

Chemical Diversity Investigation of Hepatotoxic Pyrrolizidine Alkaloids in Qianliguang (*Senecio scandens*) and Related Species by UHPLC-QTOF-MS¹

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ABSTRACT

Objective: Qianliguang (*Senecio scandens*) is a common Chinese medicinal herb. Qianliguang-containing herbal proprietary products are registered as over-the-counter remedies in China and exported to Western countries. The presence of hepatotoxic pyrrolizidine alkaloids (PAs) has raised concerns about the safety of using Qianliguang and its products. The present study aims at investigation of different types of PAs present in Qianliguang collected from representative locations in China.

Methods: In this study, a simple but specific UHPLC-QTOF-MS method for the determination of toxic PAs was developed, based on the characteristic fragment ions specific to different types of PAs. It was successfully applied for the identification and distinguishing of PAs present in Qianliguang and related *Senecio* species growing in different locations of China.

Results: Significant diversity of the PA types and quantities were revealed among the samples tested. The estimated total amounts of toxic PAs in three of the samples exceed the toxic limits of PA intake restricted by WHO, demonstrating the timely and highly demand for regulating both types and quantities of PAs present in Qianliguang.

Conclusions: This study provides the methodology for simultaneous identification and quantification of PAs present in herbs without requiring corresponding standards, which could be further used for more systematic investigations of the PA distribution in Qianliguang and other PA-containing herbs.

Key Words: Qianliguang (*Senecio scandens*), Pyrrolizidine alkaloids, UHPLC-QTOF-MS, Qualitation, Quantification

INTRODUCTION

The popular and persistent misconception that nature is always 'good, clean and healthy' denies that there are harmful naturally-occurring chemicals, and consequently some toxins-containing medicinal herbs, foods, and dietary supplements are wrongfully accepted as safe. Recent years have witnessed increasing concern about the safety of herbal supplements and botanical products. Among the natural toxins, pyrrolizidine alkaloids (PAs) are probably one of the most significant groups, because PAs are widely distributed across the plant kingdom in up to 13 distantly related angiosperm families covering about 3% of flowering plants worldwide, and about a half of them are hepatotoxic and/or tumorigenic to humans^[1-3]. PAs are esters of three types of necines: retronecine (including its 7-enantiomer), otonecine, and platynecine^[4, 5]. The former two types containing an unsaturated necine bases and are hepatotoxic and can cause

hepatic veno-occlusive disease and may ultimately lead to liver cancer in humans, while the platynecine-type PAs with a saturated necine base are generally non-toxic^[6]. To date, about 660 PAs have been identified from more than 6000 plant species, and mainly from three families: Asteraceae (Compositae), Boraginaceae and Fabaceae (Liguminosae)^[7]. A large number of these plant species have been traditionally used as herbal remedies worldwide.

Qianliguang, derived from the aerial parts of *Senecio scandens* Buch.-Ham. (Asteraceae family) (Figure 1)^[8], is a common Chinese medicinal (CM) herb documented in Pharmacopoeia of P.R. China. This medicinal herb is commonly used to treat bacterial dysentery, enteritis, conjunctivitis, and respiratory tract infections^[9-12]. It has also been used either solely or in compound formulae with other herbs in 12 proprietary CM formulations authorized by the State Food and Drug Administration of China as over-the-counter (OTC) remedies, such as Qian Bai Biyan Pian

Grant sponsor: Research Grant Council of Hong Kong (GRF Grants no. 471310 and 469712) and CUHK Direct Grant (2041744). This article is not an official U.S. Food and Drug Administration (FDA) guidance or policy statement. No official support or endorsement by the U.S. FDA is intended or should be inferred.

¹ Hepatotoxic Pyrrolizidine Alkaloids in Qianliguang (*Senecio scandens*)



Figure 1. *Senecio scandens* Buch.-Ham. (Qianliguang)^[8]

(QBBP)^[9, 13]. These OTC CM products are also exported as medicinal plant remedies to many Western countries such as the United Kingdom^[14]. However, recently the presence of PAs has raised public awareness of the safety of both Qianliguang and QBBP^[15, 16]. Furthermore, it is important to note that hepatotoxic PA profiles vary significantly in Qianliguang growing in different regions and environments^[17–20]. Since whether the PA-containing herb causes hepatotoxicity should depend on the types and also the quantities of PAs present, the safety of Qianliguang consumption relies primarily on well-controlled dosages and proper quality control of the PA profile in the herb.

However, the regulations on PAs in Qianliguang are not well defined, only established in two countries with significant variations. Such recommended dose thresholds are solely based on individual PA poisoning cases and thus vary significantly. For instant, the United Kingdom Medicines and Healthcare Product Regulatory Agency restricted that dose limit of PAs in QBBP should be 1 mg/day (or 16.7 µg/kg/day for a 60-kg human) for 2 weeks and to 0.1 µg/day (1.67 µg/kg/day) for a longer period^[14]. In China, only very recently Pharmacopoeia of P.R. China (2010 edition) stated that adonifoline in Qianliguang should not exceed 0.004% (40 µg/g of dried herb)^[9]. However, it is well known that adonifoline is not the only toxic PA present in Qianliguang^[21–24]. Moreover, studies on the presence of PAs in Qianliguang growing in China are scarce, mainly due to the lack of analytical standards for most of the PAs. To date, among 660 naturally occurring PAs, only about 10 PAs are commercially available. It is impossible or impractical to obtain all standard compounds in order to identify and quantify all toxic PAs in plants/herbal products. Therefore certain PAs, which are present in high levels in some herbs but do not have standards available, cannot be detected and quantitatively measured. Previously, one study reported a sum-parameter method for quantifying the total retronecine-type PAs in Qianliguang by hydrolyzing retronecine-type PAs into their common necine base (retronecine) for the quantification^[25]. However, using this method the identity of individual PAs is unknown, and only retronecine-type not the toxic otonecine-type PAs can be determined.

Our group has previously established the characteristic mass fragments for identifying and distinguishing different types of PAs, such as ions at m/z 120 and 138 for retronecine-type PAs (RET-PAs); m/z 150 and 168 for otonecine-type PAs (OTO-PAs); and m/z 122 and 140 for platynecine-type PAs (PLA-PAs)^[26]. We have also demonstrated that these characteristic mass fragmentation ions can be utilized to determine the presence of PAs in herbal plants and herb-derived products, and to specify the types of PAs^[27–32]. In the present study, this method were further applied to the systematic investigation of different types of PAs present in Qianliguang samples collected from representative locations in China, and two additional plant samples, which are different species in *Senecio* genus and also used as medicinal herbs.

MATERIAL AND METHODS

1. Chemicals

Senecionine and seneciphylline were purchased from Extrasynthese (Genay, France). Senkirikine was from Chromadex (California, USA). Retrorsine was obtained from Sigma (Milwaukee, USA). The mixture of platyphylline and neoplatyphylline (w/w, 3:1, purity > 98%) was a gift from Prof. Hai-Shen Chen from the Department of Phytochemistry, The Secondary Military Medical University, China. Clivorine and integerrimine were isolated in our laboratory by the previously reported method^[33, 34]. Riddelliine was a gift from Dr. Po-Chuen Chen, the U.S. National Toxicology Program (NTP).

2. Plant materials

Five samples of Qianliguang (*Senecio scandens*) were collected from: (i) Conghua, Guangdong Province in September 2007 (SS1); (ii) Nanning, Guangxi Province in July 2005 (SS2); (iii) Damao Mountain, Hong Kong in 2006 (SS3); (iv) Dapu, Hong Kong in 2006 (SS4); and (v) Bozhou, Anhui Province in July 2005 (SS5). Another two samples of *Senecio* species, *Senecio cannabifolius* var *integrifolius* Kitam. (SC6) and *Senecio nemorensis* L. (SN7) were collected from Changbai Mountain, Jilin Province in August 2005 and August 2008, respectively. All the plants used for this study were authenticated by Prof. Zhong-Zhen Zhao, a pharmacognosy professor, School of Chinese Medicine, Hong Kong Baptist University. All the voucher specimens were deposited at School of Chinese Medicine, Hong Kong Baptist University.

3. Sample preparation

The water extract of each herbal sample was prepared by boiling the sample powder (1000 g) with 6 L distilled water under reflux for 1 h. The same extraction procedure was repeated two more times. The aqueous extracts were combined and centrifuged at 3000 g for 10 min. The supernatants were concentrated and then lyophilized to afford a powdered water extract (~26% yield). 1.5 g of the water extract was then dissolved in 50 ml of 0.01% aqueous sulfuric acid solution and centrifuged at 3000 g for 10 min.

An aliquot (25 mL) of the supernatant was adjusted to pH 2–3 with 0.1% aqueous sulfuric acid solution, then extracted with 25 mL of hexane for three times to remove the non-alkaloid components. The resultant aqueous layer was adjusted to pH 9–10 with ammonium hydroxide, and then extracted with 25 mL of dichloromethane for three times. The dichloromethane extracts were combined and dried under reduced pressure by a rotary evaporator. The residue was dissolved in 1 mL of methanol and filtered with a 0.45 μm PTFE membrane filter prior to UHPLC-MS analysis. Reference standards of seneciphylline and senkirkine were dissolved individually in methanol to obtain the corresponding stock solution with a concentration of 500 μM , and diluted to appropriate concentrations for the construction of the calibration curve prior to the UHPLC-MS analysis.

4. UHPLC-MS analysis

UHPLC was performed with a Waters UHPLC system which was equipped with a binary solvent delivery system and coupled to a Bruker MicroOTOF-Q Mass Spectrometer with an ESI source. The chromatography was performed on a Waters Acquity UHPLC BEH shield RP18 column (2.1 \times 100 mm, 1.7 μm). A gradient mobile phase system containing (A) 0.03% aqueous diethylamine and (B) acetonitrile was used as follows: 0–1 min, 95%–90% of A; 1–16 min, 90%–65% of A; 16–18 min, 65%–35% of A; 18–18.1 min, 35%–0% of A, and then kept for 2 min. The flow rate was 0.35 mL/min and the injection volume was 2 μL . The mass spectrometer was operated in positive ion mode under full-scan conditions. The operating parameters were set as follows: spray voltage 4500 V, nebulizer 1.5 bar, dry heater 190 $^{\circ}\text{C}$, dry gas 8.0 L/min, collision energy 25.0 eV, scan range m/z 50–1000.

RESULTS AND DISCUSSION

1. UHPLC-MS analysis of reference PAs

In the present study, RET-PAs (riddelliine, retrorsine, seneciphylline, integerrimine and senecionine), OTO-PAs (senkirkine and clivorine) and PLA-PAs (platyphylline and neoplatyphylline) were analyzed to optimize the UHPLC-MS conditions and also obtain the retention times (t_{R}) and MS characteristics for the identification of corresponding PAs in the samples. Under the stated conditions, each PA exhibited the expected protonated molecular ion $[M+H]^+$ along with two specific fragmentation ions: for RET-PAs at m/z 120.0813 and 138.0919, for OTO-PAs at m/z 150.0919 and 168.1025, and for PLA-PAs at m/z 122.0970 and 140.1075, respectively (Figure 2). These characteristic fragmentation ions are in good agreement with our previously established MS patterns for identifying and distinguishing the types of PAs of interest^[26]. To be simplified, the MS data of these characteristic fragmentation ions shown below are described as the nominal mass m/z 120 (for m/z 120.0813), m/z 138 (for m/z 138.0919), m/z 150 (for m/z 150.0919), m/z 168 (for m/z 168.1025), m/z 122 (for m/z 122.0970) and m/z 140 (for m/z 140.1075). The structures of the fragment ions specific to the three types of PAs are shown in Figure 3.

2. Qualitative analysis of Qianliguang and related *Senecio* species

The qualitative identifications of PAs in the extracts of Qianliguang and related *Senecio* species were obtained from the extracted ion chromatograms (EICs), based on the characteristic fragment ions specific to three different types of PAs. In the EICs of Qianliguang (SS1) collected from Guangdong Province (Figure 4A), two peaks, eluted at 4.2 min and 5.2 min, had the characteristic fragmentation ions of OTO-PAs at both m/z 150 and 168, suggesting that two OTO-PAs were present in this herbal sample. The PA eluted at 5.2 min had the protonated molecular ion at m/z 366.1917 and was unequivocally identified as senkirkine by the comparison of retention time, MS characteristics and molecular weight (MW) to those of the reference standard (Table 1). The MW of the PA eluted at 4.2 min was 2 units less than senkirkine, suggesting the loss of two protons, thus it was tentatively identified as dehydrosenkirkine. In addition, there are other two PAs, eluted at 7.4 min and 12.2 min, possessing the characteristic ions of PLA-PAs at m/z 122 and 140. The peak at 12.2 min had the protonated molecular ion at m/z 340.2124, which was 2 units more than that of platyphylline (or neoplatyphylline). Thus it was tentatively identified as dihydroplatyphylline (or dihydroneoplatyphylline). The other PLA-PA, eluted at 7.4 min, had the protonated molecular ion at m/z 240.1600, which matched that of six PLA-PAs previously isolated from *Senecio* species, namely 7 (or 9)-angeloylplatynecine, 7 (or 9)-senecioylplatynecine, and 7 (or 9)-tigloylplatynecine (Supplemental Figure 1)^[35]. Therefore, this PLA-PA could be one of these six PAs.

For the other four Qianliguang samples (SS2–SS5) collected from different locations in China, OTO-PAs senkirkine and dehydrosenkirkine were also detected in SS2–4 but not in SS5 (Table 1). No other types of PAs were detected in SS2 (collected from Guangxi Province). In the two Qianliguang samples collected from Hong Kong (SS3 and SS4), a RET-PA eluted at 6.5 min exhibited two characteristic ions at m/z 120 and 138, and was detected in both of them. It had the same MW (349 Da) as riddelliine and other six RET-PAs previously identified in various *Senecio* species (structures shown in Supplemental Figure 2)^[24, 36–40]. Due to the different retention time of riddelliine (t_{R} 6.9 min), this RET-PA could be one of the other six PAs. Additionally, in sample SS3, seneciphylline eluted at 9.2 min was unequivocally identified by comparison with the reference standard. Two other RET-PAs eluted at 8.2 min and 8.8 min were found in SS3. They had a same MW of 385 Da, which is 2 units more than that of jaconine^[36], and thus were tentatively identified as dihydrojaconine and its isomer. In sample SS4, there were two additional PLA-PAs, eluted at 7.4 min and 11.7 min, with the same MW of 239 Da. As described for sample SS1, they might be 7 (or 9)-angeloylplatynecine, 7 (or 9)-senecioylplatynecine, or 7 (or 9)-tigloylplatynecine^[37]. In the Qianliguang collected from Anhui (SS5), seneciphylline, neoplatyphylline and senecionine were eluted at 9.1 min, 10.7 min and 11.0 min, respectively, and were unequivocally identified by comparison with the reference standards. Another PA, eluted at 3.4 min, had a MW

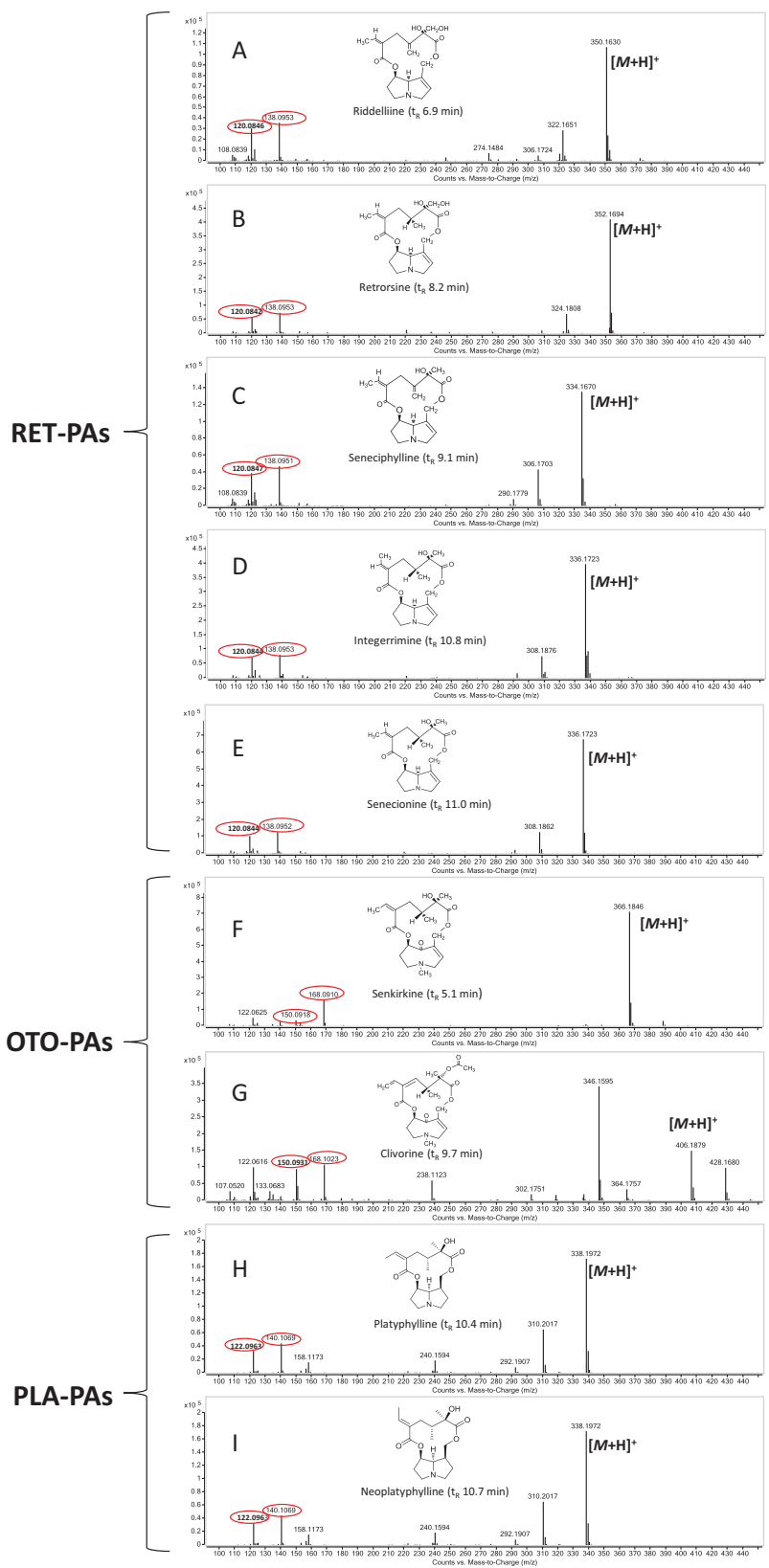


Figure 2. Mass spectra of (A) Riddelliine, (B) Retrorsine, (C) Seneciphylline, (D) Integerrimine, (E) Senecionine, (F) Senkirkine, (G) Clivorine, (H) Platyphylline, (I) Neoplatyphylline

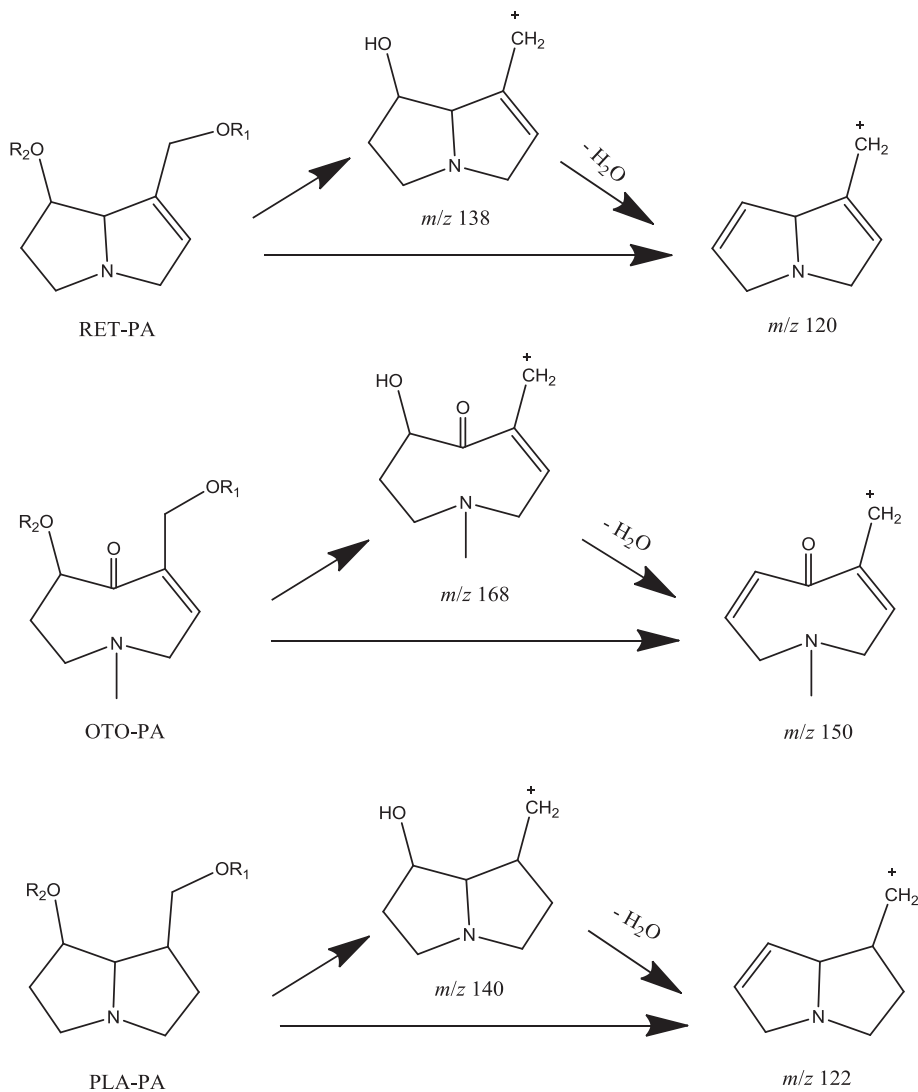


Figure 3. Structures of the characteristic fragmentation ions for three types of PAs

of 365 Da and the two characteristic ions of RET-PAs. Its MW was identical to that of adonifoline^[24], which makes it a candidate for this PA.

For both of two herbs of *Senecio* species (SC6 and SN7), a peak was eluted at 6.9 min with the two characteristic ions of RET-PAs at m/z 120 and 138 (Figure 4B and 4C). It had the same MW, 351 Da, as three RET-PAs (jacobine, retrorsine and usaramine) reported from *Senecio* species^[35]. Due to the different chromatographic property of retrorsine (t_R 8.2 min), usaramine and jacobine were more likely candidates for this PA. Another RET-PA present in both samples was eluted at 13.4 min, with a MW of 335 Da, corresponding to that of integerrimine (t_R 10.8 min) and senecionine (t_R 11.0 min). Therefore it was tentatively identified as an isomer of integerrimine or senecionine. In addition, another isomer of integerrimine or senecionine with the same MW of 335 Da but eluted at 13.8 min, was detected in SN7 (Table 1). Also in SN7, a PLA-PA was eluted at 14.1 min with the protonated molecular ion at m/z 338.1967 corresponding to that of platyphylline (or

neoplatyphylline) but having a different retention time. Therefore it was tentatively identified as an isomer of platyphylline or neoplatyphylline. In SC6, there was another PLA-PA eluted at 11.7 min with a MW of 239 Da, which was the same as that detected in sample SS4.

As described above, the diverse chemical profiles of the PAs present in Qianliguang and related *Senecio* species, which were collected from different locations and harvested at differential times of the year, were observed. It is important to note that among the unequivocally identified PAs, senecionine (RET-PA), seneciphylline (RET-PA), and senkirkinine (OTO-PA) are known hepatotoxins and tumorigens, and have been reported to induce liver tumors in experimental animals^[41]. On the other hand, adonifoline was only found in one sample (SS5), while additional 15 PAs were identified in different samples. Notably, some of toxic PAs had significantly higher quantities than adonifoline in the samples tested, indicating that the regulation of only adonifoline in Qianliguang by Pharmacopoeia of P.R. China (2010 edition) is obviously not adequate.

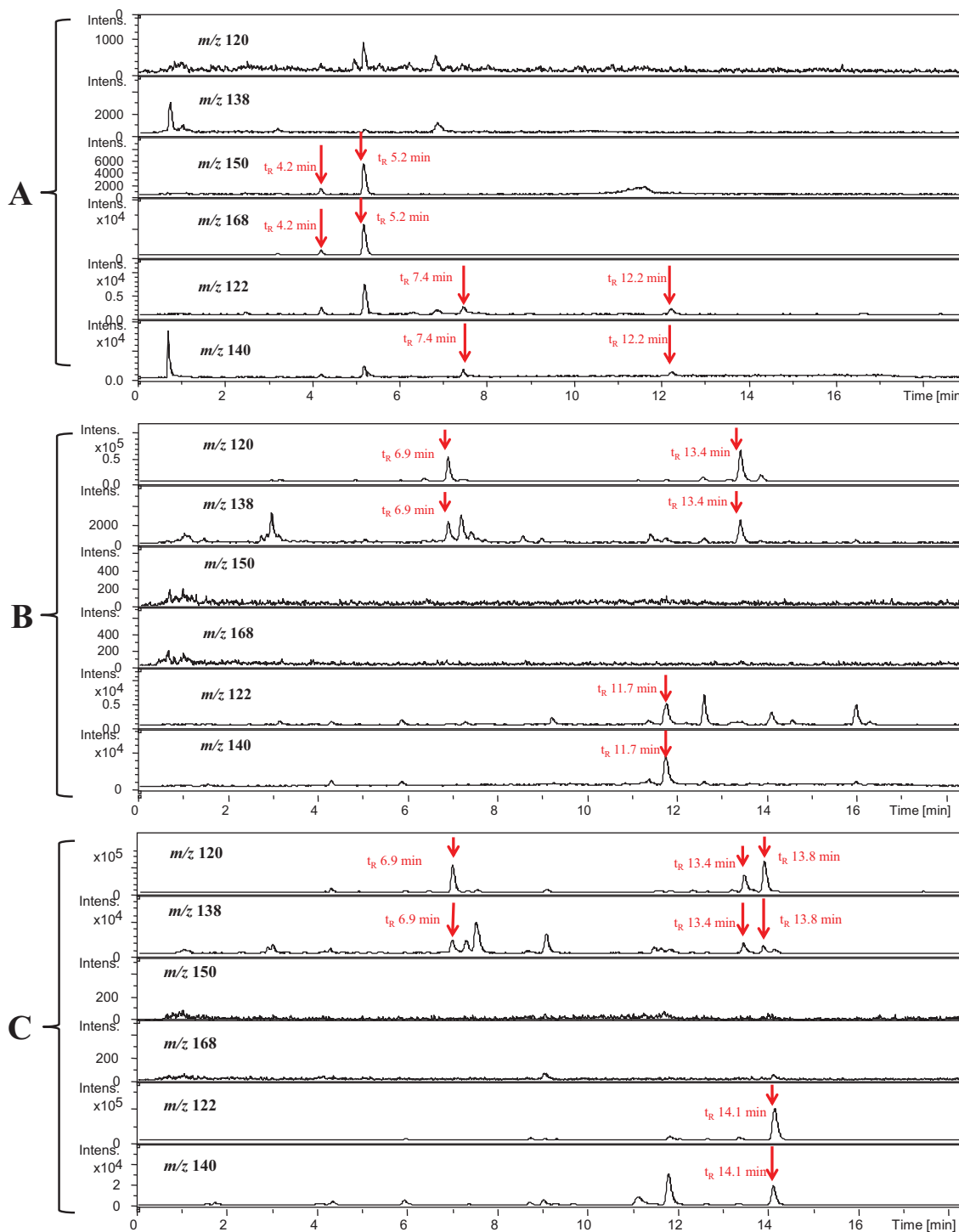


Figure 4. EICs of Qianliguang samples: (A) Qianliguang (SS1) collected from Guangdong Province; (B) *Senecio cannabinifolius* var *integrifolius* Kitam. (SC6) collected from Jilin Province; (C) *Senecio nemorensis* L. (SN7) collected from Jilin Province

3. Quantification of toxic PAs in Qianliguang and related *Senecio* species

Because the hepatotoxicity of PA-containing herbs occurs only with the unsaturated PAs^[3], i.e. RET-PAs and OTO-PAs, we therefore further quantified these two toxic types of PAs in all samples tested. Due to the unavailability of most of the reference standards, RET-PAs and OTO-PAs were semi-quantified using seneciphylline and senkirkine as the representative standard for each type, respectively. The peaks

corresponding to the protonated molecular ion of seneciphylline at m/z 334.1649, senkirkine at m/z 366.1911, and internal standard (clivorine) at m/z 406.1860 were extracted. The calibration curves were constructed using the peak area ratio (analyte/internal standard) over the concentration ranges of 0.03–10 μM for seneciphylline and 0.03–30 μM for senkirkine. The regression equations were $y = 1.322x + 0.0708$, $R^2 = 0.9997$ for seneciphylline, and $y = 0.7233x + 0.4126$, $R^2 = 0.9952$ for senkirkine, with good linearity at

Table 1. Identified PAs in Qianliguang and related *Senecio* species.

t_R (min)	$[M+H]^+$	Characteristic fragment ions (m/z)	PA Type	Identification	SS1	SS2	SS3	SS4	SS5	SC6	SN7
3.4	366.1917	120.0813, 138.0919	RET-PA	Adonifoline					✓		
4.2	364.1760	150.0919, 168.1025	OTO-PA	Dehydrosenkirkine	✓	✓	✓	✓			
5.2	366.1917	150.0919, 168.1025	OTO-PA	Senkirkine	✓	✓	✓	✓			
6.5	350.1604	120.0813, 138.0919	RET-PA	Seneciphylline epoxide, Seneciphylline <i>N</i> -oxide, Spartioidine <i>N</i> -oxide, Swazin, Jacozine, or Erucifoline, Usaramine or Jacobine			✓	✓			
6.9	352.1760	120.0813, 138.0919	RET-PA	Usaramine or Jacobine						✓	✓
7.4	240.1600	122.0970, 140.1075	PLA-PA	7 (or 9)-angeloylplatynecine, 7 (or 9)- senecioplplatynecine, or 7 (or 9)- tigloylplatynecine	✓			✓			
8.2	386.1815	120.0813, 138.0919	RET-PA	Dihydrojaconine			✓				
8.8	386.1815	120.0813, 138.0919	RET-PA	Isomer of Dehydrojaconine			✓				
9.2	334.1654	120.0813, 138.0919	RET-PA	Seneciphylline			✓		✓		
11.0	336.1811	120.0813, 138.0919	RET-PA	Senecionine					✓		
10.7	338.1967	122.0970, 140.1075	PLA-PA	Neoplatyphylline					✓		
11.7	240.1600	122.0970, 140.1075	PLA-PA	7 (or 9)-angeloylplatynecine, 7 (or 9)- senecioplplatynecine, or 7 (or 9)- tigloylplatynecine				✓		✓	
12.2	340.2124	122.0970, 140.1075	PLA-PA	Dihydroplatyphylline or Dihydroneoplatyphylline	✓						
13.4	336.1811	120.0813, 138.0919	RET-PA	Isomer of Senecionine/Integerrimine						✓	✓
13.8	336.1811	120.0813, 138.0919	RET-PA	Isomer of Senecionine/Integerrimine							✓
14.1	338.1967	122.0970, 140.1075	PLA-PA	Isomer of platyphylline/ neoplatyphylline							✓

the concentration ranges tested. The limit of quantification (LOQ) was 0.03 μM for both PAs. The protonated molecular ions of RET-PAs and OTO-PAs identified in qualitative analysis were extracted and then quantified. The results are presented in Table 2 and Figure 5. Two OTO-PAs, namely senkirkine and dehydrosenkirkine, were found to be the major toxic PAs present in Qianliguang from Guangdong (SS1), Guangxi (SS2), Damao Mountain, Hong Kong (SS3). By contrast, Qianliguang from Anhui (SS5), and two herbs of *Senecio* species (*Senecio cannabifolius* var *integrifolius* Kitam. (SC6) and *Senecio nemorensis* L. (SN7)) contained only RET-PAs. More importantly, quantity of toxic PAs in these samples varied significantly. Three samples, including SS1,

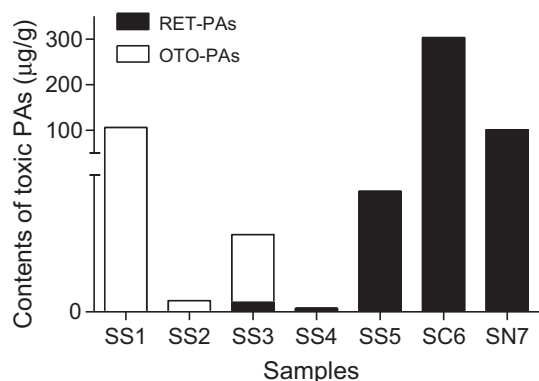


Figure 5. Contents of total toxic PAs in Qianliguang collected from different areas (SS1-SS5), *S. cannabifolius* var *integrifolius* (SC6), and *S. nemorensis* (SN7)

SC6 and SN7 contained high quantities (100–300 $\mu\text{g/g}$) of toxic PAs, while Qianliguang from Dapu, Hong Kong (SS4) had a lowest amount (about 0.2 $\mu\text{g/g}$) of toxic PAs. It is also worthy to note that the two samples of different *Senecio* species (SC6 and SN7) contained significantly high amounts of toxic RET-PAs. *S. cannabifolius* var *integrifolius* Kitam. (SC6) also called ‘Daye Fanhuncao’ is widely used in China as medicinal herbs for the treatment of maladies such as phlegm and asthma. *S. nemorensis* L. (SN7) called ‘Linyin Qianliguang’ is used for the treatment of carbuncles and furuncles. Therefore, such herbal plants in *Senecio* genus have also posed potential risk for the PA-intoxication and require related regulations.

Based on the estimated quantity of toxic PAs in the tested herbs and the dosage (0.25–0.50 g/kg/day) of Qianliguang recommended by Pharmacopoeia of P. R. China^[9], amount of total toxic PAs by the intake of Qianliguang and *Senecio* species investigated in this study was calculated and shown in Table 2. Among them, the estimated dosages of SS1, SC6 and SN7 exceeded the toxic dose of PA intake in humans (15 $\mu\text{g/kg}$, b.w./day based on the use of comfrey, a PA-containing herb) specified by the World Health Organization (WHO)^[42]. Furthermore, if calculated by the daily consumption of QBBP recommended by Pharmacopoeia of P.R. China (i.e. 9–12 tablets of QBBP, equivalent to 21.8–29.1 g of Qianliguang)^[8], the daily intake of SS1, SC6 and SN7 exceeded the short term limit of PAs in QBBP restricted by the United Kingdom Medicines and Healthcare Product Regulatory Agency (1000 $\mu\text{g/day}$ for less than 2 weeks)^[14],

Table 2. Estimated amounts of toxic PAs in Qianliguang and related *Senecio* species.

Amounts	SS1	SS2	SS3	SS4	SS5	SC6	SN7
RET-PAs (µg/g)	Nil	Nil	0.48	0.16	6.18	303.4	101.3
OTO-PAs (µg/g)	105.9	0.57	3.47	0.03	Nil	Nil	Nil
Total toxic PAs (µg/g)	105.9	0.57	3.95	0.19	6.18	303.4	101.3
Calculated dosage (µg/kg. b.w.*/day)**	26.48 ~ 52.95	0.14 ~ 0.28	0.98 ~ 1.97	0.05 ~ 0.10	1.54 ~ 3.09	75.85 ~ 151.70	25.32 ~ 50.65
Calculated intake (µg/day)***	2308.62 ~ 3081.69	12.42 ~ 16.58	86.11 ~ 114.94	4.14 ~ 5.52	134.72 ~ 179.83	6614.12 ~ 8828.94	2208.34 ~ 2947.83

*b.w.: body weight;

**Calculated by the dosage (0.25–0.50 g/kg/day) of Qianliguang recommended by the Pharmacopoeia of P. R. China;

***Calculated by the daily consumption of QBBP recommended by the Pharmacopoeia of P.R. China (i.e. 9–12 tablets of QBBP, equivalent to 21.8–29.1 g of Qianliguang)

and SS1, SS3, SS5, SC6 and SN7 exceeded the long term limit (100 µg/day for longer than 2 weeks). These results suggested a potential high risk resulting from the intake of such herbs and related formulations.

According to our previous hepatotoxicity study on Qianliguang^[43], no significant hepatotoxic effects were observed in rats fed with Qianliguang at a dose equivalent to the daily intake recommended by Pharmacopoeia of P. R. China, in which the determined total toxic PAs was 7.19 µg/g of herb (3.48 µg/kg. b.w./day, far below the toxic dose of PA intake restricted by WHO, 15 µg/kg. b.w./day). However, if significant overdose occurs, as demonstrated at a dose of about 8 times higher than that recommended (26.73 µg/kg. b.w./day), Qianliguang did produce typical PA-mediated hepatotoxicity in rats. It is worthy to note that content of toxic PAs in several Qianliguang [SS1 (105.9 µg/g), SS6 (303.4 µg/g) and SS7 (101.3 µg/g), Table 2] tested in the present study were significantly high and more than 8 times of that of Qianliguang tested in previous study^[43]. Therefore, it is reasonable to anticipate that these herbs (SS1, SS6 and SS7) are likely to induce hepatotoxicity at the common dosages used in clinic. Therefore, because significant variations in the distribution and quantity of PAs was observed not only within herb Qianliguang growing in different regions and environments but also among the *Senecio* species, more extensive and systematic investigation of toxic PAs in Qianliguang herbal materials and derived formulated products are warranted for the prevention of Qianliguang related PA-intoxication.

In conclusion, an UHPLC-QTOF-MS method based on the characteristic fragment ions specific to different types of PAs was successfully applied for the investigation of PA distribution in Qianliguang from different locations of China and related *Senecio* species. Significant variations in the types and quantities of PAs were found among the samples tested, showing that regulation of the toxic PAs in Qianliguang and related herbal products, including dietary supplements, is essential. Moreover, this study also provides the methodology for simultaneous identification and quantification of PAs present in herbs without requiring the availability of all the reference standards. This method could be further used for more systematic investigations of the PA distribution in Qianliguang and other PA-containing herbs.

CONFLICTS OF INTEREST

The authors have declared no conflict of interest.

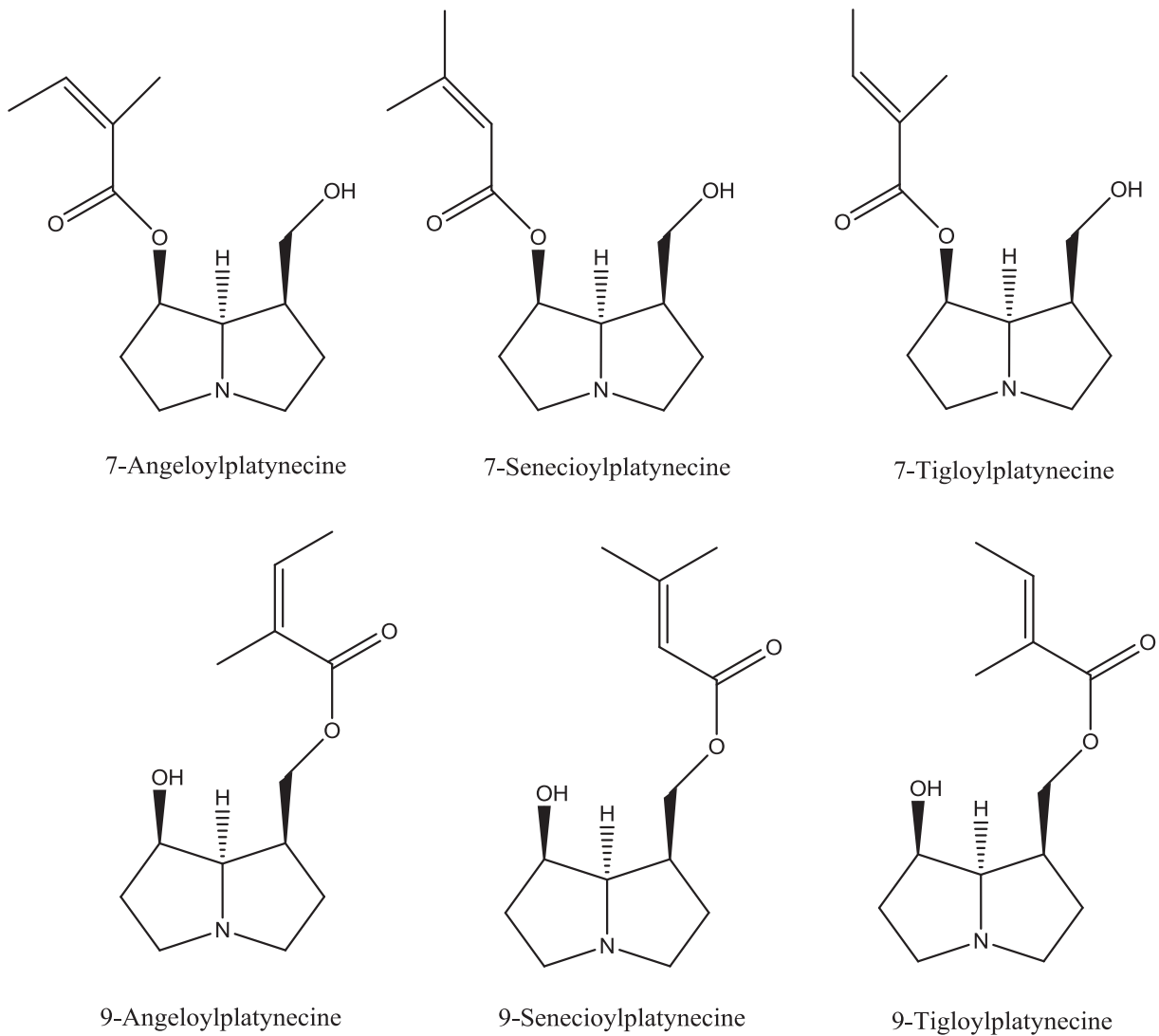
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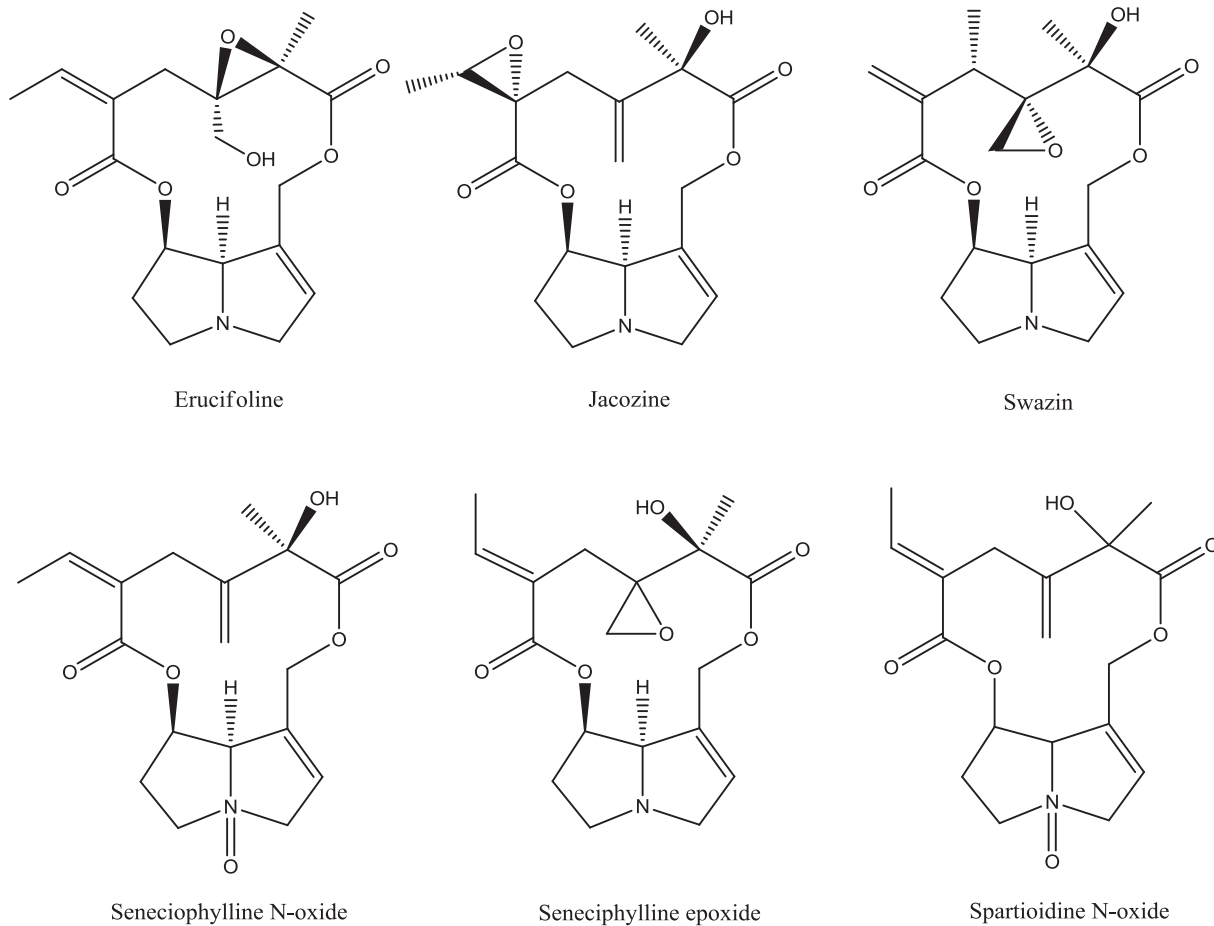
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Appendix A: Supporting information

Supplementary data associated with this article can be found in the online version at



Supplemental Figure 1. Six PLA-PAs with MW of 239 from *Senecio* species.



Supplemental Figure 2. Six RET-PAs with MW of 349 from *Senecio* species.