Journal of Pharmaceutical and Biomedical Analysis 56 (2011) 165–172

Contents lists available at ScienceDirect

Journal of Pharmaceutical and Biomedical Analysis

journal homepage: www.elsevier.com/locate/jpba

Authentication of *Senecio scandens* and *S. vulgaris* based on the comprehensive secondary metabolic patterns gained by UPLC–DAD/ESI-MS

Xuejing Yang[a], Li Yang[b], Aizhen Xiong[b], Dingxiang Li[b], Zhengtao Wang[a,b,*]

[a] Key Laboratory of Modern Chinese Medicines, Ministry of Education, Department of Pharmacognosy, China Pharmaceutical University, Nanjing, China
[b] The MOE Key Laboratory for Standardization of Chinese Medicines and the SATCM Key Laboratory for New Resources and Quality Evaluation of Chinese Medicines, Institute of Chinese Materia Medica, Shanghai University of Traditional Chinese Medicine, Shanghai, China

ARTICLE INFO

Article history:
Received 22 February 2011
Received in revised form 26 April 2011
Accepted 6 May 2011
Available online 12 May 2011

Keywords:
Senecio scandens
S. vulgaris
Secondary metabolic patterns
UPLC–DAD/ESI-MS

ABSTRACT

A secondary metabolic pattern using ultra-performance liquid chromatography (UPLC)–DAD/ESI-MS was constructed to gain chemical information for authentication of *Senecio scandens* (SS) and *Senecio vulgaris* (SV), the two representative species containing hepatotoxic pyrrolizidine alkaloids (HPAs). The metabolic pattern showed three groups of bioactive constituents: phenolic/aromatic acids, flavonoid glycosides and the HPAs. 47 peaks were identified including 19 phenolic/aromatic acids, 10 flavonoid glycosides and 18 PAs by direct comparison with the available reference compounds or deduced from the UV absorption and their ESI-MS fragmentation patterns.

The two species could be authenticated diagnostically by their metabolic profiling of the three chromatographic fingerprints. Although both SS and SV contain PAs as the characteristic constituents, only 2 PAs, adonifoline and adonifoline *N*-oxide were detected in SS, while other 16 PAs were detected in SV, including the highly toxic senecionine, retrorsine, seneciphylline and their corresponding *N*-oxides. The concentration of PAs in SV is also higher than that in SS. The number and concentration of the phenolic compounds in SS were higher than in SV. Jacaranone derivatives were only detected in SS and jacaranone ethyl ester was detected as the predominant constituent.

In the fingerprint of the *n*-butanol extracts, 10 quercetin and kaempferol glycosides derivatives were detected. 9 were found in SS and only 2 in SV. PAs, jacaranone derivatives and flavonoid glycosides can serve as the metabolic markers to distinguish the *Senecio* plants from each other, and provide evidence for their clinical application in the consideration of safety and efficacy.

1. Introduction

Herbal medicines (HM) have been used for thousands of years in China and many Asian countries, such as Korea, Japan, India, as well as in western societies as alternative medicines or health products. It is recognized that the biological activities of HM are contributed by their polyvalent effects of the co-existing multiple components which may be therapeutic or toxic [1], and thus great attention has been paid to the quality assessment of HM raw materials and finished products in order to guarantee the efficacy and safety when being utilized in clinical practices. In recent years, chromatographic fingerprint (CFP) showed advantage over the single marker substance-based quality control methods, as CFP can demonstrate the entire metabolic profiling image of HM containing complex and structural diverse secondary metabolites [2].

In recent years, *Senecio* plants (Asteraceae) have been seriously concerned due to their pyrrolizidine alkaloids (PAs) content, which are a type of the most hepatotoxic toxins in nature and are usually called hepatotoxic pyrrolizidine alkaloids (HPAs) [3,4]. Considering the risk of the human exposure of these alkaloids, in 1989 the World Health Organization issued a Health and Safety Guide on utilization of PAs-containing herbs [5]. As a result, the use of *Senecio* plants has been strictly limited by several developed western countries.

Nevertheless, in China, more than ten *Senecio* species have been used in traditional or folk medication, and *S. scandens* Buch.- Ham. ex D. Don (SS) is recorded as Senecionis Scandentis Herba (Qianliguang) in Chinese Pharmacopoeia [6]. SS is widely distributed in China, as well as Bhutan, India, Japan, Myanmar, Nepal, Phillipines, Sri Lanka, Thailand [7] and used in traditional and folk medicine for its anti-inflammatory, antipyretic, and detoxification effects [8]. In Chinese medicine, SS is used in single or as an ingredient in over hundreds finished products such as Qianbaibi tablets, Qianliguang tablets, and Qingresanjie tablets. It was reported to

* Corresponding author at: Institute of Traditional Chinese Materia Medica, Shanghai University of Traditional Chinese Medicine, 1200 Cailun Road, Shanghai 201210, China. Tel.: +86 21 51322507; fax: +86 21 51322519.
E-mail addresses: wangzht@hotmail.com, wangzht@shutcm.edu.cn (Z. Wang).

0731-7085/$ – see front matter © 2011 Elsevier B.V. All rights reserved.
doi:10.1016/j.jpba.2011.05.004

contain adonifoline as the main HPAs [9] and a variety of phenolics such as chlorogenic acids [10], jacaranone derivatives [11,12], and flavonoids like hyperoside [13].

Another representative species is *Senecio vulgaris* Linnaeus (common groundsel) (SV), a temperate weed widely distributed in Eurasia, early introduced Africa and adventives from Europe into northeast and west China [7,14]. In Europe, it played a certain role as emmenagogue and in case of functional amenorrhoea [15]. This worldwide important plant has been studied for centuries and focused on PAs. It was reported to contain many PAs including highly toxic potential senecionine, integerrimine, retrorsine, usamarine, seneciphylline, spartodine, riddelline and the corresponding N-oxides [15], but only few other types of compounds reported.

In our continuing research on *Senecio* plants [16,17], it was interestingly found that SS showed much lower toxicity than SV which may be interpreted by the differences in the number and concentration of HPAs, as well as other types of constitutions between the two species.

In view of this consideration, the present study aimed at developing a metabolic profiling pattern using UPLC–DAD/ESIMS analytical method by constructing chromatographic fingerprints of different types of constituents. The established method was successfully applied to authenticate SS and SV from each other for the purpose of quality assessment of the *Senecio* plants to ensure the safety and efficacy when they are used as herbal medicines.

2. Experimental

2.1. Chemicals and materials

HPLC grade acetonitrile was purchased from Fisher Scientific (Santa Clara, CA, USA). HPLC-grade acetic acid was purchased from Merck (Darmstadt, Germany). HPLC-grade ammonium formate was purchased from Sigma–Aldrich Co. (Fairfield, USA). Water was purified by a Milli-Q academic water purification system (Millipore, Bedford, MA, USA).

Ethanol, hydrochloric acid, ammonia water, diethyl ether, dichloromethane, *n*-butanol and methanol were all analytical grade and purchased from the Sinopharm Chemical Reagent Company (Shanghai, China). Protocatechuic acid, *p*-hydroxy benzeneacetic acid, chlorogenic acid, caffeic acid, jacaranone ethyl ester, hyperoside, adonifoline, seneciophylline, senecionine and retrorsine were provided by Shanghai R&D Centre for Standardization of Chinese Medicines and their purities were more than 98% by HPLC analysis based on a peak area normalization. Adonifoline *N*-oxide and senecionine *N*-oxide were synthetically prepared by oxidation of adonifoline and senecionine, respectively, according to Ref. [17].

Eight batches of each of *S. scandens* and *S. vulgaris* from different localities of China were collected and authenticated by author (Supplemental material 1). The voucher specimens were deposited in the Laboratory of Institute of Chinese Materia Medica, Shanghai University of Traditional Chinese Medicine.

2.2. Instrumentation and chromatographic conditions

The chromatographic analyses were performed on a Waters Acquity UPLC BEH C_{18} column (2.1 mm × 100 mm, 1.7 μm), connected with a LC-18 guard column (Vanguard 2.1 mm × 5 mm Waters Corp., Milford, MA, USA). The column and sample temperatures were maintained at 30 °C and 15 °C, respectively.

The analyses of phenolic/aromatic acids and flavonoid glycosides were carried out using a Waters Acquity UPLC and ACQUITY SQD system including a binary solvent manger, a sampler manager, a column compartment and a photodiode array detector, equipped with a Waters Empower 2 data station. Acetonitrile (A) and 0.4% acetic acid (B) were used as the gradient elution system of diethyl ether extracts and *n*-butanol extracts. Linear gradient 5–7% A at 0–2 min, 7–10% A at 2–4 min, 10–16% A at 4–8 min, 16% A at 8–11 min, 16–95% A at 11–30 min was used for diethyl ether extracts, while 5–25% A at 0–12 min was used for *n*-butanol extracts. The flow rate was 0.4 ml/min and 2 μl of samples solution was injected. The effluent was introduced into a DAD detector (scanning range 210–400 nm) and subsequently into an electrospray source (ESI). The optimized MS conditions are listed as follows: source temperature, 120 °C; desolvation temperature, 350 °C; probe voltage (capillary voltage), 3.0 kV; cone voltage, 44 kV; extractor voltage, 3 V. Nitrogen was used as the source of desolvation gas (500 l/h) and cone gas (20 l/h). The ESI-MS spectra were acquired in negative ionization modes recorded on a mass range of *m*/*z* 115–800.

The analyses of alkaloids were carried out using a Waters Acquity UPLC and Micromass ZQ system also including a binary solvent manger, sampler manager and column compartment, equipped with Waters Masslynx V4.1 data station. A linear gradient elution of acetonitrile (A) and 10 mM ammonium formate modified by the addition of 0.1% (v/v) 25% ammonia solution (B) was used. The gradient programmer was used according to the following profile: 5–20% A at 0–5 min, 20–40% A at 5–7 min, 40–90% A at 7–8.5 min. The solution flow rate was 0.5 ml/min and 2 μl of sample solution was injected in each run. The effluent was directly introduced into an electrospray source (ESI). The optimized MS conditions are listed as follows: source temperature, 150 °C; desolvation temperature, 450 °C; probe voltage (capillary voltage), 3.5 kV; cone voltage, 45 kV. Nitrogen was used as the source of desolvation gas (900 l/h) and cone gas (50 l/h). The ESI-MS spectra were acquired in positive ionization mode recorded on mass range of *m*/*z* 150–650.

ESI-MS^n spectra were acquired using a LCQ ion trap mass spectrometer (ThermoFinnigan, San Jose, CA, USA). The mass conditions for phenolic/aromatic acids and flavonoid glycosides were as follows: negative ionization mode; capillary temperature, 275 °C; capillary voltage, 19 V; mass range, *m*/*z* 80–1000; collision gas, helium; relative collision energy, 35–45%. The mass conditions for alkaloids were as follows: positive ionization mode; capillary temperature, 300 °C; source voltage, 5 kV; capillary voltage, 9 V; mass range, *m*/*z* 100–800; collision gas, helium; relative collision energy, 35–40%.

2.3. Sample preparation

The samples were pulverized to pass through a 40 mesh sieve and dried at 50 °C to constant weight. Each sample powder (5 g) was macerated in 100 ml of 80% (v/v) aqueous ethanol solution for 1 h, and then extracted under reflux on a water bath for 2 h. After that, it was filtered and the solution was evaporated to dryness.

Sample for analysis of phenolics/aromatic acids: The residue was suspended in 50 ml of 2% hydrochloric acid solution and filtered. The solution was partitioned twice with 50 ml of diethyl ether. The diethyl ether extracts were combined, evaporated to dryness, and recovered with methanol, filtered through a 0.2 membrane to produce the sample solution (1) for phenolic fingerprint analysis.

Sample for analysis of pyrrolizidine alkaloids: after extraction of the diethyl ether, the acid aqueous layer was adjusted to pH 9–10 with strong ammonia and partitioned twice with 50 ml of dichloromethane. The dichloromethane extracts were combined, evaporated to dryness and the residue was dissolved in methanol, filtered through a 0.2 membrane to produce the sample solution (2) for analysis of alkaloids.

Sample for analysis of flavonoid glycosides: the alkaline aqueous layer, after extraction of alkaloids, was re-adjusted to pH 7 with 20% hydrochloric acid and partitioned twice with 50 ml of *n*-butanol. After combination and evaporation, the residue was dissolved in methanol, and filtered, to produce sample solution (3) for analysis of flavonoid glycosides.

3. Results and discussion

3.1. Optimization of pretreatment method

Due to the chemical diversities of the components in *Senecio* plants, an extraction procedure was designed to get three fractions demonstrating different types of constitutions. Firstly, various solvents including methanol, ethanol, and water were compared. 80% aqueous ethanol (v/v) was selected for obtaining the maximum mass of the target compounds. Then the total extract was acidified following partitioning with hydrophobic solvent to get the phenolic constituents. Ethyl acetate and diethyl ether were compared for this purpose. Diethyl ether was proven to give ideal selectivity for enriching the aromatic phenolics. Then the acid layer was alkalified with ammonia and the free alkaloids were extracted by dichloromethane. Finally the alkaline layer was neutralized with 20% hydrochloric acid and partitioned with *n*-butanol to obtain the more hydrophilic flavonoid glycosides.

3.2. Optimization of chromatographic conditions

Different columns ACQUITY UPLC^{TM} BEH C_{18}, HSS T_3, BEH C_8 and BEH SHIELD RP_{18} were tested, and an UPLC BEH C_{18} column (2.1 mm × 100 mm, 1.7 μm) was selected for the fingerprints.

Mobile phases using methanol–water or acetonitrile–water were compared, and acetonitrile–water could achieve better resolutions. The retention behavior of those three types of compounds on the reversed-phase UPLC column was significantly affected by the pH of the mobile phase, so different buffers including acetic acid and ammonium formate were also tested. As a result, acetonitrile (A)–water containing 0.4% acetic acid (B) was chosen for fingerprints of phenolics/aromatic acids extracts and flavonoid glycosides extracts. Acetonitrile (A)–water containing 10 mM ammonium formate modified by the addition of 0.1% (v/v) 25% ammonia (B) was chosen for fingerprinting of pyrrolizidine alkaloids extracts.

Scanning at 254 nm and 360 nm showed more peaks and higher resolution for phenolics/aromatic acids extracts and flavonoid glycosides extracts.

3.3. Identification of phenolics, flavonoid glycosides and pyrrolizidine alkaloids in the fingerprints of the two Senecio species

The peaks in the fingerprints were authenticated by comparison of the chromatographic behaviors, the UV absorption patterns and aided by their mass spectral fragmentations. Some of the peaks were unambiguously identified by direct comparison with the available reference compounds. The identified or tentatively authenticated structures are shown in Fig. 1.

Totally 19 phenolics/aromatic acids including cinnamic and quinic acids and jacaranone derivatives, were characterized in the fingerprints of the diether extracts of the two *Senecio* species. Their chromatographic behaviors and spectral data are summarized in Table 1.

Protocatechuic acid (peak 2), chlorogenic acid (peak 3), *p*-hydroxy benzeneacetic acid (peak 4), caffeic acid (peak 6), and jacaranone ethyl ester (peak 9) were identified by comparing with the available reference compounds.

In mass spectra, the structures with quinic acid unit usually displayed the product ions at *m/z* 191, 173 and 127 which corresponded to $[M-H]^-$, $[M-H-H_2O]^-$ and $[M-H-CO-2H_2O]^-$, respectively. The hydroxycinnamic acid part usually displayed the predominant ions, e.g., 179 for caffeic acid, 163 for *p*-hydroxycinnamic acid and 193 for ferulic acid units, respectively. Eight peaks (peaks 3, 6, 12, 13, 17, 20, 22 and 23) were identified as chlorogenic acid derivatives as all of them displayed the typical caffeic acid/ferulic acid structure with maximum absorption at around 325 nm and a shoulder peak at around 295 nm. Two peaks (peaks 8 and 11) were identified as hydroxycinnamic acid derivatives as all of them showed absorption maxima at 310 nm [18–20].

Peak 5 with maximum absorption at 221, 260 and 293 nm was similar to the UV spectra of protocatechuic acid (peak 2, λ_{max} 221, 260, 295 nm) and was tentatively attributed to vanillic acid. Peak 7 with a $[M-H]^-$ ion at *m/z* 197 was identified as syringic acid according to the reported data [21].

Peaks 4, 7, 10 and 24 showed similar UV spectra with *p*-hydroxy benzeneacetic acid (peak 5, λ_{max} 222, 275 nm), indicating the presence of *p*-hydroxy benzeneacetic acid derivatives [22].

The two jacaranone derivatives (peaks 1 and 9) showed characteristic absorption at λ_{max} 229 nm. Peak 9 produced a $[M-H]^-$ ion at *m/z* 195 and $[M-H-C_2H_4-H_2O]^-$ ion at *m/z* 149 was unequivocally identified as jacaranone ethyl ester by comparing with the reference compound. Peak 1 showed a $[M-H]^-$ ion at *m/z* 167 and $[M-H-H_2O]^-$ ion at *m/z* 149 was assigned as 1-hydroxy-4-oxo-2,5-cyclohexadiene-1-acetic acid, the hydrolyzed product of jacaranone ethyl ester.

In the chromatographic fingerprints of the *n*-butanol extracts, 10 flavonoid glycosides were characterized from the two *Senecio* species as summarized in Table 2. All those 10 peaks showed absorption maxima at 255 for quercetin or at 265 nm for kaempferol derivatives [18,23]. By referring to the reported flavonoid glycosides form *Senecio plants* [13,24], they were tentatively identified as flavonol 3-*O*-conjugates of sugars.

According to the structure of aglycone, those compounds were divided into two groups: 5 compounds (peaks 1, 2, 3, 4, and 9) were identified or tentatively assigned as quercetin glycoside with λ_{max} 255 nm (band II) and fragment ions of aglycone at *m/z* 301, 273, 257, 179, 151 [18,23,25]; another 5 compounds (peaks 5, 6, 7, 8 and 10) were tentatively assigned as kaempferol glycosides based on λ_{max} 265 nm (band II) and fragment ions at *m/z* 285, 267, 241, 199 and 151 [10,25,26].

In the fingerprints of alkaloids extract, a total of 18 PAs were detected from SS and SV. Their obtained retention times, protonated molecular ions and characteristic fragment ions are summarized in Table 3. The detected PAs could be classified into three categories: retronecine-type, otonecine-type and platynecine-type [27]. Among the 18 PAs, 15 belong to the retronecine-type including 6 *N*-oxides. Compared with the reference compounds, six PAs were unequivocally identified as adonifoline *N*-oxide (peak 1), adonifoline (peak 3), senecionine *N*-oxide (peak 7), retrorsine (peak 12), seneciphylline (peak 14) and senecionine (peak 17), respectively.

Based on this MS/MS fragmentation pattern, peaks 8, 10, 13 and 16 displayed the diagnostic ions at ions *m/z* 120 and 138 and were tentatively identified as usaramine, riddelline, spartioidine and integerrimine. Accordingly, peaks 2, 4, 5 and 6, yielding the diagnostic ions at ions *m/z* 118, 136, 120 and 138 belonging to retronecine-type-PA-*N*-oxide, were identified as retrorsine *N*-oxide, spartioidine *N*-oxide, seneciophylline *N*-oxide and integerrimine *N*-oxide. Peak 9 showed the diagnostic ions at *m/z* 150 and 168 of the otonecine-type PAs and was identified as neosenkirkine. Peaks 11 and 15 showed the diagnostic ions at

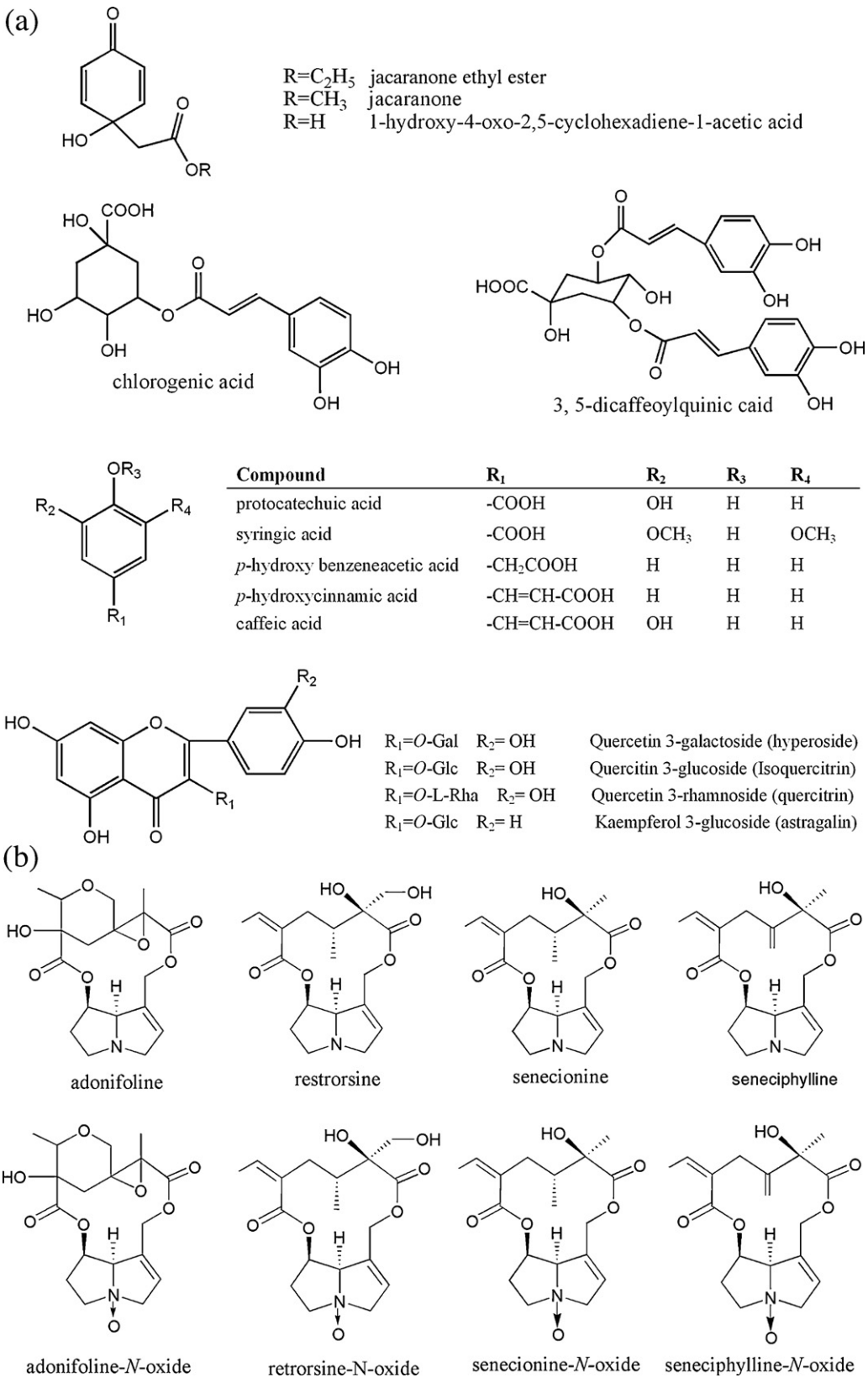

Fig. 1. Structures of major compounds in *S. scandens* and *S. vulgaris*.

Table 1
Phenolics determined by UPLC–DAD–MS characteristic in *S. scandens* and *S. vulgaris*.

Peak no.	Rt (min)	λ_{max} (nm)	MW	$[M-H]^-$	Fragment ions	Tentative assignment	Identification	Species
1	1.21	229	168	167	149	1-Hydroxy-4-oxo-2,5-cyclohexadiene-1-acetic acid	UV, MS	SS
2	1.95	221, 260, 295	154	153		Protocatechuic acid	UV, MS, R	SS/SV
3	3.80	326, 296sh, 221	354	353	191, 179, 707, 173, 127	3-*O*-Caffeoylquinic acid (chlorogenic acid)	UV, MS, R	SS/SV
4	3.98	222, 275	152	151		*p*-Hydroxy benzeneacetic acid	UV, MS, R	SS/SV
5	4.20	221, 260, 293,	178	177		Vanillic acid	UV	SS/SV
6	4.55	324, 294sh, 221	180	179		Caffeic acid	UV, MS, R	SS/SV
7	4,79	221, 274	198	197		Syringic acid	UV, MS	SS/SV
8	5.29	310, 234	338	337	191, 163, 173, 127	Coumaroylquinic acid	UV, MS	SS
9	5.66	229	196	195		Jacaranone ethyl ester	UV, MS, R	SS
10	6.29	227, 275				*p*-Hydroxy benzeneacetic acid derivative	UV	SV
11	6.42	309, 224	164	163		*p*-Hydroxycinnamic acid	UV, MS	SS/SV
12	7.50	324, 294sh, 220				Chlorogenic acid derivative	UV	SS
13	7.68	329, 299sh, 243	368	367	193, 191,173,127	Feruloylquinic acid	UV, MS	SS
14	8.50	312, 231				*p*-Hydroxycinnamic acid derivative	UV	SS
15	8.61	255, 256sh, 354	464	463	301, 273, 257, 179, 151	Quercetin-3-galactoside (hyperoside)	UV, MS, R	SS
16	9.09	255, 265sh, 355	464	463	301, 273, 257, 179, 151	Quercitin-3-glucoside (Isoquercitrin)	UV, MS	SS/SV
17	10.83	327, 297sh, 221	516	515	353, 191, 179,172, 127	Dicaffeoylquinic acid	UV, MS	SS/SV
18	11.14	265, 349	448	447	285, 267, 241, 199, 151	Kaempferol-3-*O*-hexoside	UV, MS	SS
19	11.19	255, 256sh, 349	448	447	301, 273, 257, 179, 151	Quercetin-3-rhamnoside (quercitrin)	UV, MS	SS
20	12.65	329, 293sh, 217, 243	516	515	353, 191, 179, 173, 127	Dicaffeoylquinic acid	UV, MS	SS/SV
21	13.38	265, 337	432	431	285, 267, 241, 199, 151	Kaempferol-3-*O*-deoxyhexoside	UV, MS	SS
22	14.20	329, 299sh, 220	530	529	367, 179, 193, 191, 161,173,127	Caffeoylferuloylquinic acid	UV, MS	SS
23	14.73	328, 298sh, 218, 244	530	529	367, 193, 191, 179, 173, 127	Caffeoylferuloylquinic acid	UV, MS	SS
24	15.57	228, 277				*p*-Hydroxy benzeneacetic acid derivative	UV	SS

sh, shoulder; R, reference compound; UV, UV spectrum; MS, mass spectrum; SS, *Senecio scandens*; SV, *S. vulgaris*.

Table 2
Flavonoid glycosides determined by UPLC–DAD–MS characteristic in *S. scandens* and *S. vulgaris*.

Peak no.	Rt (min)	λ_{max} (nm)	MW	$[M-H]^-$	Fragment ions	Tentative assignment	Identification	Species
1	7.37	255, 265sh, 355	610	609	463, 301, 273, 257, 179, 151	Quercetin-3-*O*-hexosyl-deoxyhexoside	UV, MS	SS
2	7.58	256, 266sh, 354	610	609	463, 301, 273, 257, 179, 151	Quercetin-3-*O*-hexosyl-deoxyhexoside	UV, MS	SS
3	7.66	255, 256sh, 354	464	463	301, 273, 257, 179, 151	Quercetin 3-*O*-galactoside (hyperoside)	UV, MS,R	SS
4	7.90	255, 265sh, 355	464	463	301, 273, 257, 179, 151	Quercitin 3-glucoside (Isoquercitrin)	UV, MS	SS
5	8.25	265, 342	578	577	431, 285, 267, 241, 199, 151	Kaempferol-3-*O*-di-deoxyhexoside	UV, MS	SV
6	8.62	265, 344	448	447	285, 255, 267, 241, 199, 151	Kaempferol 3-*O*-hexoside	UV, MS	SS
7	8.77	265, 347	594	593	431, 447, 285, 267, 241, 199, 151	Kaempferol-3-*O*-hexoside-7-*O*-deoxyhexoside/Kaempferol-3-*O*-deoxyhexoside-7-*O*-hexoside	UV, MS	SS
8	9.11	265, 345	448	447	285, 267, 241, 199, 151	Kaempferol 3-*O*-hexoside	UV, MS	SS
9	9.20	255, 256sh, 347	448	447	301, 273, 257, 179, 151	quercetin 3-*O*-rhamnoside (quercitrin)	UV, MS	SS/SV
10	10.65	265, 342	432	431	285, 267, 241, 199, 151	Kaempferol-3-*O*-deoxyhexoside	UV, MS	SS

sh, shoulder; R, reference compound; UV, UV spectrum; MS, mass spectrum; SS, *Senecio scandens*; SV, *S. vulgaris*.

Table 3
PAs determined by UPLC–MS characteristic in *S. scandens* and *S. vulgaris*.

Peak no.	Rt (min)	MW	$[M+H]^+$	Diagnostic ions (*m*/*z*)	Type	Tentative assignment	Identification	Species
1	1.89	381	381.8	138, 136, 120, 118	RETNO	Adonifoline *N*-oxide	MS, R	SS
2	3.22	367	367.9	138, 136, 120, 118	RETNO	Retrorsine *N*-oxide	MS	SV
3	3.37	365	365.9	138, 120	RET	Adonifoline	MS, R	SS
4	3.47	349	349.8	138, 136, 120, 118	RETNO	Spartioidine *N*-oxide	MS	SV
5	3.59	349	349.9	138, 136, 120, 118	RETNO	Seneciophylline *N*-oxide	MS	SV
6	4.23	351	351.9	138, 136, 120, 118	RETNO	Integerrimine *N*-oxide	MS	SV
7	4.40	351	351.9	138, 136, 120, 118	RETNO	Senecionine *N*-oxide	MS, R	SV
8	4.44	351	351.9	138, 120	RET	Usaramine	MS	SV
9	4.69	365	365.9	168, 150	OTO	Neosenkirkine	MS	SV
10	5.56	349	350.0	138, 120	RET	Riddelline	MS	SV
11	6.13	337	337.9	140, 122	Saturated-RET	Neoplatyphylline	MS	SV
12	6.19	351	351.9	138, 120	RET	Retrorsine	MS, R	SV
13	6.40	333	333.9	138, 120	RET	Spartioidine	MS	SV
14	6.54	333	333.9	138, 120	RET	Seneciophylline	MS, R	SV
15	6.83	337	337.9	140, 122	Saturated-RET	Platyphylline	MS	SV
16	6.87	335	335.9	138, 120	RET	Integerrimine	MS	SV
17	7.00	335	335.9	138, 120	RET	Senecionine	MS, R	SV
18	7.09	335	335.9	138, 120	RET	Unknown	MS	SV

RET, retronecine-type; OTO, otonecine-type; Saturated-RET, platynecine-type; RETNO, retronecine-typ-PA-*N*-oxides; R, reference compound; MS, mass spectrum; SS, *Senecio scandens*; SV, *S. vulgaris*.

m/*z* 122 and 140, characteristic of platynecine-type PAs, and were tentatively identified as neoplatyphylline and platyphylline, respectively [15,17,27].

3.4. Authentication of SS and SV by their secondary metabolic patterns

The representative UPLC chromatograms of the phenolics, flavonoid glycoside and pyrrolizidine alkaloids of SS and SV are illustrated in Figs. 2–4. In the fingerprints of the phenolics/aromatic acids (Fig. 2), 9 compounds were detected as the co-existing components in both species, including peaks 2, 3, 4, 5, 6, 7, 11, 17 and 20. Among those, peaks 3 and 17 were obviously stronger in SS than in SV. Peak 10 was the only characteristic peak of SV, while peaks 8, 12, 13, 14, 22, 23 and 24 were characteristic for SS. More importantly, the two jacaranone derivatives, 1 and 9 were diagnostic for SS.

For the 10 flavonoid glycosides identified in the fingerprints of the *n*-butanol extract from SS and SV (Fig. 3) only quercitrin (peak 9) was detected in both SS and SV. Peak 5 was specific for SV and the other 8 peaks were characteristic for SS, in which peaks 2, 3 and 4 showed the predominant accumulation.

The representative selected ion chromatograms (SIC) of pyrrolizidine alkaloids in SS and SV are shown in Fig. 4. Among the 18 PAs identified, only adonifoline (peak 1) and its *N*-oxide (peak 3) b were detected in SS, while all the other 16 PAs were detected in SV, including senecionine, seneciophylline, spartioidine, platyphylline, integerrimine and their *N*-oxide derivatives.

As mentioned above, SS has been used in Chinese medicine for its antiinflammatory, antibacterial and antipyretic effects, which can be explained by the abundant bioactive phenolic compounds. For instance, protocatechuic acids showed antioxidant and antihyperlipidaemic activities [28], *p*-hydroxy benzeneacetic acids displayed antibacterial, antimicrobial, molluscicidal and oestrogenic activity [29], and hydroxycinnamic acids possessed significant antioxidant, hypoglycaemic, antiviral and hepatoprotective activities [30]. Flavonoids showed antimicrobial, antifugal,

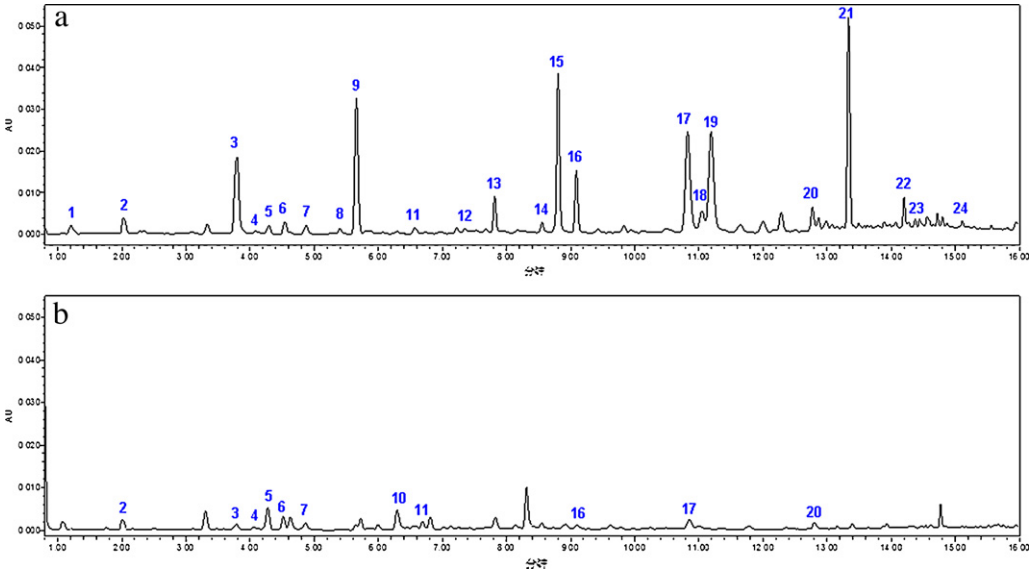

Fig. 2. Representative UPLC chromatograms at wavelength 254 nm of the fingerprints of the diethyl ether extracts showing the phenolics/aromatic acids. (A) *S. scandens* (Xinxiang, Henan) and (B) *S. vulgaris* (Tonghua, Jilin). Peak numbers correspond to Table 1.

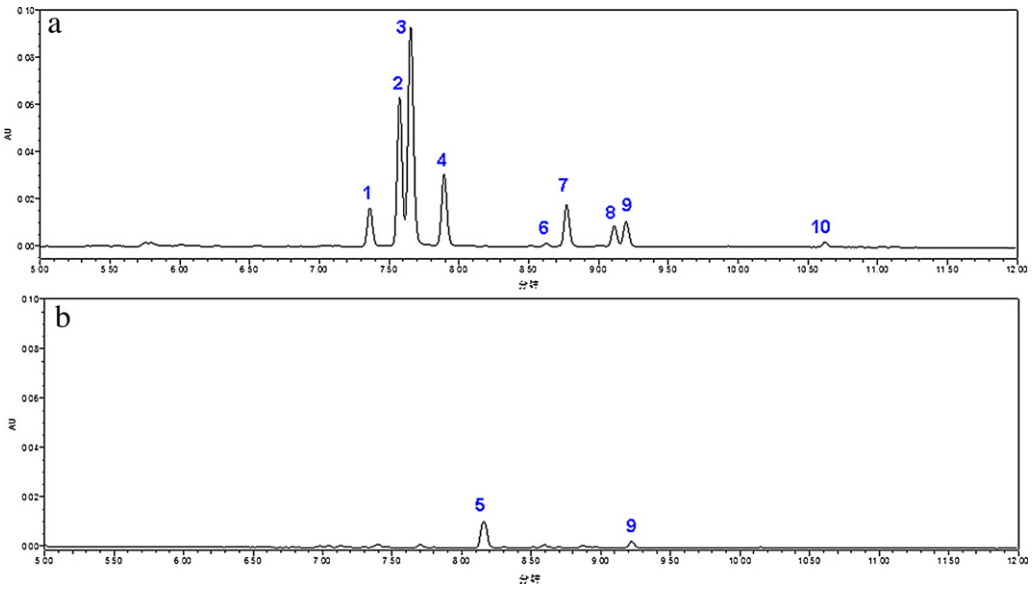

Fig. 3. Representative UPLC chromatograms at wavelength 360 nm of the fingerprints of the *n*-butanol extracts showing the flavanoids. (A) *S. scandens* (Xinxiang, Henan) and (B) *S. vulgaris* (Tonghua, Jilin). Peak numbers correspond to Table 2.

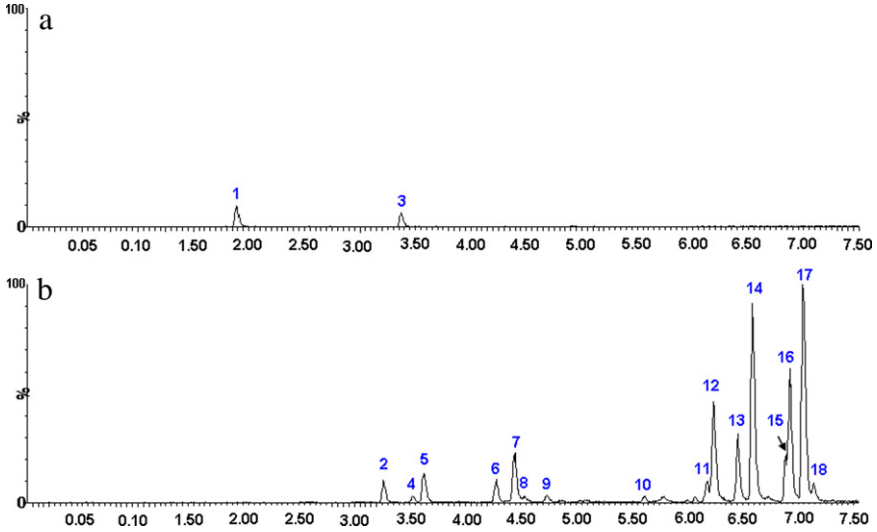

Fig. 4. Representative selected ion chromatograms of the fingerprints of the pyrrolizidine alkaloids. (A) *S. scandens* (Xinxiang, Henan) and (B) *S. vulgaris* (Tonghua, Jilin) displaying the $[M+H]^+$ ions at *m*/*z* 240, 254, 306, 320, 334, 336, 338, 350, 352, 354, 356, 366, 368, 370 and 382 for all the identified PAs from SS and SV. Peak numbers correspond to Table 3.

antitumor, antioxidant, cytotoxic, and hypoglycemic activities [31].

Furthermore, the characteristic jacaranone derivatives were reported to possess antitumor, antibacterial and antiviral activity [11,12,32].

The significant difference in pyrrolizidine alkaloids between SS and SV was in accordance with the references: the content of total alkaloids in SV was up to 0.16% of the aerial part [15], while the content of total retronecine-type PAs in SS was only 0.0118% of the whole herb [9]. In our previous *in vivo* and *in vitro* tests, senecionine (characteristic for SV) exhibited much stronger liver injury than adonifoline (diagnostic for SS) (data not shown).

The intra-species and inter-species variations were evaluated by a PCA analysis using MVSP 3.1 based on a full 16 (samples) × 47 (constituent characters) data matrix. The result showed that the two species were significantly discriminated from each other, and the intra-species differences were much smaller than that of the inter-species (Supporting material 2).

4. Conclusion

The comprehensive secondary metabolic patterns using UPLC–DAD/ESI-MS were developed and applied for authentication of *Senecio scandens* (SS) and *S. vulgaris* (SV), two representative species of HPAs-containing plants. 19 phenolics/aromatic acids, 10 flavonoid glycosides and 18 pyrrolizidine alkaloids were identified, among which 5 phenols, one flavonoid glycosides and 6 pyrrolizidine alkaloids were unambiguously identified using available references, while the others were characterized tentatively by comparing their chromatographic behaviors, the UV absorptions and the MS fragmentations with the reported data. The two species, each with eight batches of samples collected from various habitats, were authenticated using the established metabolic patterns. SS was specific in containing more phenolics/aromatic acids and flavonoid glycosides and at higher concentrations but only few and lower content of pyrrolizidine alkaloids. On the contrary, SV was specific in containing abundant pyrrolizidine alkaloids but few

and lower concentration of phenolics/aromatic acids and flavonoid glycosides.

In summary, using the combined secondary metabolic patterns of UPLC–DAD/ESI-MS, or even some selected diagnostic chemical markers, such as jacaranones, senecionine, adonifoline, SS and SV can be discriminated from each other for the purpose to ensure the safety and efficacy when they are applied as herbal products.

Acknowledgements

The financial supports of the Natural Science Foundations of China (30701104), National S&T Major Special Projects (2009ZX09502-003, 2009ZX09502-013, 2009ZX09502-021 and 2009ZX09502-024), Shanghai Rising-Star Program (09QA1405500) and Project of International Corporation from S&T Commission of Shanghai Municipality (09540704700) are gratefully acknowledged.

Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.jpba.2011.05.004.

References

[1] P.S. Xie, S.B. Chen, Y.Z. Liang, X.H. Wang, R.T. Tian, R. Upton, Chromatographic fingerprint analysis—a rational approach for quality assessment of traditional Chinese herbal medicine, J. Chromatogr. A 1112 (2006) 171–180.
[2] Z.Y. Liang, P.S. Xie, K. Chan, Quality control of herbal medicines, J. Chromatogr. B 812 (2004) 53–70.
[3] A.R. Mattocks, Toxicity of pyrrolizidine alkaloids, Nature 217 (1968) 723–728.
[4] P.R. Cheeke, Toxicity and metabolism of pyrrolizidine alkaloids, J. Anim. Sci. 66 (1998) 2343–2350.
[5] International Programme on Chemical Safety Pyrrolizidine Alkaloids, Health and Safety Guide No. 26, World Health Organisation, Geneva, 1989.
[6] The Committee of Pharmacopeia of the People's Republic of China, Pharmacopeia of the People's Republic of China, Chemical Industry Press, Beijing, 2010.
[7] Y.L. Chen, Flora of China, vol. 77, Science Press, Beijing, 1999, pp. 225–303.
[8] E. Roeder, Medicinal plants in China containing pyrrolizidine alkaloids, Pharmazie 55 (2000) 711–726.
[9] F. Zhang, C.H. Wang, A.Z. Xiong, W. Wang, L. Yang, C.J. Branford-White, Z.T. Wang, S.W. Annie Bligh, Quantitative analysis of total retronecine esters-type pyrrolizidine alkaloids in plant by high performance liquid chromatography, Anal. Chim. Acta 605 (2007) 94–101.
[10] C.F. Zhang, L.X. Chen, M. Zhang, Z.T. Wang, HPLC fingerprint of Herba Senecionis Scandentis and its ESI-MS analysis, China J. Chin. Mater. Med. 33 (2008) 2093–2096.
[11] X.Y. Tian, Y.H. Wang, Q.Y. Yang, S.S. Yu, W.S. Fang, Jacaranone analogs from *Senecio scandens*, J. Asian Nat. Prod. Res. 11 (2009) 63–68.
[12] X.Y. Tian, Y.H. Wang, Q.Y. Yang, X. Liu, W.S. Fang, S.S. Yu, Jacaranone glycosides from *Senecio scandens*, J. Asian Nat. Prod. Res. 8 (2006) 125–132.
[13] L.X. Chen, H.Y. Ma, M. Zhang, C.F. Zhang, Z.T. Wang, Studies on constituents in herb of *Senecio scandens*, China J. Chin. Mater. Med. 31 (2006) 1872–1875.
[14] C.E. Parker, S. Verma, K.B. Tomer, R.L. Reed, D.R. Buhler, Determination of *Senecio* alkaloids by thermospray liquid chromatography/mass spectrometry, Biomed. Environ. Mass Spectrom. 19 (1990) 1–12.
[15] E. Roder, Medicinal plants in Europe containing pyrrolizidine alkaloids, Pharmazie 50 (1995) 83–98.
[16] Y. Chen, L.L. Ji, H.T. Wang, Z.T. Wang, Intracellular glutathione plays important roles in pyrrolizidine alkaloids-induced growth inhibition on hepatocytes, Environ. Toxicol. Pharmacol. 28 (2009) 262–357.
[17] A.Z. Xiong, J.G. Li Yang, Y.Q. Gao, C.H. He, Z.T. Wang, Wang, Simultaneous determination of senecionine, adonifoline and their metabolites in rat serum by UPLC–ESIMS and its application in pharmacokinetic studies, J. Pharm. Biomed. Anal. 50 (2009) 1070–1074.
[18] L. Bravo, L. Goya, E. Lecumberri, LC/MS characterization of phenolic constituents of mate (*Ilex paraguariensis*, St. Hil.) and its antioxidant activity compared to commonly consumed beverages, Food Res. Int. 40 (2007) 393–405.
[19] L. Bengoechea, T. Hernandez, C. Quesada, B. Barolome, I. Estrella, C. Gomez-Cordoves, Structure of hydroxycinnamic acid derivatives establish by high-performance liquid chromatography with photodiode-array detection, Chromatographia 41 (1995) 94–98.
[20] Z.X. Lou, H.X. Wang, S. Zhu, M. Zhang, Y. Gao, C.Y. Ma, Z.P. Wang, Improved extraction and identification by ultra performance liquid chromatography tandem mass spectrometry of phenolic compounds in Burdock leaves, J. Chromatogr. A 1217 (2010) 2441–2446.
[21] A. Romani, A. Baldi, N. Mulinacci, E.F. Vincieri, M. Tattini, Extraction and identification procedures of polyphenolic compounds and carbohydrates in phillyrea (*Phillyrea* angustifolica L.) leaves, Chromatographia 42 (1996) 571–577.
[22] S.C. Gouveia, P.C. Castilho, Characterization of phenolic compounds in *Helichrysum melaleucum* by high-performance liquid chromatography with on-line ultraviolet and mass spectrometry detection, Rapid Commun. Mass Spectrom. 24 (2010) 1851–1868.
[23] P.Z. Liu, B. Yang, H. Kallio, Characterization of phenolic compounds in Chinese hawthorn (*Crataegus pinnatifida* Bge. var. major) fruit by high performance liquid chromatography–electrospray ionization mass spectrometry, Food Chem. 121 (2010) 1188–1197.
[24] L. Li, C.F. Zhang, M. Zhang, Phenolic constituents from the herb *Senecio laetus*, Pharm. Clin. Res. 4 (2009) 294–297.
[25] Y. Wang, L. Yang, Y.Q. He, C.H. Wang, E.W. Welbeck, S.W. Annie Bligh, Z.T. Wang, Characterization of fifty-one flavonoids in a Chinese herbal prescription Longdan Xiegan Decoction by high-performance liquid chromatography coupled to electrospray ionization tandem mass spectrometry and photodiode array detection, Rapid Commun. Mass Spectrom. 22 (2008) 1767–1778.
[26] M.J. Simirgiotis, G. Schmeda-Hirschmann, Direct identification of phenolic constituents in Boldo Folium (*Peumus boldus* Mol.) infusions by high-performance liquid chromatography with diode array detection and electrospray ionization tandem mass spectrometry, J. Chromatogr. A 1217 (2010) 443–449.
[27] S.L. Li, G. Lin, P.P. Fu, C.L. Chan, M. Li, Z.H. Jiang, Z.Z. Zhao, Identification of five hepatotoxic pyrrolizidine alkaloids in a commonly used traditional Chinese medicinal herb, Herba Senecionis Scandentis (Qianliguang), Rapid Commun. Mass Spectrom. 22 (2008) 591–602.
[28] R. Harini, K.V. Pugalendi, Antioxidant and antihyperlipidaemic activity of protocatechuic acid on streptozotocindiabetic rats, Redox Rep. 15 (2010) 71–80.
[29] J. Orjala, C.A. Erdelmeier, A.D. Wright, O. Rali, Sticher, Five new prenylated p-hydroxybenzoic acid derivatives with antimicrobial and molluscicidal activity from *Piper aduncum* leaves, Planta Med. 59 (1993) 546–551.
[30] V. Marques, A. Farah, Chlorogenic acids and related compounds in medicinal plants and infusions, Food Chem. 113 (2009) 1370–1376.
[31] V.E. Fernand, D.T. Dinh, S.J. Washington, S.O. Fakayode, J.N. Losso, R.O. van Ravenswaay, I.M. Warner, Determination of pharmacologically active compounds in root extracts of *Cassia alata* L. by use of high performance liquid chromatography, Talanta 74 (2008) 896–902.
[32] Y. Jo, J. Suh, M.H. Shin, J.H. Jung, K.S. Im, Jacaranone and related compounds from the fresh fruits of *Ternstroemia japonica* and their antioxidative activity, Arch. Pharmacol. Res. 28 (2005) 885–888.