

Original Article

Elemental impurities in Yataprasen Thai Polyherbal Formulation: source-dependent variation, and analytical method validation

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ABSTRACT — The global consumption of complementary and alternative medicine (CAM) has risen dramatically. However, safety concerns persist owing to contamination with elemental impurities. In this study, we optimized and validated an analytical method for quantifying four toxic metals, As, Cd, Pb, and Hg, in Yataprasen (YTPS) extract, a complex Thai traditional polyherbal formulation. Samples were obtained from natural collection sites and commercial sources, and the contributions of 13 individual herbs to total impurities were evaluated. Inductively coupled plasma mass spectrometry (ICP-MS) was applied following four acid-digestion protocols. Quantification involved external calibration and standard addition, with validation covering LOD, LOQ, linearity, precision, and recovery. Toxicological risk was assessed in accordance with the ICH Q3D(R2) guideline. Digestion with 1 mL of HNO₃ gave the highest accuracy and recovery. Cd and Pb levels showed little variation across methods. Validation demonstrated excellent accuracy (93.6–107.5% recovery), strong linearity ($R^2 > 0.998$), and low detection limits ($<1.5 \mu\text{g/kg}$). A significant difference in elemental impurity concentration was observed between the two sources, with the naturally collected YTPS exhibiting markedly higher levels of all four metals than the commercial one. While external calibration was sufficient for commercial samples, standard addition was required for naturally sourced samples to overcome matrix effects. Component analysis identified *Allium sativum* L., *Cymbopogon nardus* (L.) Rendle, and *Melia azedarach* L. as the major contributors to the impurity burden. Source-dependent variation in elemental impurity concentration was observed in YTPS extracts, with natural collection posing a greater toxicological concern. The validated analytical workflow provides a robust platform for quality control and regulatory assessment of traditional polyherbal formulations.

Key words: Elemental impurity, Complementary and alternative medicine, ICH Q3D, ICP-MS, Yataprasen

INTRODUCTION

Complementary and alternative medicine (CAM) has seen a sharp rise in global use because of its cultural roots, accessibility, and affordability. Many people believe in its effectiveness and safety, valuing its holistic approach that addresses both physical and mental health. In some countries, traditional practices are integrated into

modern healthcare systems, such as hospitals and drug-stores, to support their use as a primary healthcare option or as an alternative to modern medicine. Patient satisfaction and a sense of autonomy significantly influence the long-term use of CAM (Tangkiatkumjai *et al.*, 2020).

Despite widespread use, the efficacy and safety of CAM continue to pose significant challenges. Most of its ingredients are derived from plants grown in natural envi-



ronments containing elemental impurities, including cadmium (Cd), mercury (Hg), lead (Pb), arsenic (As), zinc (Zn), copper (Cu), nickel (Ni), and chromium (Cr). These elements originate from natural and anthropogenic sources, such as the deterioration of Earth's crust due to atmospheric exposure, industrial activities, mining, sewage discharge, pesticides used in agriculture, and byproducts from coal-burning plants (Jomova *et al.*, 2025). Elemental impurities and microorganisms found in natural sources, such as soil, water, or air, are often transferred into raw materials through root absorption *via* passive diffusion or active transport, through deposition, or during raw material processing. Microorganisms usually adhere to plant parts or are introduced during the collection, handling, and manufacture of raw materials (Hlihor *et al.*, 2022; Meng *et al.*, 2022). Such contaminants pose health risks to consumers, potentially leading to adverse effects or even death (Luo *et al.*, 2021).

As, Cd, Pb, and Hg, are toxic to humans. They enter the body through several routes, including inhalation, ingestion, and skin absorption. After entering the body, they produce harmful effects by replacing essential metals in their natural binding sites, leading to abnormal cellular functions or oxidative stress that damages biological macromolecules and DNA (Jomova *et al.*, 2025).

Several studies have examined the effects of exposure to specific levels of elemental impurities over a certain period on human health. Long-term exposure to low levels is also a significant health concern. Low blood Cd, Pb, and Hg levels are associated with chronic kidney disease (Barregard *et al.*, 2022). Additionally, chronic exposure to As has led to cardiovascular disease, hepatic dysfunction, endocrine dysregulation, renal impairment, neurological disorders, hematological abnormalities, immune disruption, and increased cancer risk (Martínez-Castillo *et al.*, 2021).

Numerous issues have been raised regarding the quality control of herbal products, particularly the safety risks posed by elemental impurities in raw materials. Such impurities are difficult to control because the plant sources are cultivated in diverse and often uncontrolled environments. Currently, there are no harmonized guidelines for elemental impurities in herbal raw materials or products. Although the Guideline for Elemental Impurities Q3D(R2) (hereinafter ICH Q3D(R2)), issued by the International Council for Harmonisation of Technical Requirements for Pharmaceuticals for Human Use, specifies the permitted daily exposure (PDE) of 24 elemental impurities across various routes of administration, it does not apply to herbal products. In addition, Chapters <232> and <233> of the United States Pharmacopoeia (USP) address

analytical procedures and elemental impurity limits for dietary supplements. However, these do not directly apply to herbal products as well. In the absence of comprehensive international regulations, individual countries or regions have developed their own guidelines that vary widely and do not align with the ICH Guideline, such as the People's Republic of China and India (Inada *et al.*, 2023). Atomic absorption spectroscopy (AAS), X-ray fluorescence spectroscopy (XRF), inductively coupled plasma mass spectrometry (ICP-MS), and inductively coupled plasma optical emission spectroscopy (ICP-OES) are employed to quantify elemental impurities. Among these techniques, ICP-MS is recommended because it enables rapid, simultaneous multielement detection and has a sensitivity of as low as parts per trillion (ppt) (Chen *et al.*, 2022).

Yataprasen (YTPS) remedy formulary is a Thai traditional formulary listed in the National List of Essential Medicine and the National Thai Traditional Medicine Formulary. It is extensively used in primary healthcare settings in Thailand to treat musculoskeletal pain and inflammation. Despite solid pre-clinical evidence of its therapeutic potential, regulations governing herbal products originating from different sources or regions remain insufficient (Angsusing *et al.*, 2024). In this study, we perform a direct comparison of four elemental impurities—As, Cd, Pb, and Hg—between plant raw materials collected from natural environments and those commercially available in Thai traditional drugstores, using National Institute of Standards and Technology (NIST) certified reference materials (CRMs) for analytical method validation.

This work integrates a comparison of herbal sources through the concentration profiling of 13 botanical ingredients and a rigorously validated ICP-MS and Hg analyzer workflow that employs four acid-digestion protocols and two quantification techniques, external calibration (EC) and standard addition (SA), anchored to CRMs to resolve matrix effects. We further reconcile apparent extract-stage exceedance with patient risk under ICH Q3D(R2) by evaluating exposures against cutaneous PDEs. This integrated approach linking dual-mode quantification, matrix-effect control, and PDE-based risk assessment for a traditional topical polyherbal medicine constitutes the central novelty of the present study. Together with a low-cost microwave digestion option, this analytical toxicological framework provides a practical blueprint for the surveillance of elemental impurities in complex herbal products.

MATERIALS AND METHODS

Chemicals and reagents

Nitric acid (HNO₃), hydrogen peroxide (H₂O₂), and As standard were obtained from FUJIFILM Wako Pure Chemical Corporation (Osaka, Japan). Cd, Pb, and Hg standards were purchased from Kanto Chemical Co., Inc. (Tokyo, Japan). Dried codfish tissue (NMIJ CRM 7402-a) for the validation of Hg analysis was sourced from the National Metrology Institute of Japan (NMIJ, Ibaraki, Japan). Frozen human urine (NIST SRM 2668, Level II) for the validation of As, Cd, and Pb analyses was obtained from the NIST (Maryland, USA). Milli-Q water with a specific resistance of 18.2 MΩ·cm (Merck Millipore, Burlington, MA, USA) was used throughout the study.

Labware

All vessels and glass test tubes were pre-treated with 2.6 M HNO₃ overnight, rinsed with purified water, and dried prior to analysis.

Preparation of standard solutions

Standard solutions of As, Cd, and Pb were prepared by diluting a 1,000 µg/L stock solution with 6% HNO₃ to the final concentrations described in Supplementary Tables S1 and S2. These solutions were used to obtain standard calibration curves and to spike samples for ICP-MS analysis in the SA technique. In the SA technique, samples were spiked with a premixed standard solution containing As, Cd, and Pb at specific concentrations, followed by the addition of 6% HNO₃ to achieve three spiking levels. Hg calibration standards with concentrations ranging from 0 to 100 ng/mL were prepared for Hg determination by atomic absorption spectrometry.

Quality assurance

Validation parameters, including accuracy, precision, linearity, limit of detection (LOD), and limit of quantification (LOQ), were evaluated to determine the validity of the analytical methods, using dried codfish tissue for total Hg determination and frozen human urine for As, Cd, and Pb determination. Although plant-based CRMs are often employed for herbal analysis, we utilized frozen human urine (NIST SRM 2668, Level II) for As, Cd, and Pb, and dried codfish tissue (NMIJ CRM 7402-a) for Hg. These materials were selected based on their certified values for the target elements, and potential matrix differences were mitigated by validating the method with the SA technique to ensure traceability across different matrices. The equations used to calculate LOD and LOQ from the calibration curves are shown below.

$$LOD = \frac{3\sigma}{S}$$

$$LOQ = \frac{10\sigma}{S}$$

where σ is the SD of the blank solution, and S is the slope of the calibration curve.

Sample collection and preparation

All 13 herbal ingredients of YTPS were authenticated by a botanist and collected from their localities in Thailand. Each plant was cleaned, dried, and ground to a fine powder, then mixed in a specific ratio, as indicated in Table 1. The commercial herbal ingredients used for manufacturing YTPS herbal spray were purchased from a traditional drugstore in Bangkok, Thailand. The dried powder was mixed in the same manner as the powder obtained from natural sources. Following dry mixing, both the mixtures and the individual plant

Table 1. Composition of Yataprasen (YTPS).

Scientific name	Part used	%w/w
<i>Putranjiva roxburghii</i> Wall.	Leaves	37
<i>Senna siamea</i> (Lam.) H.S. Irwin & Barneby	Leaves	9
<i>Baliospermum solanifolium</i> (Burm.) Suresh	Leaves	9
<i>Cymbopogon nardus</i> (L.) Rendle	Rhizomes and leaves	9
<i>Tamarindus indica</i> L.	Leaves	9
<i>Melia azedarach</i> L.	Leaves	9
<i>Boesenbergia rotunda</i> (L.) Mansf.	Rhizomes and roots	2
<i>Allium sativum</i> L.	Bulbs	2
<i>Alpinia galanga</i> (L.) Willd.	Rhizomes	2
<i>Piper nigrum</i> L.	Fruits	2
<i>Ferula assa-foetida</i> L.	Resin from roots	2
<i>Aloe vera</i> (L.) Burm.f.	Resin	2
<i>Allium ascalonicum</i> L.	Bulbs	2

components were macerated in 95% ethanol for 48 hr, filtered, and solvent-evaporated to obtain crude extracts (Angsusing *et al.*, 2024).

Determination of elemental impurities in crude extracts

Approximately 30 mg of the crude extract was added to a polytetrafluoroethylene (PTFE) vessel. To this was added (i) 0.5 mL of 60% HNO₃, (ii) 1 mL of 60% HNO₃, (iii) 0.5 mL of 60% HNO₃ with 0.1 mL of H₂O₂, or (iv) 1.0 mL of 60% HNO₃ with 0.2 mL of H₂O₂. The vessel was sealed and subjected to microwave-assisted digestion in a conventional household microwave oven for three 1-min heating cycles separated by 1-min intervals between cycles. Milli-Q water was added to the vessel to make a final volume of 5 mL.

The concentrations of As, Cd, and Pb in the extract samples were determined by ICP-MS (Agilent 8800 ICP-MS/MS, Agilent Technologies, Tokyo). Instrumental conditions were optimized to maximize the signal intensity of ⁸⁹Y while reducing the Ce oxide ratio (CeO/Ce) to below 2%. Cold vapor atomic absorption spectrometry using an RA-5A Hg analyzer (Nippon Instruments, Osaka, Japan) was employed to determine Hg concentration. Details of operating conditions are summarized in Table 2. On the basis of the data obtained, the daily YTPS exposure level was estimated and compared with the cutaneous PDE values for As, Cd, Pb, and Hg established in the ICH Q3D(R2) guideline (Parente, 2025).

Data analysis and statistical methods

The results were expressed as means ± standard deviation (SD) of three independent sample preparations, each measured in triplicate (*n* = 3). All analytical data were analyzed with GraphPad Prism software version 9.5.0

(California, USA) to detect differences between samples using analysis of variance (ANOVA) followed by post hoc tests with a significance level of *p* < 0.05.

RESULTS

Sample digestion optimization

Table 3 lists the concentrations of Cd and Pb in the samples subjected to various acid-digestion protocols. Cd concentrations ranged from 226 ± 11 to 326 ± 45 µg/kg. Pb concentrations were substantially higher, ranging from 4.03 × 10⁴ ± 1.4 × 10³ to 4.36 × 10⁴ ± 2.2 × 10³ µg/kg. Statistical evaluation was performed using one-way ANOVA, followed by Tukey's post hoc test, with *p* < 0.05 considered statistically significant. Statistical analysis revealed no significant difference in Pb concentration among the samples treated with different volumes or types of acid-digestion solutions. In contrast, Cd concentration in the sample digested with 1.0 mL HNO₃ showed a significant difference (*p* = 0.0453). The analytical data are summarized in Supplementary Table S3.

Quality assurance

CRMs were used to validate the analytical methods for quantifying As, Cd, Pb, and Hg. Urine CRM was analyzed for As, Cd, and Pb, whereas dried codfish CRM was analyzed for Hg. For all elements, *R*² > 0.99, recoveries were 93.6–107%, and %RSD ≤ 6. LOD and LOQ were <1.32 and <4.00 µg/kg, respectively (Table 4). Analytical data for the EC technique are summarized in Supplementary Table S4.

Elemental impurity concentrations in polyherbal extracts

Two techniques—EC and SA—were used to deter-

Table 2. Instrument operating conditions.

ICP-MS	
Nebulizer	MicroMist
Plasma power	1600
Argon gas flow rate (L/min)	15.0
Auxiliary gas flow rate (L/min)	0.8
Nebulizer gas flow rate (L/min)	1.0
Integration time (sec)	0.1
Collision gas/flow rate (mL/min)	H ₂ /1.0
Monitored isotope	⁷⁵ As, ¹¹³ Cd, ²⁰⁸ Pb
Hg analyzer	
Sample flow rate (L/min)	0.2
Signal monitoring period (min)	2
Reducing agent	10% SnCl ₂
Volume of reducing agent (mL)	0.5

Table 3. Influence of acid-digestion conditions on Cd and Pb quantification in YTPS extract.

Entry	Treatment		Concentration	
	HNO ₃ (mL)	H ₂ O ₂ (mL)	Cd (µg/kg)	Pb (mg/kg)
1	0.5	-	248 ± 13	43.6 ± 2.2
2	1.0	-	326 ± 45*	43.1 ± 4.4
3	0.5	0.1	236 ± 8	40.3 ± 1.4
4	1.0	0.2	226 ± 11	40.9 ± 3.2

Data were obtained from three independent sample preparations, each measured in triplicate (*n* = 3). The data were analyzed using GraphPad Prism version 9.5.0 employing one-way ANOVA with Tukey's post hoc test (statistical significance denoted by * *p* < 0.05).

Table 4. Results of ICP-MS and Hg analyzer validation for As, Cd, and Pb detection in NIST SRM urine and Hg detection in dried codfish CRM.

Element	Range (µg/kg)	R ²	Recovery		LOD (µg/kg)	LOQ (µg/kg)
			%Recovery	%RSD		
As†	0-5.33	0.9997	99.1	6.0	0.186	0.619
Cd†	0-0.8	0.9996	107	5.9	0.030	0.100
Pb†	0-2.67	0.9998	93.6	1.2	0.076	0.254
Hg‡	0-100	0.9983	98.0	5.4	1.32	4.00

†Values represent the means of three independent measurements of samples prepared from NIST SRM, using the standard addition technique. ‡Values represent the means of three independent measurements of samples prepared from dried codfish CRM, using the external calibration technique.

mine As, Cd, and Pb concentrations in polyherbal extracts obtained from natural and commercial sources. Hg concentration was quantified using the EC technique. The concentrations determined by both techniques were compared for significant differences using an unpaired, two-tailed Welch's t-test with statistical significance set at $p < 0.05$. In commercial YTPS, no statistically significant differences in As, Cd, and Pb concentrations were observed between the two quantification techniques. In contrast, in naturally sourced YTPS, statistically significant differences were noted between the two quantification techniques across all elements. For naturally sourced YTPS, the EC technique yielded higher As concentrations than the SA technique. Hg was measured using the EC technique. For the commercial YTPS, comparisons showed no significant difference for As, Cd, or Pb. Overall, elemental impurity concentrations were markedly higher in extracts from naturally sourced YTPS than in commercial YTPS, and technique-dependent differences were evident only in the naturally sourced extracts. The average values and significance levels are shown in Fig. 1. Raw data, p -values, and Welch's t-test estimation plots are summarized in Supplementary Table S5 and Fig. 1.

Elemental impurity concentrations in individual herbal ingredients

As with the polyherbal extracts, two techniques—EC and SA—were used to determine As, Cd, and Pb concentrations in extracts containing individual herbal ingredients purchased from commercial sources. Hg concentration was quantified using EC technique. Elemental impurity analysis using the EC technique gave concentration values of 12.8 ± 9.7 to 309 ± 18 , 0.409 ± 0.39 to 22.3 ± 4.2 , 6.33 ± 3.7 to $371 \pm 1.3 \times 10^2$, and 33.6 ± 18 to $9.41 \times 10^3 \pm 1.2 \times 10^3$ µg/kg for As, Cd, Pb, and Hg, respectively. Elemental impurity analysis using the SA technique showed slightly lower concentrations rang-

ing from 18.2 ± 3.2 to 232 ± 41 µg/kg, 0.443 ± 0.6 to 24.4 ± 4.5 µg/kg, and 19.2 ± 23 to $401 \pm 1.5 \times 10^2$ µg/kg for As, Cd, and Pb, respectively (Table 5). As concentration was highest in *A. sativum*, Cd and Pb concentrations were highest in *C. nardus*, and Hg reached maximum concentration in *M. azedarach*. Differences between the techniques were assessed using an unpaired, two-tailed Welch's t-test ($p < 0.05$). All samples, except those containing As, showed no statistically significant difference. The contributions of each extract to the overall elemental impurity burden in the YTPS formulation are shown as a heat map in Fig. 2. Means, p -values, and significance levels of elemental impurities in extracts of individual herbal ingredients, including Welch's t-test estimation plots, are summarized in Supplementary Table S6 and Fig. S1.

Toxicological risk assessment

YTPS topical spray contains 10% w/v of extract. The indication for musculoskeletal pain is three sprays per application, three times a day. As each spray delivers 0.5 mL, 4.5 mL is administered daily, yielding 0.45 g of extract per day. As specified in the ICH Q3D(R2) guideline for topical products, cutaneous PDEs were calculated on the basis of the maximum daily dose. The PDEs and the estimated exposure values for YTPS topical spray are shown in Fig. 3. Raw data are provided in Supplementary Table S7.

DISCUSSION

This study presents a validated ICP-MS-based analytical workflow for measuring trace elemental impurities in the YTPS extract, a complex Thai polyherbal formulation, using ICP-MS, and for interpreting results relative to the cutaneous PDEs established in the ICH Q3D(R2) guideline at the labeled dose. The distinctive feature of this workflow is the integration of (i) systematic optimization of sample digestion, (ii) dual quantification by EC

Table 5. Elemental impurities in extracts of individual herbal ingredients.

Scientific name	As ($\mu\text{g/kg}$)			Cd ($\mu\text{g/kg}$)			Pb ($\mu\text{g/kg}$)			Hg ($\mu\text{g/kg}$)	
	External calibration	Standard addition	<i>p</i> -value	External calibration	Standard addition	<i>p</i> -value	External calibration	Standard addition	<i>p</i> -value	External calibration	External calibration
<i>P. roxburghii</i>	83.9 $\pm$ 20	40.8 $\pm$ 3.8	0.0031**	1.06 $\pm$ 0.82	0.443 $\pm$ 0.60	0.1748 ns	55.9 $\pm$ 8.2	57.3 $\pm$ 6.7	0.7762 ns	371 $\pm$ 48	
<i>S. siamea</i>	144 $\pm$ 38	99.6 $\pm$ 35	0.0636 ns	1.21 $\pm$ 1	0.469 $\pm$ 0.51	0.1553 ns	127 $\pm$ 5.9	134 $\pm$ 20	0.4343 ns	344 $\pm$ 65	
<i>B. solanifolium</i>	87.6 $\pm$ 13	42.8 $\pm$ 10	0.0003***	3.91 $\pm$ 1.9	4.67 $\pm$ 2.2	0.5448 ns	371 $\pm$ 13 $\times$ 10 ¹	401 $\pm$ 15 $\times$ 10 ¹	0.7180 ns	278 $\times$ 10 ¹ $\pm$ 70	
<i>C. nardus</i>	27.2 $\pm$ 6.8	18.2 $\pm$ 3.2	0.0381*	22.3 $\pm$ 4.2	24.4 $\pm$ 4.5	0.4230 ns	41.2 $\pm$ 8.8	35.6 $\pm$ 2.9	0.1877 ns	103 $\pm$ 2.8	
<i>T. indica</i>	174 $\pm$ 13	128 $\pm$ 20	0.0011**	0.945 $\pm$ 0.4	0.536 $\pm$ 0.36	0.1360 ns	99.6 $\pm$ 67	93.2 $\pm$ 64	0.8684 ns	581 $\pm$ 6.6	
<i>M. azedarach</i>	55.1 $\pm$ 16	33.1 $\pm$ 6.8	0.0181*	1.41 $\pm$ 1.1	1.75 $\pm$ 0.51	0.5273 ns	179 $\pm$ 60	166 $\pm$ 70	0.7315 ns	941 $\times$ 10 ¹ $\pm$ 12 $\times$ 10 ²	
<i>B. rotunda</i>	77.3 $\pm$ 8.5	50.3 $\pm$ 14	0.0082**	0.409 $\pm$ 0.39	1.48 $\pm$ 0.33	0.0013**	54.2 $\pm$ 2.5	38.5 $\pm$ 12	0.0387*	209 $\pm$ 55	
<i>A. sativum</i>	309 $\pm$ 18	232 $\pm$ 41	0.0041**	11.2 $\pm$ 2.8	14.3 $\pm$ 1.4	0.0425*	97.8 $\pm$ 35	119 $\pm$ 69	0.5383 ns	609 $\pm$ 29	
<i>A. galanga</i>	41.1 $\pm$ 6	29.9 $\pm$ 5.8	0.0125*	4.46 $\pm$ 3.3	3.39 $\pm$ 1.8	0.5062 ns	103 $\pm$ 25	94.9 $\pm$ 20	0.5264 ns	368 $\pm$ 32	
<i>P. nigrum</i>	48.7 $\pm$ 13	40.3 $\pm$ 12	0.2776 ns	1.34 $\pm$ 1.5	0.625 $\pm$ 0.80	0.3351 ns	37.2 $\pm$ 15	57.9 $\pm$ 37	0.2456 ns	377 $\pm$ 57	
<i>F. assa-foetida</i>	47.3 $\pm$ 21	35.1 $\pm$ 18	0.2968 ns	1.36 $\pm$ 1.2	1.62 $\pm$ 0.72	0.6616 ns	35.7 $\pm$ 18	22.6 $\pm$ 11	0.1612 ns	33.6 $\pm$ 18	
<i>A. vera</i>	28.9 $\pm$ 8.4	35.5 $\pm$ 10	0.2487 ns	3.60 $\pm$ 1.1	2.30 $\pm$ 0.75	0.0379*	58.8 $\pm$ 18	63.2 $\pm$ 20	0.7075 ns	710 $\pm$ 54	
<i>A. ascalonicum</i>	12.8 $\pm$ 9.7	19.2 $\pm$ 7.6	0.2795 ns	1.93 $\pm$ 0.48	1.96 $\pm$ 0.73	0.9240 ns	6.3 $\pm$ 3.7	19.2 $\pm$ 23	0.2310 ns	44 $\pm$ 4.8	

Data were obtained from three independent sample preparations, each measured in triplicate (*n* = 3).

Elemental impurities in Yataprasen

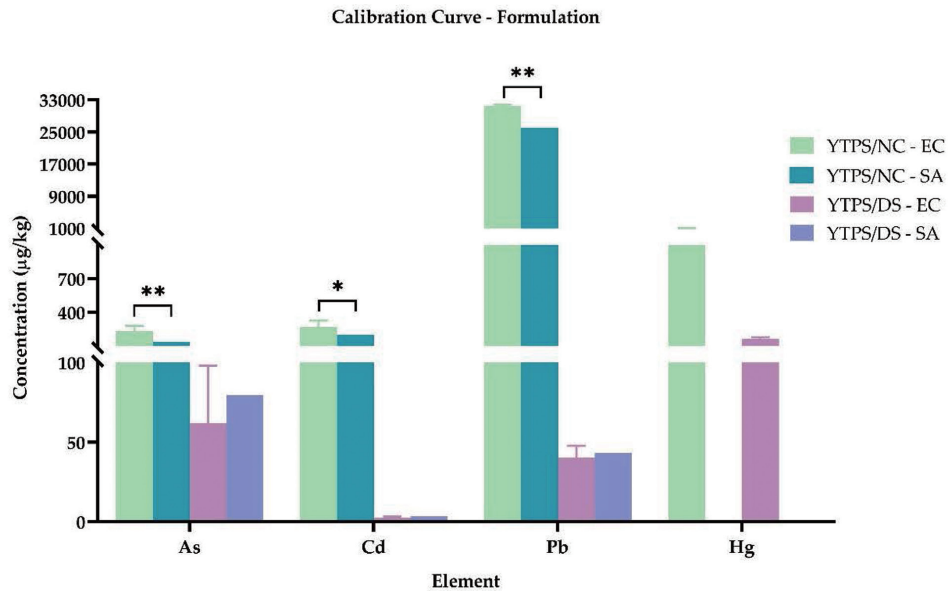


Fig. 1. Comparative bar chart of As, Cd, Pb, and Hg in the YTPS formulation measured by two quantification techniques, external calibration (EC) and standard addition (SA), for samples obtained from natural collection (NC) and drugstores (DC). Bars show the means $\pm$ SD of sample concentrations. Data represent three independent sample preparations, each measured in triplicate ($n = 3$). The data were analyzed by GraphPad Prism version 9.5.0 with an unpaired, two-tailed Welch's t-test (statistical significance denoted by * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$, **** $p < 0.0001$).

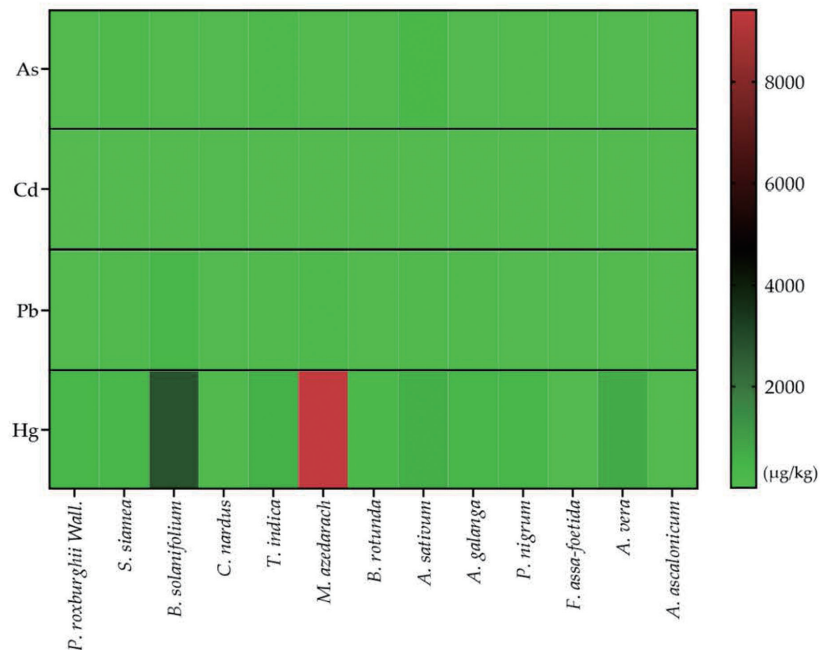


Fig. 2. Heatmap of average elemental impurity concentrations ($\mu\text{g/kg}$) in individual ingredient extracts obtained from a drugstore. Data represent three independent sample preparations, each measured in triplicate ($n = 3$).

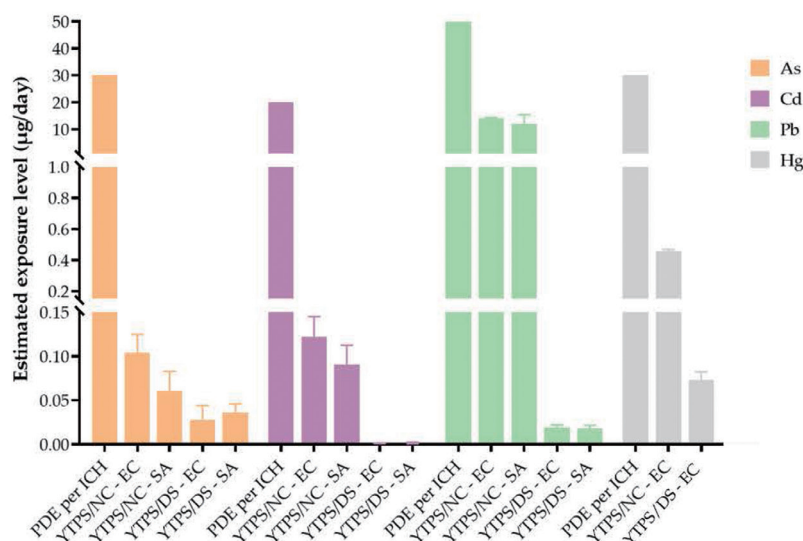


Fig. 3. Bar chart comparing permitted daily exposure (PDE) specified by ICH and exposure levels of As, Cd, Pb, and Hg in the YTPS formulation ($\mu\text{g/day}$), measured by two quantification techniques, external calibration (EC) and standard addition (SA), for samples obtained from natural collection (NC) and sold in drugstores (DC). Bars show the means $\pm$ SD of sample concentrations. Data represent three independent sample preparations, each measured in triplicate ($n = 3$). The data were analyzed by GraphPad Prism version 9.5.0.

and SA anchored to CRMs to control matrix effects, and (iii) PDE-based toxicological interpretation tailored to a traditional topical formulation. We optimized the sample acid-digestion protocol, compared the EC and SA techniques to resolve matrix effects, and established analytical method validation using CRMs.

The results indicated that the four acid-digestion protocols had no significant influence on Pb concentration. In contrast, Cd concentration was significantly higher with digestion using 1.0 mL of HNO_3 . This suggests that Pb is robust to modest changes in digestion chemistry, whereas Cd may be more sensitive to acid strength in this matrix. Our findings were similar to those of Potočnik, who reported slightly higher overall multi-element and Cd recoveries with HNO_3 digestion than with $\text{HNO}_3/\text{H}_2\text{O}_2$ digestion in some plant samples (Potočnik *et al.*, 2021). HNO_3 is extensively used for sample digestion because it extracts Pb and Cd more efficiently than acid combinations. In addition, plant-based matrix digestion with more than 30% v/v HNO_3 resulted in high recoveries of As, Cd, Pb, and Hg (Catenza and Donkor, 2022). A simplified, reproducible digestion method can be adopted for routine Pb surveillance, with focus on Cd response.

Both EC and SA techniques were valuable for diagnosis. As concentration determined by EC was signifi-

cantly higher than that determined by SA in the naturally sourced YTPS, whereas As concentration determined by the two techniques did not differ in the commercial product. Cd was below the detection limit by EC, confirmed by the low concentration determined using the SA technique. These results indicate that matrix effects are pronounced in naturally sourced extracts but are minimized by SA owing to sample matrix homogeneity. Likely contributors include botanical heterogeneity, differences in herbal powder particle size, and residual ethanol, which can cause carbon-based matrix effects in ICP-MS (Sánchez *et al.*, 2021). Prior work on plant-based samples recommends SA or matrix-matched calibration and closed-vessel digestion to control interferences while maintaining accuracy. In a similar study using complex plant matrices, SA was recommended because the values were more accurate than those obtained with EC (Kukusamude *et al.*, 2024). For processed commercial materials, EC appears sufficient, simplifying QC throughput. Notably, our simplified microwave digestion protocol achieved acceptable performance for most elements, supporting its use in resource-limited laboratories.

Method validation results met the required criteria, including good linearity ($R^2 > 0.998$), accuracy, precision (recovery 94–107%, %RSD ≤ 6), and LOD/LOQ in

the sub- $\mu\text{g/kg}$ to low $\mu\text{g/kg}$ range (AOAC international, 2016). Back-calculated checks confirmed the adequacy of the linear model across the working range. Blank control, carryover checks, and short-term solution stability tests supported the reported detection capability and ensured that LOQ remained below acceptance limits. Accuracy was confirmed using two CRMs, human urine for As, Cd, and Pb, and codfish tissue for Hg, ensuring traceability across matrices. Collectively, these parameters indicated a fit-for-purpose method suitable for surveillance and routine QC of herbal products.

Elemental impurity levels were markedly higher in the naturally sourced extract than in the commercial extract. Pb levels in the naturally sourced extract were near the upper range reported for finished products and overlapped with values in botanicals from polluted regions, consistent with other reports of high Pb with moderate As and Cd contamination in Thai traditional medicine (Wachirawongsakorn, 2016). Human and agricultural activities, including improper waste management, overuse of fertilizers and pesticides, irrigation with potentially contaminated water, and industrial discharges, lead to pollution with elemental impurities in water, soil, and air. Soil and rivers surrounding agricultural areas of naturally grown plants were reported to be contaminated with As, Cd, and Hg. An increase in electronic waste and inadequate recycling oversight also pose hazards. Elemental impurities originating from geogenic and human sources, such as irrigation with impacted waters, transfer contaminants to soils and then plants, potentially creating a synergistic pollution pathway. In the case of naturally sourced materials, limited process control, species-specific uptake variability, and soil conditions, including temperature, moisture, organic matter, pH, and nutrients, further increase the burden (Luo *et al.*, 2021). Post-harvest handling plays a significant role in contaminating herbal materials, and insufficient hygiene practices during material handling, cleaning, storage, transportation, and manufacturing processes amplify contamination risk. In contrast, sources that follow good agricultural and collection practices (GACP) and good manufacturing practice (GMP) have low levels of impurities.

Among the 13 commercial ingredients, *A. sativum* has the highest As concentration; *C. nardus*, the highest Cd and Pb concentrations; and *M. azedarach*, the highest Hg concentration. These results indicate disproportionate contributions by specific botanicals to the total burden, highlighting priority targets for supplier qualification and raw-material screening. Similar As concentrations in *A. sativum* in Thailand have been reported, whereas Cd concentration is slightly higher in our study, regardless of Hg

analysis (Phuengphai *et al.*, 2022). Impurities are hardly detected in *C. nardus* because the whole plant is not commonly used, even though its essential oil is generally used in fragrance and cosmetics (Hubai and Kováts, 2024). However, the entire *C. nardus* plant accumulates metals in its tissues (Ultra *et al.*, 2022). Findings for the related species *C. citratus*, reveals that *C. nardus* absorbs Cd and Pb from soil and similarly accumulates metals when grown in contaminated areas (Hubai and Kováts, 2024). *M. azedarach* leaves are used as a biomonitor for air pollution because they are capable of accumulating various elemental impurities (Bozdoğan Sert, 2016). *M. azedarach* is also used in the phytoremediation of soils contaminated with elemental impurities through stem and root uptake (Khamis *et al.*, 2014). Likewise, *S. siamea* accumulates elemental impurities via foliar uptake (Gajbhiye *et al.*, 2022). One study found that *T. indica* occasionally exceeded the safety limits for elemental impurities, likely due to environmental contamination (Bisht *et al.*, 2022). All toxic metals in *B. rotunda* are within toxic reference limits, and the plant has a relatively high concentration of As (Ubonnuch *et al.*, 2013). Studies of *A. galanga* conducted in Thailand demonstrated that the impurities were below the safety limit (Ubonnuch *et al.*, 2013; Limmatvapirat *et al.*, 2011). Interestingly, *A. galanga* exhibited a similar profile of low impurity levels (Limmatvapirat *et al.*, 2011). Black pepper, *P. nigrum*, is a well-known spice consistently reported to contain low levels of elemental impurities (Winiarska-Mieczan *et al.*, 2023). The elemental impurity levels in *F. assa-foetida* are within acceptable ranges (Alshwyeh *et al.*, 2024). *A. vera* accumulates elemental impurities, particularly Pb and Cd from soil, which are beneficial for phytoremediation. A wide range of toxic metal impurities have been reported; few exceeded the Pb limit, likely because of soil quality (Iqbal *et al.*, 2013). *A. ascalonicum* has low concentrations of elemental impurities, falling within all health safety standards (Phuengphai *et al.*, 2022). To the best of our knowledge, there are no elemental impurity data for *P. roxburghii* and *B. solanifolium*. These findings echo a report that certain taxa accumulate As, Cd, Pb, or Hg disproportionately owing to species-specific transporters, chelation capacity, and rhizosphere interactions (Afonne and Ifediba, 2020).

Although ICH Q3D formally targets synthesized drugs, its framework frequently provides information on national caps and finished product limits for herbal medicines (Inada *et al.*, 2023). Elemental impurity control for topical products is most appropriately evaluated relative to the specific route of administration and PDEs in ICH Q3D(R2), harmonizing with the exposure-based lim-

its adopted in USP Chapters <232>/<233> and WHO. At the YTPS dosage of 4.5 mL/day (= 0.45 g of extract/day; 10% w/v), the cutaneous limits are As 50, Cd 20, Pb 50, and Hg 30 µg/g daily. By converting extract concentrations into product units, we found that the elemental impurities in both naturally sourced and commercial formulations are below these route-specific limits, supporting acceptable risk at the recommended dosage. This exposure-based risk framing is more clinically relevant than comparing raw material content limits because it links analytical results to plausible patient intake. This pattern reflects a concentration effect during extraction, as bulk plant mass is reduced to a smaller mass of extract, and any non-volatile, water- or acid-stable metals present in the raw material can become enriched per gram of extract even if the total elemental amount per patient does not increase. Our data showed that elemental impurities in raw material used for natural extracts had higher concentrations than those in commercial sources (Supplementary Table S6), even though all finished products were complied with safety limits at a dosage of 0.45 g of extract/day.

Risk framing based on ICH Q3D(R2) cutaneous PDE levels is appropriate for labeling the topical dosage of the YTPS spray at 0.45 g of extract/day. The commercial extract showed lower concentrations, consistent with lower toxicological concern for typical use. For naturally sourced extracts, the higher concentrations of elemental impurities warrant attention on source control and batch testing. Although many jurisdictions lack harmonized elemental impurity limits for herbal products, converging pharmacopeial frameworks for dietary supplements and traditional medicines suggest adopting analytical acceptance criteria aligned with PDE levels and route-specific adjustments (Parente, 2025).

The strengths of this study include comparing two quantification techniques, EC and SA, which assess matrix effects; CRM-anchored validation across two matrices; and transparent statistics (ANOVA/Tukey and Welch's t-tests) that support inferences. Limitations include a lack of regional sampling and a focus on four elements. Broader geographic sampling, inclusion of additional contaminants, and speciation analysis of As and Hg would provide deeper toxicological insight.

This study demonstrated significant variations in elemental impurity contamination between naturally sourced and commercially available YTPS extracts. Different digestion protocols and validated analytical methods using ICP-MS were employed to accurately quantify As, Cd, Pb, and Hg. The higher contamination levels in natural sources highlight the need for enhanced mon-

itoring and regulatory control of traditional herbal products. Because only one formulation and a limited number of collection sites were examined, our data cannot fully separate the influence of environmental contamination (e.g., soil and water quality at the production site) from species-specific uptake capacity of the component herbs. However, the average levels of Pb in naturally collected herbs were greater than those seen in cultivated herbs, thus indicating that location and the methods of harvest have more impact on total amounts of Pb present in a product than do any steps taken during production. Additional sample site collection across regions and cultivation conditions will be required to generalize this observation. Furthermore, the absence of Pb isotope analysis in this study precluded the identification of the specific provenance of the lead based on isotopic signatures. Additional studies in the future using Pb isotope-ratio analyses would add additional information to our existing research by facilitating the separation of environmental contamination sources from those associated with processing. The methodology established herein offers a reliable framework for routine surveillance of elemental impurities in herbal medicines.

Source origin emerged as the principal driver of elemental impurities, and extract-stage exceedance did not translate to risk when judged relative to dose-based PDE levels. We proposed PDE-based process controls, targeted raw-material screening of high-contributing botanicals (*A. sativum*, *C. nardus*, and *M. azedarach*), and examined the matrix effect on quantification to maintain compliance with THP/ASEAN and ICH Q3D(R2). Adopted in routine QC, this approach reduces false non-conformance, focuses on upstream mitigation, and strengthens confidence in the safety of traditional herbal products.

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Conflict of interest

The authors declare that there is no conflict of interest.

Data availability

Data are included in the article and available as supplementary materials.

Author contributions

Conceptualization: J.A., Y.T., C.C., Y.O.; Funding acquisition: Y.O.; Investigation: J.A., Y.T., Y.O.; Supervision: Y.O.; Visualization: J.A., Y.T., Y.O.; Writing—original draft: J.A., Y.T., Y.O.; Writing—review & editing: J.A., Y.T., C.C., Y.O.

Ethical approval and consent to participate

Not applicable.

Patient consent for publication

Not applicable.

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