



Short communication

An integrated strategy of UPLC-Q-TOF/MS and HPTLC/PAD-DESI-MSI for the analysis of chemical variations: A case study of Tibetan medicine Tiebangchui

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The dried roots of *Aconitum pendulum* Busch are the major source of a commonly used Tibetan medicine, namely Tiebangchui (TBC) (Fig. S1). However, raw TBC contains a large amount of high toxic diester-diterpenoid alkaloids (DDAs), and the improper use would cause arrhythmia, irreversible cardiovascular damages, and even death. “Paozhi”, an appropriate processing method, is the essential prerequisite for the safe clinical application of TBC. Water-decocting and sand-frying are commonly used processing methods for toxicity attenuation and efficacy preservation for TBC. During heating, highly toxic DDAs are converted into moderately toxic monoester-diterpenoid alkaloids (MDAs)/lipo-diterpenoid alkaloids (LDAs) and then into non-toxic hydramine-diterpene alkaloids (HDAs) through hydrolysis, dehydrolysis, esterification, and transesterification, which are well accepted [1,2]. However, recent studies revealed that the chemical constituents of TBC are different from those of other *Aconitum* medicines, and the chemical analysis of TBC and its processed products has been rarely conducted [3]. Therefore, this study aims to establish a combined method of ultraperformance liquid chromatography-quadrupole time-of-

flight mass spectrometry (UPLC-Q-TOF/MS) and high-performance thin-layer chromatography/paper-based analytical device-desorption electrospray ionization mass spectrometry imaging (HPTLC/PAD-DESI-MSI) for a visualized analysis of chemical variations in raw TBC and its water-decocted and sand-fried products. The chemical mechanisms were proposed and further rationalized through density functional theory (DFT) calculations.

UPLC-Q-TOF/MS was firstly used to characterize the chemical constituents of raw, water-decocted, and sand-fried TBC. Herein, a total of 70 chemical compounds were identified from the UPLC-Q-TOF/MS spectra by comparing empirical molecular formula, accurate mass weights, characteristic fragments, cleavage pathways, and retention times with those of the published data of TBC or other *Aconitum* species [4]. In the full-scan mass spectra, compounds including DDAs, MDAs, LDAs and HDAs were all exhibited $[M+H]^+$ in positive mode. The base peak intensity (BPI) chromatograms (Fig. S2), representative fragmentation pathways (Fig. S3), and all the identified chemical constituents of raw TBC and its different processed products (Table S1) were presented in Supplementary Information Supplementary data.

HPTLC-DESI-MSI enables a quick, efficient, and untargeted analysis of chemical compounds of raw TBC and its different processed products under reproducible and flexible conditions simultaneously. Based on molecular weight, band intensity, and Rf value, 41 chemical compounds were identified from raw, water-decocted, and sand-fried TBC in the mass range of m/z 100–1000 by HPTLC-DESI-MSI (Fig. 1 and Table S1). Among them, 36 compounds were corresponded to the identified metabolites by UPLC-Q-TOF/MS. Additionally, five more alkaloids (Compounds 71–75) were detected by HPTLC-DESI-MSI compared with UPLC-Q-TOF/MS results (Table S1). These alkaloids were tentatively referred to by molecular weight, band intensity and Rf values through comparison with published studies and determination of possible chemical changes before and after processing.

PAD-DESI-MSI using filter paper as the layer was applied to recognize rapidly the *Aconitum* alkaloids of TBC and its different processed products. A total of 58 compounds were putatively

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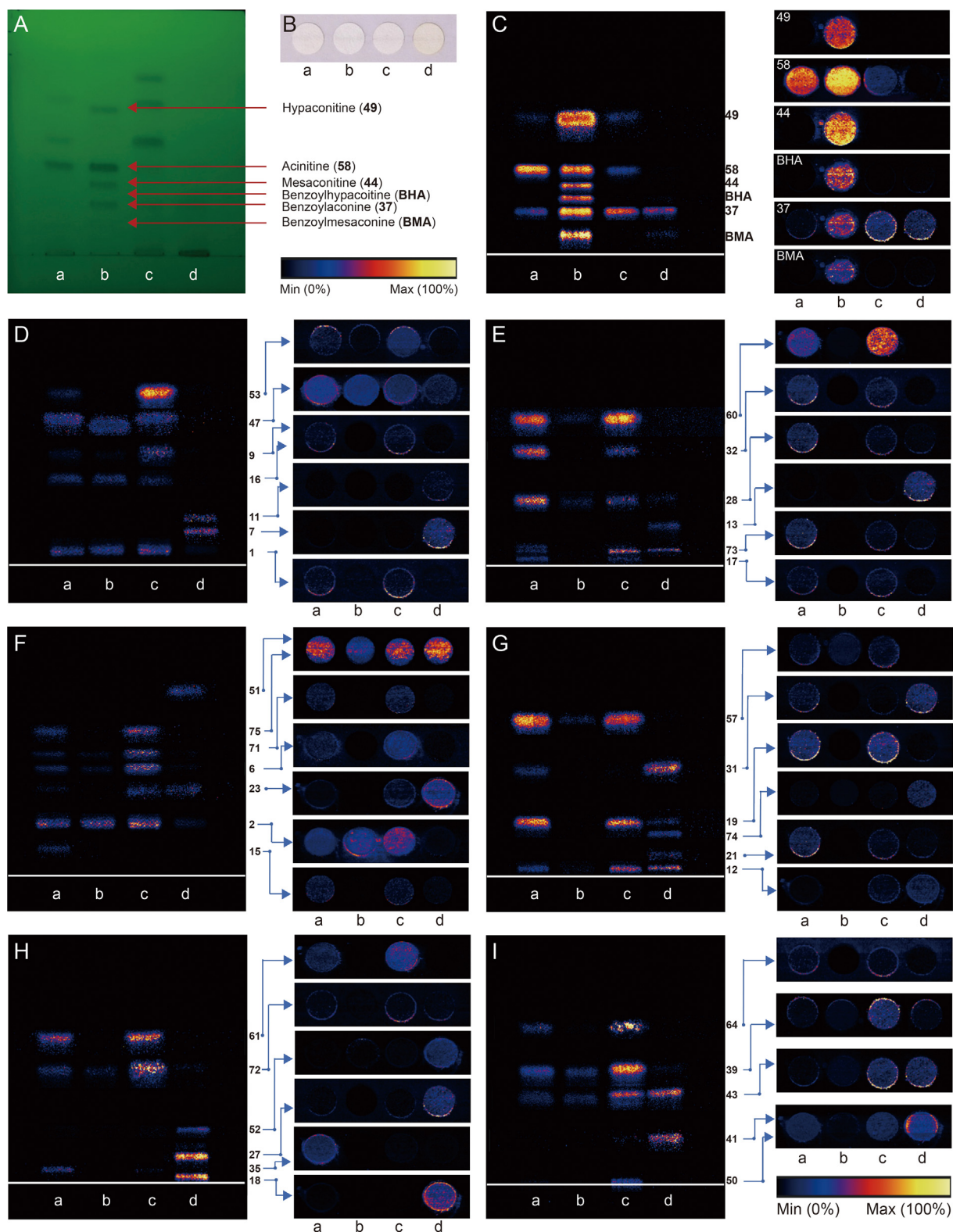


Fig. 1. High-performance thin-layer chromatography/paper-based analytical device-desorption electrospray ionization mass spectrometry imaging (HPTLC/PAD-DESI-MSI) of reference standards, raw Tiebangchui (TBC) and its different processed products. (A) HPTLC of raw TBC and its different processed products under 254 nm UV. (B) PAD of raw TBC and its different processed products. (C–I) HPTLC/PAD-DESI-MSI for different compounds in (a) Raw TBC, (b) mixed reference solution, (c) water-decocted TBC, and (d) sand-fried TBC.

identified in the mass range of m/z 100–1,000 according to the molecular weight, intensities of the alkaloids in peaks of UPLC-Q-TOF/MS spectra and bands of HPTLC-DESI-MS images (Fig. 1 and Table S1). Among them, 36 alkaloids were in good accordance with the results of UPLC-Q-TOF/MS and HPTLC-DESI-MSI. In addition, 17 alkaloids were only consistent with the UPLC-Q-TOF/MS results. Moreover, the five additional alkaloids (Compounds 71–75), which detected in HPTLC-DESI-MSI, were found the related results in PAD-DESI-MSI. The R_f values and the corresponding band/round intensities improved the reliability of the additional results.

The intensities of peak in UPLC-Q-TOF/MS spectra, band in HPTLC plates, and image in filter papers had positive correlations

with the content of each component. By comparing the intensities of the alkaloids in raw TBC and its different processed products, significant intensity decreases of DDAs (e.g., aconitine, 58; deoxyaconitine, 60), and the obviously intensity increases of MDAs (e.g., benzoylaconine, 37), LDAs (e.g., 8-lino-14-benzoylaconine, 67) were observed after water-decocting and sand-frying. The results indicated that the heating process led to the hydrolysis of DDAs and the accumulation of MDAs/LDAs. Moreover, the intensity of HDAs like senbusine A (2), and aconine (12) increased after heating, indicating further hydrolysis of MDAs into HDAs. Besides, the intensities of DDAs such as aconitine (58), 3-deoxyaconitine (60), and indaconitine (57) were obviously visible in raw and water-decocted

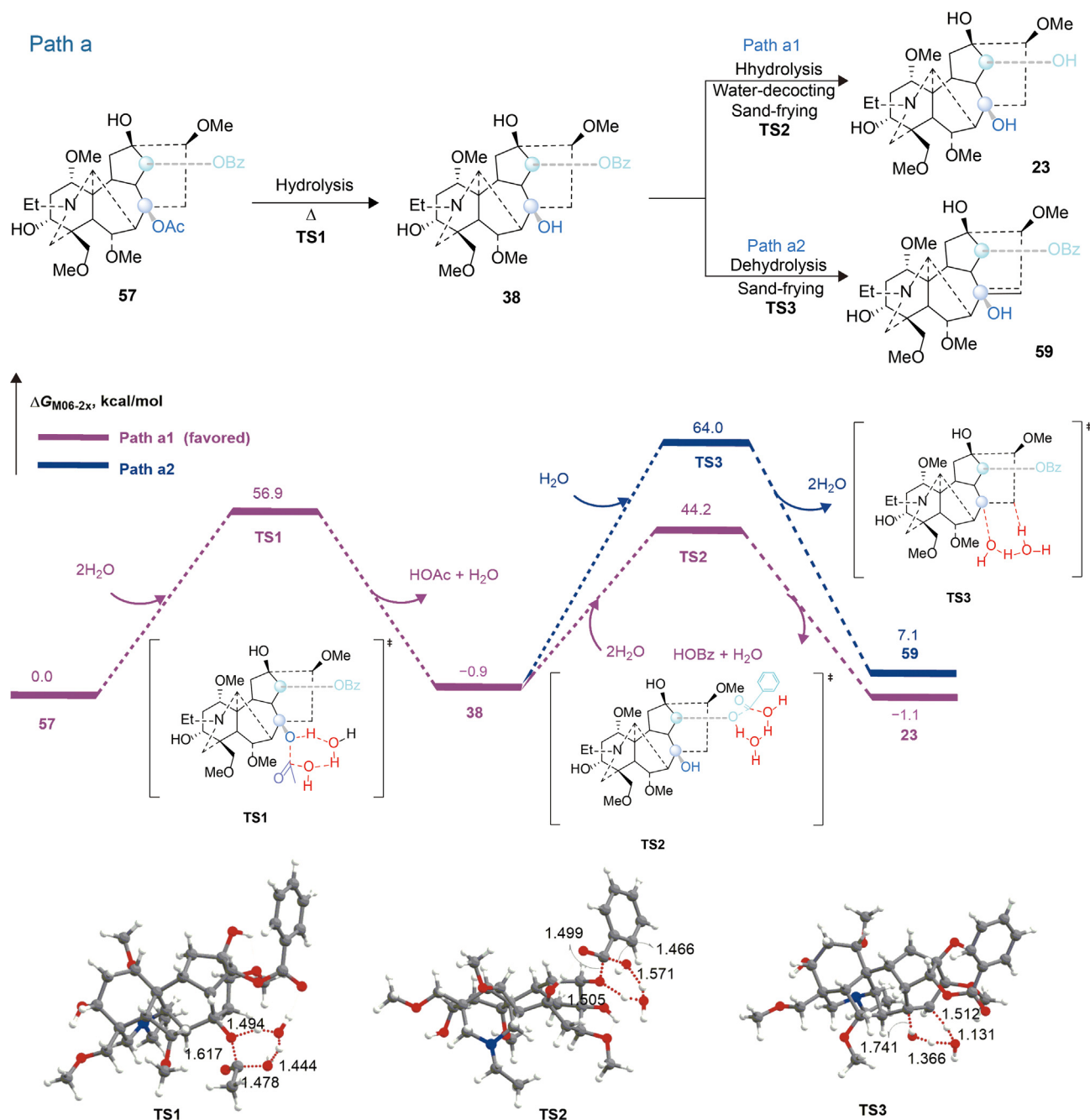


Fig. 2. Density functional theory (DFT)-computed Gibbs free energy profiles for the hydrolysis and dehydrolysis of indaconitine 57 at the M06-2X/6-31G (d,p)/SMD = water level. Energies and bond distances are given in kcal/mol and angstroms (Å), respectively. For clarity, hydrogen atoms on the optimized geometries of transition state (TS) are not shown.

TBC but hardly detected in the sand-fried product, suggesting that higher processing temperatures promoted further chemical transformation of these ester-type alkaloids. More importantly, MDAs including pyromesaconitine (**41**), pyroaconitine (**52**), 16-*epi*-pyroaconitine (**48**), and 16-*epi*-pyroaconine (**18**) were only observed in the sand-fried TBC. Hence, *in situ* formation needs higher heating temperatures, consistent with the UPLC-Q-TOF/MS identification results.

DFT calculations were further used to rationalize these chemical transformations, and the representative chemical transformations, which can provide more information of the reactions among raw and processed TBCs, as well as different TBC products, are showed in Fig. 2. The intensity of indaconitine (**57**) showed an obvious decline in processed TBC, and the hydrolysates pseudaconine (**23**) was detected in both water-decocted and sand-fried TBC (Figs. 1, and S2). However, the dehydrated product mithaconitine (**59**) was only detected in sand-fried TBC (Fig. S2). This phenomenon was explained by DFT calculations. **57** was firstly hydrolyzed to **38** under heating condition, then 45.1 kcal/mol was needed for the C14-OBz bond cleavage of **38** (transition state **TS** 2, Fig. 2 Path a1). However, the ΔG^\ddagger of **TS**3 increased (64.9 kcal/mol), thereby promoting the dehydrolysis from **38** into **59** (Fig. 2 Path a2). The theoretical calculations supported that higher energy was required to deliver **59**, which was in accordance with that was mainly detected in the sand-fried TBC. This results also indicated the importance of temperature during “Paozhi”. More DFT calculations of hydrolysis, dehydrolysis, esterification, and transesterification of TBC alkaloids during “Paozhi” of TBC were showed in Supplementary Figs. S4 and S5 Figs. S4 and S5 [1,5]. Fig. S6 further explanation for the transformation rate differences between the hydrolysis of aconitine (**58**) and the hydrolysis of 3-dexyaconine (**60**) with the only difference of the presence of C3-OH Supplementary Fig. S6. For more materials and methods details, see Supplementary Information data.

This study developed an integrated method of UPLC-Q-TOF/MS and HPTLC/PAD-DESI-MSI for the rapid characterization of alkaloids in raw and different processed products of TBC. By utilizing this combined method, a series of TBC alkaloids was identified, and the differences of chemical compositions, transformations among raw, water-decocted, and sand-fried TBC have been comprehensively revealed. DFT calculations further rationalized the proposed transformation mechanisms and indicated the importance of time and temperature during “Paozhi” procedure. The established method compensates for the deficiencies with each other and give a complete and reliable analytical result. The integrated approach also provided opportunities to unveil the “black box” of “Paozhi” and can be extended to the identification of chemical components,

quality control and safe use of toxic traditional Tibetan medicines and their processed products.

CRediT author statement

Yue Liu: Methodology, Validation, Investigation, Visualization, Writing - Original draft preparation; **Mengjia Li:** Investigation; **Xing Fu:** Investigation; **Yi Zhang:** Conceptualization, Supervision; **Ce Tang:** Conceptualization, Writing - Review and Editing, Supervision.

Declaration of competing interest

The authors declare that there are no conflicts of interest.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at <https://doi.org/10.1016/j.jpha.2023.11.014>.

References

- [1] Z. Qiu, X. Zhang, X. Wei, et al., Online discovery of the molecular mechanism for directionally detoxification of Fuzi using real-time extractive electrospray ionization mass spectrometry, *J. Ethnopharmacol.* 277 (2021), 114216.
- [2] Y. Liu, X. Yang, C. Zhou, et al., Unveiling dynamic changes of chemical constituents in raw and processed Fuzi with different steaming time points using desorption electrospray ionization mass spectrometry imaging combined with metabolomics, *Front. Pharmacol.* 13 (2022), 842890.
- [3] C. Li, Z. Zhou, T. Xu, et al., *Aconitum pendulum* and *Aconitum flavum*: A narrative review on traditional uses, phytochemistry, bioactivities and processing methods, *J. Ethnopharmacol.* 292 (2022), 115216.
- [4] H. Lei, Y. Zhang, J. Ye, et al., A comprehensive quality evaluation of Fuzi and its processed product through integration of UPLC-QTOF/MS combined MS/MS-based mass spectral molecular networking with multivariate statistical analysis and HPLC-MS/MS, *J. Ethnopharmacol.* 266 (2021), 113455.
- [5] Y. He, L. Chen, Y. Liu, et al., New understanding of aconitine hydrolysis pathway: Isolation, identification and toxicity evaluation based on intermediate products, *Arab. J. Chem.* 15 (2022), 104255.