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Characterization of steroidal saponins in crude extracts from *Dioscorea zingiberensis* C. H. Wright by ultra-performance liquid chromatography/electrospray ionization quadrupole time-of-flight tandem mass spectrometry

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ABSTRACT

Steroidal saponins are the major bioactive constituents of *Dioscorea zingiberensis* C. H. Wright (*D. zingiberensis*). In this work, ultra-performance liquid chromatography/electrospray ionization quadrupole time-of-flight tandem mass spectrometry (UPLC/Q-TOF-MS/MS) was applied to the separation and characterization of steroidal saponins in crude extracts from *D. zingiberensis*. The results showed that fragment ions from glycosidic and cross-ring cleavages gave a wealth of structural information related to aglycone skeletons, sugar types and the sequence of sugar units. According to the summarized fragmentation patterns, identification of steroidal saponins from *D. zingiberensis* could be fulfilled, even when reference standards were unavailable. As a result, a total of thirty-one saponins with five aglycone skeletons, including fourteen new trace saponins, were identified or tentatively elucidated in crude extracts from *D. zingiberensis* based on their retention times, the mass spectrometric fragmentation patterns, and MS and MS/MS data.

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1. Introduction

A steroidal saponin molecule consists of an aglycone and several glycosyl moieties. Steroidal saponins are mainly present in *Liliaceae*, *Dioscoreaceae*, *Agavaceae* and *Smilacaceae* and can be classified into spirostanol, isospirostanol, furostanol, pseudospirostanol and cholestanol saponins according to their skeletons [1]. The medicinal plant *Dioscorea zingiberensis* C. H. Wright (*D. zingiberensis*) is widely distributed in Shanxi, Hunan, Hubei and Sichuan provinces of China. Its rhizome has been known as a traditional Chinese medicine (TCM) for a long time, and used as a folk treatment for cough, anthrax, rheumatic heart disease, rheumarthritis, tumefaction and sprain [2]. The water-soluble steroidal saponins from *D. zingiberensis* are the main bioactive components, which have been used in China for many years for the treatment of coronary heart disease [3]. Furthermore, dioscin, one of steroidal saponins from *D. zingiberensis*, whose cytotoxic activity against the cancer cell line K562 *in vitro* has been reported [4]. Many steroidal saponins have been isolated and identified from *D. zingiberensis* [5–10], and the five main steroidal aglycones are shown in Fig. 1a–e. Moreover, the common sugars present in steroidal saponins from *Dioscoreaceae* are hexose (glucose) and 6-deoxyhexose (rhamnose), and generally, glucosyls are connected with the hydroxyl groups at C-3 and/or C-26 positions of steroidal aglycones.

Up to now, characterization of steroidal saponins from *D. zingiberensis* mainly depends on the NMR spectra of the pure compounds obtained by preparative isolation and purification [6–10]. But this method wastes time and energy, especially for some trace compounds, which are very difficult to be purified and characterized. LC–MS has proven to be a very convenient and efficient technique for identification of steroidal saponins in plant extracts in recent years [11–15]. Ion trap (IT) MS allows MS^n for structural elucidation of steroidal saponins, but this analyzer provides nominal mass accuracy and may not well confirm the detailed identities of the product ions at most times [12–15]. The application of Q-TOF-MS can yield empirical chemical formula based on the accurate masses of molecular ions and detailed fragmentation information, which removes ambiguities out of the interpretation, confirms the identities of the fragment ions and facilitates structural elucidation [16–21]. Recently, ultra-performance liquid chromatography

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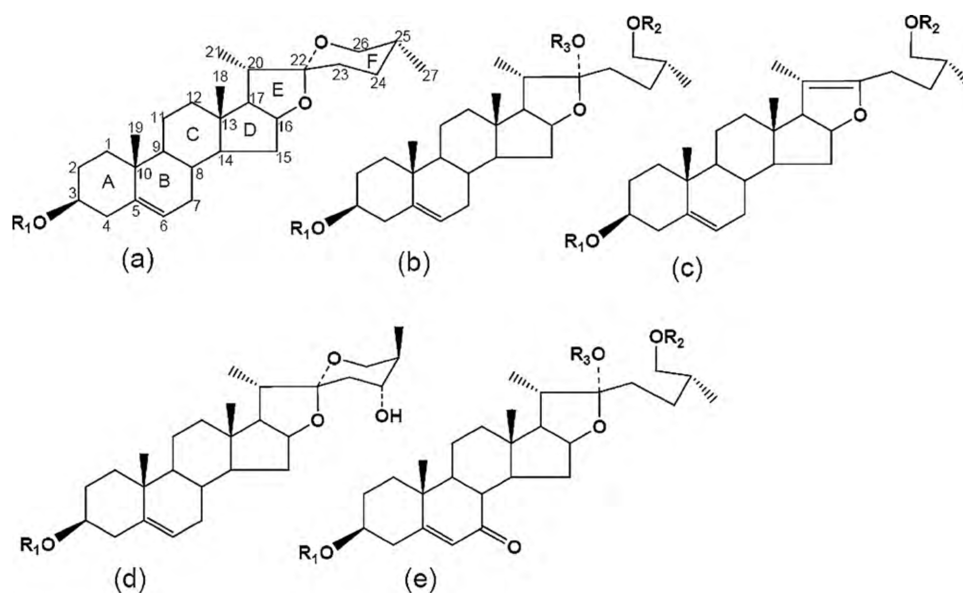

Compounds	R1	R2	R3	MW
[a]zingiberensis saponin	Glc(1→3)Glc(1→4)[Rha(1→2)]-Glc	--	--	1046.5298
[a]deltonin	Glc(1→4)[Rha(1→2)]-Glc	--	--	884.4769
[a]dioscin	Rha(1→4)[Rha(1→2)]-Glc	--		868.4821
[a]prosapogenin A of dioscin	Rha(1→2)-Glc	--	--	722.4241
[a]diosgenin diglucoside	Glc(1→4)-Glc	--	--	738.4191
[b]protobioside	Rha(1→2)-Glc	Glc	H	902.4875
[b]funkioside	H	Glc	H	594.3768
[b]peak 5	Glc(1→4)-Glc	Glc	H	918.4824
[b]peak 7	Glc	Glc	H	756.4296
[c]zingiberenin G	Glc(1→3)Glc(1→2)[Rha(1→4)]-Glc	Glc	--	1208.5829
[d]zingiberogenin	H	--	--	430.3083
[d]zingiberenin A2	Rha(1→2)-Glc	--	--	738.4191
[e]zingiberenin H	Glc(1→3)Glc(1→4)[Rha(1→2)]-Glc	Glc	H	1240.5724

Glc=β-D-glucopyranosyl, Rha=α-L-rhamnopyranosyl

Fig. 1. Structures of the steroidal saponins from *D. zingiberensis*.

(UPLC) has been introduced as a rapid and efficient tool for complex sample analysis [16,18–21]. Generally, the column is packed with particles of less than 2 μm size and operated at high pressure up to 600 bar, which results in high resolution and superior peak capacity in short analysis time. The combination of UPLC and Q-TOF-MS/MS offers high chromatographic resolution with accurate mass measurement for both MS and MS/MS experiments, then, significant advantages for rapid screening target compounds in complex matrices are achieved.

In this work, the structural characteristics of the steroidal saponins in the ethanolic extracts from the dried rhizomes of *D. zingiberensis* have been investigated by UPLC/Q-TOF-MS/MS in both negative and positive ion modes. The fragmentation patterns of reference standards were investigated and the steroidal saponins in the extracts were identified or tentatively characterized according to the retention times, MS and MS/MS data. This UPLC/Q-TOF-MS/MS method has been successfully used to characterize of steroidal saponins in the crude extracts from *D. zingiberensis*. It also provides an excellent approach for rapid screening of steroidal saponins in plant extracts.

2. Experimental

2.1. Reagents and materials

Acetonitrile (HPLC grade) was purchased from Fisher Scientific Co. (Loughborough, UK). Formic acid (HPLC grade) was purchased from Acros Co. Ltd. (NJ, USA). Water (18.2 MΩ) was purified on a Milli-Q system (Millipore, Billerica, USA). Other reagents were commercially available of analytical purity. The dried rhizomes of *D. zingiberensis* were purchased from a drug store in Zaoyang City (Hubei Province, China). Standards of zingiberensis saponin, deltonin, dioscin, prosapogenin A of dioscin and diosgenin diglucoside were isolated and purified from *D. zingiberensis* in our laboratory. Their structures were confirmed by UV, ESI-MS and ^{1}H, ^{13}C NMR and comparison with the literature [4,6,10,22].

2.2. UPLC/Q-TOF-MS/MS analysis

UPLC/Q-TOF-MS/MS analysis was performed on a Waters ACQUITY™ UPLC coupled with a Q-TOF Premier, a quadrupole and

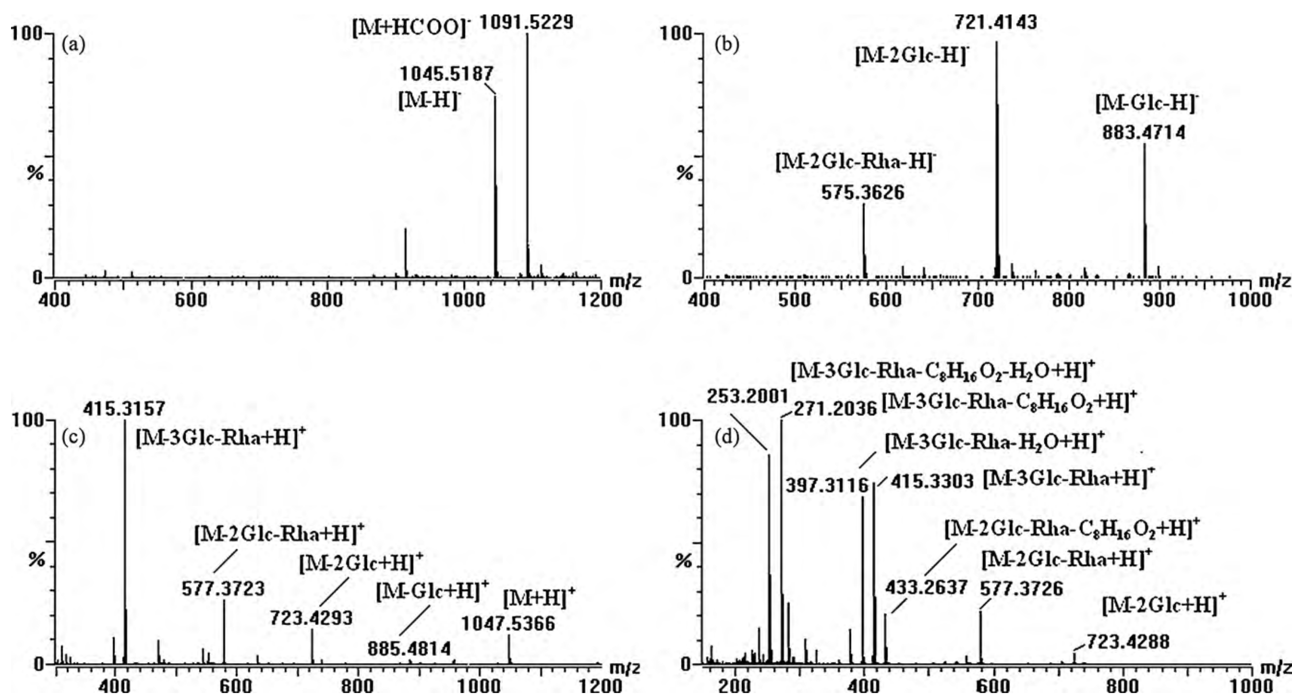

Fig. 2. MS and MS/MS spectra of zingiberensis saponin (peak 24). (a) (−)ESI-MS spectrum of zingiberensis saponin; (b) (−)ESI-MS/MS spectrum of the ion at *m/z* 1045.5187; (c) (+)ESI-MS spectrum of zingiberensis saponin; (d) (+)ESI-MS/MS spectrum of the ion at *m/z* 1047.5366.

orthogonal acceleration time-of-flight tandem mass spectrometer (Waters Co., USA), which was equipped with a LockSpray interface and an ESI interface. The system was controlled under MassLynx V4.1 software (Waters Co., USA). High-purity nitrogen was used as the nebulizer and auxiliary gas. Argon was used as the collision gas. The ESI capillary voltage was set at +3.0 kV for positive ion mode and −3.0 kV for negative ion mode. The source and desolvation temperature were set at 120 °C and 350 °C, respectively. The desolvation and cone gas flows were 800 L/h and 50 L/h, respectively. The sample cone voltage was set at 35 V for positive ion mode and 40 V for negative ion mode. The collision energy (CE) was set at 25–30 eV for positive ion mode and 30–45 eV for negative ion mode. Mass accuracy was maintained using a lock spray with leucine enkephalin for positive ion mode ($[M+H]^+$ = 556.2771) and negative ion mode ($[M-H]^-$ = 554.2615) at a concentration of 50 pg/mL and a flow rate of 10 μL/min as reference. The mass scan was over the range of *m/z* 100–1500 for positive ion mode and *m/z* 200–2000 for negative ion mode.

UPLC/Q-TOF-MS/MS was used to control the purity of the standard steroidal saponins. The chromatographic column was a Waters ACQUITY^{TM} UPLC BEH C18 (100 mm × 2.1 mm, 1.7 μm). Separation was carried out in isocratic mode using 55% (v/v) acetonitrile and 45% (v/v) aqueous solution containing 0.1% (v/v) formic acid. The flow rate was set at 0.25 mL/min. All samples were kept at 25 °C and injection volume was 1.0–5.0 μL for each analysis.

For on-line analysis of the extracts from *D. zingiberensis*, a linear gradient was developed. Acetonitrile and acidified water (0.1% formic acid, v/v) were used as the mobile phases A and B, respectively. The mobile phase was programmed as follows: 0–30 min, 10–55% A; 30–35 min, 55–75% A. Other conditions were the same as those described for standard steroidal saponins.

2.3. Sample preparation

The dried rhizomes of *D. zingiberensis* were crushed and 300 g portions were refluxed thrice with 1800 mL of 70% (v/v) ethanol at 80 °C, and each reflux time was 2 h. The mixture was filtered using analytical filter paper each time and the combined ethanol aqueous extracts were evaporated by rotary evaporation under vacuum at 60 °C. Then, the residue was freeze-dried. An aliquot (25 mg) of the dry sample was dissolved in 70% ethanol (5 mL) and filtered through a membrane filter (0.22 μm) prior to UPLC/Q-TOF-MS/MS analysis.

3. Results and discussion

3.1. The proposed fragmentation pathway for zingiberensis saponin

As the five reference standards shared the same aglycone skeleton (Fig. 1a) and similar fragmentation, zingiberensis saponin was used as an example to discuss the fragmentation patterns for these reference standards in detail. In (−)ESI-MS, as shown in Fig. 2a, the standard gave an $[M-H]^-$ ion at *m/z* 1045.5187. Because of the presence of formic acid in the mobile phase, the standard also gave a strong $[M+HCOO]^-$ ion at *m/z* 1091.5229. In Fig. 2b, three main fragment ions at *m/z* 883.4714, 721.4143 and 575.3626 were observed in the collision induced dissociation (CID) spectrum of the deprotonated molecular ion at *m/z* 1045.5187. The fragment ions at *m/z* 883.4714 and 721.4143 could be attributed to the loss of one and two hexoses from $[M-H]^-$ ion (*m/z* 1045.5187), respectively. The fragment ion at *m/z* 575.3626 was corresponding to the loss of two hexoses and one deoxyhexose from $[M-H]^-$ ion (*m/z* 1045.5187). The fragments of zingiberensis saponin revealed the characteristic cleavage of glycosidic bonds, and the fragmentation pattern directly provided the detailed structural information about the sequence of sugars. The similar MS/MS behaviors of this type of saponins in IT MS were also reported in the literature [13].

In (+)ESI-MS, the mass spectrum of zingiberensis saponin is shown in Fig. 2c. The mass spectrum was dominated by the fragment ion $[aglycone+H]^+$ (*m/z* 415.3157). In addition, the protonated molecular ion $[M+H]^+$ (*m/z* 1047.5366) and other fragments (*m/z* 885.4814, 723.4293, 577.3723) via consecutive neutral loss of two glucosyls and one rhamnosyl from $[M+H]^+$ (*m/z* 1047.5366) were also observed. In order to clarify the fragmentation pattern for

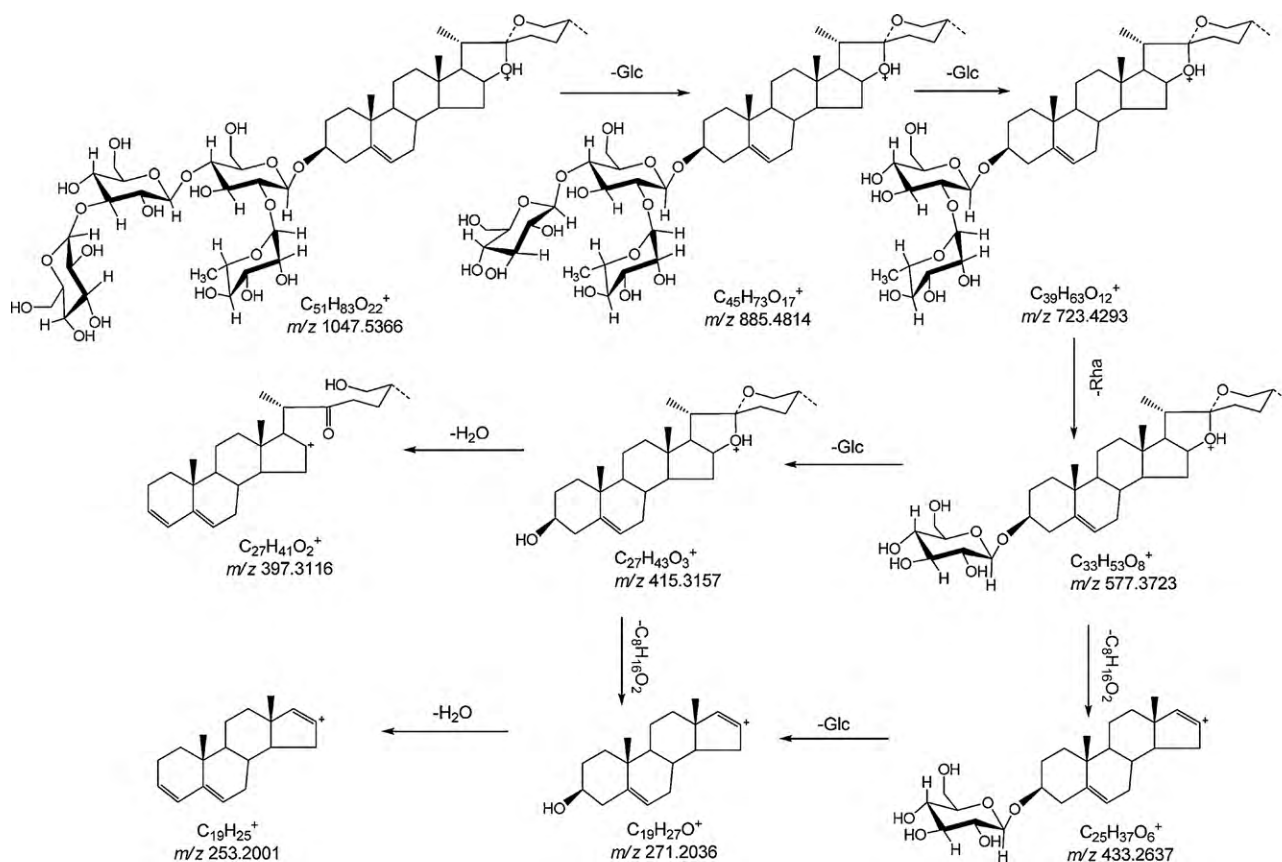

Fig. 3. The proposed fragmentation pathway for zingiberensis saponin.

Table 1
Steroidal saponins determined by UPLC/Q-TOF-MS in the crude extracts from *D. zingiberensis*.

Peak	RT (min)	Formula	Selected ion	m/z experimental	m/z calculated	Error (mDa)	Error (ppm)	i-FIT
1	10.62	$C_{57}H_{91}O_{29}$	$[M-H]^-$	1239.5599	1239.5646	−4.7	−3.8	1.1
2	12.38	$C_{57}H_{93}O_{28}$	$[M-H]^-$	1225.5811	1225.5853	−4.2	−3.4	0.4
3	12.73	$C_{51}H_{83}O_{23}$	$[M-H]^-$	1063.5280	1063.5325	−4.5	−4.2	0.4
4	12.91	$C_{51}H_{83}O_{22}$	$[M-H]^-$	1047.5317	1047.5376	−5.9	−5.6	2.9
5	12.96	$C_{45}H_{73}O_{19}$	$[M-H]^-$	917.4735	917.4746	−1.1	−1.2	4.2
6	13.36	$C_{45}H_{73}O_{18}$	$[M-H]^-$	901.4760	901.4797	−3.7	−4.1	2.3
7	13.57	$C_{39}H_{63}O_{14}$	$[M-H]^-$	755.4220	755.4218	0.2	0.3	7.4
8	14.21	$C_{51}H_{83}O_{22}$	$[M+H]^+$	1047.5444	1047.5376	6.8	6.5	2.9
9	15.25	$C_{51}H_{83}O_{23}$	$[M+H]^+$	1063.5310	1063.5325	−1.5	−1.4	4.0
10	15.33	$C_{51}H_{83}O_{22}$	$[M+H]^+$	1047.5415	1047.5376	3.9	3.7	2.9
11	15.73	$C_{45}H_{73}O_{18}$	$[M+H]^+$	901.4797	901.4797	0	0	1.5
12	16.03	$C_{57}H_{93}O_{27}$	$[M+H]^+$	1209.5879	1209.5904	−2.5	−2.1	0.6
13	16.18	$C_{57}H_{93}O_{27}$	$[M+H]^+$	1209.5933	1209.5904	2.9	2.4	2.0
14	16.68	$C_{51}H_{83}O_{23}$	$[M+H]^+$	1063.5359	1063.5325	3.4	3.2	0.7
15	17.20	$C_{45}H_{73}O_{18}$	$[M+H]^+$	901.4802	901.4797	0.5	0.6	2.2
16	17.52	$C_{45}H_{73}O_{17}$	$[M+H]^+$	885.4868	885.4848	2.0	2.3	3.4
17	17.76	$C_{45}H_{71}O_{17}$	$[M-H]^-$	883.4659	883.4691	−3.2	−3.6	2.4
18	17.83	$C_{45}H_{73}O_{17}$	$[M+H]^+$	885.4866	885.4848	1.8	2.0	6.1
19	18.14	$C_{39}H_{63}O_{13}$	$[M+H]^+$	739.4246	739.4269	−2.3	−3.1	8.5
20	18.29	$C_{45}H_{73}O_{17}$	$[M+H]^+$	885.4838	885.4848	−1.0	−1.1	0.4
21	18.62	$C_{33}H_{53}O_{9}$	$[M-H]^-$	593.3690	593.3690	0	0	6.0
22	25.25	$C_{51}H_{81}O_{22}$	$[M-H]^-$	1045.5172	1045.5220	−4.8	−4.6	1.0
23	25.75	$C_{51}H_{81}O_{22}$	$[M-H]^-$	1045.5203	1045.5220	−1.7	−1.6	1.0
24	27.24	$C_{51}H_{81}O_{22}$	$[M-H]^-$	1045.5187	1045.5220	−3.3	−3.2	1.0
25	28.42	$C_{45}H_{71}O_{17}$	$[M-H]^-$	883.4674	883.4691	−1.7	−1.9	2.3
26	28.89	$C_{45}H_{71}O_{16}$	$[M-H]^-$	867.4738	867.4742	−0.4	−0.5	0.1
27	29.84	$C_{45}H_{71}O_{18}$	$[M-H]^-$	899.4666	899.4640	2.6	2.9	8.8
28	30.39	$C_{39}H_{61}O_{12}$	$[M-H]^-$	721.4163	721.4163	0	0	4.7
29	31.22	$C_{39}H_{61}O_{13}$	$[M-H]^-$	737.4110	737.4112	−0.2	−0.3	6.9
30	32.14	$C_{39}H_{61}O_{12}$	$[M-H]^-$	721.4147	721.4163	−1.6	−2.2	9.1
31	33.32	$C_{33}H_{53}O_{8}$	$[M+H]^+$	577.3711	577.3740	−2.9	−5.0	1.5

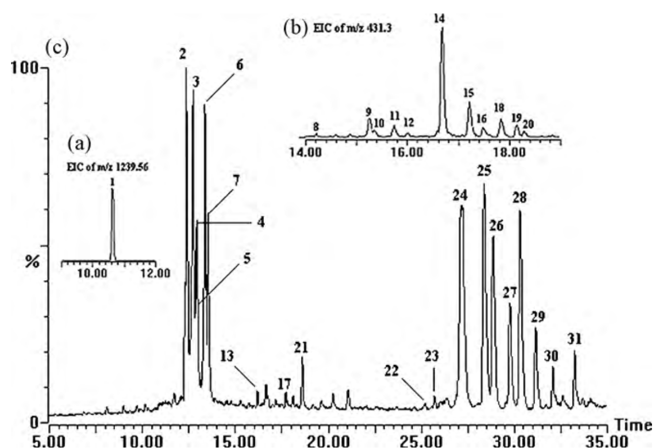

Fig. 4. Chromatograms of the extracts from *D. zingiberensis* analyzed by UPLC/Q-TOF-MS. (a) EIC of m/z 1239.56 in negative ion mode; (b) EIC of m/z 431.3 in positive ion mode; (c) TIC of the extracts from *D. zingiberensis* in negative ion mode. Peak numbers are consistent with those in Tables 1 and 2.

zingiberensis saponin, the CID spectra were measured by (+)ESI-MS/MS. Fig. 2d shows the CID spectrum of the protonated molecular ion $[M+H]^+$ (m/z 1047.5366) for zingiberensis saponin. The fragment ions at m/z 723.4288, 577.3726, 415.3303 and 397.3116 resulted from consecutive loss of two glucosyls, one rhamnosyl, one glucosyl and one molecule of water from the protonated molecular ion $[M+H]^+$(m/z 1047.5366). Loss of one neutral fragment $C_8H_{16}O_2$ from the fragment ion at m/z 577.3726 produced the fragment ion at m/z 433.2637. The fragment ions at m/z 271.2036 and 253.2001 originated from consecutive loss of 144 and 18 Da from the fragment ion at m/z 415.3303. The elimination of 144 Da (formula $C_8H_{16}O_2$) might be produced by cleavage of E-ring of the aglycone [11,15,23], and the 18 Da unit derived from loss of a molecule of water. Furthermore, the fragment ions at m/z 271.2036 and 253.2001 have been reported to be the diagnostic fragment ions of this type of steroidal saponins [11]. The main fragmentation of zingiberensis saponin and possible mechanisms are shown in Fig. 3.

3.2. Characterization of steroidal saponins in *D. zingiberensis*

The UPLC/Q-TOF-MS chromatograms of the 70% ethanol aqueous extracts from rhizomes of *D. zingiberensis* are shown in Fig. 4. The total ion chromatogram (TIC) in negative ion mode of the ethanol aqueous extracts is shown in Fig. 4c. Minor compounds were highlighted by extracted ion chromatograms (EICs). The EICs of m/z 1239.56 in negative ion mode and m/z 431.3 in positive ion mode are shown in Fig. 4a and b, respectively. The structures of steroidal saponins were identified based on accurate molecular ion (m/z), molecular formula and MS/MS data (Tables 1 and 2). The accurate mass data of the molecular ions were processed through the software MassLynx V4.1, which provided a list of possible elemental formulae using the Elemental Composition Editor. The widely accepted accuracy threshold for confirmation of elemental composition has been established at 5 ppm. Total thirty-one steroidal saponins, with five aglycone skeletons (Fig. 1a–e) and different saccharide chains, were characterized.

3.2.1. Characterization of peaks 22–31

According to the MS and MS/MS data (Table 2) of peaks 22–31, they were considered to share the same aglycone skeleton (Fig. 1a).

Comparing the retention times and fragmentation with reference standards, peaks 24, 25, 26, 28 and 29 were unambiguously identified as zingiberensis saponin, deltonin, dioscin, prosapogenin A of dioscin and diosgenin diglucoside, respectively. Their structures are shown in Fig. 1.

Peak 27 (RT = 29.84 min) produced a strong deprotonated molecular ion $[M-H]^-$ (m/z 899.4666) in (−)ESI-MS. In (+)ESI-MS, three main fragments (m/z 739.4285, 577.3649, 415.3106) attributed to consecutive loss of three glucosyls from the protonated molecular ion (m/z 901.4852) were observed. For peak 31 (RT = 33.32 min), a protonated molecular ion $[M+H]^+$ (m/z 577.3711) and a main fragment (m/z 415.3255) were observed in (+)ESI-MS. The fragment (m/z 415.3255) could be attributed to the loss of one glucosyl from the protonated molecular ion (m/z 577.3711). According to the retention times, fragmentation and reported structures in the literature [24–26], peaks 27 and 31 were identified as diosgenin triglucoside and trillin, respectively.

Peak 30 (RT = 32.14 min) showed a deprotonated molecular ion $[M-H]^-$ (m/z 721.4147) in (−)ESI-MS. In (+)ESI-MS, two main fragments (m/z 577.3762, 415.3234) corresponding to consecutive loss of one rhamnosyl and one glucosyl from the protonated molecular ion (m/z 723.4332) were observed. Considering the retention time and fragmentation, peak 30 was identified as prosapogenin B of dioscin, which had been isolated from *Dioscorea panthaica* [27], the same genus plant with *D. zingiberensis*.

Two peaks (peaks 22 and 23), having the same formula, MS and MS/MS data (Tables 1 and 2) with zingiberensis saponin, were detected and characterized to be two isomers of zingiberensis saponin. Their structural difference could be deduced to be the sequence of sugar units in the aglycone. To our knowledge, these two saponins were reported for the first time in *Dioscoreaceae*. For definite identification of these unknown saponins, further investigation was needed.

3.2.2. Characterization of peaks 2–7 and 21

Based on the MS and MS/MS data (Table 2) of peaks 2–7 and 21, they could be considered to have the same aglycone skeleton (Fig. 1b). To demonstrate the details of the procedure, peak 6 was chosen as an example to elucidate the structures of these steroidal saponins.

As shown in Fig. 5a, peak 6 readily produced a strong deprotonated molecular ion $[M-H]^-$ (m/z 901.4760) in (−)ESI-MS. In (−)ESI-MS/MS, loss of one deoxyhexose from the deprotonated molecular ion (m/z 901.4760) yielded the fragment ion at m/z 755.4267 (Fig. 5b). In (+)ESI-MS, three main fragment ions at m/z 885.4748, 739.4151 and 577.3759 could be attributed to the consecutive loss of one molecule of water, one deoxyhexose and one hexose from the ion $[M+H]^+$ (Fig. 5c). The fragmentation patterns, which are common features for this kind of furostanol steroidal saponins, suggested the presence of a hydroxyl group at the C-22 position of the aglycone as reported [13,23,28]. Fig. 5d shows the CID spectrum of m/z 885.4748 $[M-H_2O+H]^+$. The fragment ion at m/z 723.4269 was produced by the loss of one glucosyl from the ion $[M-H_2O+H]^+$(m/z 885.4748). In addition, some common features, such as loss of one neutral fragment $C_8H_{16}O_2$, one deoxyhexose and one hexose were observed, consisting with the reference standards. So, peak 6 was identified as protobioside (Fig. 1), which had been isolated from *D. zingiberensis* [10].

Peak 2 produced a strong deprotonated molecular ion $[M-H]^-$ (m/z 1225.5811) in (−)ESI-MS. In (+)ESI-MS, five fragments (m/z 1063.5331, 901.4808, 739.4202, 577.3616, 415.3234) by consecutive loss of one rhamnosyl and four glucosyls from the ion $[M-H_2O+H]^+$ (m/z 1209.5472) were obtained. For peak 3, a strong deprotonated molecular ion $[M-H]^-$ (m/z 1063.5280) in (−)ESI-MS and four fragments (m/z 901.4752, 739.4193, 577.3646, 415.3229) attributed to consecutive loss of one rhamnosyl and three glucosyls from the ion $[M-H_2O+H]^+$(m/z 1047.4989) in (+)ESI-MS were observed. So, peaks 2 and 3 were tentatively identified as parvifloside and protodeltonin, respectively, which had been isolated from *D. zingiberensis* [6,10].

Table 2

MS and MS/MS data of (−)ESI mode and (+)ESI mode, and the identification results of steroidal saponins from *D. zingiberensis* by UPLC/Q-TOF-MS/MS.

Peak	RT (min)	(−)ESI-MS *m/z*	(−)ESI-MS/MS *m/z*	(+)ESI-MS *m/z*	(+)ESI-MS/MS *m/z*	Structural elucidation
1	10.62	1239.5[M−H]$^-$ 1285.5[M+HCOO]$^-$	–	**1223.5[M−H$_2$O+H]$^+$** 1061.5[M−H$_2$O−Glc+H]$^+$ 899.4[M−H$_2$O−2Glc+H]$^+$ 753.4[M−H$_2$O−2Glc-Rha+H]$^+$ 591.3[M−H$_2$O−3Glc-Rha+H]$^+$ 429.3[M−H$_2$O−Rha-4Glc+H]$^+$	1061.5[M−H$_2$O−Glc+H]$^+$ 899.4[M−H$_2$O−2Glc+H]$^+$ 737.4[M−H$_2$O−3Glc+H]$^+$ 591.3[M−H$_2$O−3Glc-Rha+H]$^+$ 429.2[M−H$_2$O−4Glc-Rha+H]$^+$ 411.2[M−2H$_2$O−4Glc-Rha+H]$^+$ 285.1[M−H$_2$O−4Glc-Rha−C$_8$H$_{16}$O$_2$+H]$^+$	Zingiberenin H
2	12.38	**1225.5[M−H]$^-$** 1271.5[M+HCOO]$^-$	1063.5[M−Glc−H]$^-$ 901.4[M−2Glc−H]$^-$ 755.4[M−2Glc-Rha−H]$^-$ 737.4[M−2Glc-Rha−H$_2$O−H]$^-$	**1209.5[M−H$_2$O+H]$^+$** 1063.5[M−H$_2$O−Rha+H]$^+$ 901.4[M−H$_2$O−Rha-Glc+H]$^+$ 739.4[M−H$_2$O−Rha-2Glc+H]$^+$ 577.3[M−H$_2$O−Rha-3Glc+H]$^+$ 415.3[M−H$_2$O−Rha-4Glc+H]$^+$	885.4[M−H$_2$O−2Glc+H]$^+$ 723.4[M−H$_2$O−3Glc+H]$^+$ 577.3[M−H$_2$O−3Glc-Rha+H]$^+$ 415.3[M−H$_2$O−4Glc-Rha+H]$^+$ 271.2[M−H$_2$O−4Glc-Rha−C$_8$H$_{16}$O$_2$+H]$^+$ 253.1[M−2H$_2$O−4Glc-Rha−C$_8$H$_{16}$O$_2$+H]$^+$	Parvifloside
3	12.73	**1063.5[M−H]$^-$** 1109.5[M+HCOO]$^-$	901.4[M−Glc−H]$^-$ 755.4[M−Glc-Rha−H]$^-$ 593.3[M−2Glc-Rha −H]$^-$	**1047.4[M−H$_2$O+H]$^+$** 901.4[M−H$_2$O−Rha+H]$^+$ 739.4[M−H$_2$O−Rha-Glc+H]$^+$ 577.3[M−H$_2$O−Rha-2Glc+H]$^+$ 415.3[M−H$_2$O−Rha-3Glc+H]$^+$	885.4[M−H$_2$O−Glc+H]$^+$ 723.4[M−H$_2$O−2Glc+H]$^+$ 577.3[M−H$_2$O−2Glc-Rha+H]$^+$ 415.3[M−H$_2$O−3Glc-Rha+H]$^+$ 397.3[M−2H$_2$O−3Glc-Rha+H]$^+$ 271.2[M−H$_2$O−3Glc-Rha−C$_8$H$_{16}$O$_2$+H]$^+$ 253.1[M−2H$_2$O−3Glc-Rha−C$_8$H$_{16}$O$_2$+H]$^+$	Protodeltonin
4	12.91	**1047.5[M−H]$^-$** 1093.5[M+HCOO]$^-$	901.4[M−Rha−H]$^-$ 755.4[M−2Rha−H]$^-$	**1031.5[M−H$_2$O+H]$^+$** 739.4[M−H$_2$O−2Rha+H]$^+$ 577.3[M−H$_2$O−2Rha-Glc+H]$^+$ 415.3[M−H$_2$O−2Rha-2Glc+H]$^+$	869.4[M−H$_2$O−Glc+H]$^+$ 725.3[M−H$_2$O−Glc−C$_8$H$_{16}$O$_2$+H]$^+$ 577.3[M−H$_2$O−Glc-2Rha+H]$^+$ 415.3[M−H$_2$O−2Glc-2Rha+H]$^+$ 397.3[M−2H$_2$O−2Glc-2Rha+H]$^+$ 271.2[M−H$_2$O−2Glc-2Rha−C$_8$H$_{16}$O$_2$+H]$^+$ 253.1[M−2H$_2$O−2Glc-2Rha−C$_8$H$_{16}$O$_2$+H]$^+$	Protodioscin
5	12.96	**917.4[M−H]$^-$** 963.4[M+HCOO]$^-$	755.4[M−Glc−H]$^-$	**901.4[M−H$_2$O+H]$^+$** 739.4[M−H$_2$O−Glc+H]$^+$ 577.3[M−H$_2$O−2Glc+H]$^+$ 415.3[M−H$_2$O−3Glc+H]$^+$	739.4[M−H$_2$O−Glc+H]$^+$ 577.3[M−H$_2$O−2Glc+H]$^+$ 415.3[M−H$_2$O−3Glc+H]$^+$ 397.3[M−2H$_2$O−3Glc+H]$^+$ 271.2[M−H$_2$O−3Glc−C$_8$H$_{16}$O$_2$+H]$^+$ 253.1[M−2H$_2$O−3Glc−C$_8$H$_{16}$O$_2$+H]$^+$	26-*O*-β-D-glucopyranosyl-(25*R*)-furost-5-en-3β,22ξ,26-triol-3-*O*-β-D-glucopyranosyl-(1 → 4)-β-D-glucopyranoside
6	13.36	**901.4[M−H]$^-$** 947.4[M+HCOO]$^-$	755.4[M−Rha−H]$^-$	**885.4[M−H$_2$O+H]$^+$** 739.4[M−H$_2$O−Rha+H]$^+$ 577.3[M−H$_2$O−Rha-Glc+H]$^+$ 415.3[M−H$_2$O−Rha-2Glc+H]$^+$	723.4[M−H$_2$O−Glc+H]$^+$ 579.3[M−H$_2$O−Glc−C$_8$H$_{16}$O$_2$+H]$^+$ 415.3[M−H$_2$O−2Glc-Rha+H]$^+$ 397.3[M−2H$_2$O−2Glc-Rha+H]$^+$ 271.2[M−H$_2$O−2Glc-Rha−C$_8$H$_{16}$O$_2$+H]$^+$ 253.1[M−2H$_2$O−2Glc-Rha−C$_8$H$_{16}$O$_2$+H]$^+$	Protobioside
7	13.57	**755.4[M−H]$^-$** 801.4[M+HCOO]$^-$	575.3[M−H$_2$O−Glc−H]$^-$	**739.3[M−H$_2$O+H]$^+$**	577.3[M−H$_2$O−Glc+H]$^+$ 433.2[M−H$_2$O−Glc−C$_8$H$_{16}$O$_2$+H]$^+$ 271.2[M−H$_2$O−2Glc−C$_8$H$_{16}$O$_2$+H]$^+$ 253.1[M−2H$_2$O−2Glc−C$_8$H$_{16}$O$_2$+H]$^+$	26-*O*-β-D-glucopyranosyl-(25*R*)-furost-5-en-3β,22ξ,26-triol-3-*O*-β-D-glucopyranoside
8	14.21	–	–	**1047.5[M+H]$^+$** 885.4[M−Glc+H]$^+$ 739.4[M−Glc-Rha+H]$^+$ 593.3[M−Glc-2Rha+H]$^+$ 431.3[M−2Glc-2Rha+H]$^+$	885.4[M−Glc+H]$^+$ 867.4[M−Glc−H$_2$O+H]$^+$ 739.4[M−Glc-Rha+H]$^+$ 593.3[M−Glc-2Rha+H]$^+$ 577.3[M−2Glc-Rha+H]$^+$ 431.3[M−2Glc-2Rha+H]$^+$ 271.2[M−2Glc-2Rha−C$_8$H$_{16}$O$_3$+H]$^+$ 253.2[M−2Glc-2Rha−C$_8$H$_{16}$O$_3$−H$_2$O+H]$^+$	Zingiberogenin ↑ [2Glc & 2Rha]

Table 2 (*Continued*)

Peak	RT (min)	(−)ESI-MS *m*/*z*	(−)ESI-MS/MS *m*/*z*	(+)ESI-MS *m*/*z*	(+)ESI-MS/MS *m*/*z*	Structural elucidation
9	15.25	1061.5[M−H]$^-$ 1107.5[M+HCOO]$^-$	–	1063.5[M+H]$^+$ 901.4[M−Glc+H]$^+$ 739.4[M−2Glc+H]$^+$ 593.3[M−2Glc-Rha+H]$^+$ 431.3[M−3Glc-Rha+H]$^+$ 413.3[M−3Glc-Rha−H_2O+H]$^+$	–	Zingiberogenin $\uparrow$ [3Glc & Rha]
10	15.33	–	–	1047.5[M+H]$^+$ 885.4[M−Glc+H]$^+$ 739.4[M−Glc-Rha+H]$^+$ 593.3[M−Glc-2Rha+H]$^+$ 431.3[M−2Glc-2Rha+H]$^+$	–	Zingiberogenin $\uparrow$ [2Glc & 2Rha]
11	15.73	899.4[M−H]$^-$ 945.5[M+HCOO]$^-$	–	901.4[M+H]$^+$ 739.4[M−Glc+H]$^+$ 593.3[M−Glc-Rha+H]$^+$ 431.3[M−2Glc-Rha+H]$^+$ 413.2[M−2Glc-Rha−H_2O+H]$^+$	–	Zingiberogenin $\uparrow$ [2Glc & Rha]
12	16.03	–	–	1209.5[M+H]$^+$ 1047.5[M−Glc+H]$^+$ 885.4[M−2Glc+H]$^+$ 739.4[M−2Glc-Rha+H]$^+$ 593.3[M−2Glc-2Rha+H]$^+$ 431.3[M−3Glc-2Rha+H]$^+$	–	Zingiberogenin $\uparrow$ [3Glc & 2Rha]
13	16.18	1207.5[M−H]$^-$ 1253.6[M+HCOO]$^-$	–	1209.5[M+H]$^+$ 1047.5[M−Glc+H]$^+$ 885.4[M−2Glc+H]$^+$ 739.4[M−2Glc-Rha+H]$^+$ **577.3[M−3Glc-Rha+H]$^+$** 415.3[M−4Glc-Rha+H]$^+$	415.3[M−4Glc-Rha+H]$^+$ 271.2[M−4Glc-Rha−$C_8H_{16}O_2$+H]$^+$ 253.1[M−4Glc-Rha−$C_8H_{16}O_2$−H_2O+H]$^+$	Zingiberenin G
14	16.68	1061.5[M−H]$^-$ 1107.5[M+HCOO]$^-$	–	1063.5[M+H]$^+$ 901.4[M−Glc+H]$^+$ 739.4[M−2Glc+H]$^+$ 593.3[M−2Glc-Rha+H]$^+$ 431.3[M−3Glc-Rha+H]$^+$ 413.3[M−3Glc-Rha−H_2O+H]$^+$	–	Zingiberogenin $\uparrow$ [3Glc & Rha]
15	17.20	899.4[M−H]$^-$ 945.5[M+HCOO]$^-$	–	**901.4[M+H]$^+$** 739.4[M−Glc+H]$^+$ 593.3[M−Glc-Rha+H]$^+$ 431.3[M−2Glc-Rha+H]$^+$ 413.3[M−2Glc-Rha−H_2O+H]$^+$	431.3[M−2Glc-Rha+H]$^+$ 413.3[M−2Glc-Rha−H_2O+H]$^+$ 271.2[M−2Glc-Rha−$C_8H_{16}O_3$+H]$^+$ 253.1[M−2Glc-Rha−$C_8H_{16}O_3$−H_2O+H]$^+$	Zingiberogenin $\uparrow$ [2Glc & Rha]
16	17.52	929.5[M+HCOO]$^-$ 883.5[M−H]$^-$	–	885.4[M+H]$^+$ 739.4[M−Rha+H]$^+$ 593.3[M−2Rha+H]$^+$ 431.3[M−2Rha-Glc+H]$^+$ 413.3[M−2Rha-Glc−H_2O+H]$^+$	–	Zingiberogenin $\uparrow$ [Glc & 2Rha]
17	17.76	**883.4[M−H]$^-$** 929.4[M+HCOO]$^-$	737.4[M−Rha−H]$^-$ 557.3[M−Rha-Glc−H_2O−H]$^-$	**885.4[M+H]$^+$** 723.4[M−Glc+H]$^+$ 577.3[M−Glc-Rha+H]$^+$ 415.3[M−2Glc-Rha+H]$^+$ 397.3[M−2Glc-Rha−H_2O+H]$^+$	723.4[M−Glc+H]$^+$ 577.3[M−Glc-Rha+H]$^+$ 415.3[M−2Glc-Rha+H]$^+$ 397.3[M−2Glc-Rha−H_2O+H]$^+$ 271.2[M−2Glc-Rha−$C_8H_{16}O_2$+H]$^+$ 253.1[M−2Glc-Rha−H_2O−$C_8H_{16}O_2$+H]$^+$	26-*O*-β-D-glucopyranosyl-3β,26-diol-25(*R*)-$\Delta^{5,20(22)}$-dien-furosta-3-*O*-[α-L-rhamnopyranosyl-(1 → 4)]-β-D-glucopyranoside or 26-*O*-β-D-glucopyranosyl-3β,26-diol-25(*R*)-$\Delta^{5,20(22)}$-dien-furosta-3-*O*-[α-L-rhamnopyranosyl-(1 → 2)]-β-D-glucopyranoside
18	17.83	–	–	885.4[M+H]$^+$ 739.4[M−Rha+H]$^+$ 593.3[M−2Rha+H]$^+$ 431.3[M−2Rha-Glc+H]$^+$ 413.3[M−2Rha-Glc−H_2O+H]$^+$	–	Zingiberogenin $\uparrow$ [Glc & 2Rha]

19	18.14	737.4[M−H]$^{-}$ 783.4[M+HCOO]$^{-}$	–	**739.4[M+H]$^{+}$** 593.3[M−Rha+H]$^{+}$ 431.3[M−Rha-Glc+H]$^{+}$ 413.3[M−Rha-Glc−H_2O+H]$^{+}$	579.3[M−$C_8H_{16}O_3$+H]$^{+}$ 271.2[M−Rha-Glc−$C_8H_{16}O_3$+H]$^{+}$ 253.1[M−Rha-Glc−$C_8H_{16}O_3$−H_2O+H]$^{+}$	Zingiberenin A_2
20	18.29	–	–	885.4[M+H]$^{+}$ 739.4[M−Rha+H]$^{+}$ 593.3[M−2Rha+H]$^{+}$ 431.3[M−2Rha-Glc+H]$^{+}$ 413.3[M−2Rha-Glc−H_2O+H]$^{+}$	–	Zingiberogenin ↑ [Glc & 2Rha]
21	18.62	593.3[M−H]$^{-}$ 639.3[M+HCOO]$^{-}$	–	**577.3[M−H_2O+H]$^{+}$** 415.3[M−H_2O−Glc+H]$^{+}$	415.3[M−H_2O−Glc+H]$^{+}$ 271.2[M−H_2O−Glc−$C_8H_{16}O_2$+H]$^{+}$ 253.1[M−2H_2O−Glc−$C_8H_{16}O_2$+H]$^{+}$	Funkioside
22	25.25	**1045.5[M−H]$^{-}$** 1091.5[M+HCOO]$^{-}$	883.4[M−Glc−H]$^{-}$ 721.4[M−2Glc−H]$^{-}$	1047.5[M+H]$^{+}$ 885.5[M−Glc+H]$^{+}$ 723.4[M−2Glc+H]$^{+}$ 577.3[M−2Glc-Rha+H]$^{+}$ 415.3[M−3Glc-Rha+H]$^{+}$ 397.3[M−3Glc-Rha−H_2O+H]$^{+}$	–	Isomer of zingiberensis saponin
23	25.75	**1045.5[M−H]$^{-}$** 1091.5[M+HCOO]$^{-}$	883.4[M−Glc−H]$^{-}$ 721.4[M−2Glc−H]$^{-}$	1047.5[M+H]$^{+}$ 885.4[M−Glc+H]$^{+}$ 723.4[M−2Glc+H]$^{+}$ 577.3[M−2Glc-Rha+H]$^{+}$ 415.3[M−3Glc-Rha+H]$^{+}$ 397.3[M−3Glc-Rha−H_2O+H]$^{+}$	–	Isomer of zingiberensis saponin
24	27.24	**1045.5[M−H]$^{-}$** 1091.5[M+HCOO]$^{-}$	883.4[M−Glc−H]$^{-}$ 721.4[M−2Glc−H]$^{-}$ 575.3[M−2Glc-Rha−H]$^{-}$	**1047.5[M+H]$^{+}$** 885.4[M−Glc+H]$^{+}$ 723.4[M−2Glc+H]$^{+}$ 577.3[M−2Glc-Rha+H]$^{+}$ 415.3[M−3Glc-Rha+H]$^{+}$ 397.3[M−3Glc-Rha−H_2O+H]$^{+}$	723.4M−2Glc+H]$^{+}$ 577.3[M−2Glc-Rha+H]$^{+}$ 433.2[M−2Glc-Rha−$C_8H_{16}O_2$+H]$^{+}$ 415.3[M−3Glc-Rha+H]$^{+}$ 397.3[M−3Glc-Rha−H_2O+H]$^{+}$ 271.2[M−3Glc-Rha−$C_8H_{16}O_2$+H]$^{+}$ 253.2[M−3Glc-Rha−$C_8H_{16}O_2$−H_2O+H]$^{+}$	Zingiberensis saponin
25	28.42	**883.4[M−H]$^{-}$** 929.4[M+HCOO]$^{-}$	721.4[M−Glc−H]$^{-}$ 575.3[M−Glc-Rha−H]$^{-}$	**885.4[M+H]$^{+}$** 723.4[M−Glc+H]$^{+}$ 577.3[M−Glc-Rha+H]$^{+}$ 415.3[M−2Glc-Rha+H]$^{+}$ 397.3[M−2Glc-Rha−H_2O+H]$^{+}$	723.4[M−Glc+H]$^{+}$ 577.3[M−Glc-Rha+H]$^{+}$ 415.3[M−2Glc-Rha+H]$^{+}$ 397.3[M−2Glc-Rha−H_2O+H]$^{+}$ 379.3[M−2Glc-Rha-2H_2O+H]$^{+}$ 271.2[M−2Glc-Rha−$C_8H_{16}O_2$+H]$^{+}$ 253.1[M−2Glc-Rha−$C_8H_{16}O_2$−H_2O+H]$^{+}$	Deltonin
26	28.89	**867.4[M−H]$^{-}$** 913.4[M+HCOO]$^{-}$	721.4[M−Rha−H]$^{-}$ 575.3[M−2Rha−H]$^{-}$	**869.4[M+H]$^{+}$** 723.4[M−Rha+H]$^{+}$ 577.3[M−2Rha+H]$^{+}$ 415.3[M−Glc-2Rha+H]$^{+}$ 397.3[M−Glc-2Rha−H_2O+H]$^{+}$	725.3[M−$C_8H_{16}O_2$+H]$^{+}$ 577.3[M−2Rha+H]$^{+}$ 433.2[M−2Rha−$C_8H_{16}O_2$+H]$^{+}$ 415.3[M−Glc-2Rha+H]$^{+}$ 397.3[M−Glc-2Rha−H_2O+H]$^{+}$ 271.2[M−Glc-2Rha−$C_8H_{16}O_2$+H]$^{+}$ 253.1[M−Glc-2Rha−$C_8H_{16}O_2$−H_2O+H]$^{+}$	Dioscin
27	29.84	**899.4[M−H]$^{-}$** 945.4[M+HCOO]$^{-}$	737.4[M−Glc−H]$^{-}$ 575.3[M−2Glc−H]$^{-}$	901.4[M+H]$^{+}$ 739.4[M−Glc+H]$^{+}$ 577.3[M−2Glc+H]$^{+}$ 415.3[M−3Glc+H]$^{+}$ 397.3[M−3Glc−H_2O+H]$^{+}$	–	Diosgenin triglucoside
28	30.39	**721.4[M−H]$^{-}$** 767.4[M+HCOO]$^{-}$	575.3[M−Rha−H]$^{-}$	**723.4[M+H]$^{+}$** 577.3[M−Rha+H]$^{+}$ 415.3[M−Glc-Rha+H]$^{+}$ 397.3[M−Glc-Rha−H_2O+H]$^{+}$	579.3[M−$C_8H_{16}O_2$+H]$^{+}$ 271.2[M−Glc-Rha−$C_8H_{16}O_2$+H]$^{+}$ 253.1[M−Glc-Rha−$C_8H_{16}O_2$−H_2O+H]$^{+}$	Prosapogenin A of dioscin

Table 2 (*Continued*)

Peak	RT (min)	(−)ESI-MS *m/z*	(−)ESI-MS/MS *m/z*	(+)ESI-MS *m/z*	(+)ESI-MS/MS *m/z*	Structural elucidation
29	31.22	**737.4[M−H]$^-$** 783.4[M+HCOO]$^-$	575.3[M−Glc−H]$^-$	**739.4[M+H]$^+$** 577.3[M−Glc+H]$^+$ 415.3[M−2Glc+H]$^+$ 397.3[M−2Glc−H_2O+H]$^+$	577.3[M−Glc+H]$^+$ 433.2[M−Glc−$C_8H_{16}O_2$+H]$^+$ 415.3[M−2Glc+H]$^+$ 397.3[M−2Glc−H_2O+H]$^+$ 271.2[M−2Glc−$C_8H_{16}O_2$+H]$^+$ 253.1[M−2Glc−$C_8H_{16}O_2$−H_2O+H]$^+$	Diosgenin diglucoside
30	32.14	**721.4[M−H]$^-$** 767.4[M+HCOO]$^-$	575.3[M−Rha−H]$^-$	**723.4[M+H]$^+$** 577.3[M−Rha+H]$^+$ 415.3[M−Glc-Rha+H]$^+$ 397.3[M−Glc-Rha−H_2O+H]$^+$	579.3[M−$C_8H_{16}O_2$+H]$^+$ 271.2[M−Glc-Rha−$C_8H_{16}O_2$+H]$^+$ 253.1[M−Glc-Rha−$C_8H_{16}O_2$−H_2O+H]$^+$	Prosapogenin B of dioscin
31	33.32	621.3[M+HCOO]$^-$	–	**577.3[M+H]$^+$** 415.3[M−Glc+H]$^+$ 397.3[M−Glc−H_2O+H]$^+$	433.2[M−$C_8H_{16}O_2$+H]$^+$ 271.2[M−Glc−$C_8H_{16}O_2$+H]$^+$ 253.1[M−Glc−$C_8H_{16}O_2$−H_2O+H]$^+$	Trillin

The boldfaced parent ions were selected to carry out MS/MS experiments.

Peak 4 gave a strong deprotonated molecular ion [M−H]$^-$ (*m/z* 1047.5317) in (−)ESI-MS. In (+)ESI-MS, three main fragments (*m/z* 739.4266, 577.3745, 415.3250) via consecutive loss of two rhamnosyls, one glucosyl and one glucosyl from the fragment ion [M−H_2O+H]$^+$ (*m/z* 1031.5270) were obtained. So, peak 4 was tentatively identified as protodioscin, which had been isolated from *Dioscorea collettii* [29], the same genus plant with *D. zingiberensis*.

For peak 21, a strong deprotonated molecular ion [M−H]$^-$ (*m/z* 593.3690) was observed in (−)ESI-MS. In (+)ESI-MS, a main fragment (*m/z* 415.3207) attributed to loss of one glucosyl from the ion [M−H_2O+H]$^+$ (*m/z* 577.3710) was observed, suggesting the presence of a glucosyl connecting to the hydroxyl group at the C-26 position of the aglycone. So, peak 21 was identified as funkioside (Fig. 1), which had been isolated from *Tamus communis* [30], the same family plant with *D. zingiberensis*.

As shown in Table 2, peak 5 produced a deprotonated molecular ion [M−H]$^-$ (*m/z* 917.4735) in (−)ESI-MS. Three main fragments (*m/z* 739.4362, 577.3750, 415.3303) via consecutive loss of three glucosyls from the ion [M−H_2O+H]$^+$ (*m/z* 901.4769) were observed in (+)ESI-MS. The fragmentation of peak 5 suggested the presence of a glucosyl connecting to the hydroxyl group at the C-26 position and a saccharide chain including two glucosyls connecting to the hydroxyl group at C-3 position of the aglycone. Peak 7 gave a deprotonated molecular ion [M−H]$^-$ (*m/z* 755.4220) in (−)ESI-MS and three fragments (*m/z* 577.3736, 433.2593, 271.2050) by consecutive loss of one glucosyl, one neutral fragment $C_8H_{16}O_2$ and one glucosyl from the fragment ion [M−H_2O+H]$^+$ (*m/z* 739.3941) in (+)ESI-MS/MS. The fragmentation of peak 7 suggested the presence of a glucosyl connecting to the hydroxyl group at the C-26 position and a glucosyl connecting to the hydroxyl group at C-3 position of the aglycone. Consequently, peaks 5 and 7 were tentatively identified as 26-*O*-β-D-glucopyranosyl-(25*R*)-furost-5-en-3β,22ξ,26-triol-3-*O*-β-D-glucopyranosyl-(1 → 4)-β-D-glucopyranoside and 26-*O*-β-D-glucopyranosyl-(25*R*)-furost-5-en-3β,22ξ,26-triol-3-*O*-β-D-glucopyranoside, respectively (Fig. 1). To our knowledge, these two new saponins were found in *Dioscoreaceae* for the first time.

3.2.3. Characterization of peaks 13 and 17

According to the MS and MS/MS data (Table 2) and retention times of peaks 13 and 17, they could be deduced to have the same aglycone skeleton (Fig. 1c). Peak 13 was chosen to demonstrate the details of elucidation procedure.

As shown in Fig. 6a, the peak 13 readily produced a strong deprotonated molecular ion [M−H]$^-$ (*m/z* 1207.5767) in (−)ESI-MS. In (+)ESI-MS, the mass spectrum was dominated by the main fragment ion [M−3Glc-Rha+H]$^+$ (*m/z* 577.3757) (Fig. 6b). In order to investigate the fragmentation of peak 13 in detail, the CID spectrum of the fragment [M−3Glc-Rha+H]$^+$ (*m/z* 577.3757) is shown in Fig. 6c. The fragment ions at *m/z* 415.3188, 271.2049 and 253.1927 resulted from consecutive loss of one hexose, 144 Da (formula $C_8H_{16}O_2$) and one molecule of water from the fragment ion at *m/z* 577.3757. However, the fragment ion at *m/z* 433 attributed to neutral loss of $C_8H_{16}O_2$ (144 Da) directly from the fragment ion at *m/z* 577.3757 was not observed. Li et al. [11] described the same fragmentation pattern in the Q-TOF-MS/MS analysis of a pair of isomers whose structural difference only present in the location of glucosyl. They concluded that the neutral loss of $C_8H_{16}O_2$ directly from the molecular ion would occur in spirostanol saponins, while the sugar moiety present in C-26 position was preferentially eliminated in furostanol saponins. So, the glucosyl present in the fragment ion at *m/z* 577.3757 connected to the hydroxyl group at the C-26 position of the aglycone. Consequently, peak 13 was characterized to be a furostanol saponin-zingiberenin G (Fig. 1), which had been isolated from *D. zingiberensis* [8].

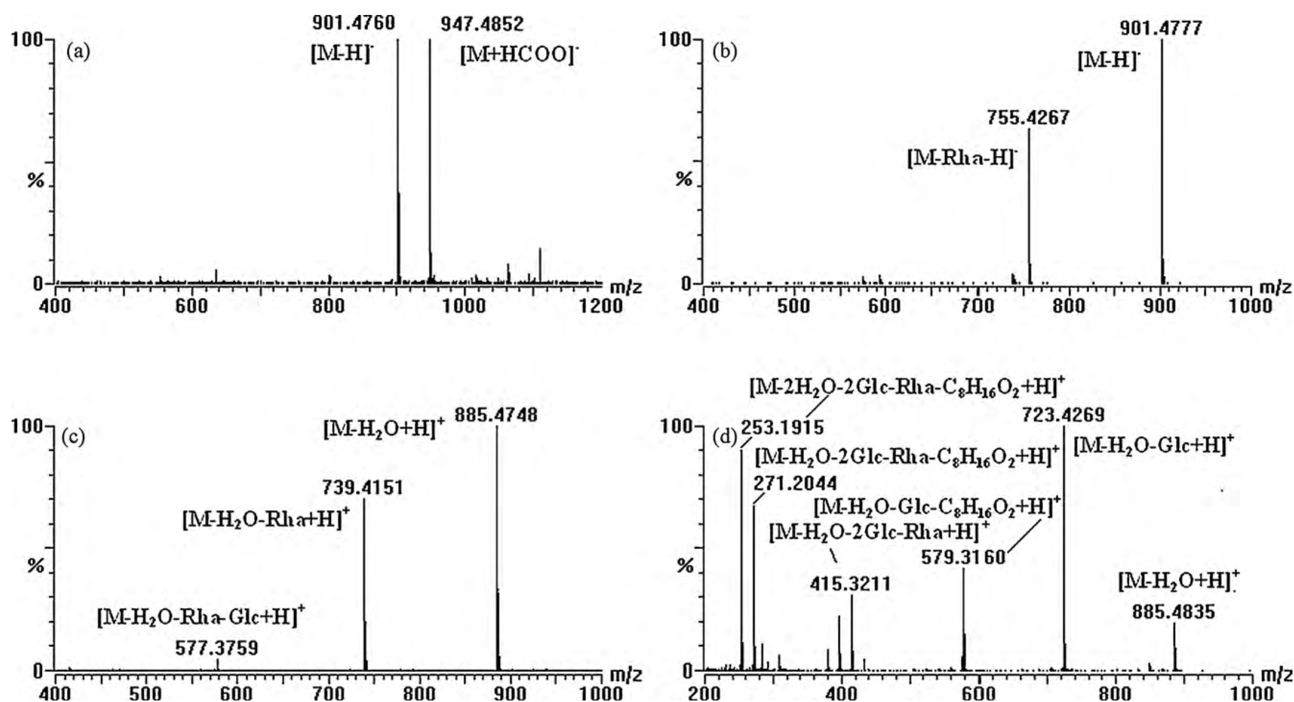

Fig. 5. MS and MS/MS spectra of peak 6. (a) (−)ESI-MS spectrum of peak 6; (b) (−)ESI-MS/MS spectrum of the ion at *m/z* 901.4760; (c) (+)ESI-MS spectrum of peak 6; (d) (+)ESI-MS/MS spectrum of the ion at *m/z* 885.4748.

Peak 17 (RT = 17.76 min) produced a deprotonated molecular ion $[M-H]^-$ (*m/z* 883.4659) in (−)ESI-MS and three fragments (*m/z* 723.4326, 577.3770, 415.3293) by consecutive loss of one glucosyl, one rhamnosyl and one glucosyl from the protonated molecular ion $[M+H]^+$ (*m/z* 885.4803) in (+)ESI-MS. So, peak 17 was tentatively identified as 26-*O*-β-D-glucopyranosyl-3β,26-diol-25(*R*)-$\Delta^{5,20(22)}$-dien-furosta-3-*O*-[α-L-rhamnopyranosyl-(1 → 4)]-β-D-glucopyranoside or 26-*O*-β-D-glucopyranosyl-3β,26-diol-25(*R*)-$\Delta^{5,20(22)}$-dien-furosta-3-*O*-[α-L-rhamnopyranosyl-(1 → 2)]-β-D-glucopyranoside, which had been isolated from *Dioscorea panthaica* [11,31], the same genus plant with *D. zingiberensis*.

3.2.4. Characterization of peaks 8–12, 14–16 and 18–20

Based on the MS and MS/MS data (Table 2) of peaks 8–12, 14–16 and 18–20, they could be considered to have the same aglycone skeleton (Fig. 1d). In positive ion mode, they had the same characteristic fragment ion $[\text{aglycone}+H]^+$ (*m/z* 431.3), and the EIC of *m/z* 431.3 is shown in Fig. 4b. Peak 19 was chosen as an exam-

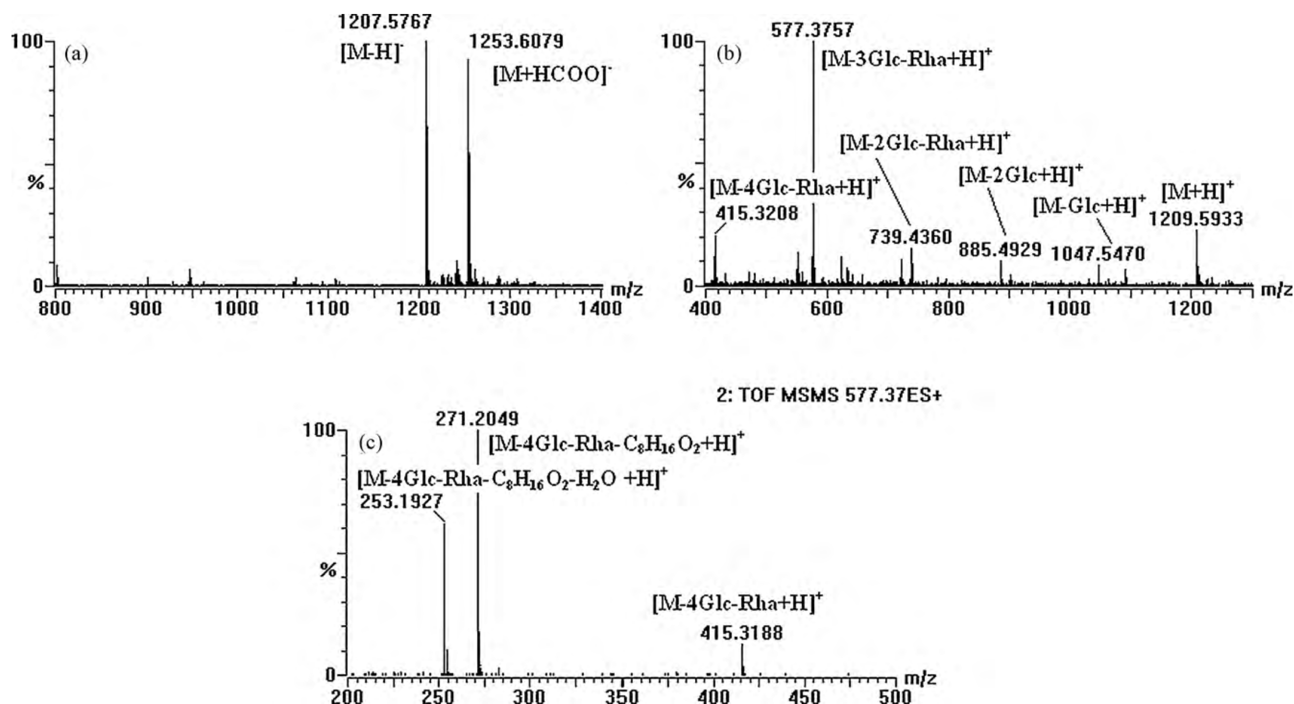

Fig. 6. MS and MS/MS spectra of peak 13. (a) (−)ESI-MS spectrum of peak 13; (b) (+)ESI-MS spectrum of peak 13; (c) (+)ESI-MS/MS spectrum of the ion at *m/z* 577.3757.

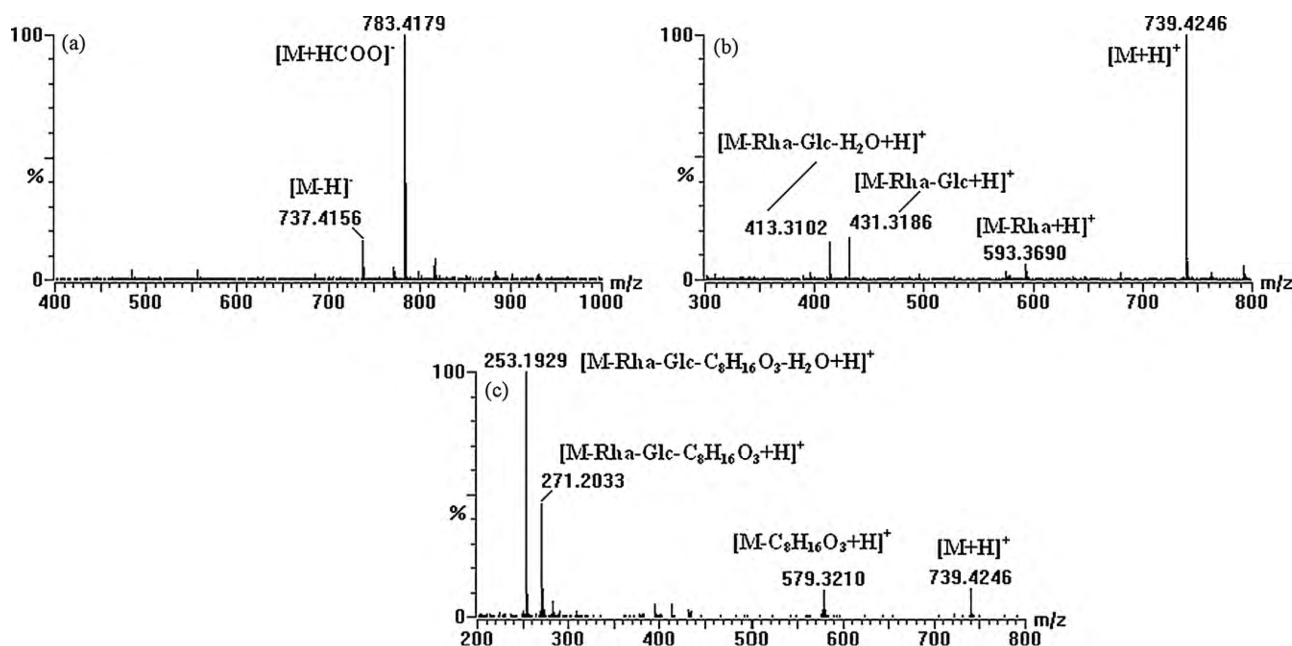

Fig. 7. MS and MS/MS spectra of peak 19. (a) (−)ESI-MS spectrum of peak 19; (b) (+)ESI-MS spectrum of peak 19; (c) (+)ESI-MS/MS spectrum of the ion at *m/z* 739.4246.

ple to discuss the fragmentation patterns for these saponins in detail.

As shown in Fig. 7a, the peak 19 showed a deprotonated molecular ion $[M-H]^-$ (*m/z* 737.4156) in (−)ESI-MS. In Fig. 7b, the mass spectrum was dominated by the protonated molecular ion $[M+H]^+$ (*m/z* 739.4246) and three main fragment ions at *m/z* 593.3690, 431.3186 and 413.3102, which resulted from the consecutive loss of one deoxyhexose, one hexose and one molecule of water from the protonated molecular ion $[M+H]^+$ (*m/z* 739.4246) in (+)ESI-MS. Fig. 7c displays the CID spectrum of *m/z* 739.4246 for peak 19. The fragment ion at *m/z* 579.3210 could be attributed to the loss of 160 Da (formula $C_8H_{16}O_3$) from the protonated molecular ion $[M+H]^+$ (*m/z* 739.4246). Considering the elimination of 160 Da which is 16 Da larger than 144 Da (formula $C_8H_{16}O_2$) and reported structure in the literature [5], it is reasonable to deduce that peak 19 has a hydroxyl substituent at the C-24 position of the F-ring of the aglycone. Consequently, peak 19 was tentatively identified as zingiberenin A_2 (Fig. 1), which had been isolated from *D. zingiberensis* [5].

Peaks 8 and 10 shared the same protonated molecular ion $[M+H]^+$ (*m/z* 1047.5) and four main fragments (*m/z* 885.4, 739.4, 593.3, 431.3) corresponding to consecutive loss of one glucosyl, two rhamnosyls and one glucosyl from the protonated molecular ion $[M+H]^+$ (*m/z* 1047.5). They were characterized to be a pair of isomers whose structural difference existed in the sequence of sugar units of the saponins. Peaks 9 and 14 had the same deprotonated molecular ion $[M-H]^-$ (*m/z* 1063.5) and four main fragments (*m/z* 901.4, 739.4, 593.3, 431.3) attributed to the consecutive loss of two

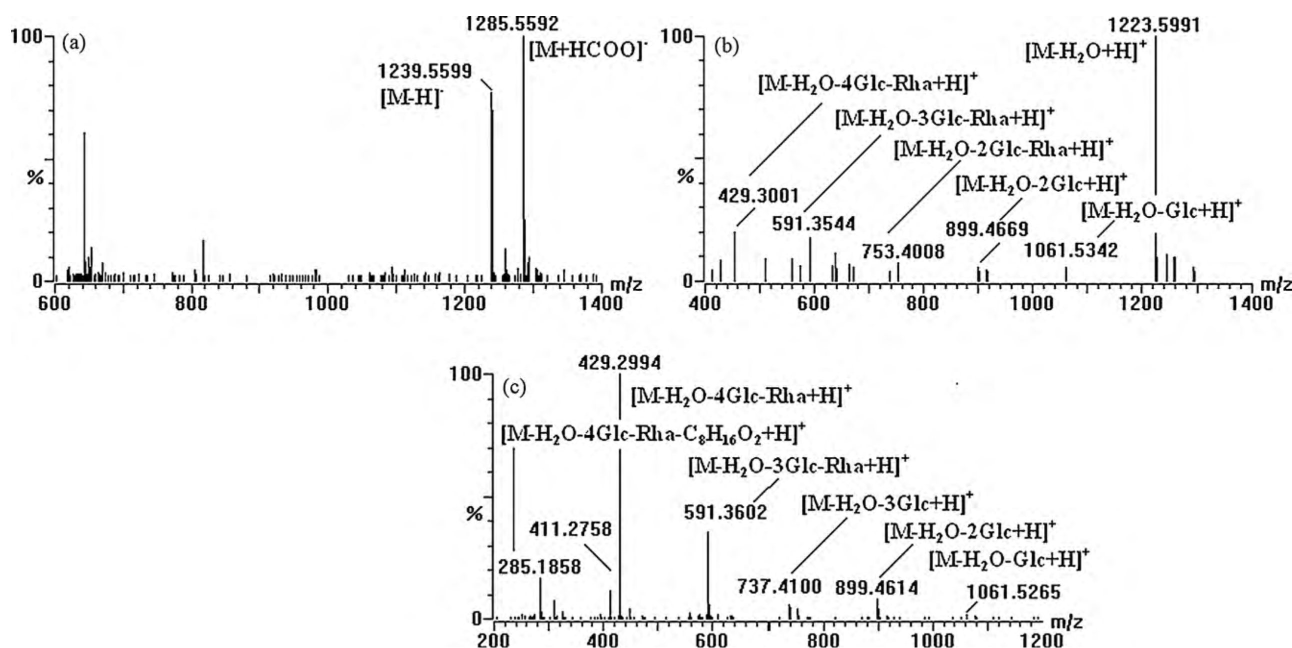

Fig. 8. MS and MS/MS spectra of peak 1. (a) (−)ESI-MS spectrum of peak 1; (b) (+)ESI-MS spectrum of peak 1; (c) (+)ESI-MS/MS spectrum of the ion at *m/z* 1223.5991.

glucosyls, one rhamnosyl and one glucosyl from the protonated molecular ion $[M+H]^+$ (*m*/*z* 1063.5). They were also characterized to be a pair of isomers whose structural difference existed in the sequence of sugar units of the saponins. Peaks 11 and 15 showed the same deprotonated molecular ion $[M-H]^-$ (*m*/*z* 899.4) and three main fragments (*m*/*z* 739.4, 593.3, 431.3) by consecutive loss of one glucosyl, one rhamnosyl and one glucosyl from the protonated molecular ion $[M+H]^+$ (*m*/*z* 901.4). They were considered to be a pair of isomers whose structural difference existed in the sequence of sugar units of the saponins. Peak 12 gave a protonated molecular ion $[M+H]^+$ (*m*/*z* 1209.5879) and five fragment ions (*m*/*z* 1047.5, 885.4, 739.4, 593.3, 431.3) attributed to consecutive loss of two glucosyls, two rhamnosyls and one glucosyl from the ion $[M+H]^+$ (*m*/*z* 1209.5879). Peaks 16, 18 and 20 displayed the same protonated molecular ion $[M+H]^+$ (*m*/*z* 885.4) and three main fragments (*m*/*z* 739.4, 593.3, 431.3) via consecutive loss of two rhamnosyls and one glucosyl from the ion $[M+H]^+$ (*m*/*z* 885.4). They were characterized to be isomers whose structural difference existed in the sequence of sugar units of the saponins.

According to the MS and MS/MS data (Table 2) of these ten compounds (peaks 8–12, 14–16, 18 and 20) above described and our deduction, they could be considered to have the same aglycone, which had been isolated from *D. zingiberensis* and named as zingiberogenin (Fig. 1) [5], and different sequence of sugars. To our knowledge, these ten saponins were found in *Dioscoreaceae* for the first time. For definite identification of these new saponins, further investigation was needed.

3.2.5. Characterization of peak 1

In (−)ESI-MS, peak 1 showed an obvious deprotonated molecular ion $[M-H]^-$ (*m*/*z* 1239.5599) in Fig. 8a. In Fig. 8b, the mass spectrum was dominated by the fragment ion $[M-H_2O+H]^+$ (*m*/*z* 1223.5991), suggesting the presence of a hydroxy group at the C-22 position of the aglycone. Fig. 8c shows the CID spectrum of *m*/*z* 1223.5991 $[M-H_2O+H]^+$ for peak 1. The mass spectrum was dominated by the fragment ion $[aglycone+H]^+$ (*m*/*z* 429.2994). In addition, the fragment ion at *m*/*z* 285.1858 (formula $C_{19}H_{25}O_2$) could be attributed to the neutral loss of 144 Da (formula $C_8H_{16}O_2$) from the fragment ion at *m*/*z* 429.2994. Considering the fragment ion at *m*/*z* 285.1858 which is 14 Da larger than *m*/*z* 271.2036 (formula $C_{19}H_{27}O$), it is reasonable to deduce that peak 1 has a carbonyl substituent at the C-7 position of the B-ring of the aglycone. Consequently, peak 1 was tentatively identified as zingiberenin H (Fig. 1), which had been isolated from *D. zingiberensis* [9].

4. Conclusions

UPLC/Q-TOF-MS/MS as a powerful tool has been applied to the characterization of steroidal saponins in *D. zingiberensis*, with advantage of avoidance of the time-consuming and tedious purification of compounds from the crude extracts. The characteristic fragmentation patterns observed in Q-TOF-MS/MS spectra allow the identification of aglycones, the nature and sequence of sugars. A total of thirty-one steroidal saponins with five aglycone skeletons were detected in the crude extracts from *D. zingiberensis*. Five saponins were unambiguously identified by comparing the retention times and the MS and MS/MS data with the reference standards. Based on previous publications and our deduction, twelve saponins were identified or tentatively characterized in the crude extracts from *D. zingiberensis*. In addition, fourteen new steroidal saponins were tentatively elucidated by Q-TOF-MS/MS from *D. zingiberensis* for the first time. However, for definite identification of these unknown saponins, further investigation is needed. In this work, UPLC/Q-TOF-MS/MS, a rapid and efficient analytical method, has been successfully developed for the characterization of steroidal saponins in the extracts from *D. zingiberensis*. Furthermore, this research provides a model for the rapid screening and structural characterization of bioactive constituents in plant extracts.

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