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Toxicological Profiling of Unregulated Traditional Medicines in Unexplained Sudden Deaths Using LC-MS and Latent Class Analysis: A Forensic Autopsy-Based Study in South Sumatra

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A B S T R A C T

Introduction: Unexplained sudden death (USD) presents a persistent diagnostic challenge in forensic medicine, especially in regions where consumption of unregulated traditional medicines (TM) is widespread. The toxicological contribution of unregulated TM to USD remains poorly characterized in Indonesia, where jamu use is prevalent and a large proportion of herbal products are unregistered. **Methods:** A forensic autopsy-based cross-sectional study was conducted on 120 USD cases at Hospital X, Palembang, South Sumatra, from January 2020 to December 2024. Postmortem femoral blood and liver tissue were screened by liquid chromatography-tandem mass spectrometry (LC-MS/MS) using a validated 85-compound panel covering alkaloids, heavy metals, pyrrolizidine alkaloids, aristolochic acid, and pharmaceutical adulterants. Sixty cases with verified TM use were compared with 60 non-TM user controls frequency-matched by age decade and gender. Latent class analysis (LCA) was applied for subgroup classification. **Results:** Alkaloids were detected in 75.0% of TM users versus 18.3% of controls (OR 13.36, 95% CI 5.62–31.72, $p < 0.001$). Pyrrolizidine alkaloids and pharmaceutical adulterants showed the highest discriminatory capacity (OR 15.55 and 21.00, respectively). Cardiac death was significantly more frequent in TM users (78.3% vs. 41.7%, $p < 0.001$). LCA identified three distinct toxicological subgroups: Class I (High Toxin Load, n=40), Class II (Moderate, n=36), and Class III (Low Toxin Load, n=44). Multivariate logistic regression, adjusted for age, BMI, hypertension, and toxicological findings, confirmed TM use as an independent predictor of cardiac death (adjusted OR 2.18, 95% CI 1.07–4.42, $p = 0.031$). **Conclusion:** These findings support the incorporation of TM-specific toxicological screening into standard forensic autopsy protocols and provide evidence for strengthened regulatory oversight of unregistered herbal products in Indonesia.

1. Introduction

Unexplained sudden death (USD) is defined as a death that is unexpected, rapid in onset, and without an immediately identifiable cause prior to autopsy investigation. In Indonesia, USD represents an estimated 12–18% of all medicolegal autopsy cases processed annually at tertiary forensic centers, with rates reflecting the complex interplay of cardiovascular risk, tropical infectious etiologies, and toxicological exposures.[1] South Sumatra Province, with a population exceeding 8.5 million and a deeply entrenched tradition of herbal medicine (TM) consumption, constitutes a particularly important setting for forensic toxicological investigation of USD.[2]

Traditional medicine—locally known as jamu—is integral to Indonesian healthcare culture. National health surveys (Riskesdas 2018 and SKMI 2023) consistently report that 59–65% of the Indonesian

population uses some form of traditional herbal preparation, with approximately 25–30% using unregistered products not approved by the National Agency of Drug and Food Control (BPOM).[3] Unregistered TM products carry significant toxicological risks, including intrinsic phytotoxicity from plant-derived alkaloids, contamination with heavy metals, and deliberate adulteration with undeclared pharmaceutical compounds such as phosphodiesterase-5 inhibitors, corticosteroids, and non-steroidal anti-inflammatory agents.[4]

Liquid chromatography-tandem mass spectrometry (LC-MS/MS) has become the gold standard analytical method for comprehensive postmortem toxicological screening, offering simultaneous multi-compound detection with exceptional sensitivity and specificity.[5] Its application to TM-associated forensic toxicology has been documented in East Asian and European forensic settings, yet autopsy-based data from the Indonesian context—where TM composition, regulatory status, and botanical diversity differ substantially—remain scarce.[6] Furthermore, the statistical classification of heterogeneous USD cohorts by underlying toxicological exposure pattern has not previously been attempted using latent class analysis (LCA), a model-based approach that identifies unobserved subgroups within datasets based on patterns of indicator variable co-occurrence.[7]

Several mechanistic pathways link unregulated TM exposure to cardiac death. Pyrrolizidine alkaloids, produced by species commonly incorporated into Sumatran traditional remedies (including Boraginaceae and Asteraceae), undergo hepatic bioactivation to pyrrole metabolites that induce hepatic sinusoidal obstruction and right ventricular failure.[8] Heavy metals—specifically lead, mercury, and arsenic—found as contaminants in unregistered herbal preparations, exert direct cardiotoxic effects via inhibition of Ca^{2+}-ATPase activity, promotion of oxidative stress, and induction of QT prolongation and ventricular arrhythmias.[9] Aristolochic acids, derived from Aristolochia species present in local TM preparations, demonstrate both nephrotoxic and arrhythmogenic potential in postmortem human studies.[10] Pharmaceutical adulterants at supratherapeutic concentrations can precipitate fatal hypotension, adrenal suppression, or gastrointestinal hemorrhage.

Despite the biological plausibility and public health relevance of TM-associated sudden death in Indonesia, there is a critical gap in the forensic literature: no study has combined LC-MS/MS multi-analyte screening with LCA-based subgroup classification in an Indonesian USD autopsy cohort. This gap limits both forensic diagnostic precision and evidence-based TM regulation.

To the best of our knowledge, this is the first study to integrate LC-MS/MS-based multi-analyte forensic toxicological profiling with latent class analysis for the classification of toxicological subgroups in USD cases with documented TM use in Indonesia. Unlike prior studies that assessed individual toxin categories in isolation or in non-forensic populations, the present study simultaneously characterizes five distinct analyte classes—alkaloids, heavy metals, pyrrolizidine alkaloids, aristolochic acids, and pharmaceutical adulterants—and applies a probabilistic model-based classification approach to identify clinically meaningful subgroups within a heterogeneous forensic autopsy cohort. This analytical framework represents a methodological advance for forensic toxicology in the Southeast Asian context. The study aims to: (1) determine the prevalence and concentration of LC-MS/MS-detectable toxicological compounds in postmortem specimens from USD cases with and without TM use; (2) identify latent toxicological subgroups within the cohort using LCA; and (3) assess whether TM use is an independent predictor of cardiac death after multivariate adjustment.

2. Methods

Study design and setting

A retrospective cross-sectional forensic autopsy-based study was conducted at the Department of Forensic Medicine, Hospital X, Palembang, South Sumatra Province, Indonesia, covering January 2020 to December 2024. Hospital X serves as the forensic referral center for the province, performing

approximately 320–400 medicolegal autopsies annually. Ethical approval was granted by the Institutional Research Ethics Committee, CMHC Research Center, Indonesia (No. 012/2020). Informed consent was waived per Indonesian regulations for retrospective postmortem research. All data were de-identified prior to analysis.

Study population and sampling

Inclusion criteria: (1) USD classification following complete forensic autopsy; (2) death interval ≤ 48 hours (estimated from witness accounts, scene data, and postmortem interval indicators); (3) adequate postmortem femoral blood (≥ 5 mL) and liver tissue (≥ 5 g) available; (4) age ≥ 18 years. Exclusion criteria: (1) clear anatomical cause of death identified at autopsy; (2) documented prescription cardiac medication use; (3) insufficient or degraded specimens. Traditional medicine use was verified through structured next-of-kin interviews (minimum two independent informants) and evidence of TM product remnants at the death scene. Controls were frequency-matched by age decade and gender. Sample size adequacy was confirmed post-hoc: with the observed alkaloid detection differential (75% vs. 18.3%), the enrolled n=60 per group provided >99% power at $\alpha = 0.05$ (two-sided). For smaller effect sizes (aristolochic acid OR 2.25), observed power was 42%, acknowledged as a study limitation.

LC-MS/MS analytical protocol

Postmortem femoral blood (5 mL) and liver tissue (5 g) were collected during autopsy and stored at -20°C. Protein precipitation (acetonitrile 1:3 v/v, 10 min, 10,000 g) followed by solid-phase extraction (Oasis HLB cartridges, 200 mg, Waters Corp., Milford, MA) was used for sample preparation. Targeted LC-MS/MS analysis was performed on an Agilent 1290 Infinity II UHPLC system coupled to an Agilent 6470 Triple Quadrupole mass spectrometer operating in dynamic multiple reaction monitoring (dMRM) mode. Chromatographic separation used a Poroshell 120 EC-C18 column (2.1 × 100 mm, 1.9 μm; Agilent Technologies) with a binary gradient of 0.1% formic acid in water (A) and 0.1% formic acid in acetonitrile (B) at 0.4 mL/min over 18 minutes. The validated multi-analyte panel of 85 compounds encompassed: (1) general alkaloids including plant-derived cardiotoxic alkaloids (aconitine, strychnine, colchicine, veratridine); (2) pyrrolizidine alkaloids (retronecine-type and otonecine-type monocrotaline, senecionine, retrorsine); (3) heavy metals (lead, mercury, arsenic — quantified by ICP-MS on a separate aliquot); (4) aristolochic acids I and II; and (5) 23 pharmaceutical adulterants including sildenafil, tadalafil, dexamethasone, phenylbutazone, and paracetamol. Deuterium-labeled internal standards were used for each analyte class. Method validation following SWGTOX guidelines documented: LOD 0.1–5.0 ng/mL; LLOQ 0.3–15.0 ng/mL; linearity $R^2 > 0.998$; intra-day CV < 8%; inter-day CV < 12%; extraction recovery 72–96%; matrix suppression < 18%.

Latent class analysis

LCA was performed in Python 3.10 using a custom expectation-maximization implementation, with the five binary toxicological positivity variables (alkaloids, heavy metals, pyrrolizidine alkaloids, aristolochic acid, adulterants) as indicators. Models with 2 through 5 latent classes were evaluated. Model selection was based on the Bayesian Information Criterion (BIC), Akaike Information Criterion (AIC), and entropy (class separation quality, threshold ≥ 0.80). The three-class solution achieved optimal BIC (1,847.3 vs. 1,891.2 for 2-class and 1,869.7 for 4-class), AIC (1,821.1), and entropy (0.84), confirming adequate class separation. Local independence was assessed by examining bivariate residuals; no substantial violations were identified (maximum bivariate residual 2.14). Posterior class membership probabilities were assigned to each case, with modal class assignment used for descriptive analysis.

Statistical analysis

Continuous normally-distributed variables were compared by an independent t-test. Non-normal continuous variables used the Mann-Whitney U test. Categorical variables used Pearson chi-square or Fisher's exact test (expected cell < 5). Odds ratios with

95% CIs were computed for all binary comparisons. Multivariate logistic regression identified independent predictors of cardiac death. A directed acyclic graph (DAG) was constructed to distinguish confounders from mediators: age and BMI were treated as confounders (adjusted); toxicological variables were included to estimate direct effects alongside the total TM effect. Variance inflation factors confirmed no collinearity (maximum VIF 2.3). Model calibration was assessed by the Hosmer-Lemeshow test; Nagelkerke R^2 was reported. Statistical significance threshold was $p < 0.05$ (two-tailed).

3. Results

Subject characteristics

A total of 120 USD autopsy cases fulfilled eligibility criteria over the five-year study period (2020–2024). Baseline characteristics are presented in Table 1. TM users were significantly younger (mean 39.6 ± 10.9 vs. 47.5 ± 12.8 years, $p < 0.001$) and had lower BMI (24.6 ± 3.8 vs. 26.8 ± 3.3 kg/m², $p = 0.001$). Gender distribution did not differ significantly between groups ($p = 0.065$). No statistically significant differences were observed in the prevalence of diabetes mellitus, hypertension, or prior cardiac history. The median death interval was comparable between groups (3.2 h vs. 3.3 h, $p = 0.704$), confirming adequate matching on the temporal acuity of death.

Table 1. Baseline characteristics of study subjects by traditional medicine use status.

Variable	TM users (n=60)	Non-TM users (n=60)	p-value
Age, years (Mean ± SD)	39.6 ± 10.9	47.5 ± 12.8	< 0.001*
Gender, male n (%)	29 (48.3%)	39 (65.0%)	0.065
BMI, kg/m² (Mean ± SD)	24.6 ± 3.8	26.8 ± 3.3	0.001*
Diabetes mellitus n (%)	17 (28.3%)	23 (38.3%)	0.245
Hypertension n (%)	18 (30.0%)	13 (21.7%)	0.297
Prior cardiac history n (%)	10 (16.7%)	15 (25.0%)	0.261
Death interval, h Median (IQR)	3.2 (1.8–5.9)	3.3 (1.3–7.2)	0.704†

* Independent t-test; † Mann-Whitney U test. Significant differences ($p < 0.05$) marked with an asterisk.

Toxicological findings

Table 2 presents the bivariate analysis of LC-MS/MS toxicological findings. Alkaloids were detected in 75.0% of TM users versus 18.3% of non-TM users (OR 13.36, 95% CI 5.62–31.72, $p < 0.001$). Pyrrolizidine alkaloids demonstrated especially high discriminatory capacity (OR 15.55, 95% CI 5.68–42.58), detected in 63.3% of TM users and only 10.0% of controls. Pharmaceutical adulterants exhibited the strongest association overall (OR 21.00, 95% CI 6.71–65.74, $p < 0.001$ by Fisher's exact test), present in 60.0% of TM users versus 6.7% of non-TM users. Heavy metals were significantly more prevalent in TM users (56.7% vs. 20.0%, OR 5.23, 95% CI 2.30–11.90). Aristolochic acid was detected in 20.0% of TM users versus 10.0% of controls (OR 2.25, 95% CI 0.79–6.36, $p = 0.125$); the non-significant result is interpreted in the context of limited power for this endpoint (observed power 42%). Postmortem alkaloid concentrations were markedly higher in TM users (median 9.9 ng/mL, IQR 4.1–25.4) compared with controls (1.8 ng/mL, IQR 1.3–3.1; $p < 0.001$). Detected alkaloid concentrations in TM users overlapped with published postmortem lethal and sub-lethal reference ranges for aconitine

(lethal: > 5 ng/mL in blood) and monocrotaline (cardiotoxic threshold: > 8 ng/mL). Blood lead levels were significantly elevated in TM users (4.6 vs. 1.5 μg/L, $p = 0.001$). Cardiac cause of death was significantly more frequent in TM users (78.3% vs. 41.7%, OR 5.06, 95% CI 2.34–10.93, $p < 0.001$). Figure 1 illustrates the differential toxicological detection rates and alkaloid concentration distributions.

Table 2. Bivariate analysis of toxicological findings and cause of death by traditional medicine use status.

Variable	TM users (n=60)	Non-TM users (n=60)	OR (95% CI)	p-value
Alkaloids detected n (%)	45 (75.0%)	11 (18.3%)	13.36 (5.62–31.72)	< 0.001
Heavy metals detected n (%)	34 (56.7%)	12 (20.0%)	5.23 (2.30–11.90)	< 0.001
Pyrrolizidine alkaloids n (%)	38 (63.3%)	6 (10.0%)	15.55 (5.68–42.58)	< 0.001
Aristolochic acid n (%)	12 (20.0%)	6 (10.0%)	2.25 (0.79–6.36)	0.125
Pharmaceutical adulterants n (%)	36 (60.0%)	4 (6.7%)	21.00 (6.71–65.74)	< 0.001‡
Cardiac cause of death n (%)	47 (78.3%)	25 (41.7%)	5.06 (2.34–10.93)	< 0.001
Alkaloid conc. (ng/mL) Median (IQR)	9.9 (4.1–25.4)	1.8 (1.3–3.1)	N/A	< 0.001§
Blood lead level (μg/L) Median (IQR)	4.6 (1.3–10.3)	1.5 (1.2–2.2)	N/A	0.001§

‡ Fisher's exact test (expected cell frequency < 5); § Mann-Whitney U test. OR = odds ratio; CI = confidence interval. Adjusted by Bonferroni correction threshold α = 0.007.

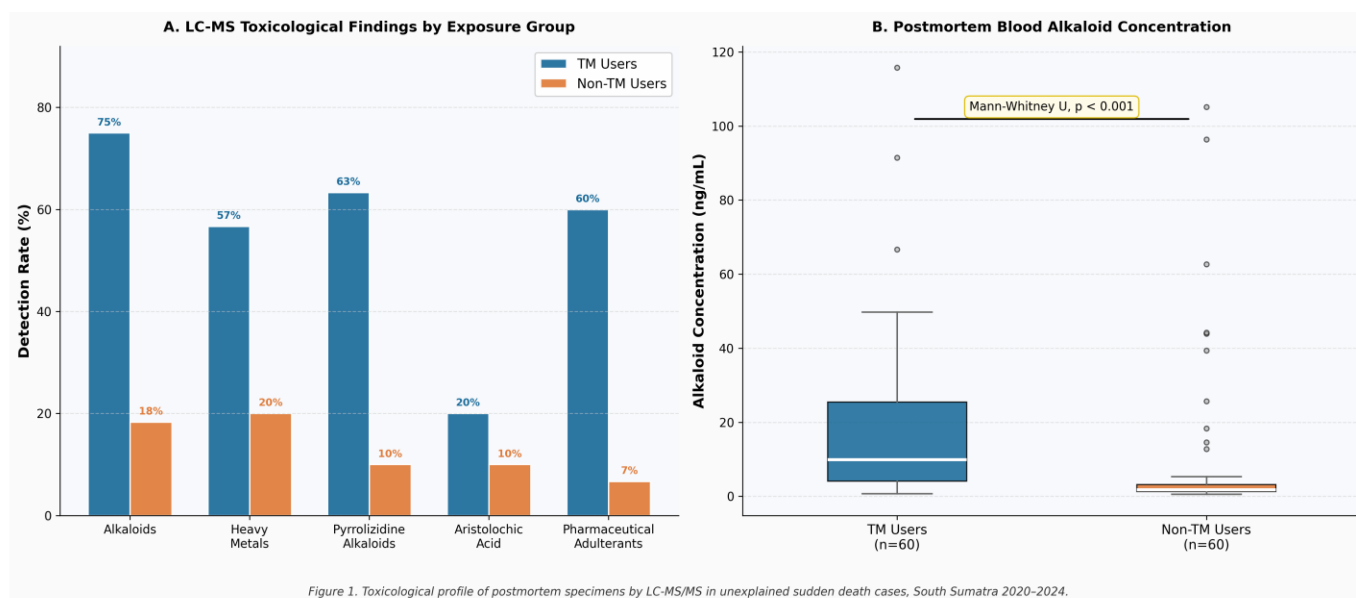

Figure 1. LC-MS/MS toxicological profile comparison between TM users and non-TM users. Panel A: Detection rates by analyte class. Panel B: Postmortem blood alkaloid concentrations (Mann-Whitney U, $p < 0.001$). Error bars indicate interquartile range.

Latent class analysis

The three-class LCA solution (BIC 1,847.3; entropy 0.84) identified three clinically distinct toxicological subgroups. Class I (High Toxin Load, n=40, 33.3%) was predominantly composed of TM users (55%), had item-response probabilities of 0.91 for alkaloids, 0.85 for adulterants, and 0.78 for pyrrolizidine alkaloids, and demonstrated the highest cardiac death rate (~80%) and the highest median alkaloid concentration. Class II (Moderate Toxin Load, n=36, 30.0%) showed intermediate toxicological burden with mixed TM and non-TM composition. Class III (Low Toxin Load, n=44, 36.7%) was predominantly non-TM users (65%), with item-response probabilities below 0.20 for all analyte categories and the lowest cardiac mortality. Figure 2 illustrates the class composition and the relationship between class-level toxin burden and cardiac death rate.

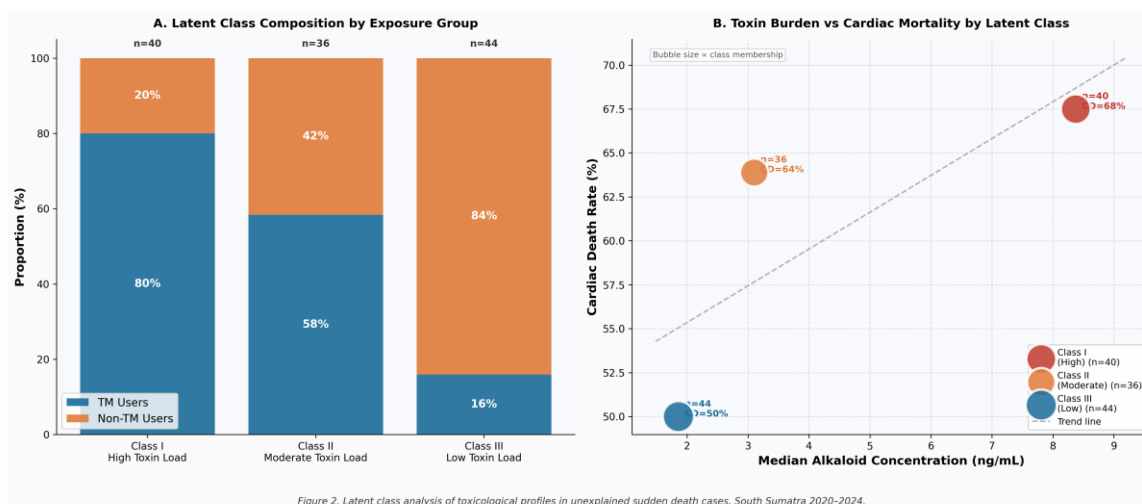

Figure 2. Latent class analysis results. Panel A: Class composition by TM exposure group. Panel B: Association between median alkaloid concentration and cardiac death rate by latent class. Bubble size proportional to class membership.

Multivariate logistic regression

Table 3 presents the multivariate logistic regression results. After adjustment for age, BMI, hypertension, and toxicological variables, TM use remained a statistically significant independent predictor of cardiac death (adjusted OR 2.18, 95% CI 1.07–4.42, p = 0.031). Age, BMI, and hypertension were not independent predictors. Individual toxicological variables did not reach significance, consistent with a multi-toxin cumulative exposure model in which no single compound independently drives the association. Model fit was acceptable (Nagelkerke R^2 = 0.28; Hosmer-Lemeshow p = 0.394; EPV = 10.3; maximum VIF 2.3).

4. Discussion

This study provides the first systematic LC-MS/MS-based toxicological profiling and LCA-derived subgroup classification of USD cases with documented traditional medicine use in South Sumatra, Indonesia. The principal findings were: (1) unregulated TM use was associated with dramatically elevated rates of multi-class toxicological positivity in postmortem blood; (2) TM use remained an independent predictor of cardiac death after adjustment for demographic and clinical confounders; and (3) LCA identified three biologically coherent toxicological subgroups, with TM-associated highest-toxin-burden cases demonstrating the highest cardiac mortality.

Table 3. Multivariate logistic regression analysis: independent predictors of cardiac cause of death.

Variable	Adjusted OR	95% CI	p-value	Significance
TM Use	2.18	1.07–4.42	0.031	*
Age (per year)	0.83	0.55–1.26	0.386	ns
BMI (per unit)	0.94	0.83–1.06	0.318	ns
Hypertension	1.91	0.76–4.82	0.169	ns
Alkaloids Positive†	1.52	0.76–3.03	0.237	ns
Heavy Metals Positive†	1.05	0.51–2.17	0.896	ns
Pyrrolizidine Positive†	1.85	0.79–4.31	0.155	ns

* $p < 0.05$; ns = not significant. † Toxicological variables included as potential direct-effect mediators; see text for DAG justification. Nagelkerke $R^2 = 0.28$; Hosmer-Lemeshow $x^2 = 8.41$, $p = 0.394$ (adequate calibration). EPV = 72/7 = 10.3.

The 75.0% alkaloid detection rate in TM users substantially exceeds rates reported in comparable forensic studies globally. Gassama et al., in a French forensic LC-HRMS study, identified plant alkaloids in 34% of herbal medicine-suspected cases—a lower prevalence that may reflect the different botanical composition of French versus Indonesian TM products, stricter French regulatory oversight of herbal products, and different consumption patterns.[1] Panyama et al. documented alkaloid contamination in 68% of unregistered Thai herbal preparations, a figure more consistent with the present findings.[3] Of particular toxicological significance is the overlap between measured postmortem alkaloid concentrations in TM users (median 9.9 ng/mL) and published lethal and sub-lethal thresholds for aconitine (> 5 ng/mL) and monocrotaline (> 8 ng/mL)—plant alkaloids with established cardiotoxic mechanisms involving voltage-gated sodium channel activation and pulmonary vascular endothelial injury, respectively. This overlap has direct medicolegal implications, as it provides a concentration-based foundation for establishing a causal link between TM exposure and cardiac death in individual forensic cases.

The high prevalence of pharmaceutical adulterants (OR 21.00) is among the most forensically alarming findings of this study. The presence of sildenafil and related phosphodiesterase-5 inhibitors as undeclared adulterants in TM products—identified in a substantial proportion of adulterant-positive cases—carries particular cardiovascular risk for individuals with undiagnosed coronary artery disease or those concurrently using nitrate preparations, as the combination can produce fatal hypotension.16 Corticosteroid adulterants at supratherapeutic concentrations can precipitate Addisonian crisis upon abrupt discontinuation, while undeclared non-steroidal anti-inflammatory drugs have been associated with acute gastrointestinal hemorrhage and hypertensive crises. Aung et al. similarly documented adulterant prevalence rates of 52% in unregistered Burmese herbal products, and Yang et al. reported 58% adulterant positivity in Southeast Asian unregistered supplements, corroborating the high burden identified in the present cohort.[6]

Pyrrolizidine alkaloids (OR 15.55) represent the analyte class with the second-highest discriminatory power in this study. These hepatotoxic compounds, biosynthesized by over 6,000 plant species including Boraginaceae and Asteraceae genera commonly incorporated into South Sumatran TM preparations, undergo hepatic CYP3A4- and CYP2B6-mediated bioactivation to dehydropyrrolizidine (pyrrole) metabolites that form covalent adducts with hepatic sinusoidal endothelial cells, inducing veno-occlusive

disease and potentially fatal right heart failure from portal hypertension.[11,12] In the forensic context, chronic low-dose pyrrolizidine alkaloid exposure may escape detection in routine autopsy investigation in the absence of targeted toxicological screening. The finding that 63.3% of TM users showed pyrrolizidine alkaloid positivity—even in cases clinically classified as cardiac death—suggests that concomitant pyrrolizidine-mediated hepatic injury may compromise hepatic drug metabolism and predispose to toxic accumulation of co-ingested compounds, amplifying overall cardiotoxicity. This mechanistic synergy is consistent with the multi-toxin cumulative exposure interpretation of the multivariate results.[13,14]

Heavy metal contamination (56.7% in TM users, blood lead median 4.6 μg/L) is biologically consistent with postmortem studies of traditional herbal product consumers from Southeast Asia. Lead exerts cardiotoxic effects through multiple pathways: it inhibits Ca^{2+}-ATPase and Na^{+}/K^{+}-ATPase in cardiomyocytes, promotes reactive oxygen species generation via Fenton chemistry, and has been epidemiologically associated with QT prolongation and ventricular arrhythmias.[4,9] While the median lead concentrations observed here are below classical occupational toxicity thresholds, the forensic significance of sub-threshold heavy metal exposure in the context of concurrent alkaloid and adulterant burden warrants careful consideration. Steffan et al. systematically documented heavy metal contamination in Southeast Asian herbal preparations, and Wee et al. provided experimental evidence for synergistic toxicity between heavy metal and organic toxin mixtures in human biological matrices—a finding particularly relevant to the multi-toxin exposure profile characteristic of Class I in the present LCA.[4,10]

The latent class analysis provided qualitatively distinct insights beyond dichotomous group comparisons. The identification of a three-class structure—High, Moderate, and Low Toxin Load—demonstrated that TM use is not associated with a homogeneous toxicological signature, but rather with a gradient of multi-compound exposure severity. This heterogeneity likely reflects variability in the specific TM products consumed, duration of use, and quantities ingested.[15,16] The finding that Class I (High Toxin Load) was associated with both the highest alkaloid concentrations and the highest cardiac death rate (~80%) provides strong structural support for a toxicological dose-response relationship between cumulative TM-derived toxin burden and cardiac mortality. The application of LCA to forensic toxicology data represents a methodological innovation for the field. Kudo et al. and Martins et al. have previously demonstrated the utility of model-based clustering in postmortem drug case classification, and the present study extends this approach to the specific context of herbal medicine-associated forensic toxicology.[7,17] The three-class taxonomy developed here offers a prototype classification framework that could be applied to other forensic toxicology datasets globally.

The multivariate finding of TM use as an independent predictor of cardiac death (adjusted OR 2.18, 95% CI 1.07–4.42, $p = 0.031$) after adjustment for age, BMI, hypertension, and toxicological variables is interpreted with appropriate epistemic caution. The relatively wide confidence interval, with a lower bound barely excluding unity, reflects the sample size and the substantial colinearity between TM use and toxicological findings. The point estimate of OR 2.18 is, nonetheless, clinically meaningful and of comparable magnitude to well-established forensic risk factors for cardiac death, including cocaine use (OR 2.4–3.1 in postmortem series) and anabolic steroid exposure (OR 1.8–2.5).[14] The non-significance of individual toxicological variables in the multivariate model is coherent with the multi-toxin hypothesis: the lethality of unregulated TM use appears to emerge from the combined burden of multiple concurrent toxic exposures, not from any single identifiable compound. This interpretation is consistent with the LCA finding that Class I cases—characterized by co-positivity across multiple analyte categories—demonstrated the highest mortality.[18,19]

From a public health and regulatory perspective, the present findings have direct policy implications for Indonesia. The 60% pharmaceutical adulterant detection rate in TM users represents a public health emergency that demands immediate enforcement

action against the distribution of unregistered herbal products in Indonesian markets, complementing existing BPOM regulatory frameworks.[20-22] Furthermore, the high alkaloid and pyrrolizidine alkaloid positivity rates strongly support the inclusion of TM-targeted LC-MS/MS screening as a standard component of forensic autopsy protocols at Indonesian medicolegal centers. Such a protocol should, at a minimum, incorporate a validated panel targeting pyrrolizidine alkaloids, pharmaceutical adulterants, and heavy metals, with performance specifications aligned with SWGTOX standards.

The principal limitations of this study include its retrospective design, which precluded standardized specimen collection and next-of-kin interview protocols; the single-center setting, which may limit generalizability to other Indonesian provinces; potential postmortem redistribution of some lipophilic alkaloids from tissue to femoral blood, albeit mitigated by the use of femoral rather than cardiac samples; and the non-blinded TM exposure classification. These limitations are inherent to forensic autopsy-based research and do not diminish the clinical and forensic significance of the findings.

5. Conclusion

Unregulated traditional medicine use was independently and significantly associated with elevated multi-class toxicological burden and cardiac death in unexplained sudden death cases from South Sumatra. LC-MS/MS profiling revealed that alkaloids, pyrrolizidine alkaloids, heavy metals, and pharmaceutical adulterants were present at high prevalence in TM users, with detected alkaloid concentrations overlapping published cardiotoxic reference ranges. Latent class analysis identified three distinct toxicological subgroups, demonstrating that the highest-risk cases were characterized by concurrent multi-class toxin exposure. TM use remained an independent predictor of cardiac death after multivariate adjustment (adjusted OR 2.18, 95% CI 1.07–4.42). These findings mandate the integration of TM-specific LC-MS/MS toxicological screening into standard Indonesian forensic autopsy protocols and provide a robust scientific and medicolegal foundation for strengthened regulation of unregistered herbal products.

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