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Liquid Chromatography

DETERMINATION OF PHENYLETHANOID GLYCOSIDES IN *LAGOTIS BREVITUBA* MAXIM. BY HIGH-PERFORMANCE LIQUID CHROMATOGRAPHY–ELECTROSPRAY IONIZATION TANDEM MASS SPECTROMETRY

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***High performance liquid chromatography coupled with electrospray ionization quadrupole-time-of-flight tandem mass spectrometry was used to profile the phenylethanoid glycosides in* Lagotis brevituba. *A total of twenty-three phenylethanoid glycosides were characterized by comparing the retention time and fragmentation with standards, accurate mass measurements, and fragmentation at low and high collision energies. Most phenylethanoid glycosides were reported in* L. brevituba *for the first time. This established method may be employed for comprehensive quality control of* L. brevituba.**

Keywords: High-performance liquid chromatography–tandem mass spectrometry; HPLC/ESI-QTOF-MS/MS; *Lagotis brevituba*; Phenylethanoid glycosides

INTRODUCTION

The genus *Lagotis* (Scrophulariaceae) is represented by approximately 30 species in the world, most distributed in Himalayas, Pamirs, Kalakoram Range, Chi-lien Range, and Hengduan Range (Editorial Committee of Flora of China 1979). *Lagotis brevituba* Maxim, grows on slopes at high altitudes of 3000–4400 meters in the Qinghai-Tibet Plateau, and has been used in Tibetan folk medicine for treatment of fever, high blood pressure, and chronic hepatitis (Editorial Committee of Tibetan Materia Medica 1991; Zong and Che 1995). The n-butanol extract of

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L. brevituba has hepatoprotective, anti-inflammatory, anti-nociceptive, anti-tumor and immunomodulatory activities (Jin and Chen 2006; Wang, Zhang, and Ma 2007; J. Guo and Huang 2011). Previous phytochemical studies have isolated phenylethanoid glycosides, flavonoids, and iridoid glycosides in the n-butanol extract of *L. brevituba* (Zhao and Shi 2004; Shi, Huang, and Lu 2006; Chi, Deng, and Wang 2009, 2010). Phenylethanoid glycosides play important roles in the pharmacological properties and in chemotaxonomy of the *Lagotis* genus. Complete taxonomy of the genus is supported by morphologic characteristics, geographic distribution, and by the structures of phenylethanoid glycosides. However, the light-induced chemical instability and time-consumed chromatographic procedures have made isolation and purification of phenylethanoid glycosides difficult. Moreover, several other plant resources, such as *L. ramalana* and *L. brachystachya*, were also used as *L. brevituba*, but no clarification about chemical differences among them was investigated. Therefore, it is highly desirable to establish a rapid and reliable method for identification of phenylethanoid glycosides directly from the extract of *L. brevituba*.

Recently, the coupling of high-performance liquid chromatography (HPLC) and mass spectrometry (MS) has been to identify compounds in medicinal plants (Zheng et al. 2008). Quadrupole-time-of-flight tandem–mass spectrometry (QTOF–MS) has been shown to provide accurate mass, formulae of compounds, fragmentation analysis, and the structural elucidation. Principles have been summarized for the characterization and structure elucidation of unknown compounds without reference standards, which represents a powerful tool in characterizing the constituents of medicinal plants (Fabre et al. 2001; Vukics et al. 2008; Vukics and Guttman 2010).

Here, a simple method using high performance liquid chromatography/electrospray ionization–quadrupole-time-of-flight–tandem mass spectrometry (HPLC/ESI–QTOF–MS/MS) was developed to identify twenty-three phenylethanoid glycosides in *L. brevituba*. Most were reported in *L. brevituba* for the first time.

EXPERIMENTAL

Reagents and Plant Materials

Acetonitrile was obtained from Fisher (LC/MS-grade, Fair Lawn, NJ USA) and formic acid from Merck (analytical-grade, Darmstadt, Germany). Water was deionized and further purified by a Milli-Q Plus water system (Millipore Ltd., Bedford, MA, USA). Other reagents and chemicals were of analytical grade. Echinacoside and verbascoside were isolated from *L. brevituba* in this laboratory and identified by ^{1}H nuclear magnetic resonance (NMR), ^{13}C NMR, two dimensional NMR, and mass spectrometry. Their purities were at least 98% by HPLC.

Lagotis brevituba plants were purchased from Qinghai Jiukang Medicine Corporation and identified by Professor Tingnong He (Northwest Institute of Plateau Biology, Chinese Academy of Sciences). Voucher specimens were deposited

at the herbarium of Northwest Institute of Plateau Biology, Chinese Academy of Sciences.

Sample Preparation

Dried and finely powdered entire plants of *Lagotis brevituba* (10 g) were extracted with methanol (3×100 mL) at room temperature. The methanol extract was concentrated under vacuum and the residue was further fractionated with n-hexane (3×100) and n-butanol (3×100). The n-butanol extract was concentrated under vacuum and then dissolved in 40% aqueous acetonitrile (100 mL). The solution was passed through a 0.22-μm filter before analysis.

Instrumentation

HPLC was performed on an Agilent 1200 series system (Agilent Technologies, Waldbronn, Germany), coupled to an autosampler, and a quaternary solvent delivery system with an online degasser. Separation was performed on Xselect CSH C_{18} column (100 mm $\times$ 2.1 mm i.d., 2.5 μm, Waters, Massachusetts, USA). The mobile phase was eluted at 220 μL/min in gradient mode with 0.1% formic acid/water (solvent A) and acetonitrile (solvent B): 0–2 min (6% B), 2–92 min (6–24% B) using a 4-μL injection volume.

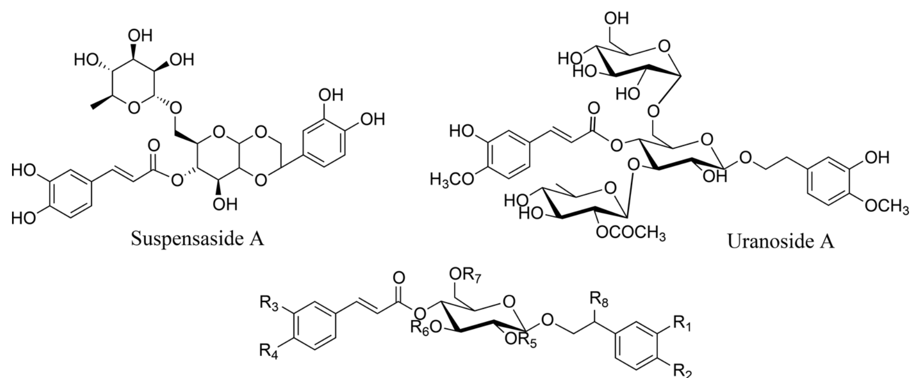
Mass spectrometry was performed using a QSTAR Elite LC/MS/MS system from Applied Biosystems/MDS Sciex (Concord, ON, Canada) coupled with an electrospray ionization (ESI) interface. Nitrogen was used in the experiment. The parameters were optimized as follows: ESI voltage, −4000 V; nebulizer gas, 60; auxiliary gas, 50; curtain gas, 35; turbo gas temperature, 500°C; declustering potential, −80 V; focusing potential, −350 V; and declustering potential 2, −10 V. Samples were analyzed with an information-dependent acquisition method which can automatically select candidate ions for tandem mass spectrometry study. The TOF mass range was set from m/z 100 to 1000 and the mass range for the product ion scan was m/z 50 to 1000. The collision energy (CE) was set from 30 to 50 eV for more structural information. The mass analyzer was calibrated using taurocholic acid (2 ng/μL) by direct injection at a flow rate of 5 μL/min. The data were acquired and processed using Analyst QS 2.0 software.

RESULTS AND DISCUSSION

A total of twenty-three phenylethanoid glycosides were characterized or tentatively identified. The structures of phenylethanoid glycosides are shown in Figure 1. The total ion current (TIC) profile is shown in Figure 2, and the mass spectral data of phenylethanoid glycosides are listed in Table 1.

Optimization of Fragmentation Voltage

Phenylethanoid glycosides are characterized by a β-glucopyranose attached directly to a hydroxyphenylethyl moiety. Hydroxyl derivatives of cinnamic acid (such as caffeoyl and feruloyl) are attached to the C4 position of β-glucopyranose.



$[M-H]^-$	R1	R2	R3	R4	R5	R6	R7	R8	Strctural elucidation
591	H	OH	H	OH	H	Rha	H	H	Osmanthuside B
607	OH	OH	H	OH	H	Rha	H	H	Lipedoside A
623	OH	OH	OH	OH	H	Rha	H	H	Verbascoside
637	OH	OH	OCH_3	OH	H	Rha	H	H	Leucosceptoside A
639	OH	OH	OH	OH	H	Glc	H	H	Plantamajoside
639	OH	OH	OH	OH	H	H	Rha	(S or R) OH	Suspensaside
651	OCH_3	OH	OCH_3	OH	H	Rha	H	H	Martynoside
653	OH	OH	OH	OH	H	H	Rha	OCH_3	Suspensaside methyl ether
667	OH	OH	OH	OH	H	H	Rha	CH_3CH_2OH	β-ethyl-OH-acteoside
771	OH	OH	OH	OH	H	Api	Glc	H	Caffeoyl calceolarioside C
783	OCH_3	OH	OCH_3	OH	H	Rha	Api	H	Leucosceptoside B
784	OH	OH	OH	OH	H	Rha	Glc	H	Echinacoside.
799	OH	OH	OCH_3	OH	H	Rha	Glc	H	Jionoside A
801	OH	OH	OH	OH	H	Glc	Glc	H	Maxoside
813	OCH_3	OH	OCH_3	OH	H	Rha	Glc	H	Cistanoside B

Figure 1. Phenylethanoid glycosides in n-butanol extract of *L. brevituba*.

Usually, a rhamnose is located to the β-glucopyranose. For a phenylethanoid glycoside with a trisaccharide group, an additional glucose is usually substituted to the β-glucopyranose. CE (collision energy) plays greatest impact on sensitivity

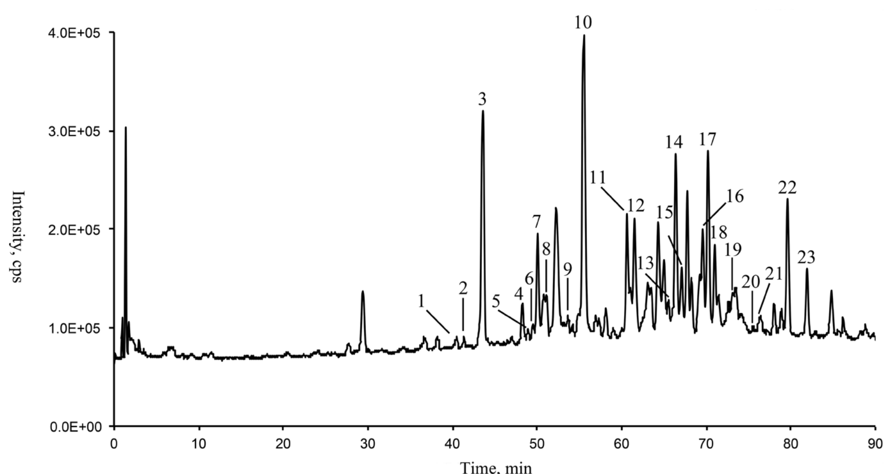


Figure 2. Chromatogram of n-butanol extract of *L. brevituba* by HPLC-TOF-MS with negative ESI.

Table 1. Identification of phenylethanoid glycosides in the n-butanol extract of *L. brevituba* by HPLC-QTOF-MS/MS

No.	Retention time (min)	$[M-H]^-$	Formula	Error (ppm)	CE (eV)	ESI-MS^2 data m/z, relative abundance, %	Structural elucidation
1	40.49	639.1936	$C_{29}H_{36}O_{16}$	1.70	30	639(100), 621(22), 487(4), 459(3), 179(16), 161(20), 151(3)	Suspensaside or its isomer
					33	639(100), 621(47), 487(8), 469(2), 459(12), 179(49), 161(78), 151(7)	
2	41.20	639.1901	$C_{29}H_{36}O_{16}$	−3.77	30	639(100), 621(42), 487(6), 459(5), 179(19), 161(24), 151(3)	Suspensaside or its isomer
					33	161(100), 639(95), 621(87), 487(12), 469(2), 459(15), 179(80), 151(11)	
3	43.56	785.2501	$C_{35}H_{46}O_{20}$	−0.40	33	785(100), 623(3), 179(2), 161(12)	Echinacoside
					40	161(100), 785(56), 623(25), 477(2), 461(1), 179(9)	
4	48.19	771.2387	$C_{34}H_{44}O_{20}$	5.09	33	771(100), 609(20), 477(3), 179(4), 161(52)	Caffeoyl calceolarioside C
					40	161(100), 771(25), 609(39), 477(6), 447(4), 315(3), 179(6)	
5	48.91	801.2402	$C_{35}H_{46}O_{21}$	−6.40	33	801(100), 161(11)	Maxoside
					45	161(100), 801(40), 639(10), 477(10), 315(10), 179(20)	
6	49.47	639.1959	$C_{29}H_{36}O_{16}$	5.30	33	639(100), 477(2), 161(100)	Plantamajoside or its isomer
					35	161(100), 639(20), 477(23), 315(2), 179(4)	
7	50.08	639.1919	$C_{29}H_{36}O_{16}$	−0.95	35	161(100), 639(17), 477(21), 315(2), 179(4)	Plantamajoside or its isomer
8	50.92	799.2720	$C_{36}H_{48}O_{20}$	7.40	35	799(100), 623(19), 477(1), 193(5), 175(10)	Jionoside A1
					45	175(100), 799(14), 623(77), 477(5), 461(3), 315(2), 193(21)	
9	52.51	653.2079	$C_{30}H_{38}O_{16}$	−0.39	33	621(100), 653(43), 487(20), 469(3), 459(11), 161(59), 179(74), 151(7)	Suspensaside methyl ether
10	55.43	623.1939	$C_{29}H_{36}O_{15}$	−5.93	33	623(100), 461(2), 161(8)	Verbascoside
					35	623(100), 461(18), 179(4), 161(83)	
					40	161(100), 623(10), 461(12), 315(2), 179(4)	

11	60.59	623.1927	$C_{29}H_{36}O_{15}$	−7.85	35	161(100), 623(86), 477(2), 461(24), 443(1), 315(1), 179(8)	Isomer of verbascoside
12	61.53	621.1879	$C_{29}H_{34}O_{15}$	9.58	33	179(100), 621(51), 487(10), 469(2), 459(13), 161(76), 151(8)	Suspensaside A
13	65.46	667.2278	$C_{31}H_{40}O_{16}$	5.97	33	621(100), 667(30), 487(15), 469(2), 459(15), 179(82), 161(90), 151(11)	*β*-Ethyl-OH-acteoside
14	66.37	637.2174	$C_{30}H_{39}O_{15}$	6.51	30	637(100), 461(28), 193(39), 175(35)	Leucosceptoside A or its isomer
					35	175(100), 637(40), 461(35), 443(4), 315(5), 193(35)	
15	68.72	607.2072	$C_{29}H_{36}O_{14}$	7.44	33	145(100), 607(45), 461(47), 443(4), 315(3), 163(12)	Lipedoside A
16	69.38	637.2184	$C_{30}H_{39}O_{15}$	8.08	35	175(100), 637(59), 461(78), 443(4), 315(5), 193(35)	Leucosceptoside A or its isomer
17	70.08	813.2880	$C_{37}H_{50}O_{20}$	7.72	33	813(100), 637(10), 491(2), 193(8), 175(44)	Cistanoside B or its isomer
					40	175(100), 813(24), 637(22), 491(6), 329(2), 193(22)	
18	70.78	813.2877	$C_{37}H_{50}O_{20}$	7.35	40	175(100), 813(32), 637(23), 491(7), 329(1), 193(20)	Cistanoside B or its isomer
19	74.46	591.2103	$C_{29}H_{36}O_{13}$	4.28	33	145(100), 591(30), 445(5), 299(3), 163(10)	Osmanthuside B
20	75.47	783.2669	$C_{36}H_{48}O_{19}$	−5.43	33	783(100), 607(22), 589(9), 461(13), 193(13), 175(34)	Leucosceptoside B
					40	175(100), 783(14), 607(25), 589(7), 475(7), 461(14), 193(25)	
21	77.19	855.2987	$C_{39}H_{52}O_{21}$	7.50	33	855(100), 813(12), 175(37), 193(12)	Uranoside A
					45	175(100), 855(8), 813(13), 637(4), 491(13), 193(21)	
22	79.81	651.2271	$C_{31}H_{41}O_{15}$	−2.75	30	651(100), 475(1), 193(2), 175(14)	Martynoside or its isomer
					33	175(100), 651(36), 475(9), 457(2), 329(1), 193(16)	
23	81.84	651.2300	$C_{31}H_{41}O_{15}$	1.69	33	175(100), 651(39), 475(10), 457(2), 329(5), 193(15)	Martynoside or its isomer

and fragmentation. Therefore, different CE values were performed in the tandem mass spectrometry experiments in order to optimize signals and obtain more structural information. For phenylethanoid glycosides with a disaccharide moiety, a collision energy of 30 eV was optimum to provide a good strong deprotonated molecular ion and 33 eV provided characteristic fragment ions for structure elucidation. For phenylethanoid glycosides with a trisaccharide moiety, 33 eV were sufficient to provide deprotonated molecular ions while CE 40 eV provided good structural information and a high relative abundance of fragment ions. In order to characterize the compounds, tandem mass spectra at low and high collision energies were employed for characterization.

FRAGMENTATION OF PHENYLETHANOID GLYCOSIDE STANDARDS IN NEGATIVE-ION MODE

In negative-ion ESI experiments, $[M-H]^-$ ions were observed in the full MS of all standard phenylethanoid glycosides. For the purpose of characterizing fragmentation behavior of reference standards, tandem mass spectra of standards were investigated, which provided structural information to elucidate the fragmentation pathways of phenylethanoid glycosides. Typical losses were the feruloyl moiety (176 Da), caffeoyl or hexose moiety (162 Da), deoxyhexose moiety (146 Da), pentose moiety (132 Da), and 42, 32, 18 Da, which indicated the presence of acetyl, methoxyl, and hydroxyl groups on the aglycone moiety.

For echinacoside, the $[M-H]^-$ ion at *m*/*z* 785 was observed as the base peak and was selected as the precursor ion for MS/MS provide fragmentation information. The accurate molecular weight was employed to distinguish isobaric compounds and fragments. A loss of 162.0340 Da loss showed that the product ion at *m*/*z* 623

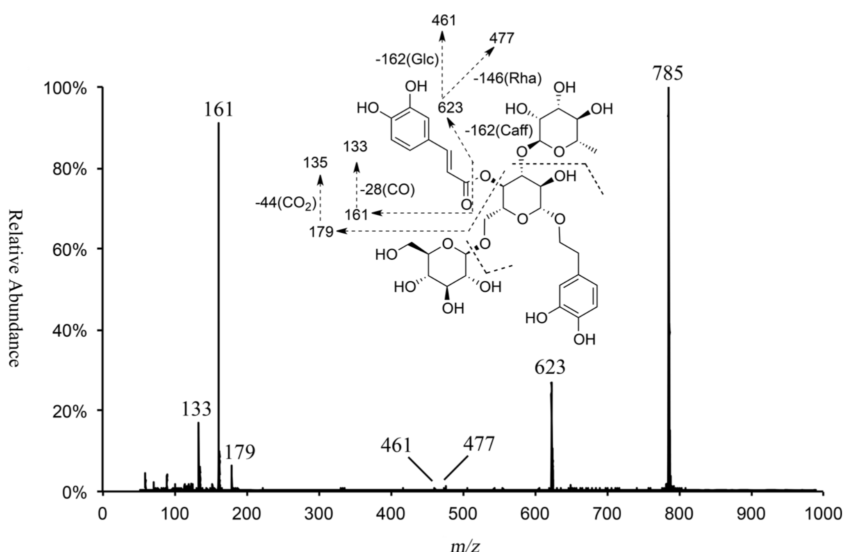


Figure 3. Tandem mass spectrum of $[M-H]^-$ of echinacoside at *m*/*z* 785.

was attributed to the neutral loss of a caffeoyl moiety ($C_9H_6O_3$, calculated 162.0322 Da, δ 10.8 ppm) rather than hexose moiety ($C_6H_{10}O_5$, calculated 162.0533 Da, δ −119.6 ppm) from $[M-H]^-$ ion at m/z 785. Intense ions at m/z 477, 461, and 315 were produced due to loss of a rhamnose (146 Da) and a glucose (162 Da) from $[M-H\text{–caffeoyl}]^-$ at m/z 623. The relative abundance of ions indicated that the loss of rhamnose moiety at the C3 position of the central β-glucopyranose was easier than the loss of the glucose moiety at C6 position. Diagnostic ions at m/z 179 and 161 were both produced from the cleavage of the caffeoyl moiety. The fragmentation behavior (shown in Figure 3) was in agreement with previous studies (Han et al. 2012; L. Li et al. 2008).

Verbascoside produced $[M-H]^-$ at m/z 623. In the tandem mass spectrum, according to an accurate 162.0295 Da loss, the product ion at m/z 461 was attributed to the neutral loss of a caffeoyl moiety ($C_9H_6O_3$, calculated 162.0322 Da, δ −16.9 ppm) rather than a hexose moiety ($C_6H_{10}O_5$, calculated 162.0533 Da, δ −147.3 ppm) from $[M-H]^-$ ion at m/z 623. The ion at m/z 315 was produced due to the loss of the rhamnose moiety from the ion at m/z 461. Diagnostic ions at m/z 179 and 161 were produced from the cleavage of the caffeoyl moiety. Fragmentation behavior was in agreement with previously reported rules (Han et al. 2012; Kirmizibekmez et al. 2005).

HPLC/ESI–QTOF–MS/MS OF PHENYLETHANOID GLYCOSIDES FROM *Lagotis brevituba*

The known phenylethanoid glycosides were identified by comparison with reference standards. For unknown compounds, structures were elucidated by fragmentation rules and comparison with mass spectral data.

Phenylethanoid glycosides of Type I are present in relatively high concentrations in *L. brevituba* and characterized by a β-glucopyranose attached directly to hydroxyphenylethyl moiety. Hydroxyl derivatives of cinnamic acid (such as caffeoyl and feruloyl) are attached to the C4 position of β-glucopyranose. Usually, a rhamnose is located at the C3 position of the β-glucopyranose. For phenylethanoid glycoside with a trisaccharide moiety, an additional glucose usually substituted at the C6 position of the β-glucopyranose.

Compound **3** showed an $[M-H]^-$ ion at m/z 785, and its tandem mass spectrum displayed ions at m/z 623, 477, 461, 179, and 161, which were the same as echinacoside. Coupled with the same retention time, compound **3** was characterized as echinacoside.

Compound **4** showed a pseudomolecular $[M-H]^-$ ion at m/z 771. The tandem mass spectrum showed high abundance characteristic fragments at m/z 179 and 161 that suggested the presence of a caffeoyl moiety. The ions at m/z 477 and 315 indicated successive loss of a rhamnose (146 Da) and a glucose (162 Da) from $[M-H\text{–caffeoyl}]^-$ ion at m/z 609. Comparing with a previous literature report, tentative identification was given to compound **4** as caffeoyl calceolarioside C (H. Guo et al. 2007).

Compound **5** exhibited a deprotonated molecule $[M-H]^-$ at m/z 801, 16 Da higher than that of echinacoside. It was presumed that compound **5** has glucose moieties at the C3 and C5 positions of central β-glucopyranose. The $[M-H\text{–caffeoyl–162–162}]^-$ ion at m/z 315 further verified this deduction. Characteristic ions

at m/z 179 and 161 with high abundances suggested the presence of caffeoyl moiety. Comparing with a previous literature report, a tentative identification was therefore given to compound **5** as maxoside (Kırmızıbekmez et al. 2009).

Compounds **6** and **7** showed the same $[M-H]^-$ at m/z 639 and the same product ions by MS/MS, which indicated that compounds **6** and **7** were isomers. High abundance fragments at m/z 179 and 161 suggested the presence of a caffeoyl moiety. The [M−H–caffeoyl–162]$^-$ ion at m/z 315 further demonstrated a glucose group. Comparing with a previous literature report, compounds **6** and **7** were tentatively identified as plantamajoside and its isomer (Qi et al. 2012).

Compound **8** gave a deprotonated molecular ion $[M-H]^-$ at m/z 799, 14 Da higher than that of echinacoside. In its mass spectrum, diagnostic ions at m/z 193 and 175 suggested the presence of a feruloyl moiety. Ions at m/z 477 and 461 indicated losses of rhamnose (146 Da) and glucose (162 Da) from the [M−H–feruloyl]$^-$ ion at m/z 623. The relative abundances revealed the position of substitutents. Based on the fragmentation patterns and previously reported data, compound **8** was tentatively identified as jionoside A1 (X. N. Li et al. 2011).

Compounds **10** and **11** gave $[M-H]^-$ at m/z 623. In the tandem mass spectrum, both showed ions at m/z 461, 315, 179, and 161, which were the same as that of verbascoside. Comparing the retention time with a standard, compound **10** was identified as verbascoside. Compound **11** was tentatively identified as an isomer of verbascoside.

Compounds **14** and **16** showed the same $[M-H]^-$ at m/z 637 and displayed the same product ions, which indicated that compound **14** and **16** were isomers. The intensive fragments at m/z 193 and 175 confirmed the existence of a feruloyl moiety. The ion at m/z 315 indicated the loss of rhamnose (146 Da) from [M−H–feruloyl]$^-$ ion at m/z 461. Based on the fragmentation behaviors above and previous reports, compounds **14** and **16** were tentatively characterized as leucosceptoside A (Kirmizibekmez et al. 2005) and its isomer.

Compound **15** exhibited $[M-H]^-$ at m/z 607, 16 Da less than that of verbascoside. Diagnostic ions at m/z 163 and 145 suggested the presence of a coumaroyl moiety. The ion at m/z 315 indicated the loss of a rhamnose (146 Da) from the [M−H–coumaroyl]$^-$ ion at m/z 461. Compound **15** was tentatively identified as ipedoside A (H. Guo et al. 2007).

Compound **17** displayed $[M-H]^-$ at m/z 813. High abundance ions at m/z 193 and 175 suggested the presence of a feruloyl group. Ions at m/z 491 and 329 indicated the loss of rhamnose (146 Da) and glucose (162 Da) from [M−H–feruloyl]$^-$ ion at m/z 637. The [M−H–feruloyl–rhamnose–glucose]$^-$ ion of compound **17** at m/z 329 was 14 Da higher than the [M−H–caffeoyl–rhamnose–glucose]$^-$ ion of echinacoside at m/z 315. It indicated that a methoxyl moiety was substituted to the phenylethyl group of compound **17**. Compound **18** showed the same $[M-H]^-$ at m/z 813 and the same fragmentation as compound **17**. Comparing with a previous report (Kobayashi et al. 1984), tentative identifications of compound **17** and **18** were cistanoside B and its isomer.

Compound **19** exhibited a deprotonated molecule $[M-H]^-$ at m/z 591. Diagnostic ions at m/z 163 and 145 suggested the presence of a coumaroyl moiety. The ion at m/z 299 indicated the loss of a rhamnose (146 Da) from the [M−H–coumaroyl]$^-$ ion at m/z 445. These data and rules in literature (Han et al. 2012) suggest that compound **19** is osmanthuside B.

Compound **20** displayed a $[M-H]^-$ at m/z 783. High abundance fragments at m/z 193 and 175 suggested the presence of a feruloyl moiety. Ions at m/z 589, 475, and 461 indicated the loss of water (18 Da), apiose (130), and rhamnose (146) from the $[M-H\text{–feruloyl}]^-$ ion at m/z 607. Compound **20** was tentatively identified as leucosceptoside B (Kirmizibekmez et al. 2005).

Compound **21** displayed $[M-H]^-$ at m/z 855, 42 Da higher than that of cistanoside B. The ion at m/z 813 in the tandem mass spectrum indicated the loss of an acetyl group (42 Da). Fragmentation patterns were the same as that of compound **17**. From a previous report, compound **21** was tentatively identified as uranoside A (Otsuka and Hiedaki 1993).

Compound **22** displayed $[M-H]^-$ at m/z 651. High abundance fragments at m/z 193 and 175 suggested the presence of a feruloyl moiety. Ions at m/z 329 indicated the loss of rhamnose (146 Da) from the $[M-H\text{–feruloyl}]^-$ ion at m/z 475. The $[M-H\text{–feruloyl–rhamnose}]^-$ ion of compound **22** at m/z 329 was 14 Da higher than the $[M-H\text{–caffeoyl–rhamnose}]^-$ ion of verbascoside at m/z 315. It revealed that a methoxyl group was added to the phenylethyl moiety of compound **22**. Compound **23** showed the same $[M-H]^-$ at m/z 651 and the same fragments as compound **22**. Using a previous report (Kirmizibekmez et al. 2005), compounds **22** and **23** were tentatively identified as martynoside and its isomer.

Type II phenylethanoid glycosides are present at relatively low concentrations in *L. brevituba* and are characterized by a β-glucopyranose attached directly to hydroxyphenylethyl moiety. The β-position of the phenylethyl group was substituted. Hydroxyl derivative of cinnamic acid (such as caffeoyl and feruloyl) was attached to C4 position of β-glucopyranose and a rhamnose was attached to C6 position of the β-glucopyranose.

Compound **12** showed $[M-H]^-$ at m/z 621. A diagnostic ion at m/z 487, formed by loss of 134 Da from $[M-H]^-$ by α-cleavage of the six-member ether ring accompanying H transformation, suggested the presence of a six-member ether ring (H. Guo et al. 2007). High abundance fragments at m/z 179 and 161 suggested the presence of a caffeoyl group. Ions at m/z 151 indicated the concurrent losses of rhamnose (146 Da) and glucose (162 Da) from $[M-H\text{–caffeoyl}]^-$ ion at m/z 459. These fragments were in agreement with that of suspensaside A (H. Guo et al. 2007). Thus, compound **12** was tentatively characterized as suspensaside A; its tandem mass spectrum is shown in Figure 4.

Compound **1** displayed $[M-H]^-$ at m/z 639. In the tandem mass spectrum, the ion at m/z 621 indicated a loss of hydroxyl moiety. The easy loss of hydroxyl group suggested that it was on the β-position of phenylethyl group. Fragments were observed at m/z 487, 469, 459, 179, 161 and 151, which was a very similar pattern to that of suspensaside A, indicating the ion at m/z 621 might be suspensaside A. Compound **2** gave the same $[M-H]^-$ at m/z 639 and the same fragmentation as compound **1**. By comparison with the literature, compounds **1** and **2** were tentatively identified as suspensaside and its isomer (H. Guo et al. 2007).

Compound **9** showed $[M-H]^-$ at m/z 653. The ion at m/z 621 indicated loss of a methoxyl group. This loss suggested that the methoxy moiety was substituted to the β-position of phenylethyl group. Fragments were observed at m/z 487, 469, 459, 179, 161, and 151, which were very similar to that of suspensaside A, suggesting the ion at m/z 621 may be suspensaside A. According to the reported data, com-

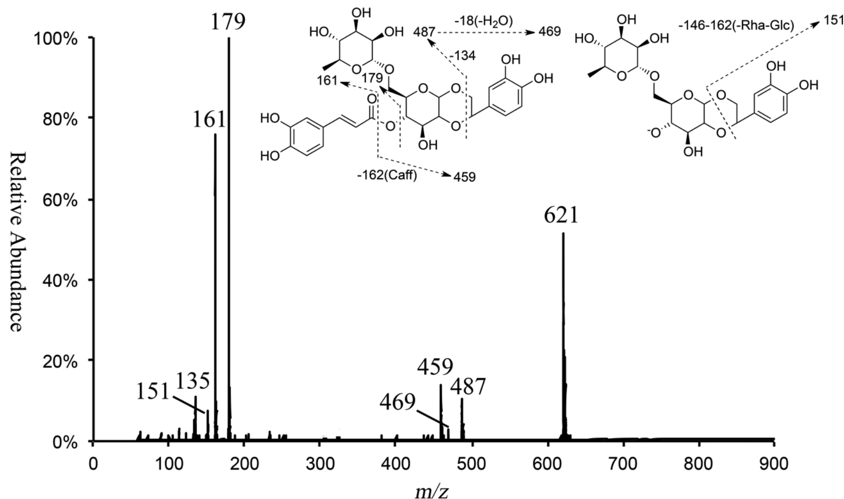


Figure 4. Tandem spectrum of $[M-H]^-$ of suspensaside A at m/z 621.

pound **9** was identified tentatively as suspensaside methyl ether (H. Guo et al. 2007). Based on a similar analysis of the tandem spectrum, compound **13** was tentatively identified as β-ethyl-OH-acteoside (Innocenti et al. 2006).

CONCLUSIONS

A rapid and reliable HPLC–QTOF–MS/MS method was established for structural identification of phenylethanoid glycosides from *L. brevituba* using accurate mass measurement and characteristic fragmentation patterns. Most of these phenylethanoid glycosides were reported in *L. brevituba* for the first time. This method may be the basis for comprehensive quality control of *L. brevituba*.

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REFERENCES

Chi, F., J. Deng, and Y. H. Wang. 2009. A new phenylethanoid glucoside from *Lagotis brevituba*. *Chin. J. Chin. Mater. Med.* 34: 2054–2056.

Chi, F., J. Deng, and Y. H. Wang. 2010. Chemical constituents from *Lagotis brevituba*. *Chin. J. Chin. Mater. Med.* 35: 869–871.

Editorial Committee of Flora of China. 1979. *Flora of China*. Beijing: Scientific Press, Vol. 67, Part 2.325–343.

Editorial Committee of Tibetan Materia Medica. 1991. *Tibetan Materia Medica*. Xining: Qinghai People's Press. 447–449 pp.

Fabre, N., I. Rustan, E. de Hoffmann, and J. Quetin-Leclercq. 2001. Determination of flavone, flavonol, and flavanone aglycones by negative ion liquid chromatography electrospray ion trap mass spectrometry. *J. Am. Soc. Mass. Spectr.* 12: 707–715.

Guo, H., A. H. Liu, M. Ye, M. Yang, and D. A. Guo. 2007. Characterization of phenolic compounds in the fruits of *Forsythia suspensa* by high-performance liquid chromatography coupled with electrospray ionization tandem mass spectrometry. *Rapid Commun. Mass. Sp.* 21: 715–729.

Guo, J., and W. Huang. 2011. Screening on effective composition of *Lagotis brevituba*. *Yunnan Journal of Traditional Chinese Medicine and Materia Medica.* 32: 61–62.

Han, L. F., B. Y. Mavis, E. W. Liu, Y. Zhang, W. Li, X. B. Song, F. H. Fu, and X. M. Gao. 2012. Structural characterisation and identification of phenylethanoid glycosides from *Cistanches deserticola* Y.C.Ma by UHPLC/ESI–QTOF–MS/MS. *Phytochem. Anal.* 23: 668–676.

Innocenti, M., L. M. Giancarlo, M. Sabrina, G. Catia, F. F. Vincieri, and M. Nadia. 2006. Electrospray ionization tandem mass spectrometric investigation of phenylpropanoids and secoiridoids from solid olive residue. *Rapid Commun. Mass Spectrom.* 20: 2013–2022.

Jin, L., and Z. Chen. 2006. Anticaneer Research of *Lagotis brevituba* Maxim. *J. Qinghai Norm. Univ.* (Natural Science). 2: 86–87.

Kirmizibekmez, H., C. Engin, M. Milena, B. Carla, Y. Erdem, and P. Sonia. 2009. Phenylethyl glycosides from *Digitalis lanata*. *Helv. Chim. Acta* 92: 1845–1852.

Kirmizibekmez, H., M. Paola, P. Sonia, P. Cosimo, D. Ali, and I. Çalıs. 2005. Identification by HPLC-PAD-MS and quantification by HPLC-PAD of phenylethanoid glycosides of five *Phlomis* species. *Phytochem. Analysis.* 16: 1–6.

Kobayashi H., K. Hiroko, M. Toshio, and F. Seigo. 1984. Studies on the consitituents of *Cistanchis Herba*, III: Isolation and structures of new phenylpropanoid glycosides, cistanosides A and B. *Chem. Pharm. Bull.* 32: 3009–3014.

Li, L., T. Rong, R. M. Yang, C. M. Liu, J. C. Young, and H. H. Zhu. 2008. Isolation and purification of phenylethanoid glycosides from *Cistanche deserticola* by high-speed counter-current chromatography. *Food Chem.* 108: 702–710.

Li, X. N., M. Y. Zhou, P. Q. Shen, J. B. Zhang, C. Chu, Z. W. Ge, and J. Z. Yan. 2011. Chemical constituents from *Rehmannia glutinosa*. *China Journal of Chin. Mater. Med.* 36: 3125–3129.

Otsuka, H. 1993. Phenylethanoids from *Linaria japonica*. *Phytochemistry.* 32: 979–981.

Qi, M., A. Z. Xiong, F. Geng, and Z. T. Wang. 2012. A novel strategy for target profiling analysis of bioactive phenylethanoid glycosides in Plantago medicinal plants using ultra-performance liquid chromatography coupled with tandem quadrupole mass spectrometry. *J. Sep. Sci.* 35: 1470–1478.

Shi, G. F., X. Y. Huang, and R. H. Lu. 2006. Chemical constituent research on *Lagotis brevituba* maxim of tibetan drugs. *China Journal of Chinese Materia Medica.* 31: 164–165.

Vukics, V., and A. Guttman. 2010. Sructural characterization of flavonoid glycosides by multistage mass spectrometry. *Mass Spectrom. Rev.* 29: 1–16.

Vukics, V., T. Ringera, A. Kery, G. K. Bonn, and A. Guttman. 2008. Analysis of heartsease (*Viola tricolor* L.) flavonoid glycosides by microliquid chromatography coupled to multistage mass spectrometry. *J. Chromatogr. A* 1206: 11–20.

Wang, Y., Y. P. Zhang, and X. M. Ma. 2007. Human stomach cancer SGC-790 1 cell apoptosis induced by n-butanol extract of *Lagotis brevitaba* Maxim. *Chin. Trad. Patent Med.* 29: 672–675.

Zhao, X. P., and J. G. Shi. 2004. Study on chemical composition of *Lagotis brevituba* Maxim. *Chin. Tradit. Herb. Drugs.* 35: 503–504.

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