QUANTIFICATION AND IN-HOUSE REFERENCE STANDARD PREPARATION OF TANSHINONE IIA FROM DANSHEN (*Salvia miltiorrhiza* Bunge) ROOTS COLLECTED IN LAI CHAU, GIA LAI, AND LAM DONG PROVINCES

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ABSTRACT

Salvia miltiorrhiza Bunge., commonly known as Đan sâm or Danshen, is a valuable medicinal herb widely used for cardiovascular disorders. The raw materials of Danshen, originating in Vietnam, however, have remained uncommon and poorly characterized. Tanshinone IIA, one of the major and attractive components of Danshen, has been used as a key bioactive marker that is required by multiple pharmacopoeias. This compound is still available only as a costly imported reference standard, which is a hindrance for both the materials' quality evaluation and the phytochemical study of Vietnamese Danshen. In this study, the reference standard of tanshinone IIA was in-house prepared from Vietnamese Danshen root materials (labelled as IH-TIIA). This standard evolved into the validated UFLC/PDA method that is used for the quantification of tanshinone IIA in roots and extracts. The procedure for preparing IH-TIIA was also applied to the tanshinone IIA isolated from all of the Vietnamese Danshen materials. All IH-TIIA samples were achieved with high purity, being 98.62% (Lai Chau), 98.68% (Gia Lai) and 98.77% (Lam Dong), respectively. The quantification results indicated a clear regional variation of tanshinone IIA yield, with the roots from Lai Chau containing the highest content (1.24%). Known to be relatively unstable, tanshinone IIA was also subjected the stability evaluation under different storage conditions. The obtained results demonstrated that the preservation at 4°C without light exposure was ideal for tanshinone IIA.

Keywords: *Salvia miltiorrhiza, tanshinone IIA, nuclear magnetic resonance, UFLC/PDA.*

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1. INTRODUCTION

Salvia miltiorrhiza Bunge. is a traditional Chinese medicinal herb prescribed for promoting blood circulation, resolving stasis, nourishing the heart, calming the spirit, and detoxifying. Thanks to these beneficial effects, Danshen has been cultivated in Vietnam and other countries for the constant yield of its typical red roots [1]. In modern medicine, its roots exist in the treatment of cardiovascular disorders such as atherosclerosis, thrombosis, and angina pectoris. Clinical studies further demonstrated the protective effects of Danshen on cartilage, anti-apoptotic and platelet aggregation inhibitory activities, and vascular function improvement [2 - 8]. Regarding the phytochemistry, Danshen has been indicated to contain lipophilic diterpenoids and phenolic acids. Among these, tanshinone IIA is the main bioactive diterpenoid that is used as a recognized marker for quality control [9]. It is included in the

pharmacopoeias of China, EU, Japan, Korea, USA, and Vietnam, specified as a minimum content of 0.20% in root materials (HPLC, dry basis). The content of tanshinone IIA is one of the most important criteria for evaluating the quality of Danshen materials. Some previous studies in Vietnam have simultaneously quantified tanshinone IIA and salvianolic acid B using modern technologies such as HPLC-MS/MS or UPLC-DAD [10 - 11]. A complete and effective procedure for preparing tanshinone IIA at the reference grade has remained left-open. However, the high cost of the reference standard has still been a limitation for Vietnamese Danshen materials evaluation. The phytochemistry of Danshen originating from Vietnam remains poorly understood, with tanshinone I, tanshinone IIA, and cryptotanshinone being the reported constituents [12]. In this study, tanshinone IIA was in-house prepared at the reference standard grade, labelled as IH-TIIA, and this product evolved in a standardized UFLC/PDA procedure for tanshinone IIA quantification of Danshen roots harvested in Lai Chau, Gia Lai, and Lam Dong provinces. With this in-house preparation and large-scale quantification, a strong, traceable solution for evaluating Vietnamese Danshen was significantly supported, reducing the dependence on imported standards and establishing a framework for pharmaceutical quality control and future pharmacological research.

2. MATERIALS AND METHODS

2.1. Plant materials

Fresh Danshen roots were collected in October 2023 from three provinces in Northern and Central Vietnam: Lai Chau (LC), Gia Lai (GL), and Lam Dong (LD) (Figure 1). The LD and GL Danshen were cultivated for 18 months following the VietGAP standard, with controlled irrigation and nutrient management; while LC Danshen (at undetermined age) was wildly collected from mountainous areas of Muong Te commune. For phytochemical comparison with Vietnamese Danshen, 1.3 kg of Danshen roots were purchased from the Dai Loc Trading, Manufacturing, and Construction Company, Ltd. LC, GL, and LD Danshen roots were botanically identified via chloroplast gene sequencing (Apollo Biotek Co., Ltd., with sequence comparisons against the NCBI database), macroscopy, and powder microscopy (the Institute of Drug Quality Control, Ho Chi Minh city, in accordance with Vietnamese Pharmacopoeia V (VPV)).

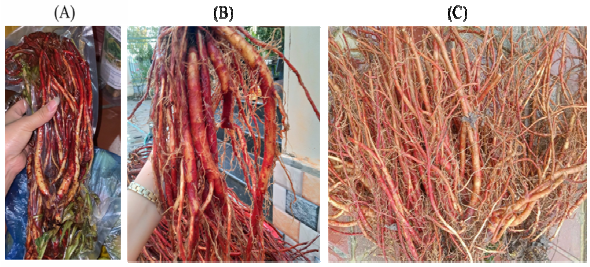


Figure 1. Danshen roots collected in Lai Chau (A), Gia Lai (B), and Lam Dong (C) provinces

2.2. Reagents and standards

Acetonitrile and methanol (HPLC-grade) were obtained from Supelco Inc. (Massachusetts, United States). Organic solvents, including *n*-hexane, chloroform, ethyl acetate, toluene, *n*-butanol, ethanol 96%, formic and sulfuric acids, were purchased from Chemsol (Vietnam). Distilled water was internally prepared. A commercial tanshinone IIA standard was supplied by the Institute of Drug Quality Control, Ho Chi Minh City (99.7817% pure (UFLC/PDA)) and was used for the quality evaluation of IH-TIIA.

2.3. UFLC/PDA analysis

The UFLC/PDA analysis was performed on a SPD-M20A (Shimadzu) system, using a Gemini-NX C18 column (250 × 4.6 mm, 5 µm) and a guard column. The mobile phase was acetonitrile-water (60: 40, v/v), 1.0 mL/min, injection volume of 20 µL, column temperature of 25°C, PDA detection at 270 nm, and a run time of 30 min.

2.4. Preparation of IH-TIIA from purchased Chinese Danshen roots

Scheme 1 of the Supplementary Materials section shows the process of preparing IH-TIIA from the purchased Chinese Danshen roots. Briefly, the roots were ground to obtain powder, which was macerated in ethanol 96% (*v/v*) at room temperature (three times of repetition, interval of

24 h) to obtain extract. The ethanolic extract was liquid-liquid extracted with a series of organic solvents (*n*-hexane, ethyl acetate, and *n*-butanol) to obtain the corresponding extracts. A residue was observed in the *n*-hexane extract, which was separated by mixing the extract with chloroform, filtering, and drying. After being eliminated, the residue, the *n*-hexane extract, was taken to the sephadex® LH-20 gel filtration and silica gel column chromatography (the gradient mobile phase of *n*-hexane–chloroform–ethyl acetate), yielding 41 fractions (A1-A41). A precipitate was observed in the A4 fraction, which was subsequently isolated and recrystallized in *n*-hexane–chloroform (1.5: 1, *v/v*) mixture at 0˚C to obtain a dark brown solid. This solid was completely dried and taken to NMR and ESI-MS recordings for structural evaluation (IH-TIIA).

2.5. Quantification of tanshinone IIA in LC, GL, and LD Danshen roots

Before the quantification of tanshinone IIA, in LC, GL, and LD Danshen roots, the method was validated via the parameters of specificity, system suitability, linearity, accuracy, precision, LOD, and LOQ. Recovery tests (110–140 % levels) and six replicate injections of the standard confirmed analytical reliability. The ethanolic extract of LC, GL, and LD Danshen roots were prepared following the same process as the Chinese one. Approximately 25 mg of each extract was dissolved in methanol (10: 5, *w/v*), sonicated for 10 min, and filtered via 0.45-µm PTFE membrane. IH-TIIA was used to prepare the calibration solutions (concentration ranging from 0.00005 to 0.05 mg/mL). Scheme 2 of the Supplementary Materials section shows the process of quantifying tanshinone IIA from LC, GL, and LD Danshen roots, while figure 2 is the summary of the preparation of IH-TIIA and the quantification of tanshinone IIA in LC, GL, and LD Danshen roots.

2.6. Establishing a complete protocol for preparing IH-TIIA from Vietnamese Danshen roots and investigation on the storage conditions of tanshinone IIA

A complete protocol for preparing in-house reference tanshinone IIA was established for further quantification of tanshinone IIA for Vietnamese Danshen roots. Specifically, the extraction process was aided by sonication, using methanol and ethanol as extraction solvents. The isolated tanshinone IIA was investigated the stability under different storage conditions (at 4°C and ambient temperature) in the solid form.

2.7. Statistical analysis

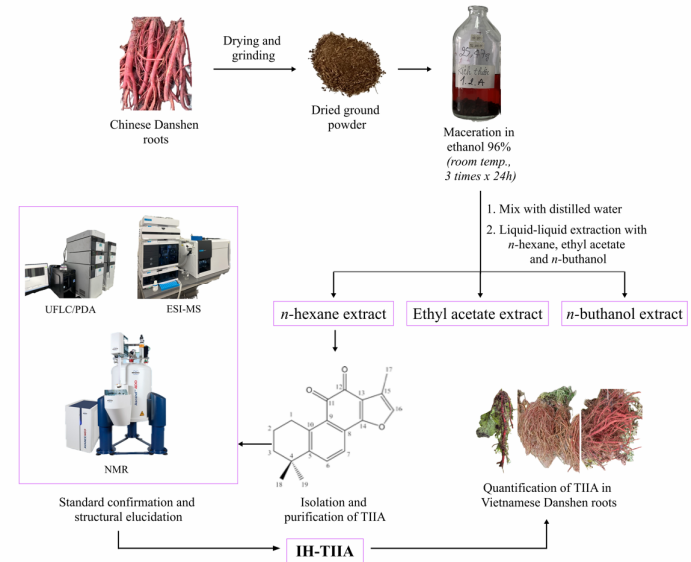


Figure 2. IH-TIIA preparation and TIIA quantification on LC, GL, and LD Danshen roots

The statistical analysis was performed in Microsoft Excel using ANOVA followed by Tukey's post-hoc test. Data are presented as mean ± SD (n = 3). The value of $p < 0.05$ was determined to be statistically significant.

3. RESULTS AND DISCUSSIONS

3.1. Botanical identification for LC, GL, and LD Danshen roots

3.1.1. Chloroplast DNA sequencing

Figures S1-S4 of the non-separative Supplementary Materials are the result of chloroplast DNA sequencing for the collected Danshen samples. Based on this result, the LD sample was identified as *Salvia miltiorrhiza*, whereas the species-level identification of the LC and GL samples was inconclusive.

3.1.2. Morphological observations

Figure 3. Morphological features of the identified Danshen samples

The LC roots were observed to be thinner and darker reddish-brown on the surface than those of the other two samples. On the other hand, the LD and GL roots had a more uniform cylindrical form and brighter periderms (Figure 1). Histological and powder microscopy revealed typical VPV diagnostic features, including multilayered cork, concentric vascular rings, lignified vessels, and resinous deposits. The vascular tissues were better organized in LD and GL samples, whereas resin was more abundant in LC roots, indicating wild-growing conditions. The purchased roots from China showed dense cork layers and highly differentiated vessels, which were possibly a consequence of a different processing manner. Figure 3 shows the morphological features of the identified Danshen samples. All samples met VPV criteria for *Salvia miltiorrhiza*, ensuring their suitability for subsequent extraction and standard preparation experiments.

3.2. Isolation, purification, and characterization of IH-TIIA and AD02

From 1.3 kg of Chinese Danshen roots, 5.55 g of *n*-hexane extract and 64.54 g of ethyl acetate extract were obtained, respectively. After the gel filtration on sephadex® LH-20, silica gel column chromatography, and further purification, 319.78 mg of a reddish-orange solid (AD02) was obtained. AD02 was subjected to thin-layer chromatography (TLC) together with the crystallized IH-TIIA at an earlier stage; the results are shown in Figure 4. Compound AD02 was structurally elucidated via ^{1}H-NMR, ^{13}C-NMR, COSY, HSQC, HMBC, and HR-ESI-MS spectra.

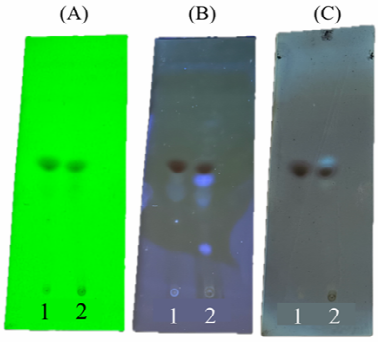


Figure 4.TLC results of crystallized IH-TIIA (1) and AD02 (2) [(A): under UV_{254} light, (B): under UV_{365} light, (C): reaction with H_2SO_4 25% reagent]

Its ^{1}H-NMR spectrum showed the signal of aromatic protons at δ_H (ppm) 7.63 (1H, d, 8.1, H-6) and 7.55 (1H, d, 8.2, H-7), an olefinic proton at δ_H (ppm) 7.22 (1H, q, 1.4, H-16), methyl protons at δ_H (ppm) 2.26 (3H, d, 1.4, H-17), 1.31 (3H, s, H-18 and H-19), and methylene protons at δ_H (ppm) 3.18 (2H, t, 6.4), 1.80 (2H, m), and 1.66 (2H, m). The ^{13}C-NMR spectrum of AD02 yielded 19 signals, including methyl carbons at δ_C (ppm) 8.8 (C-17), 31.8 (C-18 and C-19), aromatic carbons at δ_C (ppm) 144.5 (C-5), 133.5 (C-6), 120.2 (C-7), 127.5 (C-8), 126.5 (C-9), and 150.1 (C-10), olefinic oxygen-bearing carbons at δ_C (ppm) 161.7 (C-14) and 141.3 (C-16), other olefinic carbons at δ_C (ppm) 119.9 (C-13) and 121.2 (C-15), methylene carbons at δ_C

(ppm) 29.9 (C-1), 19.1 (C-2), and 37.9 (C-3), carbonyl carbons at δ_C (ppm) 183.7 (C-11) and 175.8 (C-12), and a quarternary carbon at δ_C (ppm) 34.7 (C-4). These signals indicated the miltiradiene-type nature of AD02's molecule, and a structural resemblance between AD02 and tanshinone IIA. Supported by the observations in its COSY and HSQC spectra, the signal assignment for each of AD02's protons and carbons was conducted. The HMBC spectrum of AD02 yielded the correlations of H-1/C-2, H-2/C-3, H-3/C-4 and C-5, H-18 and H-19/C-4, allowing the determination of dimethylation at C-4 and the ring A of the miltiradiene backbone. Additionally, correlations were observed between H-6/C-5 and C-7, as well as between H-7/C-8 and C-14, indicating the presence of the aromatic B ring of AD02. Together with the correlations of H-16/C-15, H-17/C-13, and C-15, the D ring of AD02's molecule was revealed to exist. With these spectral points, the structural resemblance between AD02 and tanshinone IIA became evident. Compared with the previous publications [9,10], spectral similarities between AD02 and tanshinone IIA were apparent (Table 1). Finally, in the HR-ESI-MS spectrum of AD02, a pseudomolecular ion peak of $[M + H]^+$ was observed at *m/z* 295.1336 (calculated for $C_{19}H_{19}O_3^+$, theoretical value of 295.1334), allowing the structural conclusion for AD02 to be tanshinone IIA (Figure 5).

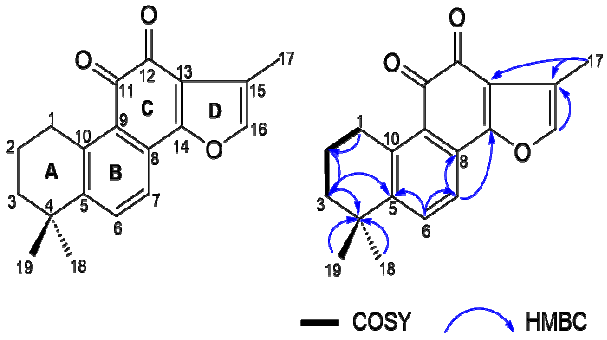
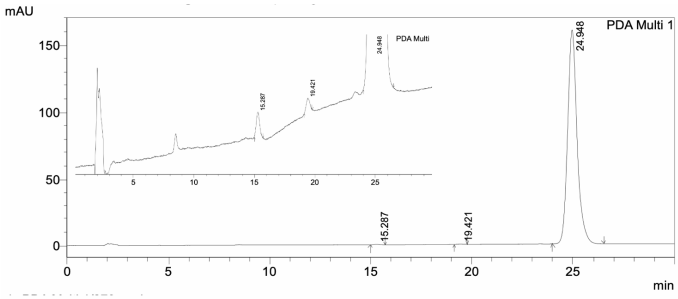


Figure 5. Major COSY, HMBC correlations, and molecular structure of AD02

Table 1. NMR data comparison between AD02 and tanshinone IIA

Position	AD02 ($CDCl_3$)		Tanshinone IIA ($CDCl_3$) [13]	
	δ_H (ppm), *J* (Hz)	δ_C (ppm)	δ_H (ppm), *J* (Hz)	δ_C (ppm)
1	3.18 (2H, t, 6.4)	29.9	3.18 (t, 6.5)	29.9
2	1.80 (2H, m)	19.1	1.80 (m)	19.1
3	1.66 (2H, m)	37.9	1.66 (m)	37.8
4	-	34.7	-	34.7
5	-	144.5	-	144.1
6	7.63 (1H, d, 8.1)	133.5	7.63 (d, 8.0)	133.3
7	7.55 (1H, d, 8.2)	120.2	7.53 (d, 8.0)	120.8
8	-	127.5	-	127.4
9	-	126.5	-	126.5
10	-	150.1	-	150.1
11	-	183.7	-	183.6
12	-	175.8	-	175.7
13	-	119.9	-	119.9
14	-	161.7	-	171.7
15	-	121.2	-	121.4
16	7.22 (1H, q, 1.4)	141.3	7.21 (q, 1.5)	141.1
17	2.26 (3H, d, 1.4)	8.8	2.26 (d, 1.5)	8.7(*)
18	1.31 (3H, s)	31.8	1.31 (s)	31.2
19	1.31 (3H, s)	31.8	1.31 (s)	31.2

Note: (*)Data from Liu et al. (2011) [14]



PeakTable @D:\Datalab\tmphuong\2024\Dan sam\15-4\C 0.05.lcd
PDA Ch1 270nm 4nm

Peak#	Ret. Time	Area	Height	Area %	Height %
1	15.287	7431	383	0.143	0.238
2	19.421	3986	201	0.077	0.125
3	24.948	5195774	160019	99.781	99.636
Total		5207191	160603	100.000	100.000

Figure 6. UFLC/PDA analysis result for AD02

Figure 6 shows the UFLC/PDA analysis result of AD02 at 270 nm. In this figure, a single peak at 24.95 min, accounting for 99.781% of the total area, was observed. The PDA purity index (0.999994) exceeded the threshold (0.896635), with no detectable impurities. The spectral homogeneity in the 200 - 400 nm range confirmed that AD02 is a pure in-house reference standard for tanshinone IIA.

3.3. Quantification of TIIA in LC, GL, and LD Danshen roots

3.3.1. Quantification method validation

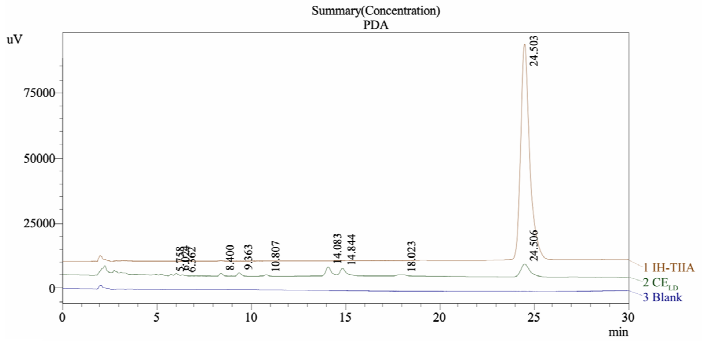


Figure 7. Chromatographic results of the TIIA quantification method validation

Table 2. Validation results of the TIIA quantification method validation

Parameters for validation	Results
System suitability	Retention time (R_t): 24.5248 ± 0.00791 mins
	Peak area: 2199175.500 ± 0.768 (mAU)
	Tailing factor: 1.431 ± 0.444
	Number of plates: 12987.823 ± 0.541
Linearity	R^2= 0.9993; y = 104228669x + 3440.5784
LOD	0.0000528 mg/mL
LOQ	0.000174 mg/mL
Accuracy	98.0–102.0 %
Precision	RSD = 1.154 % (n = 6)

Note: System suitability was evaluated using six injections of a 0.5 mg/mL standard solution; Linearity was established across six calibration levels; LOD and LOQ were calculated using the 3.3σ/S and 10σ/S approaches; Acceptance criteria included: RSD of R_t ≤ 1%, RSD of peak area ≤ 2%, tailing factor 0.8 - 1.5, recovery 98 - 102%, and precision RSD ≤ 2%.

Figure 7 and table 2 show the validation results for the TIIA quantification method. These results indicated that the established method was suitable for quantifying TIIA in Danshen root samples.

3.3.2. Quantification of TIIA in Vietnamese Danshen roots

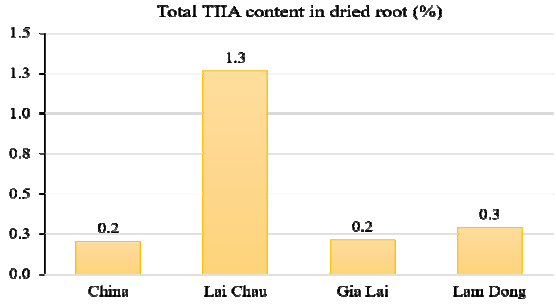


Figure 8. Total TIIA content of Chinese, Lai Chau, Gia Lai, and Lam Dong provinces Danshen roots

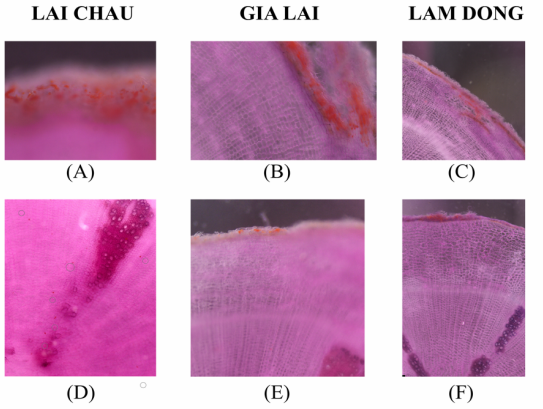


Figure 9. Representative histological sections (10X) of cork (A-C) and parenchyma tissues (D - F) from LC root (A, D), GL root (B, E), and LD root (C, F)

Figure 8 shows the total TIIA content of the Chinese, LC, GL, and LD Danshen roots. This figure shows that the roots from Lai Chau had the highest total TIIA content (1.3%), higher than that of the Chinese roots. Besides, the dense resinous deposits in cork and parenchyma tissues were clearly observed in the histological sections of these samples (Figure 9). These results suggest that the geographical origin of the roots affected both the amount of TIIA and the quality of Danshen. According to the Vietnamese Pharmacopoeia V (2019) [15], Chinese Pharmacopoeia (2020) [16], Japanese Pharmacopoeia XVIII (2021) [17], and Korean Pharmacopoeia XI (2020), *Salvia miltiorrhiza* roots must contain not less than 0.20% of TIIA (HPLC, dry basis). From Figure 8, it was evident that all root samples in this study exceeded this threshold, confirming their compliance with pharmacopoeial standards and their suitability as medicinal raw materials for further pharmaceutical research.

3.4. Establishing a complete protocol for preparing IH-TIIA from Vietnamese Danshen roots

A schematic overview of the preparation workflow is shown in the Scheme 2 and figure S5 of the Supplementary Materials section.

3.4.1. Yield of crude ethanolic extract from Danshen roots

The maceration using ethanol 96% as the extraction solvent was chosen due to the its better scalability. Figure 10 shows the total content of tanshinone IIA, the amount of extracted tanshinone IIA, and the extraction yield (%) of LC, GL, and LD Danshen roots. Observing in this figure that LC roots had the highest tanshinone IIA content (1.2%), but only 0.09% of the compound was extracted. In contrast, the GL roots, which had lower tanshinone IIA content (0.2%), achieved the highest amount of extracted tanshinone IIA (82.1%). The tanshinone IIA chromatographic peak of the samples matched that of IH-TIIA, confirming the component (Figure S6 of the Supplementary Materials section).

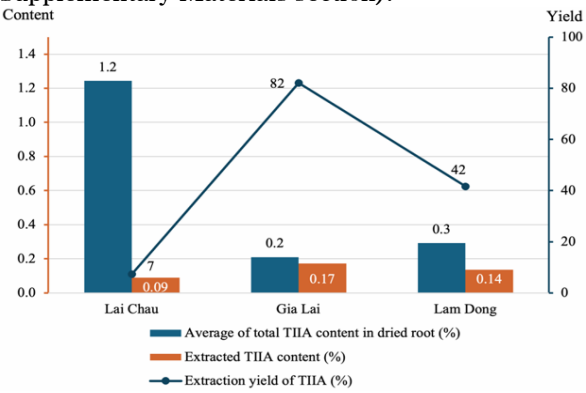


Figure 10. The total content of tanshinone IIA, the amount of extracted tanshinone IIA, and the extraction yield (%) of LC, GL, and LD Danshen roots

3.4.2. Efficiency of tanshinone IIA enrichment for the crude ethanolic extracts

A two-step liquid–liquid extraction procedure, consisting of water/*n*-hexane partition (labelled as Hex1) and then 90% MeOH-H_2O/*n*-hexane partition (labelled as Hex2), was applied for the enrichment of tanshinone IIA for crude ethanolic extracts. Via UFLC/PDA with IH-TIIA as the reference, the amount of enriched tanshinone IIA was determined, and the results are shown in figure 11 and table S1 of the Supplementary Materials section. Observing figure 11 showed that the highest enrichment was obtained from the LD sample (tanshinone IIA content of 16.0%, peak area of 74.0%, and yield of 87.2%), followed by the GL and LC ones.

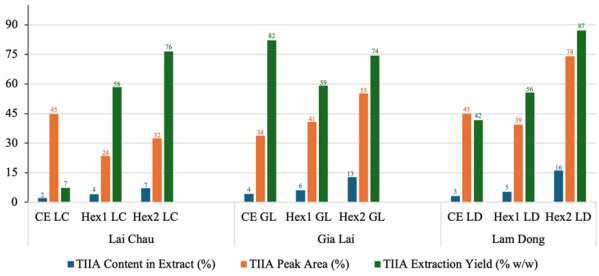


Figure 11. The amount of tanshinone IIA, chromatographic peak area, and extraction yield (%, *w/w*) of the Hex1, Hex2, and crude extracts (CE) of LC, GL, and LD Danshen roots

(n = 3, p < 0.05)

3.4.3. Purification and structural confirmation of isolated tanshinone IIA

The Hex2 extract was eluted by the column chromatography gradient mobile phase of *n*-hexane-ethyl acetate-chloroform. The fractions that had a precipitate were joined, and the precipitate was isolated, crystallized at 0°C in *n*-hexane-chloroform (1.5: 1, *v/v*) to afford a reddish-orange solid. This solid was structurally elucidated and confirmed as tanshinone IIA via UFLC/PDA, NMR, and ESI-MS techniques. Figure 12 is the UFLC results of the tanshinone IIA purification.

Structural confirmation of isolated tanshinone IIA from LC, GL and LD roots was obtained by ^{1}H-, ^{13}C-NMR, and HR-ESI-MS spectra, which showed a full consistency with tanshinone IIA (Table S2 of the Supplementary Materials sections). Specifically, the spectra of all samples exhibited the characteristic aromatic/olefinic proton region (δ_H (ppm) 7.2 - 7.6) and quinoid carbons δ_C (ppm) 183.6 (C-11) and 175.8 (C-12). The $[M]^+$ pseudomolecular ion peak was observed at *m/z* 295.1330 to 295.1335 for all of the samples, in accordance with the molecular formula of tanshinone IIA. The UFLC/PDA analysis confirmed high chromatographic purity for all preparations: 98.68% (GL), 98.76% (LD), and 98.62% (LC) (Figures S7 - S9 of the Supplementary Materials section). These results demonstrated that the tanshinone IIA isolated from LC, GL, and LD Danshen roots totally matches the reference standard grade as the prepared IH-TIIA from Chinese Danshen roots.

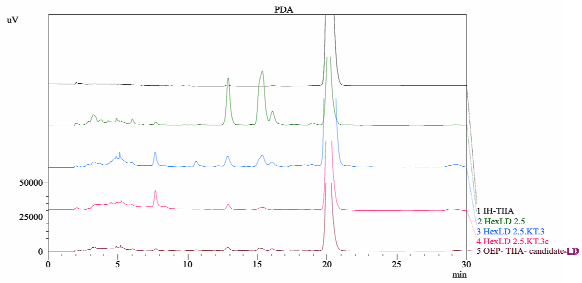


Figure 12. The UFLC results of the purification of tanshinone IIA from LD roots

Stability control for TIIA in root materials and after purification

A single-point comparison between freshly obtained and four-year-stored Chinese Danshen roots (Figure 13) revealed a pronounced decrease in the tanshinone IIA content, from 0.205 2.050 % in the fresh sample to 0.00105 6.62243 % in the stored one. Despite not being a longitudinal study, this substantial reduction aligns with the known instability of diterpenoid quinones in crude botanical matrices, where moisture, enzymatic activity, microbial takes place, and oxidation-accelerated degradation. In contrast, the crystalline form is less susceptible to such matrix-driven loss. These findings underscore the necessity of using freshly prepared IH-TIIA and avoiding prolonged storage of crude root material for accurate quantification and quality control.

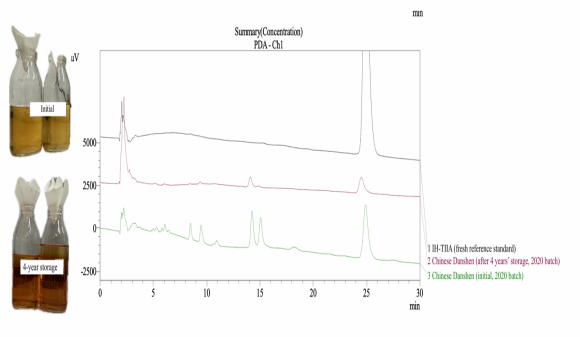


Figure 13. Degradation of Chinese Danshen roots after four years of storage

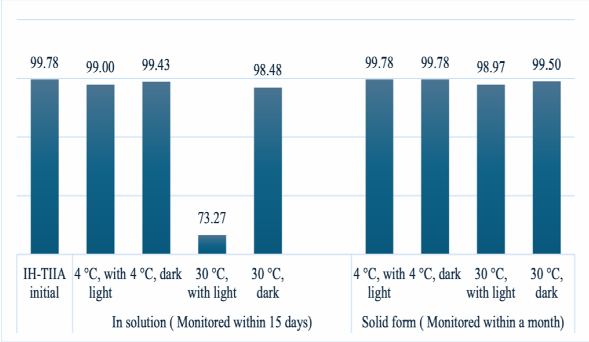


Figure 14. Stability of IH-TIIA in solution and solid forms under different storage conditions

Figure 14 shows the stability of purified IH-TIIA in solution and solid forms under different storage conditions, while Tables S3 and S4 of the Supplementary Materials section summarize the chromatographic purity of IH-TIIA, expressed as the percentage of the tanshinone IIA peak area (mean ± SD, n = 3), under various storage conditions. The initial reference sample exhibited a purity of 99.78167 ± 0.00306%. Stability testing showed that solid IH-TIIA remained essentially unchanged after 1 month, with 99.78% at 4°C and 98.97% at 30°C. In solution, refrigerated samples were well preserved (99.00% under light, 99.43% in the dark), whereas storage at 30°C caused severe photodegradation (73.27% with light) but only minor loss in the dark (98.48%). These results demonstrate that solid-state IH-TIIA is intrinsically stable, while the solution form is susceptible to thermal and photolytic stress. Collectively, the findings underscore that refrigeration and light protection are indispensable for maintaining the integrity of IH-TIIA, reinforcing the need for stringent storage protocols when preparing and handling in-house reference standards.

From the results, it was inferred that IH-TIIA is best preserved in solid form at refrigerated temperatures (equal or below 4°C), ensuring long-term stability and analytical reliability. In contrast, solution samples require strict cold storage to prevent rapid degradation. In solution, IH-TIIA remained highly stable at 4°C (99.00 to 99.43%). At 30°C, however, a strong photodegradation was observed, with purity decreasing to 73.268 ± 0.142% under light, while samples stored in the dark retained 98.4768 ± 0.0213%. In solid form, IH-TIIA exhibited an excellent stability, maintaining approximately 99% purity across all conditions up to six months, including storage at 30°C, with minimal variability. Collectively, the stability profiles demonstrate that solid IH-TIIA is highly suitable for long-term storage across a broad temperature range (4 to 30°C), whereas solution standards require strict control of temperature and light, with refrigeration (≤ 4°C) and light protection being critical to preserving analytical integrity.

4. CONCLUSION

This study established a protocol for isolating tanshinone IIA from Danshen roots collected in Lai Chau, Gia Lai, and Lam Dong provinces, producing an in-house reference standard of (IH-TIIA) with a high purity (> 98.6%) and confirmed structural identity. Using IH-TIIA in a validated UFLC/PDA method, reliable quantification of tanshinone IIA was achieved, from which significant regional variation was indicated. The approach further enabled evaluation of purification efficiency and demonstrated the stability of TIIA under controlled storage (4°C). This work provides a cost-effective, reproducible alternative to imported standards, and delivers a practical framework with direct implications for pharmaceutical standardization and quality control.

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