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Characterization and identification of baccharane glycosides in Impatientis Semen by rapid-resolution liquid chromatography with electrospray ionization quadrupole time-of-flight tandem mass spectrometry

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ABSTRACT

Baccharane glycosides represent a group of rare saponins in plant kingdom and have been regarded as chemical marker for quality control of Impatientis Semen. Based on the structural skeleton, the baccharane glycosides were classified into three types: hosenkol A, hosenkol B and hosenkol C type. In this study, a rapid-resolution liquid chromatography coupled with electrospray ionization quadrupole time-of-flight tandem mass spectrometry (RRLC/ESI-Q-TOF MS/MS) was performed to investigate the fragmentation behaviours of baccharane glycosides from Impatientis Semen. In full scan mass spectrum, the accurate determination of molecular formula was obtained by the predominant ion $[M+COO]^-$ in negative mode. In the MS/MS spectrum, fragmentation reactions of the $[M+H]^+$ acquired in positive mode were recorded to provide abundant structural information on the aglycone and glycosyl moieties. The characteristic ion for hosenkol A and hosenkol B type glycosides was at *m/z* 399, while for hosenkol C type glycosides the diagnostic ion was at *m/z* 381. Neutral losses of monosaccharide, disaccharide, H_2O and C_3H_4 were observed for stepwise structural characterization. As a result, 19 compounds including 9 target saponins and 10 non-target saponins were rapidly screened out in ethanol extract of Impatientis Semen, and 5 of them were found to be novel baccharane glycosides.

1. Introduction

Impatiens balsamina L. (family Balsaminaceae), has been largely cultivated in China for ornamental and medicinal purpose. In Chinese Pharmacopoeia, the dried seeds of *I. balsamina* L. are documented as the source of Impatientis Semen, which have traditionally been prescribed for the treatment amenorrhea, abdominal mass, bone choking throat and sores [1,2].

Various chemical constituents, including fatty acids [3], diterpenes [4], flavonoids [5], triterpenes [6], saponins [7–9] have been isolated from Impatientis Semen. Among these constituents, saponins were proved to effectively inhibit Concanavalin A induced T-cell proliferation [10]. This type of secondary metabolites, also named baccharane glycosides, have been found only in very few species such as Polypodiaceae (*Lemmaphyllum microphyllum* C. Presl [11]), Meliaceae (*Aglaia silvestris* (M. Roemer) Merrill [12], *Aglaia foveolata* Pannell [13]), *etc.*

The baccharane glycosides were naturally believed as chemical markers for quality control of Impatientis Semen because of its rarity in plant kingdom. To the best of our knowledge, there were only three papers concerned with the analysis of these compounds in Impatientis Semen: Tan et al. [14] developed a reversed-phase high performance liquid chromatography with evaporative light scattering detection (RP-HPLC-ELSD) method to quantify two baccharane glycosides; Pei et al. [15] applied a high performance liquid chromatography with evaporative light scattering detection (HPLC-ELSD) method to determine four baccharane glycosides; Li et al. [16] employed a high performance liquid chromatography (HPLC) with electrospray ionization mass spectrometric detection (ESI-MSD) and evaporative light scattering detection (ELSD) method to detect eight baccharane glycosides. The above mentioned works focused merely on quantitative analysis of major saponins in Impatientis Semen and those minor saponins were neglected, the reason could be largely ascribed to the unavailability of sufficient reference compounds due to the difficulty of isolation and identification of such high-polar and thermal sensitive structures occurred in Impatientis Semen at low concentration.

Rapid resolution liquid chromatography (RRLC) coupled with hybrid quadrupole time-of-flight tandem mass spectrometry (Q-TOF MS/MS) now has been widely used in many fields, such as food stuffs [17] and herbal medicine [18–22]. Compared with the low-resolution MS methods such as quadrupole, triple quadrupole and ion trap mass spectrometry, Q-TOF MS/MS has the ability to

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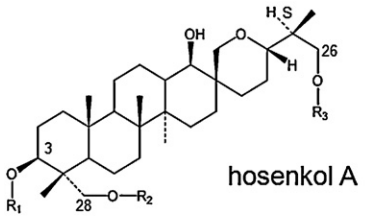
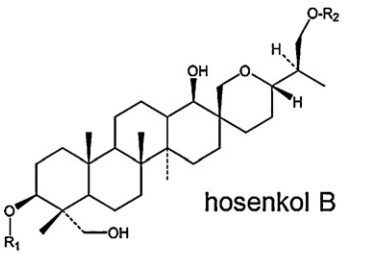
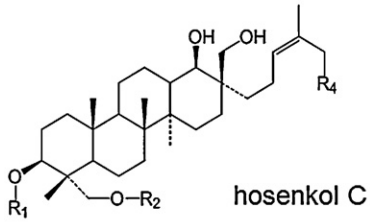


Name	Structure	MW
*hosenkoside A	R1=Rb R2=Ra R3=H	978
*hosenkoside D	R1=Ra R2=Ra R3=H	816
*hosenkoside K	R1=Rb R2=Ra R3=Ra	1140
*hosenkoside K	R1=Rc R2=Ra R3=H	948
*hosenkoside M	R1=Rc R2=Ra R3=Ra	1110
hosenkoside J	R1=Rb R2=H R3=H	816



Name	Structure	MW
*hosenkoside B	R1=Rb R2=Ra	978
*hosenkoside F	R1=Rc R2=Ra	948
hosenkoside E	R1=Rb R2=H	816
hosenkoside H	R1=Rc R2=H	786
hosenkoside I	R1=Ra R2=Ra	816



Name	Structure	MW
*hosenkoside C	R1=Rb R2=Ra R4=OH	978
*hosenkoside G	R1=Rc R2=Ra R4=OH	948
hosenkoside O	R1=Rb R2=Ra R4=H	962

Ra=β-D-glcose
Rb=β-D-glucopyranosyl(2→1)-β-D-glucopyranosyl
Rc= β-D-giucopyranosyl(2→1)-β-D-xylopyranosyl

Fig. 1. Chemical structures of the identified known baccharane glycosides from Impatientis Semen. *Compounds were identified by comparison with reference compounds.

operate with relatively higher mass resolution and to make exact mass measurements for both precursor and fragment ions, moreover it can also provide a degree of selectivity since it is able to discriminate between interference and among mass peaks having similar nominal masses but different exact masses. Consequently, the role of Q-TOF MS/MS instruments is crucial for efficiently identify non-target compounds in complex matrices when the reference compounds were unavailable [21].

In this work, the relationship between structure characteristic and MS/MS fragmentation ions of nine baccharane glycosides was investigated in detail. On the basis of the indicative data, the RRLC/Q-TOF MS/MS method was used to directly elucidate the baccharane glycosides in the extract of Impatientis Semen. A total of 19 baccharane glycosides were identified or tentatively characterized, 5 of them appeared to be novel compounds.

2. Materials and methods

2.1. Samples, chemicals and reagents

The seeds of *I. balsamina* L. were gathered from the Jangsu province, China. A voucher specimen was deposited in the Department of Pharmacognosy, China Pharmaceutical University, Nanjing, China.

The baccharane glycoside standards, including hosenkosides A, B, C, D, F, G, K, L, and M were isolated from the Impatientis Semen in our laboratory. Their structures (Fig. 1) were unequivocally elucidated by spectroscopic methods (IR, MS, ^{1}H NMR and ^{13}C NMR). The purity of the standards was determined to be higher than 95% by normalization of the peak area by HPLC-ELSD. Stock solutions (0.1 mg/ml) of the nine saponins were prepared individually in methanol.

Acetonitrile (ACN) and methanol (both HPLC grade) were purchased from Merck (Darmstadt, Germany). Formic acid was purchased from Tedia (Fairfield, OH, USA). Deionized water was prepared by passing distilled water through a Milli-Q system (Millipore, Milford, MA, USA).

2.2. Sample preparation

The Impatientis Semen were pulverized into powder (1.0 g, 60 mesh) and weighed, then defatted with petroleum ether (70–90 °C) in a Soxhlet extractor for 4 h. After that, the residue was placed into a stopper conical flask containing 30 ml 70% ethanol, immersed at the room temperature for 6 h, and then extracted by ultrasonication for 30 min. The supernatant was filtered and 15 ml of the successive filtrate was evaporated to dryness with a rotary evaporator. After that, the residue was reconstituted in 2 ml water with a test tube. The resultant solution was loaded onto the SPE cartridge. Under gentle vacuum, the cartridges with absorbed extracts were purified twice by 3 ml distilled water, and then the cartridges were washed twice with 3 ml of methanol and evaporated in vacuum nearly to dryness, after that, the residue was reconstituted in 10 ml methanol with a volumetric flask [16].

2.3. LC conditions

Chromatographic analysis was performed on an Agilent 1200 Series LC system (Agilent Technologies, Germany) equipped with a binary pump, an online degasser, an autoplate-sampler and a thermostatically controlled column compartment. Chromatographic separation was carried out at 25 °C on an Shim-pack CLC-ODS column (6.0 mm × 15 cm, 5 μm). The mobile phase consisted of 0.1% formic acid water (A) and ACN (B) using a gradient elution of 23–37% B at 0–26 min. The flow rate was kept at 0.8 ml/min, and was split at the column outlet to allow about 50% eluent to flow into the mass spectrometer. The sample volume injected was set at 1 μl.

Table 1
MS/MS data and proposed fragmentation pathways of the nine standard saponins.

Type	Standard	Precursor ions $[M+H]^+$	Molecular formula	Error (ppm)	Fragment ions	Elem. Comp.	Error (ppm)	Pathways
Hosenkol A type	Hosenkoside A	979.5487	$C_{48}H_{83}O_{20}$	−0.95	817.4906	$C_{42}H_{73}O_{15}$	4.65	$[M+H-Glc]^+$
					637.4323	$C_{36}H_{61}O_9$	−2.03	$[M+H-2Glc-H_2O]^+$
					475.3784	$C_{30}H_{51}O_4$	−0.45	$[M+H-3Glc-H_2O]^+$
					457.3656	$C_{30}H_{49}O_3$	4.43	$[M+H-3Glc-2H_2O]^+$
					439.3561	$C_{30}H_{47}O_2$	2.18	$[M+H-3Glc-3H_2O]^+$
					427.3570	$C_{29}H_{47}O_2$	0.13	$[M+H-3Glc-2H_2O-CH_2O]^+$
					399.3298	$C_{27}H_{43}O_2$	−10.15	$[M+H-3Glc-3H_2O-C_3H_4]^+$
	Hosenkoside D	817.4942	$C_{42}H_{73}O_{15}$	6.05	637.4298	$C_{36}H_{61}O_9$	1.9	$[M+H-Glc-H_2O]^+$
					475.3777	$C_{30}H_{51}O_4$	1.03	$[M+H-2Glc-H_2O]^+$
					457.3695	$C_{30}H_{49}O_3$	−4.12	$[M+H-2Glc-2H_2O]^+$
					439.3554	$C_{30}H_{47}O_2$	3.78	$[M+H-2Glc-3H_2O]^+$
					399.3270	$C_{27}H_{43}O_2$	−3.12	$[M+H-3Glc-3H_2O-C_3H_4]^+$
	Hosenkoside K	1141.6001	$C_{54}H_{93}O_{25}$	−0.05	979.5470	$C_{48}H_{83}O_{20}$	0.23	$[M+H-Glc]^+$
					817.4941	$C_{42}H_{72}O_{15}$	0.37	$[M+H-2Glc]^+$
					637.4319	$C_{36}H_{61}O_9$	−1.4	$[M+H-3Glc-H_2O]^+$
					475.3789	$C_{30}H_{51}O_4$	−1.5	$[M+H-4Glc-H_2O]^+$
					457.3676	$C_{30}H_{49}O_3$	0.05	$[M+H-4Glc-2H_2O]^+$
					439.3561	$C_{30}H_{49}O_3$	2.18	$[M+H-4Glc-3H_2O]^+$
					427.3529	$C_{29}H_{47}O_2$	9.75	$[M+H-4Glc-2H_2O-CH_2O]^+$
					399.3244	$C_{27}H_{43}O_2$	3.41	$[M+H-4Glc-3H_2O-C_3H_4]^+$
	Hosenkoside L	949.5330	$C_{47}H_{81}O_{19}$	3.86	637.4306	$C_{36}H_{61}O_9$	0.64	$[M+H-Xyl-H_2O]^+$
					475.3803	$C_{30}H_{51}O_4$	−4.46	$[M+H-Xyl-Glc-H_2O]^+$
					457.3601	$C_{30}H_{49}O_3$	−16.44	$[M+H-Xyl-2Glc-2H_2O]^+$
					439.3501	$C_{30}H_{47}O_2$	−15.836	$[M+H-Xyl-2Glc-3H_2O]^+$
					427.3601	$C_{29}H_{47}O_2$	−7.14	$[M+H-Xyl-2Glc-2H_2O-CH_2O]^+$
					399.3317	$C_{27}H_{43}O_2$	14.88	$[M+H-Xyl-2Glc-3H_2O-C_3H_4]^+$
	Hosenkoside M	1111.5865	$C_{53}H_{91}O_{24}$	2.68	931.5267	$C_{47}H_{79}O_{18}$	−0.65	$[M+H-Glc-H_2O]^+$
					799.4850	$C_{42}H_{71}O_{14}$	−1.46	$[M+H-Glc-Xyl-H_2O]^+$
					637.4294	$C_{36}H_{61}O_9$	2.53	$[M+H-2Glc-Xyl-H_2O]^+$
					475.3773	$C_{30}H_{51}O_4$	1.87	$[M+H-3Glc-Xyl-H_2O]^+$
					457.3686	$C_{30}H_{49}O_3$	−2.14	$[M+H-3Glc-Xyl-2H_2O]^+$
					439.3557	$C_{30}H_{47}O_2$	3.1	$[M+H-3Glc-Xyl-3H_2O]^+$
					427.3588	$C_{29}H_{47}O_2$	−4.09	$[M+H-3Glc-Xyl-2H_2O-CH_2O]^+$
					399.3269	$C_{27}H_{43}O_2$	−2.87	$[M+H-3Glc-Xyl-3H_2O-C_3H_4]^+$
Hosenkol B type	Hosenkoside B	979.5429	$C_{48}H_{83}O_{20}$	4.42	799.4892	$C_{42}H_{71}O_{14}$	−6.72	$[M+H-Glc-H_2O]^+$
					637.4330	$C_{36}H_{61}O_9$	−3.13	$[M+H-2Glc-H_2O]^+$
					475.3770	$C_{30}H_{51}O_4$	2.5	$[M+H-3Glc-H_2O]^+$
					457.3703	$C_{30}H_{49}O_3$	−5.87	$[M+H-3Glc-2H_2O]^+$
					439.3563	$C_{30}H_{47}O_2$	1.73	$[M+H-3Glc-3H_2O]^+$
					427.3613	$C_{29}H_{47}O_2$	−9.95	$[M+H-3Glc-2H_2O-CH_2O]^+$
					399.3254	$C_{27}H_{43}O_2$	0.9	$[M+H-3Glc-3H_2O-C_3H_4]^+$
	Hosenkoside F	949.5395	$C_{47}H_{81}O_{19}$	−3	769.4741	$C_{41}H_{69}O_{13}$	−1.08	$[M+H-Glc-H_2O]^+$
					637.4298	$C_{36}H_{61}O_9$	1.9	$[M+H-Glc-Xyl-H_2O]^+$
					475.3795	$C_{30}H_{51}O_4$	−2.77	$[M+H-2Glc-Xyl-H_2O]^+$
					457.3656	$C_{30}H_{49}O_3$	4.43	$[M+H-Xyl-2Glc-2H_2O]^+$
					439.3556	$C_{30}H_{47}O_2$	3.32	$[M+H-Xyl-2Glc-3H_2O]^+$
					427.3650	$C_{29}H_{47}O_2$	18.58	$[M+H-Xyl-2Glc-2H_2O-CH_2O]^+$
					399.3296	$C_{27}H_{43}O_2$	−9.65	$[M+H-Xyl-2Glc-3H_2O-C_3H_4]^+$
Hosenkol C type	Hosenkoside C	979.5395	$C_{48}H_{83}O_{20}$	1.89	817.4930	$C_{42}H_{73}O_{15}$	2.38	$[M+H-Glc]^+$
					655.4418	$C_{36}H_{63}O_{10}$	0.49	$[M+H-2Glc]^+$
					475.3786	$C_{30}H_{51}O_4$	0.28	$[M+H-3Glc-H_2O]^+$
					439.3578	$C_{30}H_{47}O_2$	−0.44	$[M+H-3Glc-3H_2O]^+$
					409.3449	$C_{29}H_{45}O$	3.9	$[M+H-3Glc-3H_2O-CH_2O]^+$
					421.3520	$C_{30}H_{45}O$	6.01	$[M+H-3Glc-4H_2O]^+$
					381.3129	$C_{27}H_{41}O$	7.45	$[M+H-3Glc-4H_2O-C_3H_4]^+$
	Hosenkoside G	949.5416	$C_{47}H_{81}O_{19}$	−4.63	817.4971	$C_{42}H_{73}O_{15}$	1.71	$[M+H-Xyl]^+$
					799.4862	$C_{42}H_{71}O_{14}$	−2.96	$[M+H-Xyl-H_2O]^+$
					655.4387	$C_{36}H_{63}O_{10}$	4.39	$[M+H-Xyl-Glc]^+$
					637.4318	$C_{36}H_{61}O_9$	−1.24	$[M+H-Xyl-Glc-H_2O]^+$
					475.3815	$C_{30}H_{51}O_4$	−6.98	$[M+H-Xyl-2Glc-H_2O]^+$
					439.3571	$C_{30}H_{47}O_2$	−0.1	$[M+H-Xyl-2Glc-3H_2O]^+$
					421.3466	$C_{30}H_{45}O$	−0.26	$[M+H-Xyl-2Glc-4H_2O]^+$
					381.3163	$C_{27}H_{41}O$	−2.91	$[M+H-Xyl-2Glc-4H_2O-C_3H_4]^+$

2.4. Q-TOF-MS/MS analysis

Detections were performed using a 6520 Q-TOF mass spectrometer (Agilent Technologies, Germany) equipped with an ESI interface. The operating parameters were: drying gas (N_2) flow rate, 10.0 l/min; drying gas temperature, 320 °C; nebulizer, 30 psig; capillary, 3500 V; skimmer, 65 V; OCTRFV, 750 V; and fragmentor voltage 120 V. For MS/MS experiments, the collision energy was 15 V to acquire signals and obtain maximal structural information from the ions of interest. The system

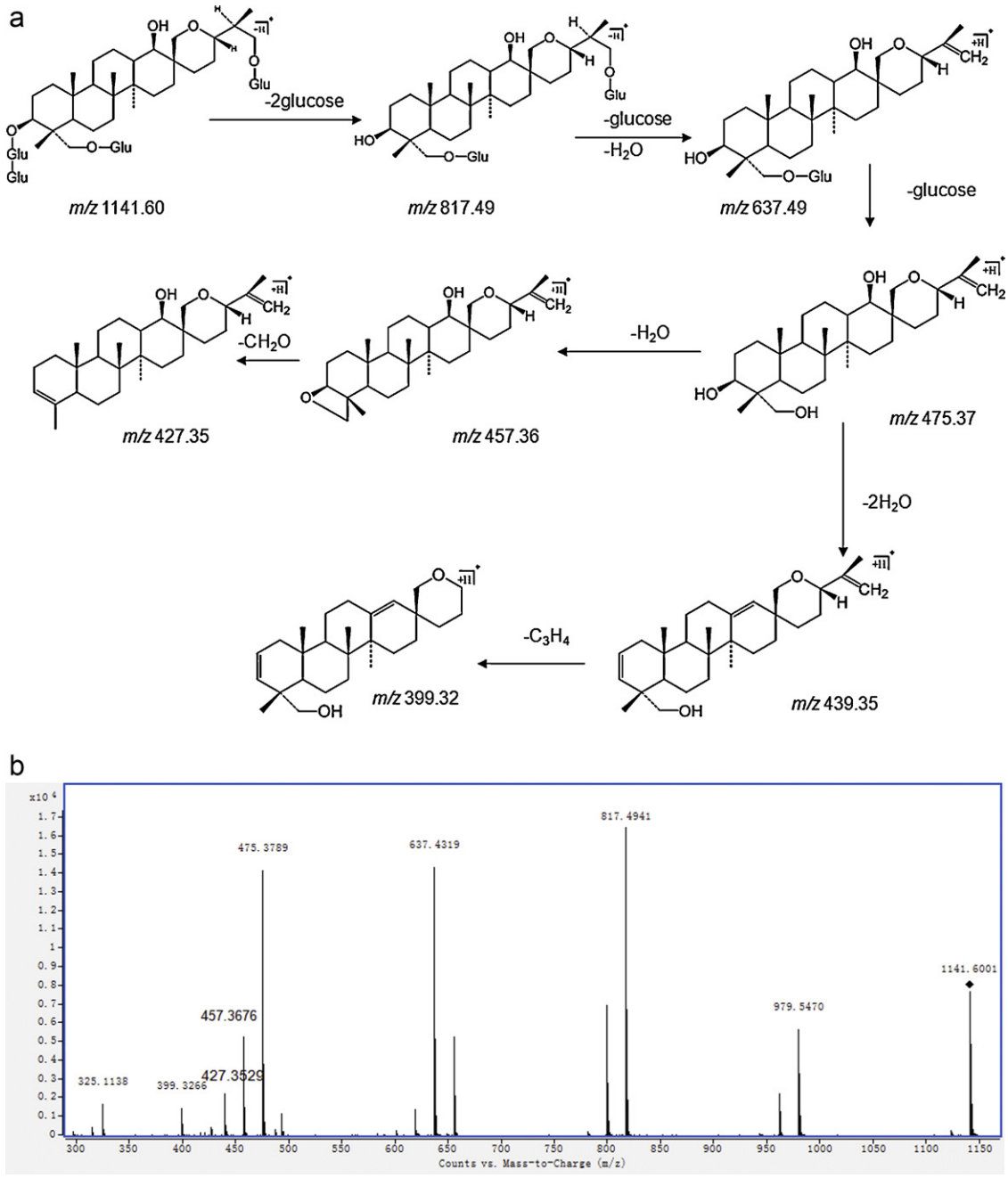


Fig. 2. The fragmentation pathway of hosenkoside K recorded using a collision energy of 15 V (a) and its CID MS/MS spectrum (b).

was operated under Masshunter workstation software version B.02.00 (Agilent Technologies). Each sample was analyzed in both positive and negative modes to provide sufficient information for structural identification. The mass range was set at *m*/*z* 100–1700.

3. Results and discussion

3.1. Characterization of the nine reference compounds

The baccharane glycosides separated from Impatientis Semen could be divided into three types: hosenkol A, hosenkol B and hosenkol C type, which mainly differ in the baccharane skeleton. The hosenkol C type possesses an unique opened pyran ring, and the distinguished feature of hosenkol A and B type lies in the chiral configuration of C-25, *viz.*, the former is *S*-configuration and the latter is *R*-configuration. Each baccharane glycosides usually consist of one or several sugar moieties linked at C-3, C-26 and/or C-28 of aglycone (Fig. 1).

The fragmentation pathways of 9 reference saponins were investigated by the RRLC–Q-TOF MS/MS method in both negative and positive modes. In negative mode, all compounds generated relatively high-abundance $[M-H]^-$ ions and few diagnostic ions even at a high collision energy up to 70 V. By contrast, in positive mode, they presented rich characteristic fragmentations and relatively lower abundance $[M+H]^+$ ions at 15 V collision energy. The results showed that negative mode provided accurate determination of molecular formula, while positive mode produced moderate

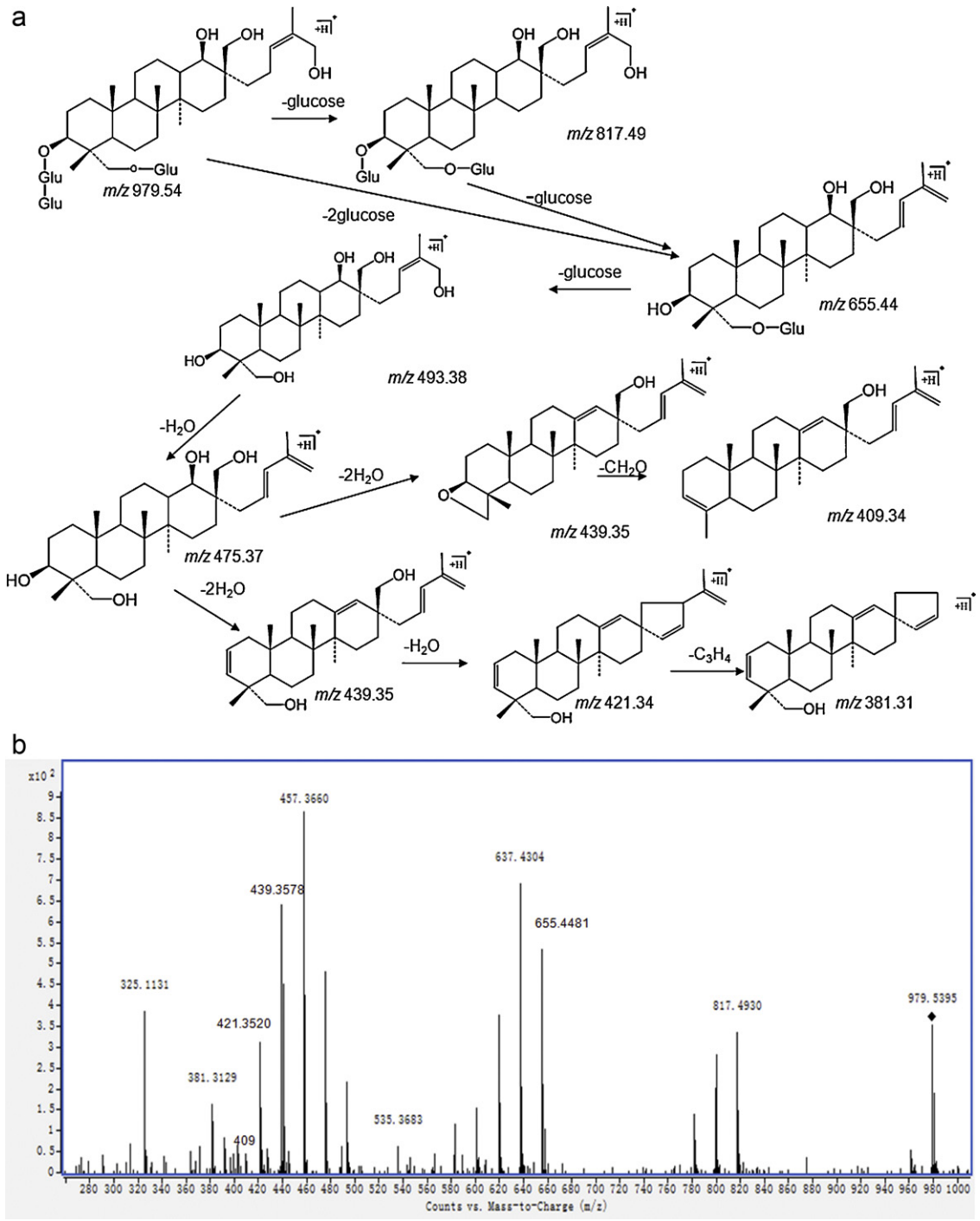


Fig. 3. The fragmentation pathway of hosenkoside C recorded using a collision energy of 15 V (a) and its CID MS/MS spectrum (b).

response mass and extensive fragmentation information. Therefore, the $[M+H]^+$ ion in positive mode was selected for MS/MS analysis.

In MS/MS spectrum, the $[M+H]^+$ ions as precursor ions in positive mode, 9 reference compounds were characterized by a series of common ions at *m/z* 475.37 ($C_{30}H_{51}O_4$), 457.37 ($C_{30}H_{49}O_3$) and 439.35 ($C_{30}H_{47}O_2$) at collision energy of 15 V. These diagnostic ions corresponded to the baccharane skeletons. The molecular ions and characteristic fragment ions of the references in the MS/MS experiments are shown in Table 1. Hosenkol A type includes hosenkosides K (**3**), M (**5**), A (**13**), L (**15**) and D (**16**), hosenkol B type comprises hosenkosides B (**1**) and F (**2**), hosenkol C type consists of hosenkosides C (**7**) and G (**9**). Each group was outlined below.

3.1.1. *Hosenkol A type*

All of the five members of hosenkol A type shared the same baccharane skeleton of hosenkol A. Fig. 2 showed the fragmentation pathway (a) and MS/MS spectrum (b) of hosenkosides K (**3**). In the (+) MS scan of hosenkosides K (**3**), the $[M+H]^+$ ion at *m/z* 1141.6001 corresponded to the formula $C_{54}H_{93}O_{25}$, which was selected as the precursor ion in the subsequent MS/MS experiment to give fragmentation information. In MS/MS spectrum, the precursor ion successive loss of four terminal glucose units at *m/z* 979.5470,

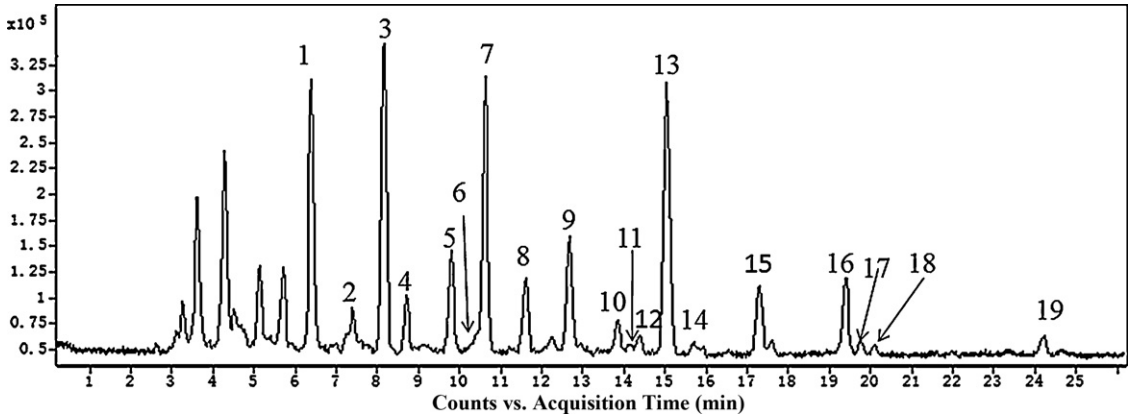


Fig. 4. Total ion chromatogram of the extract of Impatientis Semen in negative ion mode.

817.4941, 637.4319, 475.3789 indicated the presence of sugar moieties at C-3, C-26 and C-28. Besides a sugar moiety fragment ion at *m/z* 325.1164 originated from $[Glc+Glc+H]^+$ ion illustrated a disaccharide linked at C-3. The successive loss H_2O from ion at *m/z* 475.3789 generated ions at *m/z* 457.3676 ($C_{30}H_{49}O_3$) and 439.3561 ($C_{30}H_{47}O_2$). The ion at *m/z* 427.3529 ($C_{25}H_{47}O_5$) generated by cleavage CH_2O from ion at *m/z* 457.3676, which was useful for differentiating some positional isomers containing the hydroxyl unit at C-28 or other position [23,24]. The characteristic ion of hosenkol A was the ion at *m/z* 399.3244 ($C_{23}H_{43}O_2$) generated by elimination 40 Da (C_3H_4) from the ion at *m/z* 439.3626, which was different from the fragmentation pathway of hosenkol C type.

3.1.2. Hosenkol B type

Hosenkol B type included hosenkosides B (**1**) and hosenkosides F (**2**) which had the common baccharane of hosenkol B. For hosenkosides B (**1**), in MS/MS spectrum, the precursor ion at *m/z* 979.5429 ($C_{48}H_{83}O_{20}$) produced $[M+H-Glc-H_2O]^+$ ion at *m/z* 799.4892 ($C_{42}H_{71}O_{14}$) by typical loss of terminal glucose unit at C-26. The $[M+H-Glc-H_2O]^+$ ion consecutive yielded the diagnostic ions of baccharane glycosides at *m/z* 475.3770 ($C_{30}H_{51}O_4$), 457.3703 ($C_{30}H_{49}O_3$), 439.3563 ($C_{30}H_{47}O_2$) by sequential loss of 324 Da, 18 Da and 18 Da. The skeleton of hosenkol A and hosenkol B were similar except the chiral configuration at C-25. So the characteristic ion of hosenkol B was also at *m/z* 399.32 ($C_{23}H_{43}O_2$).

3.1.3. Hosenkol C type

Hosenkol C type comprised hosenkosides C (**7**) and hosenkosides G (**9**), and they had the same baccharane of hosenkol C. Fig. 3 showed the fragmentation pathway (a) and MS/MS spectrum (b) of hosenkosides C (**7**). The $[M+H]^+$ ion of hosenkosides C (**7**) was obtained at *m/z* 979.5395 ($C_{48}H_{83}O_{20}$), then direct elimination of 162 Da from the $[M+H]^+$ ion yielded the product $[M+H-Glc]^+$ ion at *m/z* 817.4930 ($C_{42}H_{73}O_{15}$), and the $[M+H-3Glc-H_2O]^+$ ion at *m/z* 475.3786 ($C_{30}H_{51}O_4$) was produced by consecutive loss of another terminal disaccharide moiety at C-3. Then successive loss H_2O from ion at *m/z* 475.3786 generated ions at *m/z* 457.3660 ($C_{30}H_{49}O_3$) and 439.3578 ($C_{30}H_{47}O_2$). The typical loss of 18 Da (*m/z* at 439.3578–421.3520) and 40 Da (*m/z* at 421.3520–381.3129) was observed in the MS/MS spectrum, illustrated between the C-24 and C-25 a double-bond existed. Apparently, the ion at *m/z* 381.3129 ($C_{27}H_{41}O_4$) was the characteristic ion of hosenkol C type.

3.2. Identification of baccharane glycosides in extracts of Impatientis Semen

Above MS/MS fragmentation behaviours of 9 models indicated most of baccharane glycosides displayed characteristic baccharane ions in positive mode, including ions at *m/z* 475.37, 457.36 and 439.35. By extracting any of these characteristic ions from the MS/MS spectrum in positive, a total of 18 compounds were screened out. Additionally, another baccharane glycoside (the 19th compound) with H instead of OH at C-26 was also detected, whose characteristic baccharane ions in positive mode were ions at *m/z* 459.3807, 441.3785 and 423.3636. Fig. 4 shows the total ion chromatograms (TICs) of the extract of Impatientis Semen in negative mode. Among the 19 compounds detected, saponins **1**, **2**, **3**, **5**, **7**, **9**, **13**, **15** and **16** were unambiguously identified by comparing the retention time (t_R) and fragmentation behaviours with those of the reference compounds, and another ten compounds were identified by their high accuracy MS spectrum and MS/MS fragmentation data (Table 2).

Two isomers (**3** and **4**) in the extracted ion chromatogram showed $[M+HCOO]^-$ ion at *m/z* 1185.59 in negative mode. In positive mode, compound **4** gave rise to a $[M+H]^+$ ion at *m/z* 1141.5967 and the MS/MS spectrum showed the ions at *m/z* 979.5379, 817.4935 and 637.4267 by consecutive loss of terminal glucose units. The ion at *m/z* 325.1175 illustrated a disaccharide linked to the aglycone. The characteristic ion at *m/z* 399.3253 indicated compound **4** belonging to the hosenkol A or B type. Since a disaccharide at C-3 was found commonly in baccharane glycosides, this compound was thus tentatively identified as the hosenkol B 3-O-(Glc-Glc)-26-O-Glc-28-O-Glc, which was a new compound.

Another two isomers (**5**, **6**) gave $[M+HCOO]^-$ ion at *m/z* 1155.57 in negative mode. In positive mode, MS/MS spectrum showed **6** generated ions *m/z* at 931.5302, 799.4791 and 637.4302 by cleavage of the terminal xylose and glucose. The characteristic ion at *m/z* 399.3300 suggested **6** belonging to hosenkol A or B type. Compared the fragment ions of **6** with those of **5** (hosenkoside M), no obvious difference was observed. Summing up above features, compound **6** was tentatively identified as hosenkol B 3-O-(Glc-Xyl)-26-O-Glc-28-O-Glc, it was also a new compound.

The last series isomers in our study included 5 compounds (**10**, **11**, **12**, **17**, **18**), and they shared the common ion $[M+HCOO]^-$ at *m/z* 861.48 in negative mode. These isomers could be divided into two groups by MS/MS spectrum, compounds **10**, **11**, **12** generated the common ion at *m/z* 325.10 by cleavage a disaccharide, whereas compounds **17**, **18** did not yield this ion. Compounds **10** and **12** generated similar fragment ions as hosenkol A or B type baccharane glycosides did, so they might be a couple of chiral isomers hosenkoside J and E [7,8]. Compound **11** had similar fragment ions to hosenkol C type. The ion at *m/z* 325.1135 illustrated a disaccharide linked to the aglycone. Additionally, it generated the characteristic ion at *m/z* 381.3167. So it was tentatively characterized as hosenkol C 3-O-(Glc-Glc), which was a new compound.

Table 2
MS/MS data and fragment pathways for identified compounds from Impatientis Semen.

Compound No.	t_R (min)	MS (−120 V)	MS (+120 V)		Error (ppm)	MS/MS (+15 V)		Identification	Reference
		$[M+HCOO^-]^-$ (m/z)	$[M+H]^+$ (m/z)	Elem. Comp.		Common fragment ions	Characteristic fragment ions		
1[a]	6.418	1023.5369	979.5485	$C_{48}H_{83}O_{20}$	−1.31	799.4741, 637.4330	475.3802, 457.3686, 439.3553, 427.3574, 399.3253, 325.1107	Hosenkoside B	[7]
2[a]	7.395	993.3562	949.5411	$C_{47}H_{81}O_{19}$	−4.68	817.4644, 655.4422	475.3779, 457.3709, 439.3519, 427.3627, 399.3231	Hosenkoside F	[8]
3[a]	8.214	1185.5918	1141.6040	$C_{54}H_{93}O_{25}$	−3.47	979.5453, 817.4958, 637.4314	475.3779, 457.3680, 439.3606, 399.3250, 325.1099	Hosenkoside K	[8]
4	8.723	1185.5909	1141.5967	$C_{54}H_{93}O_{25}$	2.93	979.5379, 817.4935, 637.4267	475.3766, 457.3671, 439.3545, 399.3253, 325.1175	Hosenkol B 3-O-(Glc-Glc)-26-O-Glc-28-O-Glc	-
5[a]	9.794	1155.5837	1111.5890	$C_{53}H_{91}O_{24}$	0.43	931.5310, 799.4828, 637.4320	475.3795, 457.3686, 439.3603, 399.3210	Hosenkoside M	[9]
6	10.45	1155.5797	1111.5854	$C_{53}H_{91}O_{24}$	3.67	931.5302, 799.4791, 637.4203	475.3771, 457.3681, 439.3557, 427.3624, 399.3300	Hosenkol B 3-O-(Glc-Xyl)-26-O-Glc-28-O-Glc	-
7[a]	10.612	1023.5372	979.5359	$C_{44}H_{83}O_{23}$	−4.02	817.4998, 637.4301	493.3944, 475.3778, 439.3582, 421.3485, 409.3491, 381.3161	Hosenkoside C	[7]
8	11.58	1023.5392	979.5476	$C_{48}H_{83}O_{20}$	−0.39	799.4824, 637.4315	475.3780, 457.3663, 439.3578, 427.3538, 399.3266, 325.1089	isomer of HosenkosideB or A	[6,7]
9[a]	12.526	993.5271	949.5491	$C_{47}H_{81}O_{19}$	11.9478	817.4971, 655.4405, 637.4352	475.3716, 457.3709, 439.3522, 409.3461, 381.3253	Hosenkoside G	[8]
10	13.849	861.4871	817.4976	$C_{42}H_{73}O_{15}$	−3.92	655.4329, 637.4208	493.3796, 457.3665, 439.3595, 427.3581.399.3259, 325.1074	Hosenkosides J or E	[7,8]
11	13.901	861.4821	817.4926	$C_{42}H_{73}O_{15}$	2.2	655.4394, 637.4335, 619.4195	493.3888, 475.3778, 457.3678, 439.3581, 421.3476, 381.3167, 325.1135	Hosenkol C 3-O-(Glc-Glc)	-
12	14.166	861.4830	817.4948	$C_{42}H_{73}O_{15}$	−0.49	799.4831, 637.4319, 619.4201	475.3774, 457.3681, 439.3570, 427.2031, 381.7559, 325.1145	Hosenkosides J or E	[7,8]
13[a]	15.035	1023.5383	979.5445	$C_{48}H_{83}O_{20}$	2.78	799.4863, 637.4280	475.3808, 457.3969, 439.3608, 399.3279, 325.1158	Hosenkosides A	[6,7]
14	15.799	831.4733	787.4822	$C_{41}H_{71}O_{14}$	2.08	637.4334	475.3781, 457.3684, 439.3550, 399.3290	Hosenkoside H	[8]
15[a]	17.101	993.5292	949.5534	$C_{41}H_{81}O_{19}$	16.47	799.4827, 637.4213	475.3810, 457.3780, 439.3559, 399.3253	Hosenkoside L	[9]
16[a]	19.287	861.4854	817.4965	$C_{42}H_{73}O_{15}$	−2.57	637.4298	475.3777, 457.3695, 439.3554, 399.3270	Hosenkoside D	[7]
17	19.775	861.4862	817.4967	$C_{42}H_{73}O_{15}$	−2.82	637.4238	475.3811, 457.3679, 439.3596, 399.3362	Hosenkoside I or its isomer	[8]
18	20.094	861.4847	817.4947	$C_{42}H_{73}O_{15}$	−0.37	655.4340	493.3870, 475.3806, 457.3749, 439.3542, 427.3566, 399.3288	Hosenkoside I or its isomer	[8]
19	24.192	1007.5434	963.5393	$C_{48}H_{83}O_{19}$	−13.5	801.4941, 639.4418	477.3953, 459.3807, 441.3785, 423.3636, 383.3320	Hosenkoside O	[9]

[a] Compounds were identified by comparison with reference compounds.

The other two compounds **17**, **18** displayed similar fragmentation with hosenkoside D. Thus, compound **17** and **18** were tentatively characterized as hosenkoside I and its isomer [8], and one of them might be a new compound.

Compound **8** presented the $[M+HCOO]^-$ ion at *m*/*z* 1023.5392 in negative mode. From MS/MS spectrum in positive, compound **8** had similar fragment ions to compounds **1** (hosenkosides B) and **13** (hosenkosides A). So compound **8** was the isomer of compounds **1** or **13**. To our knowledge, this baccharane glycoside was not reported before.

Compound **14** gave a $[M+HCOO]^-$ ion at *m*/*z* 831.4733 in negative mode. The observation of ions at *m*/*z* 637.4334, 475.3781 suggested that two glucoses linked to the aglycone. The presence of 457.3684, 439.3550, 399.3290 suggested the structure of compound **14** had an aglycone of hosenkol A or B, and it were tentatively characterized as hosenkoside H [8].

Compound **19** produced the $[M+HCOO]^-$ ion at *m*/*z* 1007.5434. In MS/MS spectrum of positive mode, the presence of the ions at *m*/*z* 801.4941, 639.4418, 477.3953, 325.1086 indicated that there were a disaccharide linked to C-3 and one glucose connected to the C-26 or C-28. A series ions at *m*/*z* 459.3807, 441.3785, 423.3636, 383.3320 suggested compound **19** possessed a similar fragmentation pattern as hosenkol C type baccharane glycosides did, with a difference of 16 Da (H instead of OH at C-26) for the characteristic ions, so it was tentatively characterized as hosenkoside O [9].

4. Conclusions

In this study, a RRLC method with ESI-Q-TOF MS/MS has been employed to study the structure and fragmentation pattern of baccharane glycosides in Impatientis Semen. Based on the retention time, accurate mass of $[M+HCOO]^-$ ion in negative mode and fragmentation pattern of MS/MS spectrum in positive mode, 19 baccharane glycosides including 5 novel ones had been identified. The RRLC/Q-TOF MS/MS method presented here has been demonstrated to be an effective tool in characterizing the components in the complex plant matrices, although further investigation is still needed to confirm the absolute configurations of isomers.

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