



Metabolic profiling of *Actaea* species extracts using high performance liquid chromatography coupled with electrospray ionization time-of-flight mass spectrometry

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ABSTRACT

Despite persistent questions about the safety of black cohosh (*Actaea racemosa* L., syn. *Cimicifuga racemosa* L.), its products continue to be one of the most popular botanical supplements in the United States market. Black cohosh products have been associated with cases of liver toxicity, but subsequent evaluation found some products to be adulterated with other related plants from the same genus. US FDA regulations require that black cohosh products be unadulterated, and correct identification of different species of *Actaea* is a key first step for their good manufacturing practice. We have developed a phytochemical method to distinguish four different groups of *Actaea*, including: species other than *A. racemosa*, Asian species, *A. racemosa*, and North American species other than *A. racemosa*. Using HPLC–TOF–ESI–MS technique and principal component analysis, we identified 15 chemical markers (1–3, 5–6, 8–10, 12, 16–21). Three marker compounds were unambiguously identified using authentic standards, and 12 marker compounds were tentatively identified by comparison of fragmentation patterns with previously reported data. The presence of these marker compounds is critical for discrimination among the four groups of closely related plants. The use of metabolic profiling to distinguish black cohosh from related species of *Actaea* has broader implications in the identification of markers to help authenticate other important medicinal plants.

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1. Introduction

Actaea (syn. *Cimicifuga*) is a small genus belonging to the Ranunculaceae, a family of plants native to temperate regions of the northern hemisphere. In North America, *A. racemosa* (black cohosh) is widely used as a dietary herbal supplement for the treatment of menopausal symptoms [1,2]. In 2008, black cohosh herbal products were ranked tenth in U.S. botanical sales [3]. In addition to black cohosh, there are eight other North American species of *Actaea*, including *A. podocarpa*, *A. pachypoda*, *A. rubra*, *A. laciniata*, *A. americana*, *A. elata*, *A. arizonica*, and *A. cordifolia*, but none is used commonly for menopausal symptoms. Asian species of *Actaea* were also widely used as traditional medicines for hundreds of years. *A. heracleifolia*, *A. dahurica*, and *A. cimicifuga* (Syn. *Cimicifuga foetida*) are listed in the Chinese Pharmacopoeia and used for their anti-inflammatory, antipyretic and analgesic effects [4]. Modern research has revealed several bioactive properties of Asian species

of *Actaea*, but no species is widely used for menopausal symptoms [5–10].

Black cohosh has different traditional usages with other North American and Asian species of *Actaea*. However, our studies have found that black cohosh products in the U.S. market are adulterated widely with other species of *Actaea*, which are lower-priced and exert unknown effect on menopausal symptoms [11]. It is critical that the black cohosh products be of high quality and be unadulterated. Health Canada has found that several cases of liver toxicity were associated with people who took black cohosh products adulterated with related herbal species [12]. The most commonly used species of *Actaea* for black cohosh adulteration include *A. americana*, *A. cimicifuga*, *A. heracleifolia*, and *A. dahurica* [13]. Therefore, correct identification of the plant and the discovery of the suitable marker compounds to distinguish different species of *Actaea* becomes a key first step for good manufacturing practices of black cohosh products. Previously, these *Actaea* species have been distinguished, with various success, using DNA, HPLC–UV (DAD), HPLC–ELSD, HPLC–MS, GC–MS, high performance TLC (HPTLC)–densitometry, Fourier transform near-infrared (FT–NIR) spectroscopy, and high-field NMR fingerprints [11,14–17]. Two marker compounds, cimicifugoside C and cimifugin, have been identified to distinguish black cohosh and other Asian species of

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Table 1
The general information of 25 samples (16 species) used in this research^a.

Name	Collection place	Parts	Collection date	Sample codes	Injection orders ^b
<i>A. racemosa</i>	Fox Lane (Lake), NY	Rhizome	5/25/2000	a1–a3	1st–3rd
<i>A. racemosa</i>	Asheville, NC	Rhizome	1999	b1–b3	4th–6th
<i>A. racemosa</i>	Fanfield, PA	Rhizome	01/03/2006	c1–c3	7th–9th
<i>A. racemosa</i>	Hunterdam Co. NJ	Rhizome	09/10/2000	d1–d3	10th–12th
<i>A. racemosa</i>	MD	Rhizome	Early 2000	e1–e3	13th–15th
<i>A. racemosa</i>	Rippowam, NY	Rhizome	5/25/2000	f1–f3	16th–18th
<i>A. racemosa</i>	Pound Ridge, NY	Rhizome	4/26/2007	g1–g3	19th–21st
<i>A. racemosa</i>	Bedford, NY	Rhizome	07/02/2006	h1–h3	22nd–24th
<i>A. racemosa</i>	Buena Vista, VA	Rhizome	10/03/2006	i1–i3	25th–27th
<i>A. americana</i>	Botanical Liaison LLC	Rhizome	2004	j1–j3	28th–30th
<i>A. laciniata</i>	OR, WA	Rhizome	08/2007	k1–k3	31st–33rd
<i>A. podocarpa</i>	Botanical Liaison LLC, AZ	Rhizome	2004	l1–l3	34th–36th
<i>A. pachypoda</i>	Sugar Cove, NC	Rhizome	Before 2004	m1–m3	37th–39th
<i>A. rubra</i>	Pineville, OR	Rhizome	Before 2004	n1–n3	40th–42nd
<i>A. elata</i>	Crone Miller Lake, OSU Forest, OR	Rhizome	08/2007	o1–o3	43rd–45th
<i>A. elata var. alpestris</i>	OR	Rhizome	10/2007	p1–p3	46th–48th
<i>A. arizonica</i>	Flagstaff Arboretum, AZ	Rhizome	2007	q1–q3	49th–51st
<i>A. cordifolia</i>	National Botanic Garden of Belgium	Rhizome	2007	r1–r3	52nd–54th
<i>A. heracleifolia</i>	Liaoning, China	Rhizome	08/2006	s1–s3	55th–57th
<i>A. mairei</i>	Guizhou, China	Rhizome	08/09/2006	t1–t3	58th–60th
<i>A. dahurica</i>	Liaoning, China	Rhizome	08/2006	u1–u3	61st–63rd
<i>A. brachycarpa</i>	Yunnan, China	Rhizome	08/2006	v1–v3	64th–66th
<i>A. simplex</i>	Yunnan, China	Rhizome	08/09/2006	w1–w3	67th–69th
<i>A. cimicifuga</i>	Dali, Yunnan, China	Rhizome	08/05/2006	x1–x3	70th–72nd
<i>A. yunnanensis</i>	Yunnan, China	Rhizome	07/2005	y1–y3	73rd–75th

^a Samples (codes from a–r) were collected from North America; Samples (codes from s–y) were collected from Asia;

^b These injection orders in this table are the same as those in Fig. 4.

Actaea [11,14]. These studies have resulted in either the different types of fingerprints to discriminate among different *Actaea* species without additional marker compounds, or marker compounds found based on available standards.

Recently, the use of HPLC coupled with time-of-flight mass spectrometry (TOF-MS) has been employed for the analysis of herbal medicines and metabolites, and proved to be powerful for the structural characterizations of constituents [18–21]. The TOF spectrometer can produce exact mass measurements. Based on these data, elemental compositions of marker molecular ions and their fragmental ions can be confirmed, which are very useful for elucidating the pathway of the mass fragmental cleavage.

In the present research, a multivariate statistical analysis method, principal component analysis (PCA), was introduced to analyze the HPLC–TOF-MS total ion current (TIC) chromatograms of *Actaea* species. We tried to find useful marker compounds like cimifugin derivatives, triterpene glycosides, and alkaloids, to distinguish different species of *Actaea*. Additionally, based on the molecular and fragmental ions analysis using a group of authentic standards which belong to different classes of aglycone structures, we aimed to first develop the proposed characteristic MS/MS fragmentation rules originated from the cleavage of the C(20)–C(27) side chain of these structures. This side chain shows the most structural variation among all the classes of triterpene glycoside aglycone skeleton. Applying these MS/MS fragmentation rules to compounds without standards, together with the data previously reported, most of the structures of triterpene glycosides and alkaloid marker compounds would be tentatively elucidated.

2. Experimental

2.1. Chemicals

HPLC–MS grade acetonitrile, methanol, water (J. T. Baker, Phillipsburg, NJ) and formic acid (Sigma–Aldrich, Louis, MO) were used for HPLC–TOF-MS analysis. Guaranteed reagent grade methanol (EMD, Gibbstown, NJ) and deionized water were used for the extraction of *Actaea* plant material. Prim-*O*-glucosylcimifugin

(**1**) was purchased from Shanghai Nature Standard Biotech Co. Ltd. (Shanghai, China). Cimifugin (**2**), cimracemoside A (**13**), actein (**14**) and 26-deoxyactein (**15**) were purchased from ChromaDex (Irvine, CA). Cimicifugoside H-1 (**4**), 23-*O*-acetylshengmanol 3-*O*- β -D-xylopyranoside (**7**), cimracemoside C (**10**), and 25-acetylcimigenol 3-xyloside (**11**) were kindly provided by Dr. Stefan Gafner (Tom's of Maine, ME).

2.2. Sample materials

Twenty-five samples of 16 species of *Actaea* were collected in North America and Asia from 1999 to 2007. The collection locations, time, codes, and injection no. of TOF mass analysis for all the samples were shown in Table 1. Among them, *A. americana* and *A. podocarpa* were purchased from Botanical Liaison LLC (Boulder, DO). *A. cordifolia* was supplied by Dirk De Meyere at the National Botanic Garden of Belgium. *A. laciniata*, *A. pachypoda*, *A. rubra*, *A. elata*, *A. elata var. alpestris*, *A. arizonica*, and nine samples of *A. racemosa* were collected in North America. While the remaining seven samples, *A. heracleifolia*, *A. mairei*, *A. dahurica*, *A. brachycarpa*, *A. simplex*, *A. cimicifuga*, and *A. yunnanensis*, were collected from Asia. Voucher specimens of *Actaea* samples used in this study were deposited at the City University of New York. Rhizomes of all *Actaea* plants were used in this study.

2.3. Sample preparation

Air-dried rhizomes of each sample of *Actaea* plants (ca. 1 g) were extracted with 80% MeOH (10 mL) at room temperature. Extraction was facilitated by sonication for 60 min in a water bath, and the sample temperature not exceeding 40 °C, and the resulting suspension was centrifuged at 3000 rpm for 15 min. The solvent was then removed, and the residue was re-extracted three times using the same method. The resulting extracts were combined and the solvent was evaporated under nitrogen. All remaining water was removed by lyophilization. The resulting extract was stored at –20 °C until analysis. Prior to HPLC–TOF-MS analysis, each extract was dissolved in 70% MeOH (30 mL). In-source MS/MS fragmen-

tation was conducted (with an aperture voltage of 60 V) on these extracts, following which extracts were diluted by 10-fold for standard MS analysis (with an aperture voltage of 0 V). All the prepared samples were filtered through a 0.45 μm nylon membrane filter prior to analysis.

2.4. Liquid chromatography

Separation was achieved by HPLC using a Waters 2695 separations module (Milford, MA), equipped with a 2998 photodiode array detector (PDA). The separations were carried out on a 100 \times 2.0 mm i.d., 2.5 μm Synergi C18 column (Phenomenex, Torrance, CA). All analyses were performed at 25 $^{\circ}\text{C}$ with a flow rate of 0.2 mL/min. The sample volume injected was 10 μL . Analysis was based on triple injections. Mobile phase was composed of 0.1% aqueous formic acid (A), acetonitrile containing 0.1% formic acid (B), and methanol containing 0.1% formic acid (C) using a stepwise gradient elution of 92%A/8%B/0%C–75%A/25%B/0%C at 0–16 min, 75%A/25%B/0%C–59%A/27%B/14%C at 16–26 min, 59%A/27%B/14%C–46%A/18%B/36%C at 26–27 min, 46%A/18%B/36%C–40.6%A/20.1%B/39.3%C at 27–44 min, 40.6%A/20.1%B/39.3%C–33%A/23%B/44%C at 44–62 min, 33%A/23%B/44%C–56%A/44%B/0%C at 62–62.5 min, 56%A/44%B/0%C–35%A/65%B/0%C at 62.5–75 min, 35%A/65%B/0%C–18%A/82%B/0%C at 75–90 min, 18%A/82%B/0%C–0%A/100%B/0%C at 90–105 min, and kept this proportion of solvent for 12 min. The UV/vis spectra were recorded from 190 to 500 nm.

2.5. Mass spectrometry

High resolution electrospray ionization mass spectrometry (HR-ESI-MS) was performed using a LCT premier XE TOF mass spectrometer (Waters, Milford, MA) equipped with an ESI interface and controlled by MassLynx V4.1 software. Mass spectra were acquired in both the positive and negative mode over the range m/z 100–1000. The capillary voltages were set at 3000 V (positive mode) and 2800 V (negative mode), respectively, and the cone voltage was 20 V. Nitrogen gas was used for both the nebulizer and in desolvation. The desolvation and cone gas flow rates were 600 and 20 L/h, respectively. The desolvation temperature was 400 $^{\circ}\text{C}$, and the source temperature was 120 $^{\circ}\text{C}$. For the dynamic range enhancement (DRE) lockmass, a solution of leucine enkephalin (Sigma–Aldrich, Steinheim, Germany) was infused by a secondary reference probe at 200 $\mu\text{g}/\text{mL}$ in acetonitrile/water (1:1) containing 0.1% formic acid with the help of a second LC pump (Waters 515 HPLC pump). The reference mass was scanned once every five scans for each positive and negative data collection. Both positive and negative ESI data were collected using a scan time of 0.2 s, with an interscan time of 0.01 s, and a polarity switch time of 0.3 s. The full chromatograms were recorded at two different aperture voltages. The most intense fragmental ions and molecular ions could be obtained, when the aperture voltage were set at 60 V and 0 V, respectively. W-optics mode was used for increased resolution.

2.6. Chemometric data analysis

The HPLC–TOF-MS data of 25 samples from 16 species of *Actaea* was analyzed by PCA analysis to identify potential discriminate variables. Peak detection and alignment, and the filtering of raw data were carried out using the Markerlynx v4.1. The parameters used included a retention time range of 8–125 min, a mass range of 100–1000 Da, and a mass tolerance of 50 mDa. Isotopic peaks were excluded for analysis; noise elimination level was set at 1.00, the intensity threshold (counts) of collection parameters was set at 500; retention time tolerance was set at 0.4 min. The retention time and m/z data pair for each peak was determined by the software.

3. Results and discussion

3.1. Metabolic fingerprinting and PCA analysis

The TIC chromatograms for the reversed-phase LC–ESI-TOF-MS analysis of 25 samples extracted from different species of *Actaea* are shown in Supplemental Fig. S1. Although some differences could be visually noted among these species of *Actaea* chromatograms, more subtle changes could be detected using a pattern recognition approach. The retention times, m/z value of mass fragmental ions, and their intensities were used to compare the phenotypic differences of the 16 species of *Actaea* using PCA, an unsupervised and therefore unbiased technique for multivariate analysis. Processed data displayed a clear differentiation of three clusters of the 16 species of *Actaea* which divided along geographic and botanical origin into Asian, North American (other than *A. racemosa*), and true black cohosh (i.e. *A. racemosa*) groups (Fig. 1A). Each sample was injected three times, e.g. a1–a3. The samples are labeled alphabetically, corresponding to different *Actaea*, and numerically, corresponding to different LC injections (Table 1). All black cohosh species formed a centralized cluster within the larger North American group, because all black cohosh samples evolutionarily originated from the same species, and only differed in collection locations and harvest time. North American and Asian species also separated into distinct clusters (Fig. 1A). Using the corresponding loading plot, markers (ion-retention time pairs) most responsible for cluster formation, were identified as useful indicators of the botanical origin of each sample (Fig. 1B). Fifteen molecular marker ions were found to be useful in distinguishing species of *Actaea* and were selected for further identification by MS/MS fragmental and UV analysis.

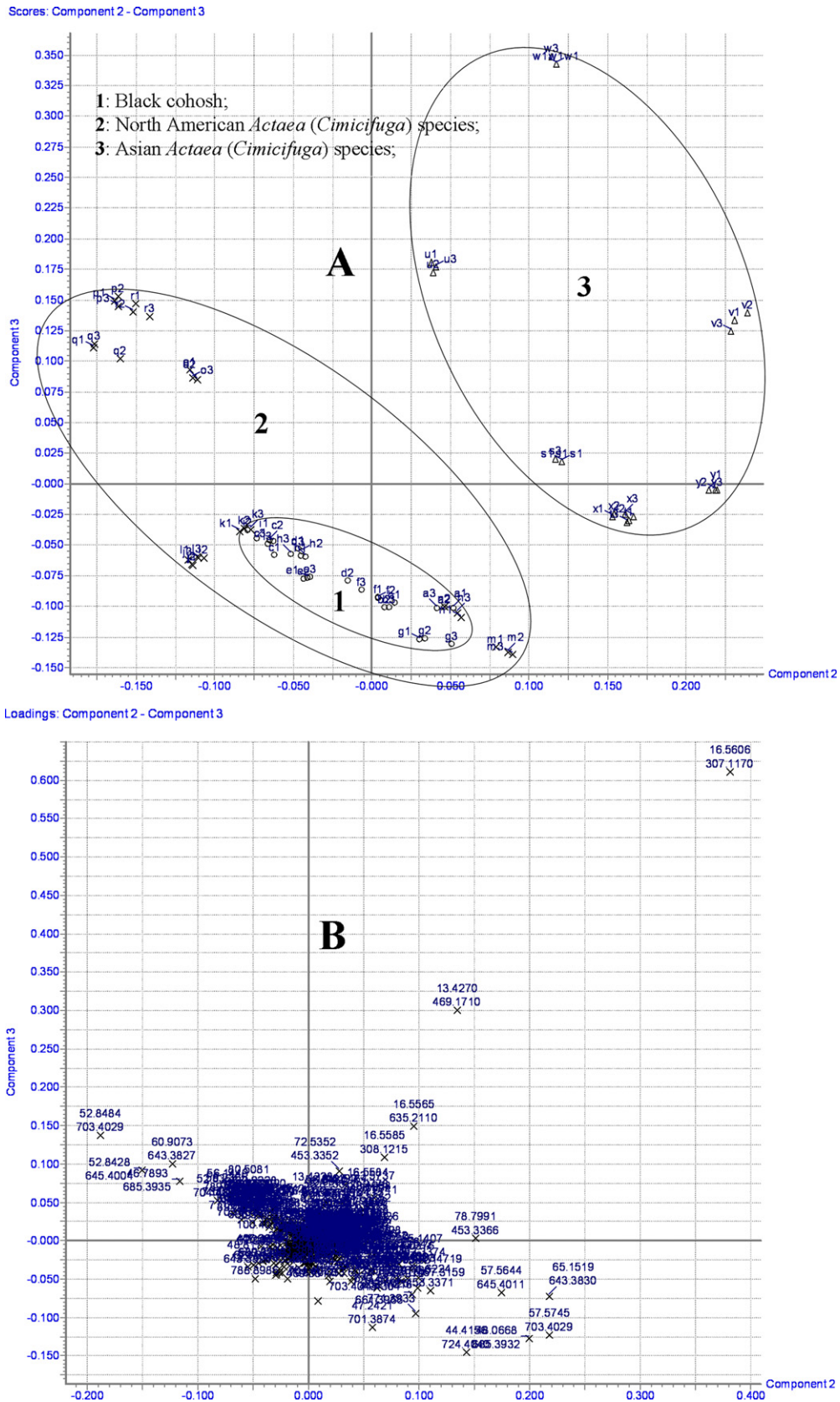
3.2. Mass fragmental analysis of standards and marker compounds

For each standard and *Actaea* species extract sample, TOF-ESI-MS/MS spectra were recorded in both positive and negative ion modes; however, the more useful ion information was obtained in positive mode. Therefore, the positive ion mode was selected for analysis. TIC chromatograms of all identified marker compounds are shown in Fig. 2.

3.2.1. Analysis of cimifugin and its derivatives

Cimifugin derivatives are a group of linear dihydrofurochromone-class compounds (Fig. 3). Most of fragmentation occurs on the dihydrofuran ring. The typical cleavages of this class of structure include fragmental losses of 18, 48, and 72 Da. These characteristic losses are useful in the identification of compounds with this skeleton [22].

Prim-O-glycosylcimifugin (**1**) and cimifugin (**2**) were identified using known standards. The UV spectra of these two compounds showed similar absorption maxima at 300.0, 254.2 (sh), 215.0 nm for **1** and 298.0, 254.3 (sh), 217.0 nm for **2** (Supplemental Fig. S2A and S2B). When the aperture voltage was set at 60 V, three cleavage fragmental ions at m/z 289.1068 $[\text{M}+\text{H}-\text{H}_2\text{O}]^+$, 259.0573 $[\text{M}+\text{H}-\text{H}_2\text{O}-2 \times \cdot\text{CH}_3]^+$, and 235.0569 $[\text{M}+\text{H}-2,2\text{-dimethylepoxyethane}]^+$ with the losses of 18, 48, and 72 Da from the protonated molecular ion at m/z 307.1160 $[\text{M}+\text{H}]^+$ were generated for **2** (Table 2). These similar losses of 18, 48, and 72 Da were also found in the fragmentation patterns of **1**, indicated by fragmental ions at m/z 451.1602, 421.1099, 397.1128 from the molecular ion at m/z 469.1703, and m/z 289.1068, 259.0580, 235.0572 from the aglycone ion at m/z 307.1161 which was generated from the ion at m/z 469.1703 by the elimination of a glucosyl moiety (Table 2).



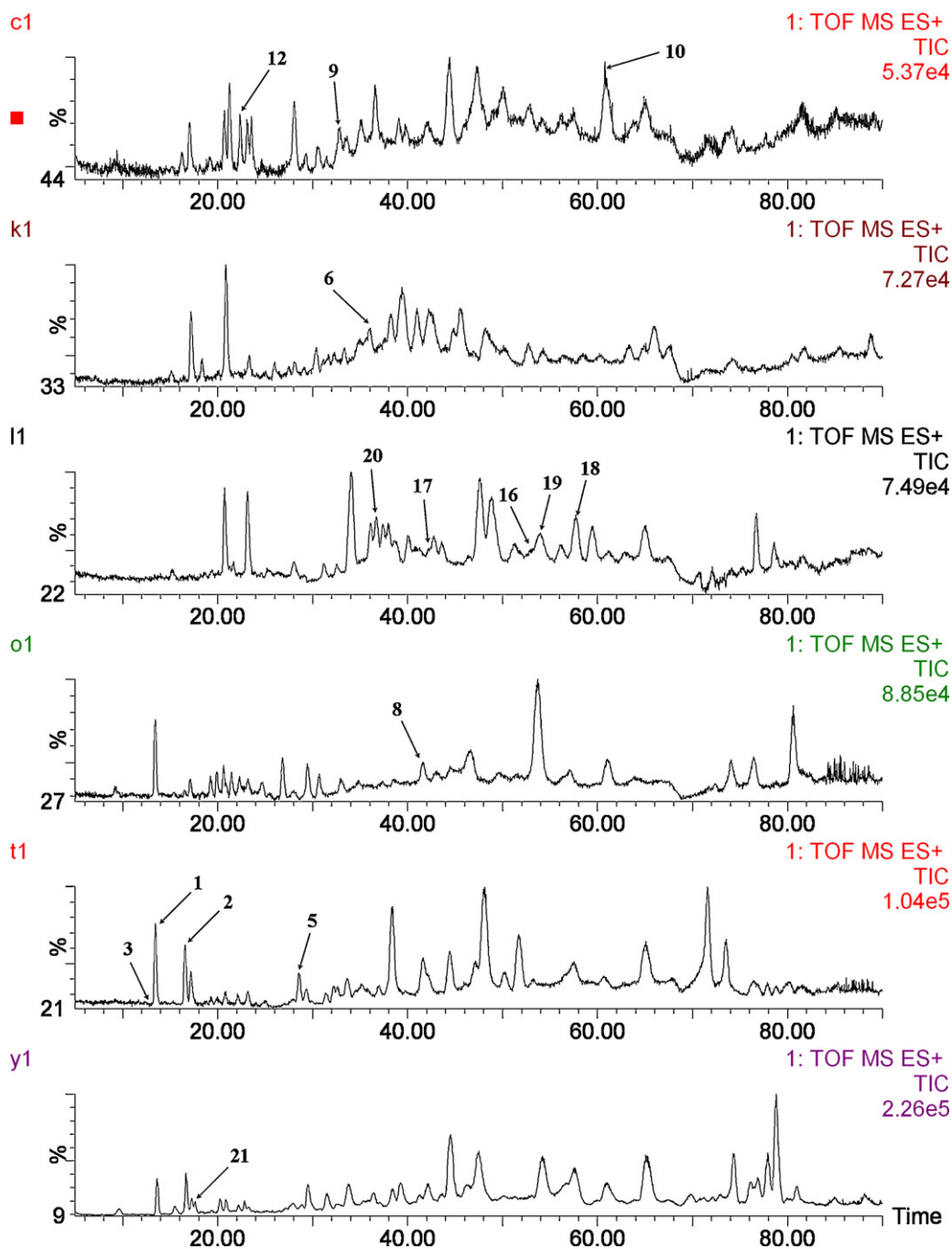


Fig. 2. Fifteen marker compounds in the TIC chromatograms.

Divaricatacid (**3**) was tentatively identified by the similarity in fragmental loss patterns as those of **1** and **2**. Additionally, **3** had a similar UV absorbance profile as **1** and **2** with maxima at 310.0, 255.0 (sh), and 217.0 nm (Table 2 and Supplemental Fig. S2C). The typical fragmental ions by the losses of 18, 48, and 72 Da at m/z 303.0852 $[M+H-H_2O]^+$, 273.0367 $[M+H-H_2O-2 \times \cdot CH_3]^+$, and 249.0362 $[M+H-2,2\text{-dimethylepoxyethane}]^+$ were also found in the spectrum. An additional two fragmental ions at m/z 231.0222 $[M+H-2,2\text{-dimethylepoxyethane}-H_2O]^+$ and 221.0414 $[M+H-2,2\text{-dimethylepoxyethane}-CO]^+$ were found in the spectrum (Table 2). All molecular and fragmental ions detected are in agreement with previous publication [22]. Although putative in identification, this is the first report for the presence of divaricatacid in *Actaea* species.

3.2.2. Analysis of triterpene glycosides

A number of different classes of triterpene glycosides, based on their aglycone structures, have been identified from *Actaea* species [23,24]. Some of these triterpene glycosides are known to have potent biological activity [25–28]. In our study, seven triterpene glycoside standards (**4**, **7**, **10**, **11**, and **13–15**) were used to aid in the characterization of marker compounds. These standards represent five classes of triterpene glycosides: 16,23-diketo-shengmanol, shengmanol, cimigenol, cimiracerol, and acetetyl-acteol (Table 3) [24].

The highly oxygenated *Actaea* triterpene glycosides are readily decomposed during ionization by the loss of neutral molecules such as water, sugar, acetic acid, and occasionally an acetyl group. The loss of an acetyl group is a rare occurrence usually observed in

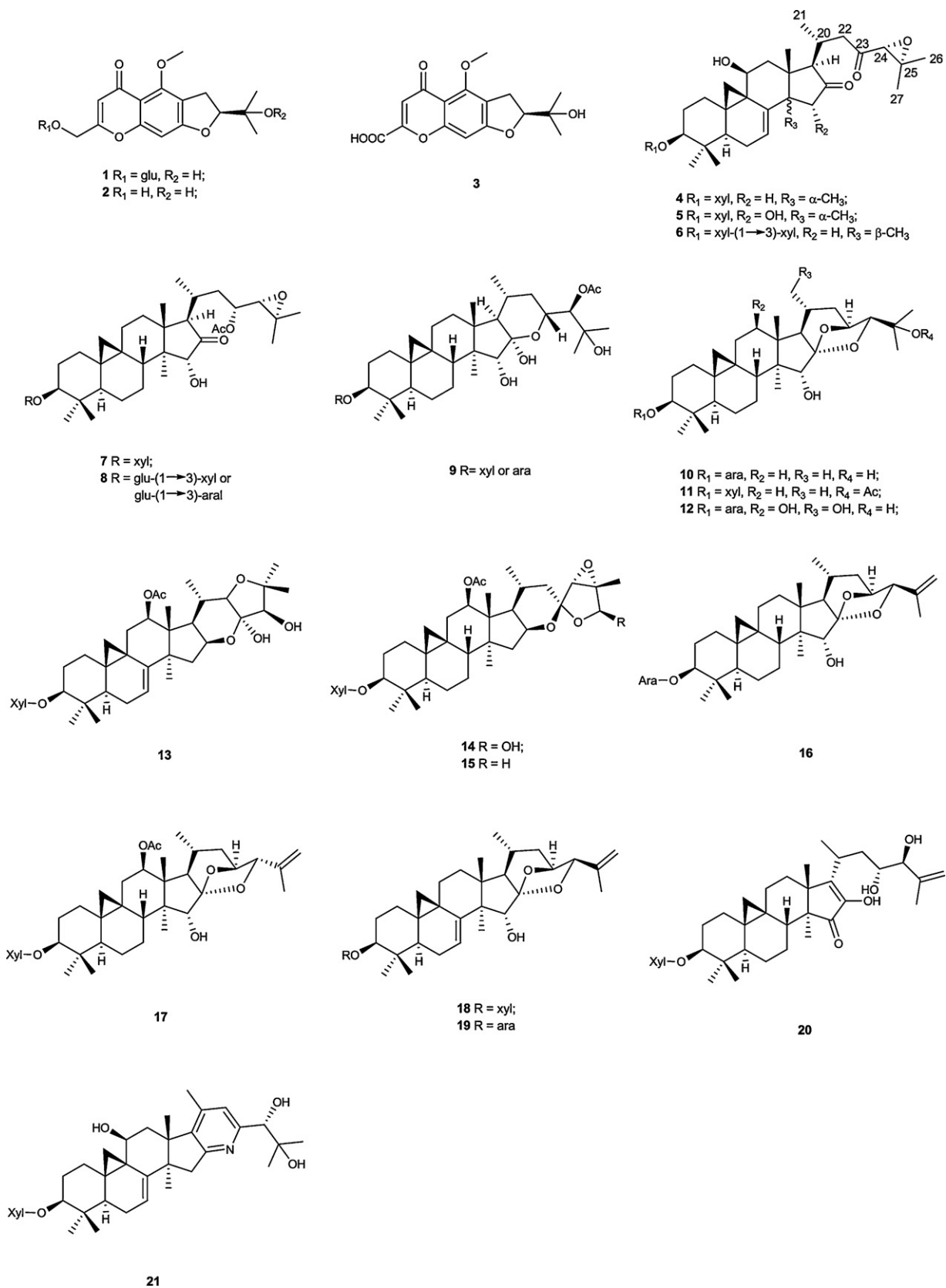


Fig. 3. Chemical structures of standards and identified marker compounds for *Actaea* species.

Table 2

The summary results of the standards and marker compounds analyzed by HPLC–ESI-TOF-MS technique.

No.	RT (min)	Marker ion exact mass (ppm)	Selected fragmental ions exact mass [adduct molecular ions–neutral molecules or radicals] [†] (molecular formula, ppm)	UV (λ_{\max} , nm)	Identification	Type of marker compounds ^a
1	13.44	469.1703 (–1.5) [M+H] ⁺ (C ₂₂ H ₂₉ O ₁₁)	451.1602 [M+H–H ₂ O] ⁺ (C ₂₂ H ₂₇ O ₁₀ , –0.4); 421.1099 [M+H–H ₂ O–2CH ₃] ⁺ (C ₂₀ H ₂₁ O ₁₀ , –8.5); 397.1128 [M+H–2,2-dimethylepoxyethane] ⁺ (C ₁₈ H ₂₁ O ₁₀ , –1.8); 307.1161 [M+H–glucosyl group] ⁺ (C ₁₆ H ₁₉ O ₆ , –6.8); 290.1126 [M+H–glucosyl group–HO] ⁺ (C ₁₆ H ₁₈ O ₅ , –9.6); 289.1060 [M+H–glucosyl group–H ₂ O] ⁺ (C ₁₆ H ₁₇ O ₅ , –5.5); 261.1102 [M+H–glucosyl group–H ₂ O–CO] ⁺ (C ₁₄ H ₁₁ O ₅ , –9.6); 259.0580 [M+H–glucosyl group–H ₂ O–2CH ₃] ⁺ (C ₁₄ H ₁₁ O ₅ , –10.0); 235.0572 [M+H–glucosyl group–2,2-dimethylepoxyethane] ⁺ (C ₁₂ H ₁₁ O ₅ , –14.5)	300.0, 254.2sh, 215.0	Prim-O-glucosylcimifugin	A
2	16.57	307.1160 (–7.2) [M+H] ⁺ (C ₁₆ H ₁₉ O ₆)	289.1068 [M+H–H ₂ O] ⁺ (C ₁₆ H ₁₇ O ₅ , –2.8); 259.0573 [M+H–H ₂ O–2CH ₃] ⁺ (C ₁₄ H ₁₁ O ₅ , –12.7); 235.0569 [M+H–2,2-dimethylepoxyethane] ⁺ (C ₁₂ H ₁₁ O ₅ , –15.7); 217.0465 [M+H–2,2-dimethylepoxyethane–H ₂ O] ⁺ (C ₁₂ H ₉ O ₄ , –16.7); 207.0612 [M+H–2,2-dimethylepoxyethane–CO] ⁺ (C ₁₁ H ₁₁ O ₄ , –21.7)	298.0, 254.3sh, 217.0	Cimifugin	A
3	12.41	321.0953 (–6.5) [M+H] ⁺ (C ₁₆ H ₁₇ O ₇)	303.0852 [M+H–H ₂ O] ⁺ (C ₁₆ H ₁₅ O ₆ , –5.6); 273.0367 [M+H–H ₂ O–2CH ₃] ⁺ (C ₁₄ H ₉ O ₆ , –11.7); 249.0362 [M+H–2,2-dimethylepoxyethane] ⁺ (C ₁₂ H ₉ O ₆ , –14.9); 231.0222 [M+H–2,2-dimethylepoxyethane–H ₂ O] ⁺ (C ₁₂ H ₇ O ₅ , –17.7); 221.0414 [M+H–2,2-dimethylepoxyethane–CO] ⁺ (C ₁₁ H ₉ O ₅ , –16.3)	310.0, 255.0sh, 217.0	Divaricatacid	A
4	38.39	639.3508 (–0.2) [M+Na] ⁺ (C ₃₅ H ₅₂ O ₉ Na)	617.3694 [M+H] ⁺ (C ₃₅ H ₅₂ O ₉ , 0.6); 599.3591 [M+H–H ₂ O] ⁺ (C ₃₅ H ₅₁ O ₈ , 1.2); 577.3390 [M+H–propa-1,2-diene] ⁺ (C ₃₂ H ₄₉ O ₉ , 2.3); 467.3165 [M+H–xylose] ⁺ (C ₃₀ H ₄₃ O ₄ , 0.9); 449.3050 [M+H–xylose–H ₂ O] ⁺ (C ₃₀ H ₄₁ O ₃ , –1.3); 427.2861 [M+H–xylose–propa-1,2-diene] ⁺ (C ₂₇ H ₃₉ O ₄ , 3.0); 395.2580 [M+H–xylose–2,2-dimethylepoxyethane] ⁺ (C ₂₆ H ₃₅ O ₃ , –1.5); 377.2475 [M+H–xylose–H ₂ O–2,2-dimethylepoxyethane] ⁺ (C ₂₆ H ₃₃ O ₂ , –1.6);		Cimicifugoside H-1	Not marker compound
5	28.56	655.3448 (–1.5) [M+Na] ⁺ (C ₃₅ H ₅₂ O ₁₀ Na)	633.3636 [M+H] ⁺ (C ₃₅ H ₅₂ O ₁₀ , –0.5); 615.3523 [M+H–H ₂ O] ⁺ (C ₃₅ H ₅₁ O ₉ , –1.6); 575.3218 [M+H–H ₂ O–propa-1,2-diene] ⁺ (C ₃₂ H ₄₇ O ₉ , –0.3); 561.3074 [M+H–2,2-dimethylepoxyethane] ⁺ (C ₃₁ H ₄₅ O ₉ , 1.8); 483.3104 [M+H–xylose] ⁺ (C ₃₀ H ₄₃ O ₅ , –1.2); 465.3004 [M+H–xylose–H ₂ O] ⁺ (C ₃₀ H ₄₁ O ₄ , –0.2); 447.2879 [M+H–xylose–2H ₂ O] ⁺ (C ₃₀ H ₃₉ O ₃ , –4.5) 443.2796 [M+H–xylose–propa-1,2-diene] ⁺ (C ₂₇ H ₃₉ O ₅ , –0.2); 425.2690 [M+H–xylose–H ₂ O–propa-1,2-diene] ⁺ (C ₂₇ H ₃₇ O ₄ , –0.5); 411.2523 [M+H–xylose–2,2-dimethylepoxyethane] ⁺ (C ₂₆ H ₃₅ O ₄ , –2.9); 393.2419 [M+H–xylose–H ₂ O–2,2-dimethylepoxyethane] ⁺ (C ₂₆ H ₃₃ O ₃ , –2.8); 375.2308 [M+H–xylose–2H ₂ O–2,2-dimethylepoxyethane] ⁺ (C ₂₆ H ₃₁ O ₂ , –4.3);		Cimicifugoside H-5	A
6	35.90	771.3942 (1.3) [M+Na] ⁺ (C ₄₀ H ₆₀ O ₁₃ Na)	749.4130 [M+H] ⁺ (C ₄₀ H ₆₁ O ₁₃ , 2.4); 731.3984 [M+H–H ₂ O] ⁺ (C ₄₀ H ₅₉ O ₁₂ , –3.1); 599.3588 [M+H–xylose] ⁺ (C ₃₅ H ₅₁ O ₈ , 0.7); 559.3263 [M+H–xylose–propa-1,2-diene] ⁺ (C ₃₂ H ₄₇ O ₈ , –1.4); 467.3158 [M+H–2xylose] ⁺ (C ₃₀ H ₄₃ O ₄ , –0.6); 449.3054 [M+H–2xylose–H ₂ O] ⁺ (C ₃₀ H ₄₁ O ₃ , –0.4); 427.2846 [M+H–2xylose–propa-1,2-diene] ⁺ (C ₂₇ H ₃₉ O ₄ , –0.5); 409.2753 [M+H–2xylose–H ₂ O–propa-1,2-diene] ⁺ (C ₂₇ H ₃₇ O ₃ , 2.4); 395.2620 [M+H–2xylose–2,2-dimethylepoxyethane] ⁺ (C ₂₆ H ₃₅ O ₃ , 8.6); 377.2483 [M+H–2xylose–H ₂ O–2,2-dimethylepoxyethane] ⁺ (C ₂₆ H ₃₃ O ₂ , 0.5).		Shengmacichun dixyloside	D
7	48.12	685.3895 (–4.8) [M+Na] ⁺ (C ₃₇ H ₅₈ O ₁₀ Na)	663.4145 [M+H] ⁺ (C ₃₇ H ₅₉ O ₁₀ , 5.6); 645.3975 [M+H–H ₂ O] ⁺ (C ₃₇ H ₅₇ O ₉ , –4.3); 603.3932 [M+H–acetic acid] ⁺ (C ₃₅ H ₅₅ O ₈ , 5.8); 585.3770 [M+H–acetic acid–H ₂ O] ⁺ (C ₃₅ H ₅₃ O ₇ , –3.6); 545.3511 [M+H–acetic acid–H ₂ O–propa-1,2-diene] ⁺ (C ₃₂ H ₄₉ O ₇ , 6.1); 531.3333 [M+H–acetic acid–2,2-dimethylepoxyethane] ⁺ (C ₃₁ H ₄₇ O ₇ , 2.1); 513.3542 [M+H–xylose] ⁺ (C ₃₂ H ₄₉ O ₅ , –7.4); 495.3451 [M+H–xylose–H ₂ O] ⁺ (C ₃₂ H ₄₇ O ₄ , 4.6); 453.3370 [M+H–xylose–acetic acid] ⁺ (C ₃₀ H ₄₅ O ₃ , 0.2); 435.3266 [M+H–xylose–acetic acid–H ₂ O] ⁺ (C ₃₀ H ₄₃ O ₂ , 0.7); 413.3070 [M+H–xylose–acetic acid–propa-1,2-diene] ⁺ (C ₂₇ H ₄₁ O ₃ , 3.4); 395.2958 [M+H–xylose–acetic acid–H ₂ O–propa-1,2-diene] ⁺ (C ₂₇ H ₃₉ O ₂ , 2.0); 381.2799 [M+H–xylose–acetic acid–2,2-dimethylepoxyethane] ⁺ (C ₂₆ H ₃₇ O ₂ , 1.3); 363.2690 [M+H–xylose–acetic acid–H ₂ O–2,2-dimethylepoxyethane] ⁺ (C ₂₆ H ₃₅ O, 0.6).		23-O-Acetylshengmanol 3-O-β-D-xylopyranoside	Not marker compound
8	41.59	847.4446 (–1.2) [M+Na] ⁺ (C ₄₃ H ₆₈ O ₁₅ Na)	825.4619 [M+H] ⁺ (C ₄₃ H ₆₉ O ₁₅ , –2.1); 807.4501 [M+H–H ₂ O] ⁺ (C ₄₃ H ₆₇ O ₁₄ , –3.7); 663.4095 [M+H–glucosyl group] ⁺ (C ₃₇ H ₅₉ O ₁₀ , –2.0); 645.4001 [M+H–glucosyl group–H ₂ O] ⁺ (C ₃₇ H ₅₇ O ₉ , –0.3); 513.3581 [M+H–glucosyl group–xylose or arabinose] ⁺ (C ₃₂ H ₄₉ O ₅ , 0.2); 495.3475 [M+H–glucosyl group–xylose or arabinose–H ₂ O] ⁺ (C ₃₂ H ₄₇ O ₄ , 0.2); 471.3484 [M+H–glucosyl group–xylose or arabinose–Ac] ⁺ (C ₃₀ H ₄₇ O ₄ , 2.1); 453.3388 [M+H–glucosyl group–xylose or arabinose–acetic acid] ⁺ (C ₃₀ H ₄₅ O ₃ , 4.2); 435.3284 [M+H–glucosyl group–xylose or arabinose–acetic acid–H ₂ O] ⁺ (C ₃₀ H ₄₃ O ₂ , 4.8); 431.3179 [M+H–glucosyl group–xylose or arabinose–Ac–propa-1,2-diene] ⁺ (C ₂₇ H ₄₃ O ₄ , 4.2); 413.3058 [M+H–glucosyl group–xylose or arabinose–acetic acid–propa-1,2-diene] ⁺ (C ₂₇ H ₄₁ O ₃ , 0.5); 381.2804 [M+H–glucosyl group–xylose or arabinose–acetic acid–2,2-dimethylepoxyethane] ⁺ (C ₂₆ H ₃₇ O ₂ , 2.6); 363.2682 [M+H–glucosyl group–xylose or arabinose–acetic acid–H ₂ O–2,2-dimethylepoxyethane] ⁺ (C ₂₆ H ₃₅ O, –1.7)		23-O-Acetylshengmanol 3-O-β-D-glucopyranoside (1–3)-β-D-xylopyranoside or arabinoside	D

Table 2 (Continued)

No.	RT (min)	Marker ion exact mass (ppm)	Selected fragmental ions exact mass [adduct molecular ions–neutral molecules or radicals] ⁺ (molecular formula, ppm)	UV (λ_{\max} , nm)	Identification	Type of marker compounds ^a
9	32.92	703.4042 (1.3) [M+Na] ⁺ (C ₃₇ H ₆₀ O ₁₁ Na)	663.4106 [M+H–H ₂ O] ⁺ (C ₃₇ H ₅₉ O ₁₀ , –0.5); 645.4000 (C ₃₇ H ₅₇ O ₉ , –0.8); 621.4014 [M+H–acetic acid] ⁺ (C ₃₅ H ₅₇ O ₉ , 2.9); 603.3905 [M+H–acetic acid–H ₂ O] ⁺ (C ₃₅ H ₅₅ O ₈ , 2.2); 585.3795 [M+H–acetic acid–2H ₂ O] ⁺ (C ₃₅ H ₅₃ O ₇ , 1.2); 563.3603 [M+H–acetic acid–H ₂ O–propa-1,2-diene] ⁺ (C ₃₂ H ₅₁ O ₈ , 6.0); 531.3677 [M+H–xylose or arabinose] ⁺ (C ₃₂ H ₅₁ O ₆ , –3.2); 513.3572 [M+H–xylose or arabinose–H ₂ O] ⁺ (C ₃₂ H ₄₉ O ₅ , –3.1); 471.3472 [M+H–xylose or arabinose–acetic acid] ⁺ (C ₃₀ H ₄₇ O ₄ , –0.8); 453.3365 [M+H–xylose or arabinose–H ₂ O–acetic acid] ⁺ (C ₃₀ H ₄₅ O ₃ , –2.0); 435.3264 [M+H–xylose or arabinose–2H ₂ O–acetic acid] ⁺ (C ₃₀ H ₄₃ O ₂ , 0.4); 399.2909 [M+H–xylose or arabinose–acetic acid–2,2-dimethylepoxyethane] ⁺ (C ₂₆ H ₃₉ O ₃ , 6.2); 395.2963 [M+H–xylose or arabinose–2H ₂ O–acetic acid–propa-1,2-diene] ⁺ (C ₂₇ H ₃₉ O ₂ , 8.3); 381.2785 [M+H–xylose or arabinose–H ₂ O–acetic acid–2,2-dimethylepoxyethane] ⁺ (C ₂₆ H ₃₇ O ₂ , –6.3); 363.2677 [M+H–xylose or arabinose–2H ₂ O–acetic acid–2,2-dimethylepoxyethane] ⁺ (C ₂₆ H ₃₅ O, –8.3)		24-O-Acetylhydroshengmanol-3-O-β-D-xylopyranoside or arabinoside	C
10	60.93	643.3813 (1.4) [M+Na] ⁺ (C ₃₅ H ₅₆ O ₉ Na)	621.4022 [M+H] ⁺ (C ₃₅ H ₅₇ O ₉ , 3.1); 603.3898 [M+H–H ₂ O] ⁺ (C ₃₅ H ₅₅ O ₈ , –0.2); 585.3795 [M+H–2H ₂ O] ⁺ (C ₃₅ H ₅₃ O ₇ , 0.7); 531.3356 [M+H–H ₂ O–2,2-dimethylepoxyethane] ⁺ (C ₃₁ H ₄₇ O ₇ , 6.4); 471.3481 [M+H–arabinose] ⁺ (C ₃₀ H ₄₇ O ₄ , 1.5); 453.3388 [M+H–arabinose–H ₂ O] ⁺ (C ₃₀ H ₄₅ O ₃ , 4.2); 435.3286 [M+H–arabinose–2H ₂ O] ⁺ (C ₃₀ H ₄₃ O ₂ , 5.3); 417.3160 [M+H–arabinose–3H ₂ O] ⁺ (C ₃₀ H ₄₁ O, –1.7); 381.2786 [M+H–arabinose–H ₂ O–2,2-dimethylepoxyethane] ⁺ (C ₂₆ H ₃₇ O ₂ , –2.1); 363.2683 [M+H–arabinose–2H ₂ O–2,2-dimethylepoxyethane] ⁺ (C ₂₆ H ₃₅ O, –1.4)		Cimiracemoside C	C
11	78.81	685.3916 (–1.8) [M+Na] ⁺ (C ₃₇ H ₅₈ O ₁₀ Na)	663.4117 [M+H] ⁺ (C ₃₇ H ₅₉ O ₁₀ , 1.4); 645.4024 [M+H–H ₂ O] ⁺ (C ₃₇ H ₅₇ O ₉ , 3.3); 603.3891 [M+H–acetic acid] ⁺ (C ₃₅ H ₅₅ O ₈ , –1.0); 585.3789 [M+H–acetic acid–H ₂ O] ⁺ (C ₃₅ H ₅₃ O ₇ , –0.3); 513.3586 [M+H–xylose] ⁺ (C ₃₂ H ₄₉ O ₅ , 1.2); 495.3496 [M+H–xylose–H ₂ O] ⁺ (C ₃₂ H ₄₇ O ₄ , 4.4); 453.3366 [M+H–xylose–acetic acid] ⁺ (C ₃₀ H ₄₅ O ₃ , –0.7); 435.3258 [M+H–xylose–acetic acid–H ₂ O] ⁺ (C ₃₀ H ₄₃ O ₂ , –1.1); 381.2778 [M+H–xylose–acetic acid–2,2-dimethylepoxyethane] ⁺ (C ₂₆ H ₃₇ O ₂ , –4.2); 363.2677 [M+H–xylose–acetic acid–H ₂ O–2,2-dimethylepoxyethane] ⁺ (C ₂₆ H ₃₅ O, –3.0).		25-O-Acetylcimigenol 3-O-β-D-xylopyranoside	Not marker compound
12	22.41	675.3727 (1.0) [M+Na] ⁺ (C ₃₅ H ₅₆ O ₁₁ Na)	635.3799 [M+H–H ₂ O] ⁺ (C ₃₅ H ₅₅ O ₁₀ , 0.6); 617.3694 [M+H–2H ₂ O] ⁺ (C ₃₅ H ₅₃ O ₉ , 0.6); 599.3583 [M+H–3H ₂ O] ⁺ (C ₃₅ H ₅₁ O ₈ , –0.2); 581.3478 [M+H–4H ₂ O] ⁺ (C ₃₅ H ₄₉ O ₇ , 0.0); 545.3130 [M+H–2H ₂ O–2,2-dimethylepoxyethane] ⁺ (C ₃₁ H ₄₅ O ₈ , 2.9); 503.3369 [M+H–arabinose] ⁺ (C ₃₀ H ₄₇ O ₆ , –0.8); 485.3271 [M+H–arabinose–H ₂ O] ⁺ (C ₃₀ H ₄₅ O ₅ , 0.8); 467.3165 [M+H–arabinose–2H ₂ O] ⁺ (C ₃₀ H ₄₃ O ₄ , 0.9); 449.3062 [M+H–arabinose–3H ₂ O] ⁺ (C ₃₀ H ₄₁ O ₃ , 1.3); 413.2722 [M+H–arabinose–H ₂ O–2,2-dimethylepoxyethane] ⁺ (C ₂₆ H ₃₇ O ₄ , 7.3); 395.2588 [M+H–arabinose–2H ₂ O–2,2-dimethylepoxyethane] ⁺ (C ₂₆ H ₃₅ O ₃ , 0.5); 377.2475 [M+H–arabinose–3H ₂ O–2,2-dimethylepoxyethane] ⁺ (C ₂₆ H ₃₃ O ₂ , –1.6)		12β,21-Dihydroxycimigenol-3-O-L-arabinoside	C
13	36.65	677.3904 (0.4) [M+H] ⁺ (C ₃₇ H ₅₇ O ₁₁)	659.3799 [M+H–H ₂ O] ⁺ (C ₃₇ H ₅₅ O ₁₀ , 0.6); 617.3688 [M+H–acetic acid] ⁺ (C ₃₅ H ₅₃ O ₉ , –0.4); 599.3578 [M+H–H ₂ O–acetic acid] ⁺ (C ₃₅ H ₅₁ O ₈ , –1.0); 581.3455 [M+H–2H ₂ O–acetic acid] ⁺ (C ₃₅ H ₄₉ O ₇ , –4.0); 527.3363 [M+H–xylose] ⁺ (C ₃₂ H ₄₇ O ₆ , –1.9); 467.3164 [M+H–xylose–acetic acid] ⁺ (C ₃₀ H ₄₃ O ₄ , 0.6); 449.3047 [M+H–xylose–acetic acid–H ₂ O] ⁺ (C ₃₀ H ₄₁ O ₃ , –2.0); 431.2951 [M+H–xylose–acetic acid–2H ₂ O] ⁺ (C ₃₀ H ₃₉ O ₂ , 0.2); 395.2586 [M+H–xylose–acetic acid–2,2-dimethylepoxyethane] ⁺ (C ₂₆ H ₃₅ O ₃ , 0.0); 377.2465 [M+H–xylose–acetic acid–H ₂ O–2,2-dimethylepoxyethane] ⁺ (C ₂₆ H ₃₃ O ₂ , –4.2); 359.2328 [M+H–xylose–acetic acid–2H ₂ O–2,2-dimethylepoxyethane] ⁺ (C ₂₆ H ₃₁ O, –13.1)		Cimiracemoside A	Not marker compound
14	47.29	677.3923 (3.2) [M+H] ⁺ (C ₃₇ H ₅₇ O ₁₁)	659.3782 [M+H–H ₂ O] ⁺ (C ₃₇ H ₅₅ O ₁₀ , –2.0); 617.3701 [M+H–acetic acid] ⁺ (C ₃₅ H ₅₃ O ₉ , 1.8); 599.3586 [M+H–H ₂ O–acetic acid] ⁺ (C ₃₅ H ₅₁ O ₈ , 0.3); 581.3487 [M+H–2H ₂ O–acetic acid] ⁺ (C ₃₅ H ₄₉ O ₇ , 1.5); 527.3391 [M+H–xylose] ⁺ (C ₃₂ H ₄₇ O ₆ , 3.4); 467.3171 [M+H–xylose–acetic acid] ⁺ (C ₃₀ H ₄₃ O ₄ , 2.1); 449.3063 [M+H–xylose–acetic acid–H ₂ O] ⁺ (C ₃₀ H ₄₁ O ₃ , 1.6); 431.2971 [M+H–xylose–acetic acid–2H ₂ O] ⁺ (C ₃₀ H ₃₉ O ₂ , 4.9)		Actein	Not marker compound
15	44.42	661.3952 (0.0) [M+H] ⁺ (C ₃₇ H ₅₇ O ₁₀)	643.3864 [M+H–H ₂ O] ⁺ (C ₃₇ H ₅₅ O ₉ , 2.8); 601.3751 [M+H–acetic acid] ⁺ (C ₃₅ H ₅₃ O ₈ , 1.8); 583.3648 [M+H–H ₂ O–acetic acid] ⁺ (C ₃₅ H ₅₁ O ₇ , 2.2); 565.3551 [M+H–2H ₂ O–acetic acid] ⁺ (C ₃₅ H ₄₉ O ₆ , 3.9); 511.3448 [M+H–xylose] ⁺ (C ₃₂ H ₄₇ O ₅ , 4.9); 451.3210 [M+H–xylose–acetic acid] ⁺ (C ₃₀ H ₄₃ O ₃ , –0.4); 433.3120 [M+H–xylose–acetic acid–H ₂ O] ⁺ (C ₃₀ H ₄₁ O ₂ , 3.0); 415.3036 [M+H–xylose–acetic acid–2H ₂ O] ⁺ (C ₃₀ H ₃₉ O, 8.4)		26-deoxyactein	Not marker compound
16	52.84	625.3726 (1.6) [M+Na] ⁺ (C ₃₅ H ₅₄ O ₈ Na)	603.3911 [M+H] ⁺ (C ₃₅ H ₅₅ O ₈ , 2.3); 585.3788 [M+H–H ₂ O] ⁺ (C ₃₅ H ₅₃ O ₇ , –0.5); 561.3434 [M+H–H ₂ O–propene] ⁺ (C ₃₂ H ₄₉ O ₈ , 1.2); 453.3376 [M+H–arabinose] ⁺ (C ₃₀ H ₄₅ O ₃ , 1.5); 435.3236 [M+H–arabinose–H ₂ O] ⁺ (C ₃₀ H ₄₃ O ₂ , –6.2); 411.2885 [M+H–arabinose–propene] ⁺ (C ₂₇ H ₃₉ O ₃ , –3.4); 397.2731 [M+H–arabinose–2-methylpropene] ⁺ (C ₂₆ H ₃₇ O ₃ , –3.0); 393.2791 [M+H–arabinose–H ₂ O–propene] ⁺ (C ₂₇ H ₃₇ O ₂ , –0.8); 379.2625 [M+H–arabinose–H ₂ O–2-methylpropene] ⁺ (C ₂₆ H ₃₅ O ₂ , –3.2)		(23R,24S)-25-anhydrocimigenol-3-O-α-L-arabinoside	D

17	42.16	683.3776 (0.7) [M+Na] ⁺ (C ₃₇ H ₅₆ O ₁₀ Na)	661.3954 [M+H] ⁺ (C ₃₇ H ₅₇ O ₁₀ , 0.3); 601.3738 [M+H-acetic acid] ⁺ (C ₃₅ H ₅₁ O ₇ , 2.5); 583.3615 [M+H-acetic acid-H ₂ O] ⁺ (C ₃₅ H ₅₃ O ₈ , -0.3); 559.3281 [M+H-acetic acid-propene] ⁺ (C ₃₂ H ₄₇ O ₈ , 1.8); 511.3444 [M+H-xylose] ⁺ (C ₃₂ H ₄₇ O ₅ , 3.9); 469.3323 [M+H-xylose-Ac] ⁺ (C ₃₀ H ₄₅ O ₄ , 1.1); 451.3189 [M+H-xylose-acetic acid] ⁺ (C ₃₀ H ₄₃ O ₃ , -5.1); 433.3082 3189 [M+H-xylose-acetic acid-H ₂ O] ⁺ (C ₃₀ H ₄₁ O ₂ , -5.8); 427.2848 [M+H-xylose-Ac-propene] ⁺ (C ₂₇ H ₃₉ O ₄ , 0.0); 409.2731 [M+H-xylose-acetic acid-propene] ⁺ (C ₂₇ H ₃₇ O ₃ , -2.9); 395.2586 [M+H-xylose-acetic acid-2-methylpropene] ⁺ (C ₂₆ H ₃₅ O ₃ , 0.0); 377.2473 [M+H-xylose-acetic acid-H ₂ O-2-methylpropene] ⁺ (C ₂₆ H ₃₃ O ₂ , -2.1)	Cimiracemoside K	D
18	57.91	623.3569 (1.4) [M+Na] ⁺ (C ₃₅ H ₅₂ O ₈ Na)	601.3752 [M+H] ⁺ (C ₃₅ H ₅₃ O ₈ , 2.0); 583.3650 [M+H-H ₂ O] ⁺ (C ₃₅ H ₅₁ O ₇ , 2.6); 559.3271 [M+H-propene] ⁺ (C ₃₂ H ₄₇ O ₈ , 0.0); 545.3136 [M+H-2-methylpropene] ⁺ (C ₃₁ H ₄₅ O ₈ , 4.0); 527.2999 [M+H-H ₂ O-2-methylpropene] ⁺ (C ₃₁ H ₄₃ O ₇ , -1.9); 469.3320 [M+H-xylosyl group] ⁺ (C ₃₀ H ₄₅ O ₄ , 0.4); 451.3220 [M+H-xylose] ⁺ (C ₃₀ H ₄₃ O ₃ , 1.8); 433.3062 [M+H-xylose-H ₂ O] ⁺ (C ₃₀ H ₄₁ O ₂ , -10.4); 427.2848 [M+H-xylosyl group-propene] ⁺ (C ₂₇ H ₃₉ O ₄ , 0.5); 409.2722 [M+H-xylose-propene] ⁺ (C ₂₇ H ₃₇ O ₃ , -5.1); 395.2585 [M+H-xylose-2-methylpropene] ⁺ (C ₂₆ H ₃₅ O ₃ , -0.5); 377.2474 [M+H-xylose-H ₂ O-2-methylpropene] ⁺ (C ₂₆ H ₃₃ O ₂ , -1.9);	(23R,24R)-7-en-25-anhydrocimigenol-3-O-β-D-xylopyranoside	D
19	53.99	623.3569 (1.4) [M+Na] ⁺ (C ₃₅ H ₅₂ O ₈ Na)	601.3745 [M+H] ⁺ (C ₃₅ H ₅₃ O ₈ , 0.8); 583.3651 [M+H-H ₂ O] ⁺ (C ₃₅ H ₅₁ O ₇ , 2.7); 559.3276 [M+H-propene] ⁺ (C ₃₂ H ₄₇ O ₈ , 0.9); 527.3028 [M+H-H ₂ O-2-methylpropene] ⁺ (C ₃₁ H ₄₃ O ₇ , 3.6); 469.3314 [M+H-arabinosyl group] ⁺ (C ₃₀ H ₄₅ O ₄ , -0.9); 451.3222 [M+H-arabinose] ⁺ (C ₃₀ H ₄₃ O ₃ , 2.2); 433.3093 [M+H-arabinose-H ₂ O] ⁺ (C ₃₀ H ₄₁ O ₂ , -3.2); 427.2842 [M+H-arabinosyl group-propene] ⁺ (C ₂₇ H ₃₉ O ₄ , -0.9); 409.2733 [M+H-arabinose-H ₂ O-propene] ⁺ (C ₂₇ H ₃₇ O ₃ , -2.4); 395.2586 [M+H-arabinose-H ₂ O-2-methylpropene] ⁺ (C ₂₆ H ₃₅ O ₃ , -0.3); 377.2469 [M+H-arabinose-2H ₂ O-2-methylpropene] ⁺ (C ₂₆ H ₃₃ O ₂ , -3.2);	(23R,24R)-7-en-25-anhydrocimigenol-3-O-α-L-arabinoside	D
20	36.75	643.3842 (3.1) [M+Na] ⁺ (C ₃₅ H ₅₆ O ₉ Na)	621.4006 [M+H] ⁺ (C ₃₅ H ₅₇ O ₉ , 0.5); 603.3908 [M+H-H ₂ O] ⁺ (C ₃₅ H ₅₅ O ₈ , 1.5); 585.3798 [M+H-2H ₂ O] ⁺ (C ₃₅ H ₅₃ O ₇ , 1.2); 579.3531 [M+H-propene] ⁺ (C ₃₂ H ₅₁ O ₉ , -0.3); 561.3434 [M+H-H ₂ O-propene] ⁺ (C ₃₂ H ₄₉ O ₈ , 1.2); 471.3478 [M+H-xylose] ⁺ (C ₃₀ H ₄₇ O ₄ , 0.8); 453.3381 [M+H-xylose-H ₂ O] ⁺ (C ₃₀ H ₄₅ O ₃ , 2.6); 435.3264 [M+H-xylose-2H ₂ O] ⁺ (C ₃₀ H ₄₃ O ₂ , 0.2); 429.2993 [M+H-xylose-propene] ⁺ (C ₂₇ H ₄₁ O ₄ , -0.7); 411.2888 [M+H-xylose-H ₂ O-propene] ⁺ (C ₂₇ H ₃₉ O ₃ , -2.7); 397.2745 [M+H-xylose-H ₂ O-2-methylpropene] ⁺ (C ₂₆ H ₃₇ O ₃ , 0.5); 379.2635 [M+H-xylose-2H ₂ O-2-methylpropene] ⁺ (C ₂₆ H ₃₅ O ₂ , 0.5)	Cimidahuside G	D
21	17.55	614.3679 (-2.3) [M+H] ⁺ (C ₃₅ H ₅₂ NO ₈)	596.3611 [M+H-H ₂ O] ⁺ (C ₃₅ H ₅₀ NO ₇ , 4.0); 578.3476 [M+H-2H ₂ O] ⁺ (C ₃₅ H ₄₈ NO ₆ , -1.0); 560.3345 [M+H-3H ₂ O] ⁺ (C ₃₅ H ₄₆ NO ₅ , -5.5); 556.3260 [M+H-H ₂ O-propa-1,2-diene] ⁺ (C ₃₂ H ₄₆ NO ₇ , -2.5); 464.3148 [M+H-xylose] ⁺ (C ₃₀ H ₄₂ NO ₃ , -3.7); 446.3062 [M+H-xylose-H ₂ O] ⁺ (C ₃₀ H ₄₀ NO ₂ , 0.7); 428.2952 [M+H-xylose-2H ₂ O] ⁺ (C ₃₀ H ₃₈ NO, -0.2)	Cimicifine A (syn. Cimicifugadine)	D

^a A: marker compounds for species other than *Actaea racemosa*; B: marker compounds for Asian species; C: marker compounds for *Actaea racemosa*; D: marker compounds for North American species other than *Actaea racemosa*.

Table 3

The characteristic molecular ions and fragmental cleavages in each standard and marker compounds in chromatogram.

No.	Aglycone class	Abundance of molecular ions relative to base peak (%) ^a	Base peak (aperture voltage: 0V)	Fragmental Base peak (aperture voltage: 60 V)	Aperture voltage: 60 V ^b		Aperture voltage: 0 V ^c	
					Characteristic fragmental ions [MW (MF, ppm) (AMBPP)] produced by loss of C ₄ H ₈ O ^d	Characteristic fragmental ions [MW (MF, ppm) (AMBPP)] produced by loss of C ₄ H ₈ ^d	Characteristic fragmental ions [MW (MF, ppm) (AIFBP)] produced by loss of C ₃ H ₄ ^d	Characteristic fragmental ions [MW (MF, ppm) (AIFBP)] produced by loss of C ₃ H ₄ ^d
4	16,23-Diketo-shengmanol	[M+Na] ⁺ 639.3508 (41) [M+H] ⁺ 617.3694 (7)	577.3390 (C ₃₂ H ₄₉ O ₉ , [M+H-propa-1,2-diene] ⁺)	395.2580 (C ₂₆ H ₃₅ O ₃ , [M+H-xylose-2,2-dimethyl epoxyethane] ⁺)	467.3132 (C ₃₀ H ₄₃ O ₄ , -6.2) (13) → 395.2580 (C ₂₆ H ₃₅ O ₃ , -1.5) (100); 449.3050 (C ₃₀ H ₄₁ O ₃ , -1.3) (19) → 377.2475 (C ₂₆ H ₃₃ O ₂ , -1.6) (78);	No	617.3694 (C ₃₅ H ₅₃ O ₉ , 0.6) (7) → 577.3390 (C ₃₂ H ₄₉ O ₉ , 2.2) (100); 467.3165 (C ₃₀ H ₄₃ O ₄ , 0.9) (6) → 427.2861 (C ₂₇ H ₃₉ O ₄ , 3.0) (53);	No
5	16,23-Diketo-shengmanol	[M+Na] ⁺ 655.3448 (100) [M+HM+H] ⁺ 633.3636 (4)	655.3448 (C ₃₅ H ₅₂ O ₁₀ Na, [M+Na] ⁺)	393.2419 (C ₂₆ H ₃₃ O ₃ , [M+H-xylose-H ₂ O-2,2-dimethyl epoxyethane] ⁺)	483.3077 (C ₃₀ H ₄₃ O ₅ , -6.8) (12) → 411.2523 (C ₂₆ H ₃₅ O ₄ , -2.9) (40); 465.2991 (C ₃₀ H ₄₁ O ₄ , -3.0) (24) → 393.2419 (C ₂₆ H ₃₃ O ₃ , -2.8) (100); 447.2879 (C ₃₀ H ₃₉ O ₃ , -4.8) (11) → 375.2308 (C ₂₆ H ₃₁ O ₂ , -4.3) (34);	No	615.3523 (C ₃₅ H ₅₁ O ₉ , -1.6) (9) → 575.3218 (C ₃₂ H ₄₇ O ₉ , -0.3) (5); 483.3104 (C ₃₀ H ₄₃ O ₅ , -1.2) (17) → 443.2796 (C ₂₇ H ₃₉ O ₅ , -0.2) (13); 465.3004 (C ₃₀ H ₄₁ O ₄ , -0.2) (9) → 425.2690 (C ₂₇ H ₃₇ O ₄ , -0.5) (7);	No
6	16,23-Diketo-shengmanol	[M+Na] ⁺ 771.3942 (100) [M+H] ⁺ 749.4130 (4)	771.3942 (C ₄₀ H ₆₀ O ₁₃ Na, [M+Na] ⁺)	377.2483 (C ₂₆ H ₃₃ O ₂ , [M+H-2xylose-H ₂ O-2-methylprop-1-ene] ⁺)	467.3116 (C ₃₀ H ₄₃ O ₄ , -9.6) (30) → 395.2620 (C ₂₆ H ₃₅ O ₃ , 8.6) (77); 449.3036 (C ₃₀ H ₄₁ O ₃ , -4.5) (52) → 377.2483 (C ₂₆ H ₃₃ O ₂ , 0.5) (100);	No	599.3588 (C ₃₅ H ₅₁ O ₈ , 0.7) (12) → 559.3263 (C ₃₂ H ₄₇ O ₈ , -1.4) (2); 467.3158 (C ₃₀ H ₄₃ O ₄ , -0.6) (13) → 427.2846 (C ₂₇ H ₃₉ O ₄ , -0.5) (7); 449.3054 (C ₃₀ H ₄₁ O ₃ , -0.4) (6) → 409.2753 (C ₂₇ H ₃₇ O ₃ , 2.4) (5);	No
7	Shengmanol	[M+Na] ⁺ 685.3895 (100) [M+H] ⁺ 663.4145 (12)	685.3895 (C ₃₇ H ₅₈ O ₁₀ Na, [M+Na] ⁺)	435.3266 (C ₃₀ H ₄₃ O ₂ , [M+H-xylose-acetic acid-H ₂ O] ⁺)	453.3370 (C ₃₀ H ₄₅ O ₃ , 0.2) (63) → 381.2799 (C ₂₆ H ₃₇ O ₂ , 1.3) (76); 435.3266 (C ₃₀ H ₄₃ O ₂ , 0.7) (100) → 363.2690 (C ₂₆ H ₃₅ O, 0.6) (67);	No	585.3770 (C ₃₅ H ₅₃ O ₇ , -3.6) (20) → 545.3511 (C ₃₂ H ₄₉ O ₇ , 6.1) (6); 453.3338 (C ₃₀ H ₄₅ O ₃ , -6.8) (73) → 413.3070 (C ₂₇ H ₄₁ O ₃ , 3.4) (9); 435.3239 (C ₃₀ H ₄₃ O ₂ , -5.5) (37) → 395.2958 (C ₂₇ H ₃₉ O ₂ , 2.0) (5);	No
8	Shengmanol	[M+Na] ⁺ 847.4446 (100) [M+H] ⁺ 825.4619 (8)	847.4446 (C ₄₃ H ₆₈ O ₁₅ Na, [M+Na] ⁺)	747.4294 (C ₄₁ H ₆₃ O ₁₂ , [M+H-H ₂ O-acetic acid] ⁺)	453.3388 (C ₃₀ H ₄₅ O ₃ , 4.2) (51) → 381.2804 (C ₂₆ H ₃₇ O ₂ , 2.6) (37); 435.3284 (C ₃₀ H ₄₃ O ₂ , 4.8) (68) → 363.2682 (C ₂₆ H ₃₅ O, -1.7) (17); 471.3472 (C ₃₀ H ₄₇ O ₄ , -0.4) (8) → 399.2929 (C ₂₆ H ₃₉ O ₃ , 7.5) (16);	No	471.3484 (C ₃₀ H ₄₇ O ₄ , 2.1) (8) → 431.3179 (C ₂₇ H ₄₃ O ₄ , 4.2) (2); 453.3375 (C ₃₀ H ₄₅ O ₃ , 1.3) (27) → 413.3059 (C ₂₇ H ₄₁ O ₃ , 0.7) (5); 603.3905 (C ₃₅ H ₅₅ O ₈ , 1.3) (9) → 563.3603 (C ₃₂ H ₅₁ O ₈ , 3.4) (2); 453.3380 (C ₃₀ H ₄₅ O ₃ , 2.4) (24) → 413.3064 (C ₂₇ H ₄₁ O ₃ , 1.9) (5); 435.3276 (C ₃₀ H ₄₃ O ₂ , 3.0) (6) → 395.2963 (C ₂₇ H ₃₉ O ₂ , 3.3) (1);	No
9	Hydro-shengmanol	[M+Na] ⁺ 703.4042 (100) [M+H] ⁺ 681.4261 (2)	703.4042 (C ₃₇ H ₆₀ O ₁₁ Na, [M+Na] ⁺)	643.3813 (C ₃₅ H ₅₆ O ₉ Na, [M+Na-acetic acid] ⁺)	453.3365 (C ₃₀ H ₄₅ O ₃ , -0.9) (64) → 381.2785 (C ₂₆ H ₃₇ O ₂ , -2.4) (39); 435.3264 (C ₃₀ H ₄₃ O ₂ , 0.2) (83) → 363.2677 (C ₂₆ H ₃₅ O, -3.0) (32);	No		No

10	Cimigenol	[M+Na] ⁺ 643.3813 (100) [M+H] ⁺ 621.4022 (7)	643.3813 (C ₃₅ H ₅₆ O ₉ Na, [M+Na] ⁺)	453.3388 (C ₃₀ H ₄₅ O ₃ , [M+H–arabinose–H ₂ O] ⁺)	603.3914 (C ₃₅ H ₅₅ O ₈ , 2.8) (32) → 531.3356 (C ₃₁ H ₄₇ O ₇ , 6.4) (5); 453.3388 (C ₃₀ H ₄₅ O ₃ , 4.2) (100) → 381.2786 (C ₂₆ H ₃₇ O ₂ , –2.1) (25); 435.3286 (C ₃₀ H ₄₃ O ₂ , 5.3) (83) → 363.2683 (C ₂₆ H ₃₅ O, –1.4) (22);	No	No	No
11	Cimigenol	[M+Na] ⁺ 685.3931 (80) [M+H] ⁺ 663.4052 (8)	453.3376 (C ₃₀ H ₄₅ O ₃ , [M+H–xylose–acetic acid] ⁺)	453.3376 (C ₃₀ H ₄₅ O ₃ , [M+H–xylose–acetic acid] ⁺)	453.3366 (C ₃₀ H ₄₅ O ₃ , –0.7) (78) → 381.2778 (C ₂₆ H ₃₇ O ₂ , –4.2) (24); 435.3258 (C ₃₀ H ₄₃ O ₂ , –1.1) (100) → 363.2677 (C ₂₆ H ₃₅ O, –3.0) (20);	No	No	No
12	Cimigenol	[M+Na] ⁺ 675.3727 (100) [M+H] ⁺ 653.3916 (3)	675.3727 (C ₃₅ H ₅₆ O ₁₁ Na, [M+Na] ⁺)	395.2588 (C ₂₆ H ₃₅ O ₃ , [M+H–arabinose–2H ₂ O –2,2-dimethyl epoxyethane] ⁺)	485.3271 (C ₃₀ H ₄₅ O ₅ , 0.8) (17) → 413.2722 (C ₂₆ H ₃₇ O ₄ , 7.3) (29); 467.3165 (C ₃₀ H ₄₃ O ₄ , 0.9) (31) → 395.2588 (C ₂₆ H ₃₅ O ₃ , 0.5) (100); 449.3062 (C ₃₀ H ₄₁ O ₃ , 1.3) (53) → 377.2475 (C ₂₆ H ₃₃ O ₂ , –1.6) (80);	No	No	No
13	Cimiracerol	[M+Na] ⁺ 699.3719 (1) [M+H] ⁺ 677.3901 (2)	659.3799 (C ₃₇ H ₅₅ O ₁₀ , [M+H–H ₂ O] ⁺)	449.3047 (C ₃₇ H ₅₅ O ₁₀ , [M+H–xylose–acetic acid–H ₂ O] ⁺)	467.3164 (C ₃₀ H ₄₃ O ₄ , 0.6) (33) → 395.2586 (C ₂₆ H ₃₅ O ₃ , 0.0) (11); 449.3047 (C ₃₀ H ₄₁ O ₃ , –2.0) (100) → 377.2465 (C ₂₆ H ₃₃ O ₂ , –4.2) (13); 431.2951 (C ₃₀ H ₃₉ O ₂ , 0.2) (46) → 359.2328 (C ₂₆ H ₃₁ O, –13.1) (4);	No	No	No
14	Actetyl-acteol	[M+Na] ⁺ 699.3724 (4) [M+H] ⁺ 677.3923 (6)	659.3782 (C ₃₇ H ₅₅ O ₁₀ , [M+H–H ₂ O] ⁺)	449.3080 (C ₃₇ H ₅₅ O ₁₀ , [M+H–xylose–acetic acid–H ₂ O] ⁺)	No	No	No	No
15	Actetyl-acteol	[M+Na] ⁺ 683.3781 (11) [M+H] ⁺ 661.3952 (100)	661.3952 (C ₃₇ H ₅₇ O ₁₀ , [M+H] ⁺)	451.3204 (C ₃₀ H ₄₃ O ₃ , [M+H–xylose–acetic acid] ⁺)	No	No	No	No
16	Anhydro-cimigenol	[M+Na] ⁺ 625.3726 (100) [M+H] ⁺ 603.3911 (27)	625.3726 (C ₃₅ H ₅₄ O ₈ Na, [M+Na] ⁺)	379.2625 (C ₂₆ H ₃₅ O ₂ , [M+H–arabinose–H ₂ O– 2–methylprop-1- ene] ⁺)	No	453.3376 (C ₃₀ H ₄₅ O ₃ , 1.5) (16) → 397.2731 (C ₂₆ H ₃₇ O ₃ , –3.0) (59); 435.3236 (C ₃₀ H ₄₃ O ₂ , –6.2) (12) → 379.2625 (C ₂₆ H ₃₅ O ₂ , –3.2) (100);	No	603.3911 (C ₃₅ H ₅₅ O ₈ , 2.3) (24) → 561.3434 (C ₃₂ H ₄₉ O ₈ , 1.2) (57); 453.3376 (C ₃₀ H ₄₅ O ₃ , 1.5) (7) → 411.2885 (C ₂₇ H ₃₉ O ₃ , –3.4) (11); 435.3293 (C ₃₀ H ₄₃ O ₂ , 6.9) (2) → 393.2791 (C ₂₇ H ₃₇ O ₂ , –0.8) (5); 601.3738 (C ₃₅ H ₅₃ O ₈ , –0.3) (15) → 559.3281 (C ₃₂ H ₄₇ O ₈ , 1.8) (10); 469.3323 (C ₃₀ H ₄₅ O ₄ , 1.1) (15) → 427.2848 (C ₂₇ H ₃₉ O ₄ , 0.0) (63); 451.3214 (C ₃₀ H ₄₃ O ₃ , 0.4) (20) → 409.2731 (C ₂₇ H ₃₇ O ₃ , –2.9) (11);
17	Anhydro-cimigenol	[M+Na] ⁺ 683.3776 (100) [M+H] ⁺ 661.3954 (7)	683.3776 (C ₃₇ H ₅₆ O ₁₀ Na, [M+Na] ⁺)	377.2473 (C ₂₆ H ₃₃ O ₂ , [M+H–xylose–acetic acid–H ₂ O–2– methylprop-1-ene] ⁺)	No	451.3189 (C ₃₀ H ₄₃ O ₃ , –5.1) (16) → 395.2588 (C ₂₆ H ₃₅ O ₃ , 0.5) (96); 433.3082 (C ₃₀ H ₄₁ O ₂ , –5.8) (12) → 377.2473 (C ₂₆ H ₃₃ O ₂ , –2.1) (100);	No	

Table 3 (Continued)

No.	Aglycone class	Abundance of molecular ions relative to base peak (%) ^a	Base peak (aperture voltage: 0 V)	Fragmental Base peak (aperture voltage: 60 V)	Aperture voltage: 60 V ^b		Aperture voltage: 0 V ^c	
					Characteristic fragmental ions [MW (MF, ppm) (AMB)] produced by loss of C ₄ H ₈ O ^d	Characteristic fragmental ions [MW (MF, ppm) (AMB)] produced by loss of C ₄ H ₈ ^d	Characteristic fragmental ions [MW (MF, ppm) (AIFBP)] produced by loss of C ₃ H ₄ ^d	Characteristic fragmental ions [MW (MF, ppm) (AIFBP)] produced by loss of C ₃ H ₆ ^d
18	Anhydro-cimigenol	[M+Na] ⁺ 623.3569 (100) [M+H] ⁺ 601.3752 (17)	623.3569 (C ₃₅ H ₅₂ O ₈ Na, [M+Na] ⁺)	377.2475 (C ₂₆ H ₃₃ O ₂ , [M+H–xylose–H ₂ O–2–methylprop-1-ene] ⁺)	No	451.3203 (C ₃₀ H ₄₃ O ₃ , –2.0) (18) → 395.2585 (C ₂₆ H ₃₅ O ₃ , –0.3) (56); 433.3062 (C ₃₀ H ₄₁ O ₂ , –10.4) (11) → 377.2474 (C ₂₆ H ₃₃ O ₂ , –1.9) (100);	No	601.3752 (C ₃₅ H ₅₃ O ₈ , 2.0) (9) → 559.3271 (C ₃₂ H ₄₇ O ₈ , 0.0) (10); 469.3320 (C ₃₀ H ₄₅ O ₄ , 0.4) (7) → 427.2848 (C ₂₇ H ₃₉ O ₄ , 0.0) (41); 451.3220 (C ₃₀ H ₄₃ O ₃ , 1.8) (8) → 409.2722 (C ₂₇ H ₃₇ O ₃ , –5.1) (9); 601.3745 (C ₃₅ H ₅₃ O ₈ , 0.8) (17) → 559.3276 (C ₃₂ H ₄₇ O ₈ , 0.9) (50); 469.3314 (C ₃₀ H ₄₅ O ₄ , –0.9) (11) → 427.2842 (C ₂₇ H ₃₉ O ₄ , –1.4) (16); 451.3222 (C ₃₀ H ₄₃ O ₃ , 2.2) (9) → 409.2733 (C ₂₇ H ₃₇ O ₃ , –2.4) (20); 621.4006 (C ₃₅ H ₅₇ O ₉ , 0.5) (22) → 579.3531 (C ₃₂ H ₅₁ O ₉ , –0.3) (9); 603.3908 (C ₃₅ H ₅₅ O ₈ , 1.8) (20) → 561.3434 (C ₃₂ H ₄₉ O ₈ , 1.2) (28); 471.3478 (C ₃₀ H ₄₇ O ₄ , 0.8) (6) → 429.2993 (C ₂₇ H ₄₁ O ₄ , –0.7) (12); 453.3381 (C ₃₀ H ₄₅ O ₃ , 2.6) (6) → 411.2888 (C ₂₇ H ₃₉ O ₃ , –2.7) (7); No
19	Anhydro-cimigenol	[M+Na] ⁺ 623.3569 (100) [M+H] ⁺ 601.3746 (17)	623.3569 (C ₃₅ H ₅₂ O ₈ Na, [M+Na] ⁺)	377.2469 (C ₂₆ H ₃₃ O ₂ , [M+H–arabinose–H ₂ O–2–methylprop-1-ene] ⁺)	No	451.3207 (C ₃₀ H ₄₃ O ₃ , –1.1) (23) → 395.2586 (C ₂₆ H ₃₅ O ₃ , 0.0) (38); 433.3093 (C ₃₀ H ₄₁ O ₂ , –3.2) (13) → 377.2469 (C ₂₆ H ₃₃ O ₂ , –3.2) (100);	No	
20	Andydro-hydroshengmanol	[M+Na] ⁺ 643.3842 (100) [M+H] ⁺ 621.4006 (12)	643.3842 (C ₃₅ H ₅₆ O ₉ Na, [M+Na] ⁺)	379.2625 (C ₂₆ H ₃₅ O ₂ , [M+H–xylose–2H ₂ O–2–methylprop-1-ene] ⁺)	No	453.3352 (C ₃₀ H ₄₅ O ₃ , –3.8) (13) → 397.2745 (C ₂₆ H ₃₇ O ₃ , 0.5) (52); 435.3264 (C ₃₀ H ₄₃ O ₂ , 0.2) (7) → 379.2635 (C ₂₆ H ₃₅ O ₂ , 0.5) (100);	No	
21	Alkaloid	[M+H] ⁺ 614.3679 (100)	614.3679 (C ₃₅ H ₅₂ NO ₈ , [M+H] ⁺)	596.3611 (C ₃₅ H ₅₀ NO ₇ , [M+H–H ₂ O] ⁺)	No	No	596.3611 (C ₃₅ H ₅₀ NO ₇ , 4.0) (100) → 556.3260 (C ₃₂ H ₄₆ NO ₇ , –2.5) (5) ^e	

^a Aperture voltage: 0 V; the abundances were calculated on base peak.

^b The abundances were calculated on fragmental base peak.

^c The abundances were calculated on base peak.

^d MW: molecular weight; AMB: abundance of molecular ions relative to base peak; MF: molecular formula; AIFBP: abundance of the ions relative to fragmental base peak (%); C₄H₈O: 2,2-dimethylepoxyethane; C₃H₄: propa-1,2-diene; C₄H₈: 2-methylpropene; C₃H₆: propene.

^e The aperture voltage used for compound **21** was 60 V.

esters and acetylaminines [29–32]. When the aperture voltage was set to 0V, most of the cleavages, such as the loss of neutral water, pentose (xylose or arabinose), acetic acid, or ketene (acetyl group) loss, were found in the spectra (Table 2). Many triterpenoids exist as isomers in *Actaea* species, but many of these isomers belong to different structural classes. These isomers were subjected to these kinds of losses, and they cannot be distinguished only by such cleavages analyses. Furthermore, standards of all these triterpene glycosides are not commercially available. To address this issue, we developed a method of MS/MS fragmental analysis from the available authentic standards to distinguish these triterpene glycosides without standards. Although an unambiguous identification of each compound cannot always be achieved, we were able to assign a structural class for most of the triterpene glycosides we analyzed. These triterpene glycosides are similar in structure and their diversity is attributed to variation in the structure of C(20)–C(27), it is in this region that we focused our fragmentation analysis (Fig. 3). In order to study this region of structural variation (C(20)–C(27)) we conducted in-source fragmentation by increasing the aperture voltage to 60 V, this provided additional information for structure elucidation. Different classes of triterpene glycosides showed different capacities to lose 2,2-dimethylepoxyethane, propa-1,2-diene, 2-methylpropene, and propene, when fragmentation occurred using different aperture voltages. Relative abundance of molecular ions and base peaks was also used to distinguish among classes of triterpene glycosides (Table 3).

16,23-diketoshengmanol class (4–6): This class of triterpene glycosides contained a highly abundant sodium adduct peak in the spectra (Table 3). At an aperture voltage of 60 V, these compounds lost 2,2-dimethylepoxyethane (C₄H₈O). For example, in the spectrum of cimicifugoside H-1 (4), the cleavage from the fragmental ion at *m/z* 467.3132 [M+H–xylose]⁺ (C₃₀H₄₃O₄) to *m/z* 395.2580 [M+H–xylose–2,2-dimethylepoxyethane]⁺ (C₂₆H₃₅O₃), and from the fragment ion *m/z* 449.3050 [M+H–xylose–H₂O]⁺ (C₃₀H₄₁O₃) to *m/z* 377.2475 [M+H–xylose–H₂O–2,2-dimethylepoxyethane]⁺ (C₂₆H₃₃O₂). The relative abundance of the product ions (*m/z* 395.2580 and 377.2475) was much greater than those of their respective parent ions (*m/z* 467.3132 and 449.3050) (Table 3). Compounds in this class also showed losses of propa-1,2-diene (C₃H₄). At an aperture voltage of 0V, it was easy to find the molecular ion at *m/z* 617.3694 [M+H]⁺ (C₃₅H₅₃O₉) losing a C₃H₄ structure to *m/z* 577.3390 [M+H–propa-1,2-diene]⁺ (C₃₂H₄₉O₉), and the aglycone fragment at *m/z* 467.3165 [M+H–xylose]⁺ (C₃₀H₄₃O₄) losing a propa-1,2-diene structure to *m/z* 427.2861 [M+H–xylose–propa-1,2-diene]⁺ (C₂₇H₃₉O₄) (Table 3). The proposed mechanisms of loss of 2,2-dimethylepoxyethane and propa-1,2-diene are explained in Supplemental Fig. S3A. The loss of 2,2-dimethylepoxyethane was due to a cleavage of the C(23)–C(24) bond and the rearrangement of the proton on C(22)–C(24). Simultaneously, a double bond between C(22) and C(23) formed. The loss of propa-1,2-diene was due to cleavages of the C(24)–C(25) and C(25)–O bonds, and the rearrangements of the H(26) and H(27) protons to C(24) and the oxygen atom attached at C(24) and C(25).

Compounds 5 and 6 were tentatively identified as cimicifugoside H-5 and shengmacichun dixyloside (Fig. 3), based on the 2,2-dimethylepoxyethane and propa-1,2-diene losses and the abundances of the much higher product ions than those of the parent ions (Table 3), together with the presence in species of *Actaea* reported by previous publication [33–35].

Shengmanol class (7 and 8): Both the 16,23-diketoshengmanol and shengmanol classes of triterpene glycosides contain a 2,2-dimethylepoxyethanyl group in their structures (Fig. 3). An important difference between them is the presence of a ketone group on C(23) in 16,23-diketoshengmanol where there is an acetate group (CH₃COO) group in shengmanol class compounds. In the mass spectra, 7 and 8 also

showed losses of 2,2-dimethylepoxyethane and propa-1,2-diene; however the abundance ratios of product ions to parent ions were much smaller compared to those in compounds 4–6 (Table 3). For example, in compound 7, the fragmental ions *m/z* 453.3370 [M+H–xylose–acetic acid]⁺ (C₃₀H₄₅O₃) and 435.3266 [M+H–xylose–acetic acid–H₂O]⁺ (C₃₀H₄₃O₂) each lose a 2,2-dimethylepoxyethane producing fragments at *m/z* 381.2799 [M+H–xylose–acetic acid–2,2-dimethylepoxyethane]⁺ (C₂₆H₃₇O₂) and *m/z* 363.2690 [M+H–xylose–acetic acid–H₂O–2,2-dimethylepoxyethane]⁺ (C₂₆H₃₅O), respectively. The relative abundance of the product ions at *m/z* 381.2799 and 363.2690 compared with parent ions at *m/z* 453.3370 and 435.3266, was much less than those of 4–6. The ratio of the abundance of the product ion to its parent ion is a useful characteristic to distinguish shengmanol and 16,23-diketoshengmanol classes of triterpene glycosides. The MS/MS spectra of 23-O-acetylshengmanol-3-O-β-D-glucopyranoside-(1-3)-β-D-xylopyranoside or arabinoside (8) also displayed this characteristic fragmentation and was identified by combining this data with previously published results [36]. The proposed mechanisms of two losses of 2,2-dimethylepoxyethane and propa-1,2-diene for shengmanol class triterpene glycosides are outlined in Supplemental Fig. S3B.

Hydroshengmanol class (9): Kusano hypothesized that hydroshengmanol class of triterpene glycosides in *Actaea* species originated biosynthetically from shengmanol glycoside [23]. The mass spectrum of 9 showed the presence of a pentose and an acetyl group in the structure (Table 2). Based on the previous publication, this compound was tentatively identified as 24β-O-acetylhydroshengmanol-3-O-β-D-xylopyranoside or arabinoside [37–39]. The characteristic fragmental loss of 2,2-dimethylepoxyethane and propa-1,2-diene is very similar to those in the shengmanol class of triterpene glycosides (Table 3). A loss of 2,2-dimethylepoxyethane occurred in response to the formation of a bond between C(24) and the oxygen present at C(25), a cleavage of the C(23)–C(24) bond, and a rearrangement of the proton on C(22) to the AcO group cleaved from C(24) (Supplemental Fig. S3C). Propa-1,2-diene was lost following the cleavages of C(24)–C(25) and C(25)–O, and rearrangements of the two protons at C(26) and C(27) to acetate and hydroxyl groups to form acetic acid and water to leave, respectively (Supplemental Fig. S3C).

Cimigenol class (10–12): This class of compounds lost a 2,2-dimethylepoxyethane moiety when the aperture voltage was increased to 60 V. The proposed mechanism for this cleavage is shown in Supplemental Fig. S3D. For compound 11, prior to the loss of 2,2-dimethylepoxyethane, a ketene was eliminated from the acetyl group to form a hydroxyl group. The loss of propa-1,2-diene observed in the 16,23-diketoshengmanol, shengmanol, and hydroshengmanol classes was not found in the fragmentation of cimigenol compounds, and helped differentiate this class of triterpene glycosides from others (Table 3). Compounds 10 and 11 were unambiguously identified as cimracemoside C and 25-O-acetylcimigenol 3-O-β-D-xylopyranoside by comparison of retention time, mass, and fragmentation patterns with those of authentic standards. Compound 12 was tentatively identified as 12β,21-dihydroxycimigenol-3-O-L-arabinoside based on the characteristic cleavages as observed in 10 and 11 and reported from a previous study [40].

Cimiracero class (13): The aglycone fragment in this class displayed the same characteristic cleavage as in the cimigenol class such as the loss of 2,2-dimethylepoxyethane, however the loss of a propa-1,2-diene was not observed. The proposed mechanism of the loss of 2,2-dimethylepoxyethane is shown in Supplemental Fig. S3E. The differentiating factor between the cimircero and cimigenol classes is the sodium adduct abundance of the molecular ion. For the cimigenol class of compounds (10–12), when the

aperture voltage was set to 0V, the abundance of sodium adduct ions was much greater than those of protonated ions, and most of the sodium adduct peaks were the base peak in the whole spectra. However, only a small abundance of the sodium adduct was found in the spectra of **13** (Table 3).

Acetyl-acteol class (14 and 15): This class of triterpene glycosides is unique in that the 2,2-dimethylepoxyethane and the propa-1,2-diene moiety losses are not present in the spectra; this differentiates this class of aglycones from all others in this study. Similar to the cimicerol class, sodium adducts are found in low abundance (Table 3).

Anhydrocimigenol class (16–19): This class of triterpene glycosides differs from the cimigenol class by the loss of water which occurred from the hydroxyl group on C(25) and the proton on C(26) (Fig. 3). In the mass spectra of this class, the unique fragmental losses of 2-methylpropene, and propene were observed, when the aperture voltage was set to 60V and 0V, respectively, whereas in the 16,23-diketoshengmanol, shengmanol, hydroshengmanol, cimigenol, and cimicerol classes, 2,2-dimethylepoxyethane and propa-1,2-diene fragmentation occurred (Table 3). This can be explained by the absence of oxygen on C(25). The proposed mechanisms of anhydrocimigenol triterpene glycosides losses of 2-methylpropene and propene are outlined in Supplemental Fig. S3F. The cleavage of the C(24)–O and C(16)–O(23) bonds leads to the formation of ketones on C(24) and C(16). The cleavage of the C(23)–C(24) bond and the rearrangement of the two protons from C(22) and C(23) to C(24) induced the loss of 2-methylpropene. The cleavage of the C(24)–C(25) bond and the rearrangement of the C(23) proton to C(25) led the loss of propene, which produced a double bond between C(23) and C(24). Compounds **16–19** showed the same mass fragmental cleavage profiles. The aglycones of **16** and **17** were tentatively identified as 25-anhydrocimigenol and 12-O-acetyl-25-anhydrocimigenol, respectively. Compounds **18** and **19**, both contain the (23R,24R)-7-en-25-anhydrocimigenol aglycone. The sugar residues of **16–18** are likely to be either xylose or arabinose due to the natural abundance of these sugars in *Actaea* triterpene glycosides; however, in a previous study, the *Actaea* triterpene arabinosides had a shorter retention time than triterpene xylosides with the same aglycone using a solvent system of either acetonitrile–water with 0.1% acetic acid or acetonitrile–methanol–water [24,41]. Therefore, using relative retention times we tentatively identified **16–18** as 25-anhydrocimigenol-3-O- α -L-arabinose [42], 12-O-acetyl-25-anhydrocimigenol-3-O- β -D-xylopyranoside (cimicerolmoside K) [33], and (23R,24R)-7-en-25-anhydro cimigenol-3-O- β -D-xylopyranoside [43], respectively. Compound **19** possessed the same molecular formula and similar mass spectral pattern as **18**; however it eluted at a shorter retention time, we therefore infer the presence of arabinose, and tentatively identified its structure as (23R,24R)-7-en-25-anhydrocimigenol-3-O- α -L-arabinoside (Table 3).

Anhydroshengmanol class (20): Similar to anhydroshengmanol class compounds, compound **20** contains a 2-methyl-3-propenyl group on carbon-24. Due to this 2-methyl-3-propenyl group, compound **20** displays the similar losses of 2-methylpropene and propene typically observed in the anhydroshengmanol class compounds (Fig. 3). Based on this data and comparison with previous studies, we tentatively identified **20** as Cimidahuside G (Table 3) [7]. The proposed mechanism of the losses of 2-methylpropene and propene is outlined in Supplemental Fig. S3G.

Alkaloid class (21): A single alkaloid, cimicifugadine (syn. cimicifine A), was identified by MS/MS which matched spectral values well with the previous reports (Table 2) [44,45]. When the aperture voltage was increased to 60 V, no 2,2-dimethylepoxyethane moiety loss was observed in the mass spectrum. However, the propa-1,2-diene moiety loss is present in the spectrum, when the aperture

voltage was set to 0V (Table 3). The proposed mechanism of the losses of propa-1,2-diene is outlined in Supplemental Fig. S3H.

3.3. Different types of marker compounds

Marker compounds refer to chemical constituents within a medicinal material that can be used to verify its potency or identity. For some medicinal plants, marker compounds can be described as active ingredients. In other instances, marker compounds of interest are chemicals that confirm the correct botanical identity of original plant material [46]. In this study, we have identified 15 marker compounds which are useful for the correct identification of *Actaea* plant material. These compounds were informative in the division of samples into four distinct categories: *Actaea* species other than *A. racemosa* (OAR), Asian species (AS), *A. racemosa* (AR), and North American species other than *A. racemosa* (NAOAR).

The classification of the four types of marker compounds was mainly based on the trend view plot produced by PCA analysis. Using the ESI probe, two types of molecular ions, the protonated ion and sodium adduct, were formed for every marker compound. The trend views were based on data from either the protonated molecular ion or sodium adduct molecular ion. Comparing these two types of trend view plots for each marker compound, the trend view that showed the presence of a marker among more samples was selected for marker evaluation.

3.3.1. Marker compounds for OAR

Marker compounds for OAR could not be detected in *A. racemosa*. This type of marker compound is useful to determine the presence of *Actaea* species other than black cohosh, which are potential adulterants in black cohosh dietary supplements. Three cimifugin derivatives (**1–3**), and one 16,23-diketoshengmanol class triterpene glycoside (**5**), were found to be useful OAR markers. For example, prim-O-cimifugin (**1**) was found in all Asian and North American species with the exception of *A. americana*, *A. laciniata*, *A. podocarpa*, and *A. racemosa* (Fig. 4A). The detection of compound **1** in black cohosh products would indicate the presence of other *Actaea* species, and therefore the adulteration of the black cohosh product.

This is the first report of the detection of OAR marker compounds **1** and **2** in *A. heracleifolia* (Fig. 4A and B). A previous study was unable to detect the presence of these two compounds in *A. heracleifolia* [24]. We associate this finding with our use of highly sensitive methods in the TOF-MS analysis we conducted.

3.3.2. Marker compounds for AS

Compound **21**, the alkaloid, was found to be a useful marker only present in Asian species of *Actaea* (AS) (Fig. 4E). This marker compound is useful in identifying the presence of Asian *Actaea* species. Compound **21** was detected in *A. heracleifolia*, *A. mairei*, *A. dahurica*, and *A. yunnanensis*. Among them, *A. heracleifolia* and *A. dahurica* are most commonly used in Chinese traditional medicine. This marker compound may be very useful in identification of Asian species of *Actaea* in black cohosh products as these species are often mistaken with black cohosh.

3.3.3. Marker compounds for AR

In order to confirm the presence of *Actaea racemosa* in black cohosh products or other *Actaea* samples, a marker compound that correlates to the presence of black cohosh and no other species would be very useful. Compound **12** was found in high concentrations in all the black cohosh samples collected from different locations, but was not detected in any other *Actaea* species. Compound **10** has been previously reported to be a useful marker in the identification of black cohosh species [14]. In this work we detected the presence of compound **10** in black cohosh as well as

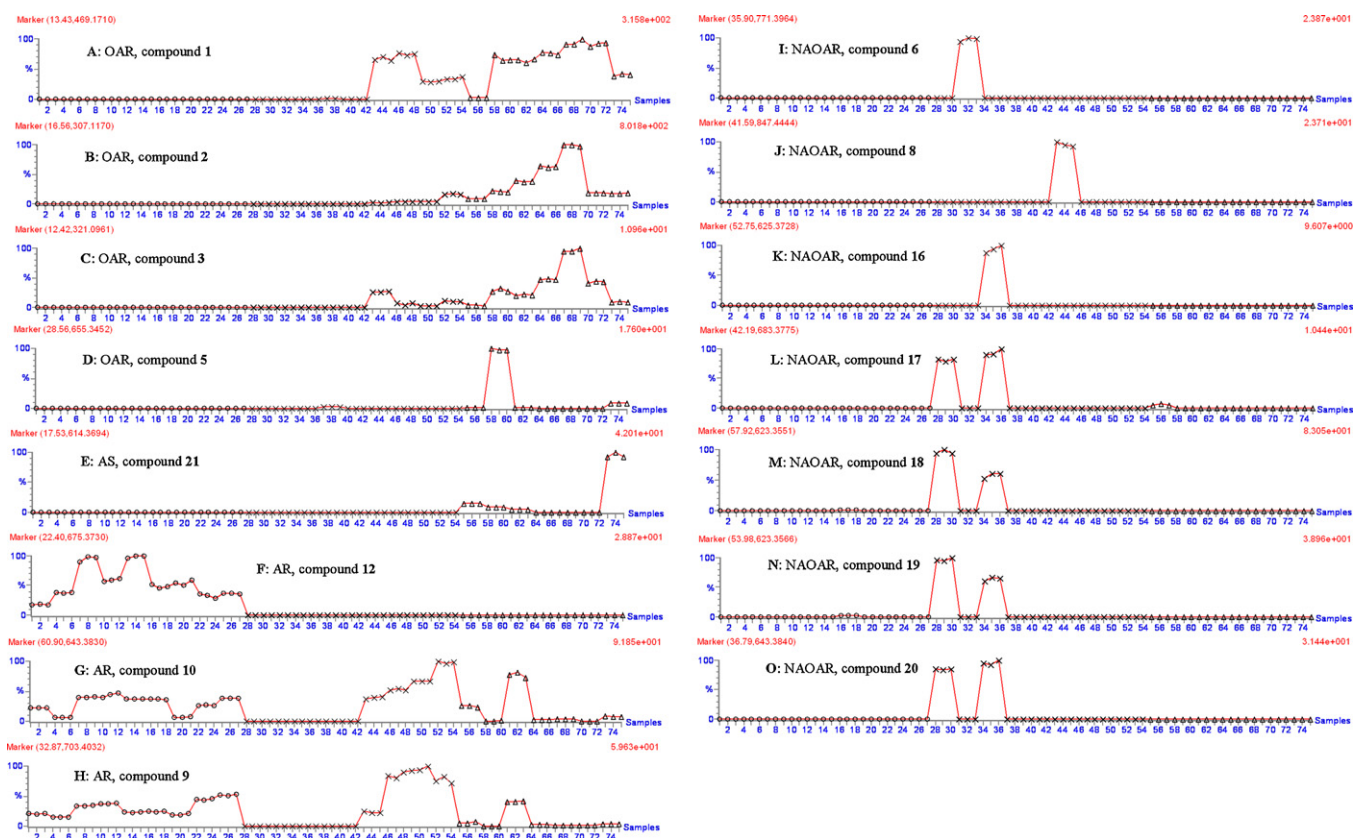


Fig. 4. Selected trend plots of marker compounds for *Actaea* species. Triple injections for each sample, from first to twenty-seventh injections (labeled “o”) are for black cohosh samples from different locations; From twenty-eighth to fifty-fourth injections (labeled “x”) are for North American species of *Actaea* other than black cohosh; From fifty-fifth to seventy-eighth injections (labeled “Δ”) are for Asian species of *Actaea*.

other North American *Actaea* species including *A. elata*, *A. elata* var. *alpestris*, *A. arizonica*, *A. cordifolia*, and some Asian *Actaea* species including *A. heracleifolia*, *A. dahurica*, and *A. yunnanensis* (Fig. 4G). On the basis of our findings we determined that compound **10** alone is not a suitable marker compound for black cohosh identification. Compound **10** was useful as an AR marker when combined with additional marker compounds in a two-marker-compound method for identification. This method is based on combining two types of marker compounds to aid in the identification of black cohosh species within a black cohosh product. Type I marker compounds are found in black cohosh species, but not in *A. americana*, *A. laciniata*, *A. podocarpa*, *A. pachypoda*, and *A. rubra* species, such as compounds **9** and **10** (Fig. 4G and H). Type II marker compounds are cimifugin derivatives (**1–3**), which are not found in *A. americana*, *A. laciniata*, *A. podocarpa*, and black cohosh species (Fig. 4A–C). If no cimifugin derivatives (**1–3**) are detected, *A. americana*, *A. laciniata*, *A. podocarpa*, and black cohosh may be present within a product. If a type I marker compound is present in a sample, but no type II marker can be found, we can determine that black cohosh is the only species present in a black cohosh product.

3.3.4. Marker compounds for NAOAR

Compounds **6**, **8**, and **16** could only be detected in *A. laciniata*, *A. elata*, and *A. podocarpa*, respectively (Fig. 4I–K), and are therefore useful for identification of each of these three North American species. Compound **20** was found in *A. americana* and *A. podocarpa* and no other species we analyzed; it is therefore a useful marker compound for these species (Fig. 4O). Marker compounds **17–19** were detected in large concentrations in *A. americana* and *A. podocarpa* species. Compound **17** was also detected in small

amounts in *A. heracleifolia*. Compounds **18** and **19** were also detected in small amounts in one sample of *A. racemosa* (sample code: f) (Fig. 4L–N). While compounds **6**, **8**, **16**, and **20** can each be used individually as marker compounds for NAOAR species, compounds **17–19** must be combined with additional markers, compounds **9** or **10**, for complete identification. If compounds **17**, **18**, or **19** are present in a sample, but neither compounds **9** or **10** cannot be found, we can determine that NAOAR species are present in a black cohosh product. (Fig. 4G, H, L, M and N).

4. Conclusion

In this study, we used HPLC–ESI–TOF–MS/MS and chemometric analysis to identify marker compounds useful in the assessment of 16 species of *Actaea*. A total of 15 marker compounds including three cimifugin derivatives, 11 triterpene glycosides, and one alkaloid, were identified using a rapid extraction process in which no sample pre-treatment was required. Discovery and identification of these marker compounds were achieved using time-of-flight mass spectrometry and multivariate principle component analysis. By varying the MS aperture voltage, we were able to achieve efficient fragmentation. Using the accurate mass measurements recorded for each of these fragments we were able to produce strong evidence for structural identification.

Principle component analysis was used to direct our search for marker compounds from 25 samples representing 16 species of *Actaea*. The utility of these marker compounds for species and group identification has been determined under the experimental conditions described. It is therefore important to adhere to these conditions in order to make full use of the marker compounds described here for accurate identifications.

Using characteristic fragmental cleavage we developed original rules for the identification of triterpene glycosides in species of *Actaea*. These fragmental rules focus on the differences observed in the C(20)–C(27) region of triterpenes, where previous publications only report simple losses of water, acetic acid, and sugars. These cleavage rules add useful ways to analyze or identify the minor triterpene glycosides in *Actaea* species.

The methods we developed have implications beyond the identification of *Actaea* species and can be useful for a number of applications. HPLC-ESI-TOF and chemometric analysis are powerful tools for the identification of chemical markers which can be very useful in the identification of components of botanical products. The adulteration of botanical supplements is a major problem that efficacy of the original product can be decreased and even toxicity can become a concern [12]. Therefore, identification of markers compounds may provide useful tools in quality control of botanical supplements.

Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.chroma.2011.01.033.

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