strategy of key steps for quality control of herbal medicines

(1) GAP & GACP, education of pesticide application for farmers, tax-incentive policies should be implemented to guarantee the basic quality of herbal medicines. (2) Standard and enforcement regarding hazardous elements content in herbal medicines should be clarified and published to especially control the hazardous elements content in herbal medicines (3) Ratification and classification of three levels of hazardous elements in herbal medicines shall be established including pollution-free (within a certain limit), green (within a strictly controlled range) and organic (totally free of toxicants). (4) A comprehensive surveillance, monitoring and tracking system covering the whole herbal medicine supply chain to regularly monitor the residual pesticide content in herbal medicine samples should be conducted throughout the whole producing process of herbal medicine including cultivation, storage, and processing.

Further discussions, consultation, and harmonization, particularly relating to recommendations one, two, and three are desirable across a variety of geographic, cultural, and legislative landscapes in order to arrive at a meaningful and effective solution, since HMs are transported and consumed internationally. Though in this study the pesticide problem is confirmed, quantified, and further illuminated, future research around mapping the distribution and transmission of pesticides via their various vectors would be a welcome and valuable contribution to the field. Additional studies would deepen our understanding of the relationships between how pesticides are applied and the proportion from different vectors that make their way into HM. As little as 30% of sprayed pesticides reach their target (Zhu et al., 2019) and already complex intelligent systems are being designed for more targeted spraying (Asaei et al., 2019). The ecological influence of pesticides such as on the soil bacterial biome could also have an effect on intrinsic aspects of HM (Huang et al., 2018). If proposal four in particular here could be informed and refined by this kind of knowledge, there is further potential to optimize conditions to reduce intrinsic factors and direct future research areas to support a common goal of reducing pesticides and risk while increasing efficacy and safety.

We acknowledge that there are substantial challenges in implementing surveillance, tracking, and vigilance for HM on an international scale as outlined by proposal four. However, solutions for end-to-end technologies of full HM supply chain information management are already emerging (Heinrich et al., 2019). The apparent historical and current trend of good-will and increasing East-West co-operation in working together on HM quality standards is manifest recently with more comprehensive adoption of International Conference for Harmonization (ICH Q4) principles by the

upcoming 11th Edition Chinese Pharmacopoeia on one side, and European Directorate for the Quality of Medicines (EDQM) consulting with Chinese experts on the other. These factors collectively appear to support the credibility, feasibility, and timing of the proposals recommended here.

The potential limitations of our study merit considerations regarding pesticide profiles and risk assessments. As per the over-limit ratio, since the pesticide limit in HMs is not currently available, MRLs were derived according to the EP equation. For those pesticides in lack of ADIs, a default MRL of 0.01 mg·kg⁻¹ was defined. Without animal toxicological experiments, these ADIs and MRLs derived may not fully describe the realistic toxicity of pesticides, resulting in uncertainty of the over-limit situation of these eight pesticides. Of the 136 pesticides detected, their indicators including ARfD, ADI, NOEL, LOAEL, and TD₅₀ were not completely available. Therefore, some pesticides were lost in the risk assessments. The dosages of each HMs were obtained from CP, generally lower than the realistic dosage of herbal application in the clinic, leading to underestimated risks. For a realistic and comprehensive estimation, pesticide application data, and herbal processing factors (PF) should also be accounted for. We may assume that our interpretations of the pesticide contamination in HMs represent an over-estimate as only cultivated HMs were investigated, so it can't be extrapolated to predict the levels of pesticides in processed HMs and herbal supplements. Further researches will be warranted to determine the leaching behavior in HM preparations, general exposure scenarios, and assumed total daily intake of pesticides from HMs. Data on these aspects will refine the formal permissible limits of pesticides in HMs (Harris et al., 2011).

5. Conclusions

In conclusion, contamination from pesticide residues in HM is extensive and severe. Over half herbal medicines contained pesticides over the EP limit, and 43% contained 35 varieties of banned pesticides, eight of which were over 500 times higher than the default Maximum Residue Limit. DDTs, carbofuran, and mevinphos were confirmed as the most risk-inducing pesticides by three different risk assessments. Herbal medicines with fructus & semen applied posed most serious risks, while pesticides were the most widely detected in radix & rhizoma. Pesticides Aldicarb, carbofuran, isocarbofos, mevinphos, and DDTs, with above-threshold concentrations contained in herbal medicines, could potentially induce carcinogenic, genotoxic, endocrine disrupting, reproductive, development, neurotoxic, nephrotoxic, and hepatotoxic pathologies, causing irreversible health disorders. It warrants further attention than previously given in light of its significant potential hazard to public health. Extrinsic contamination may be the neglected culprit in the adverse effects of HM. A globalized and regulated solution for surveilling, monitoring, and certifying the HM supply chain is proposed here with a goal of improved global health.

Supplementary information

Appendix which include the following tables and figures:

Table S1.1: List of 1000 herbal medicine samples for LC-MS

Table S1.2: List of 771 herbal medicine samples for GC-MS

Table S2.1: List of 136 pesticides and relative indicators

Table S2.2: Standard curve and R² of 98 pesticides by LC-MS

Table S2.3: Standard curve and R² of 44 pesticides by GC-MS

Table S2.4: Ion trace and retention time of 98 pesticides by LC-MS

Table S2.5: Ion trace and retention time of 44 pesticides by GC-MS

Table S3: Eight ADI-vacant pesticides and 35 banned pesticides of MRLs calculated according to EP

equation

Table S4: Weighting system of risk-ranking score of pesticides in herbal medicines

Table S5: Detection rate and over-limit ratio of pesticides, and detection rate of banned pesticides

Table S6: Number of detected pesticides, of over-limit pesticides and of banned pesticides in single herbal medicine

Table S7.1: Detection frequency of different numbers of pesticides in five medicinal properties by LC-MS & GC-MS

Table S7.2: Detection frequency of six pesticide types in five medicinal properties by LC-MS & GC-MS

Figure S1: Highest ranking pesticides and herbal medicines and proportion of risk-ranking scores

Figure S2: Violin plot of patient health impact assessment of pesticides in herbal medicines

Figure S3: Heatmap of risk-ranking scores (S) of 136 pesticides in 182 herbal medicines

Figure S4: Heatmap of impact score (mean IS_{i,h}) of 136 pesticides in 182 herbal medicines

Figure S5: Comparative patient Impact Score (IS_h) and Risk Index (RI) of 182 herbal medicines

Abbreviations

HM: herbal medicines

LC/MS-MS: liquid chromatography-tandem mass spectrometry

GC/MS-MS: gas chromatography-tandem mass spectrometry

SRM: selected reaction monitoring

- 814 *LOD: limit of detection*
- 815 *LOQ: limit of quantitation*
- 816 EP: European Pharmacopoeia
- 817 MRL: Maximum Residue Limit
- 818 DDT: dichlorobiphenyl trichloroethane
- 819 HQ: hazard quotient
- 820 HI: hazard index
- 821 S: risk-ranking score
- 822 RI: Risk Index
- 823 IS: impact scores
- 824 DALY: disability-adjusted life years
- 825 ADI: Acceptable Daily Intake
- 826 ARfD: Acute Reference Dose
- 827 EU: European Union
- 828 US: United States
- 829 DT₅₀: half-life of degradation time
- 830 CNS: Central Nervous System
- 831 POPs: Persistent Organic Pollutants
- 832 GAP: Good Agriculture Practice
- 833 GACP: Good Agricultural and Collection Practice
- 834 ICH: International Conference for Harmonization
- 835 EDQM: European Directorate for the Quality of Medicines

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837 **Declaration of competing interest**

838 All authors declare no competing interests.

839

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Highlight

Extrinsic contamination in herbal medicines is severe with extremely toxic and banned pesticides detected.

DDTs, carbofuran, and mevinphos were confirmed as the most risk-inducing pesticides.

Crataegi fructus posed most serious risks, while pesticides were most widely detected in radix & rhizoma.

A globalized and regulated solution for surveilling, monitoring, and certifying the HM supply chain is in urgent need.

Declaration of interests

☐ ☒ The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

☐ The authors declare the following financial interests/personal relationships which may be considered as potential competing interests:

All authors declare no competing interests.