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UHPLC-Q-TOF-MS/MS-guided dereplication to study chemical constituents of *Hedera nepalensis* leaves in northern Vietnam

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Abstract

Hedera nepalensis is commonly used as a traditional medicine for the treatment of several diseases. In this research, the efficient characterization of chemical constituents, especially triterpenoid saponins, contained in *H. nepalensis* extract was established by applying ultra-high-performance liquid chromatography-quadrupole time-of-flight tandem mass spectrometry method (UHPLC-Q-TOF-MS/MS). As a result, a total of 45 compounds including 21 triterpene saponins were tentatively elucidated based on MS and MS/MS data, in which eight structures have been reported for the first time. This study provided an efficient analysis strategy to rapidly determine the chemical constituents and laid a foundation for future study of *H. nepalensis* planted in Northern Vietnam and other *Hedera* species.

Keywords *Hedera nepalensis*, Dereplication, Triterpene saponins, UHPLC-Q-TOF-MS/MS

Introduction

Hedera nepalensis belongs to *Hedera*, a 15 species genus of the Araliaceae family which includes about 70 genera and 700 species of flowering plants (Simab et al. 2011). The *Hedera* genus is well known for its economic importance (Ackerfield and Wen 2002). *H. nepalensis* is native to Vietnam, as well as China, India, Nepal, Bhutan, Afghanistan, Laos, Thailand, and Myanmar, at altitudes of approximately 1000–3000 (m) (Bashir et al. 2012).

H. nepalensis crude methanolic extract was investigated for the presence of important phytochemicals, including steroids, alkaloids, cardiac glycosides, and saponins (Simab et al. 2011). Twelve saponins named HN-saponins A, B, D1, D2, E, F, H, I, K, M, N, and P were isolated from *H. nepalensis* stem and bark (Haruhisa et al. 1985). α-hederin and hederacoside C were discovered in the most species of the *Hedera* genus (Leonid and Vladimir 2017).

A triterpene saponin structure is composed of a triterpene skeleton (aglycone) and sugar chain(s). Frequently, a series of triterpene saponins presented in a plant with the same aglycone but varied sugar chains. It is often difficult to isolate and identify a saponin compound due to its high polarity and structural similarity, especially when the sugar chain contains more than three sugar residues (Er-Fei et al. 2018). Hence, another method to identify and characterize known or new chemical structures is required.

UHPLC-Q-TOF-MS/MS is an increasingly potent and significant method for determining the chemical structures (Kumaria et al. 2016). UHPLC-Q-TOF-MS/MS has been applied to characterize phytochemicals in medicinal plants, and obtained considerable results. In 2011, UHPLC-Q-TOF-MS/MS techniques were performed to detect the targeted metabolites from the aqueous

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extracts of *Eurycoma longifolia* (Chua et al. 2011). In 2014, from the crude extracts of *Physalis alkekengi* calyx, 46 physalins were systematically analyzed by an integrated approach using UHPLC-QTOF-MS/MS (Huang et al. 2014). In addition, to distinguish between the chemical components of *Nigella glandulifera* Freyn et Sint and *Nigella sativa* L. seeds for quality control, UHPLC-Q-TOF-MS/MS was applied (Qi et al. 2014). In 2016, 31 saponins in *Shizhu ginseng* were identified applying UHPLC-Q-TOF-MS/MS (Sun et al. 2016). In 2018, UHPLC-Q-TOF-MS/MS guided dereplication of *Pulsatilla chinensis* was successfully identified a total of 22 triterpenoid saponins with four aglycone skeletons (Miao-miao et al. 2018).

The methanolic extracts of *H. nepalensis* (leaves and stems) were screened for different bioactivities such as brine shrimp cytotoxicity, antibacterial, potato disk antitumor, and phytotoxic activities (Samia et al. 2007). In addition, *H. nepalensis* was shown to contain various active compounds including saponins, affording antitumor, antioxidant (Simab et al. 2011; Samia et al. 2012), and antidiabetic (Saleem et al. 2014) activities.

The aim of the present study is to characterize the chemical constituents (especially triterpene saponins) of *H. nepalensis* leaves planted in Vietnam applying UHPLC-Q-TOF-MS/MS technique. The results of this preliminary work can assist in clarifying the chemical compositions of *H. nepalensis* and accelerating the new drug discovery from this valuable species.

Experimental

Chemicals and reagents

Deionized water for HPLC; HPLC grade acetonitrile, HPLC grade methanol, analytical grade formic acid ($\geq$ 98%) were obtained from Scharlau (Barcelona, Spain).

Sample preparation

Hedera nepalensis was collected from Sapa, Lao Cai province, Vietnam, and identified by botanist Tran Huu Dang MSc, Southern Institute of Ecology, Vietnam Academy of Science and Technology. A voucher specimen (Code: NaPro0619) was deposited in the Center for Research and Technology Transfer, Vietnam Academy of Science and Technology. The leaves were washed gently, let dry naturally, and cut into small pieces. 100.0 mg of leaves pieces was accurately weighed into a tube with a cover, and 2.0 mL methanol–water (8:2, v/v) solvent was added. After 10 min of ultrasonication, the sample was heated to 50 °C for 5 min. The extract was pipetted into a 10.0 mL volumetric flask after being centrifuged. The extraction stage continues with the residue. The solution was precisely scaled up to 10.0 mL using the solvent solution after five times of extraction. The sample was filtrated through a 0.45-µm filter membrane before injecting for UHPLC-Q-TOF-MS/MS analysis.

UHPLC-Q-TOF analysis

The UHPLC analysis was performed on an ExionLC™ UHPLC system (AB SCIEX, USA). The chromatographic separation was carried out on a Hypersil GOLD C18 column (150 × 2.1 mm, 3 µm) (Thermo Fisher Scientific, USA) at room temperature. The mobile phase consisted of water containing 0.1% formic acid (A) and acetonitrile containing 0.1% formic acid (B) with a linear gradient elution (0–4 min, 2–20% B; 4–30 min, 20–68% B; 30–32 min, 68–98% B; 32–40 min, 98% B) at a flow rate of 0.4 (mL/min). The sample volume injected was set at 5.0 (µL).

High-resolution MS and MS/MS spectra were acquired on an AB SCIEX $X500_R$ QTOF mass spectrometer (AB SCIEX, USA) coupled to the UPLC via an electrospray ionization (ESI) interface in both negative and positive ion modes. The operating parameters were optimized as follows: the ion source temperature, 500 °C; curtain gas, 30 psi; nebulizer gas (GS 1), 45 psi; heater gas (GS 2), 45 psi. The mass ranges for the TOF MS and TOF MS/MS were set at m/z 70–2000 and 50–1500, respectively. For the negative mode, ion spray voltage was set at − 4.5 kV, the declustering potential (DP) was − 70 V, the collision energy (CE) was performed at − 20 eV, and the collision energy spread (CES) was 10 eV. For the positive mode, ion spray voltage was set at 5.5 kV, the DP was 80 V, the CE was 20 eV, and the CES was 10 eV.

SCIEX OS software version 1.2.0.4122 (AB SCIEX, USA) was used to record and process the raw data.

Results and discussion

Differentiation of aglycones using positive ionization mode

Under the positive ESI mode, hederagenin, oleanolic acid, 30-norhederagenin, and akebonic acid showed the characteristic ion $[M+H]^+$ at m/z 473.3631, 457.3682, 457.3318, and 441.3369, respectively. The losses of water and HCOOH mainly occurred in these aglycones. The chemical structures and fragmentation pathways of the four aglycones were illustrated in Figs. 1, 2, 3 and 4, respectively.

Structural characterization of *H. nepalensis* triterpene saponins sugar linkages

The sugar chains of triterpenoid saponins generally substitute at C-3 and/or C-28 position(s) of an aglycone.

In the positive ESI mode, based on the characteristic fragment ions, the composition of sugar chains can be deduced as follows: the loss of glucopyranosyl (Glc) is 162 Da, rhamnopyranosyl (Rha) is 146 Da,

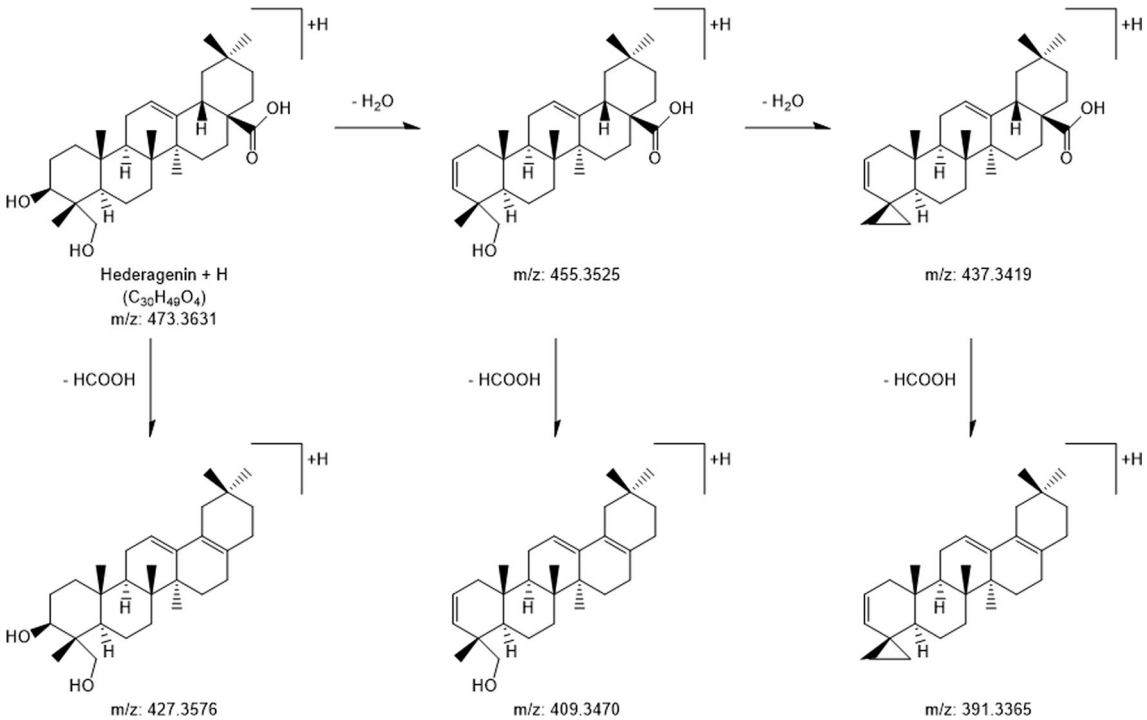


Fig. 1 ms/ms fragmentation pathway of hederagenin aglycone in positive mode

arabinopyranosyl (Ara) is 132 Da, and glucuronopyranosyl (Glu) is 176 Da. The sugar moieties at C-3 and C-28 are gradually removed, from C-3 to C-28 and from end to inner.

Deprotonated ion $[M\text{-}H]^-$ and characteristic solvent adducts ion $[M+HCOO]^-$ can be observed in the negative ESI mode, revealing the molecular mass and chemical composition of a triterpene saponin. Typically, the sugar chain at C-28 tends to be entirely eliminated, then the positions and composition of oligosaccharides chains can be readily differentiated, follows by an abundant fragment ion as a base peak.

Characterization of standard compounds

Two standard compounds, α-hederin and hederacoside C, were examined by UHPLC-Q-TOF-MS/MS in order to clarify the MS fragmentation patterns of triterpene saponins.

At $T_R = 20.41$, the MS spectra of α-hederin showed a deprotonated molecular ions $[M\text{-}H]^-$ at m/z 749.4473 in the negative mode and a pseudomolecular ion $[M+H]^+$ at m/z 751.4633 in the positive mode.

Hederacoside C ($T_R = 12.49$) yielded $[M\text{-}H]^-$ ion at m/z 1219.6110 and $[M+H]^+$ ion at m/z 1221.6313 in the negative and positive ionization modes, respectively.

A summary of the significant fragment ions identified in the mass spectra of the two reference triterpene saponins was provided in Additional file 1: Table S1. The typical MS and MS/MS spectra of α-hederin and hederacoside C were shown in Fig. 5.

Total ion chromatogram (TIC) of the sample in both positive and negative modes was shown in Additional file 1: Fig. S1a and Fig. S1b .

Structural characterization of triterpene saponins

Twenty-one triterpene saponins were elucidated and characterized from the *H. nepalensis* extract. The chemical structures were illustrated in Fig. 6, and the MS data are listed in Table 1. Non-saponin compounds were listed and proved in Additional file 1: Table S2.

The MS spectra of **compound 15** ($T_R = 8.29$ min) yielded a parent ion $[M+HCOO]^-$ at m/z 1281.6111 and a deprotonated molecular ions $[M\text{-}H]^-$ at m/z 1235.6070 in the negative mode, primarily fragmented into ions at m/z 765 and 469, showed the loss of 1 Rha and 2 Glc at C-28, 1 Glc and 1 Ara at C-3, and the aglycone corresponded to hederagenin. Besides, **compound 15** showed $[M+NH_4]^+$ ion at m/z 1254.6511, and daughter ions at m/z 1075, 943, 797, 635, and 473 because of the successive loss of Glc-Ara-Rha-Glc-Glc. Therefore, **compound 15** was determined as Hederasaponin G.

At $T_R = 11.09$ min, **compound 20** showed the formula of $C_{60}H_{98}O_{27}$ ($[M\text{-}H]^-$ at m/z 1249.6253), and provided fragment ions at m/z 779 and 469, corresponding to the

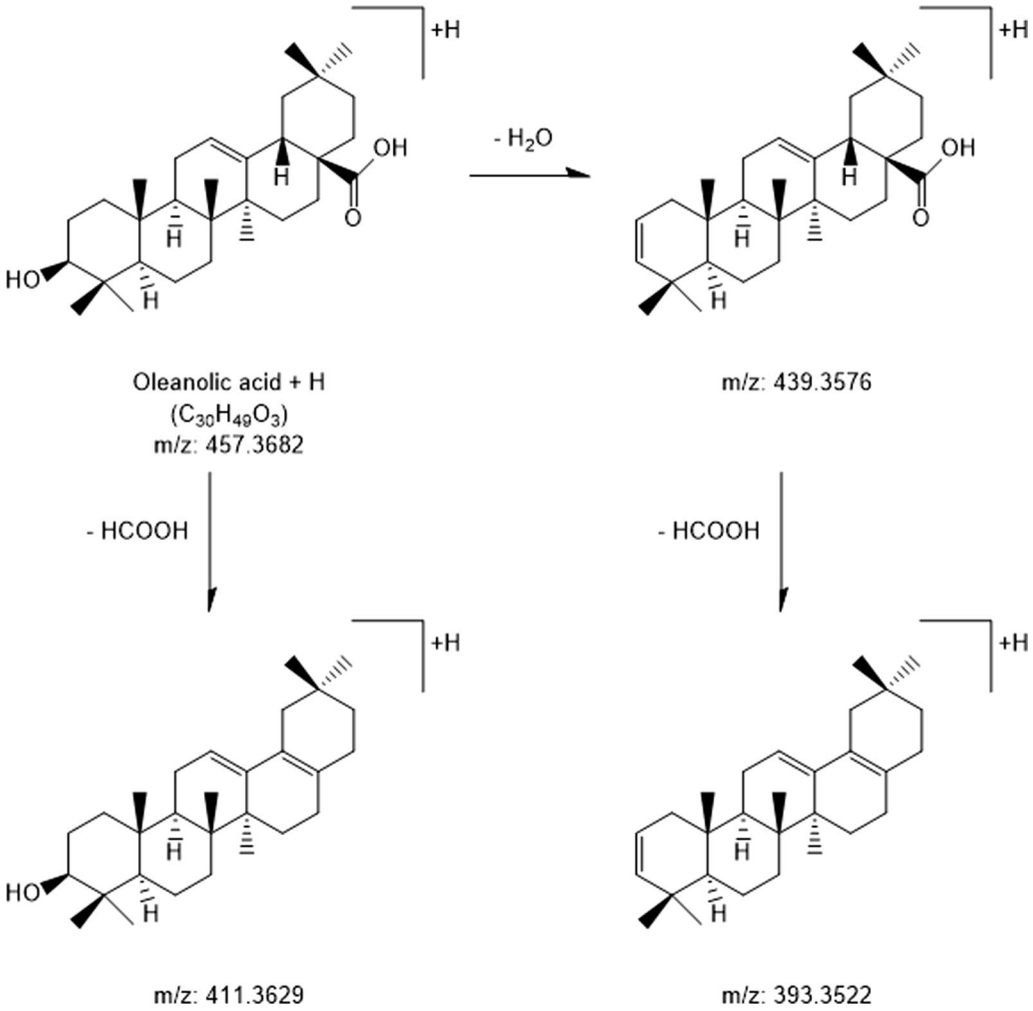


Fig. 2 ms/ms fragmentation pathway of oleanolic acid aglycone in positive mode

loss of 2 Glc and 1 Rha at C-28, and a Rha-Glc sugar chain at C-3 of the hederagenin aglycone. Hence, **compound 20** was tentatively characterized as hederagenin 3-O-[α-L-rhamnopyranosyl-(1 → 2)-β-D-glucopyranoside], 28-O-[α-L-rhamnopyranosyl-(1 → 4)-β-D-glucopyranosyl-(1 → 6)-β-D-glucopyranosyl] ester.

In the negative mode, **compound 21** (T_R = 11.29 min) yielded $[M+HCOO]^-$ ion at m/z 1249.5838 and $[M-H]^-$ ion at m/z 1203.5787, primarily fragmented to 733 and 469, which indicated the loss of 1 Rha with 2 Glc at C-28, and a Rha-Ara sugar chain at C-3. Besides, in the positive mode, the MS spectra of **compound 21** showed $[M+NH_4]^+$ ion at m/z 1222.6232, and fragments m/z 1073, 927, 765, 603, and 457, which indicated the elimination of Glc-Glc-Rha-Ara-Rha, respectively. Therefore, **compound 21** was suggested to be 30-norhederagenin 3-O-[α-L-arabinopyranosyl-(1 → 2)-α-L-rhamnopyranoside], 28-O-[β-D-glucopyranosyl-(1 → 6)-β-D-glucopyranosyl-(1 → 2)-α-L-rhamnopyranosyl] ester **or** 30-norhederagenin 3-O-[α-L-arabinopyranosyl-(1 → 2)-α-L-rhamnopyranoside], 28-O-[β-D-glucopyranosyl-(1 → 2)-(β-D-glucopyranosyl-(1 → 4))-α-L-rhamnopyranosyl] ester.

The MS/MS spectra of **compounds 22** (T_R = 12.57 min) **and 25** (T_R = 13.38 min) exhibited identical pseudomolecular ions $[M+H]^+$ at m/z 619.4214 and 619.4208, respectively, and produced identical aglycone ions at m/z 391, 437, 455, and 473, which corresponded to hederagenin. Thus, **compound 22** and **compound 25** were characterized as hederagenin 3-O-α-L-rhamnopyranoside and hederagenin 28-O-α-L-rhamnopyranoside, or vice versa.

Compound 23 (T_R = 12.64 min) produced $[M-H]^-$ at m/z 1219.6122 in the negative mode, and provided fragment ions at m/z 749, 603, and 469. Comparing the MS/MS spectra and retention time information with

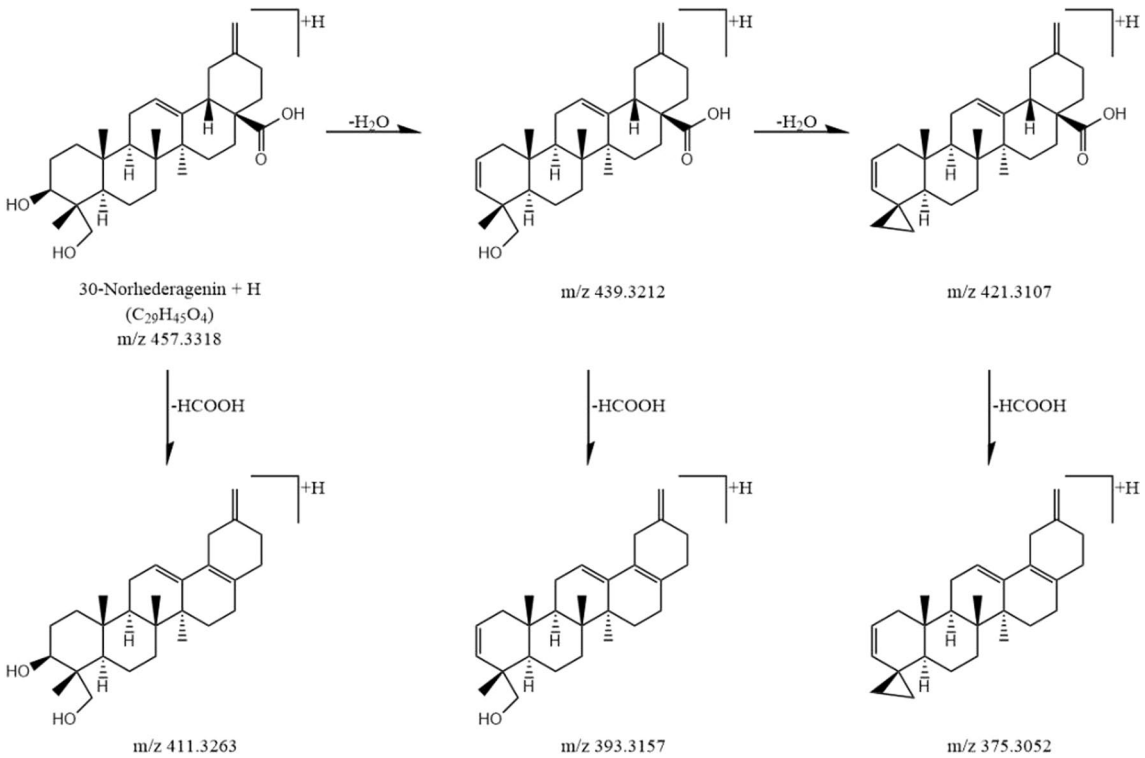


Fig. 3 ms/ms fragmentation pathway of 30-norhederagenin aglycone in positive mode

the reference standards, **compound 23** was undoubtedly determined as Hederacoside C.

At $T_R = 13.27$ min, **compound 24** yielded $[M+HCOO]^-$ and $[M\text{-}H]^-$ ions at m/z 1119.5594 and 1073.5553 in the negative mode, respectively. **Compound 24** primarily fragmented into ions at m/z 749, 603, and 469, indicated that hederagenin aglycone lost 2 Glc at C-28, and a Rha-Ara sugar chain at C-3. As a result, **compound 24** was tentatively identified as hederagenin 3-O-[α-L-rhamnopyranosyl-(1 → 2)-α-L-arabinopyranoside], 28-O-[β-D-glucopyranosyl-(1 → 6)-β-D-glucopyranosyl] ester.

The MS spectrum of **compound 26** at $T_R = 13.75$ min showed $[M\text{-}H]^-$ ion at m/z 1187.5867 ($C_{58}H_{91}O_{25}$, calcd. MW. = 1187.5850), while the MS/MS spectrum presented fragment ions at m/z 717, 581, and 469. Comparing with the result of Yue-Wei et al., **compound 26** was determined as Ciwujianoside B (Yue-Wei et al. 2017).

In the negative mode, **compound 27** ($T_R = 13.85$ min) produced a deprotonated molecular ion $[M\text{-}H]^-$ at m/z 1261.6227, primarily fragmented to 749, 603, and 469, which indicated the loss of 1 Rha, 2 Glc and an acetyl group at C-28, and a Rha-Ara sugar chain at C-3. In addition, **compound 27** showed a pseudomolecular ion $[M+H]^+$ at m/z 1263.6378, and hederagenin aglycone ions at m/z 409, 437, 455, and 473 in the positive mode. The fragmentation of the **compound 27** primarily yielded daughter ions at m/z 1117, 985, 839, and 473, because of the successive loss of Rha-Ara-Rha, and the loss of 2 Glc containing an acetyl group. Therefore, **compound 27** was predicted to be hederagenin 3-O-[α-L-Rhamnopyranosyl-(1 → 2)-α-L-arabinopyranoside], 28-O-[α-L-rhamnopyranosyl-(1 → 4)-6-O-acetyl-β-D-glucopyranosyl-(1 → 6)-β-D-glucopyranosyl] ester.

At $T_R = 14.33$, in the negative mode, **compound 28** yielded an $[M\text{-}H]^-$ ion at m/z 763.4302, and provided fragment ions at m/z 631 and 455, corresponding to the loss of 1 Ara and 1 Glu of oleanolic acid aglycone. Therefore, **compound 28** was tentatively identified as oleanolic acid 3-O-α-L-arabinopyranosyl-(1 → 3)-β-D-glucuronopyranoside (Momordin I).

In the negative mode, **compound 29** ($T_R = 14.57$ min) yielded a parent ion $[M+HCOO]^-$ at m/z 1349.6404 and a deprotonated molecular ion $[M\text{-}H]^-$ at m/z 1303.6346, with fragment ions at m/z 791 and 469, which indicated the elimination of 2 Glc, 1 Rha with an acetyl group at C-28, and 1 Rha, 1 Ara with an acetyl group at C-3 of hederagenin. Besides, in the positive mode, **compound 29** provided an $[M+H]^+$ ion at m/z 1305.6486, produced hederagenin aglycone ions at m/z 409, 437, 455, and 473. The fragment ions were 1173, 985, 839, 635,

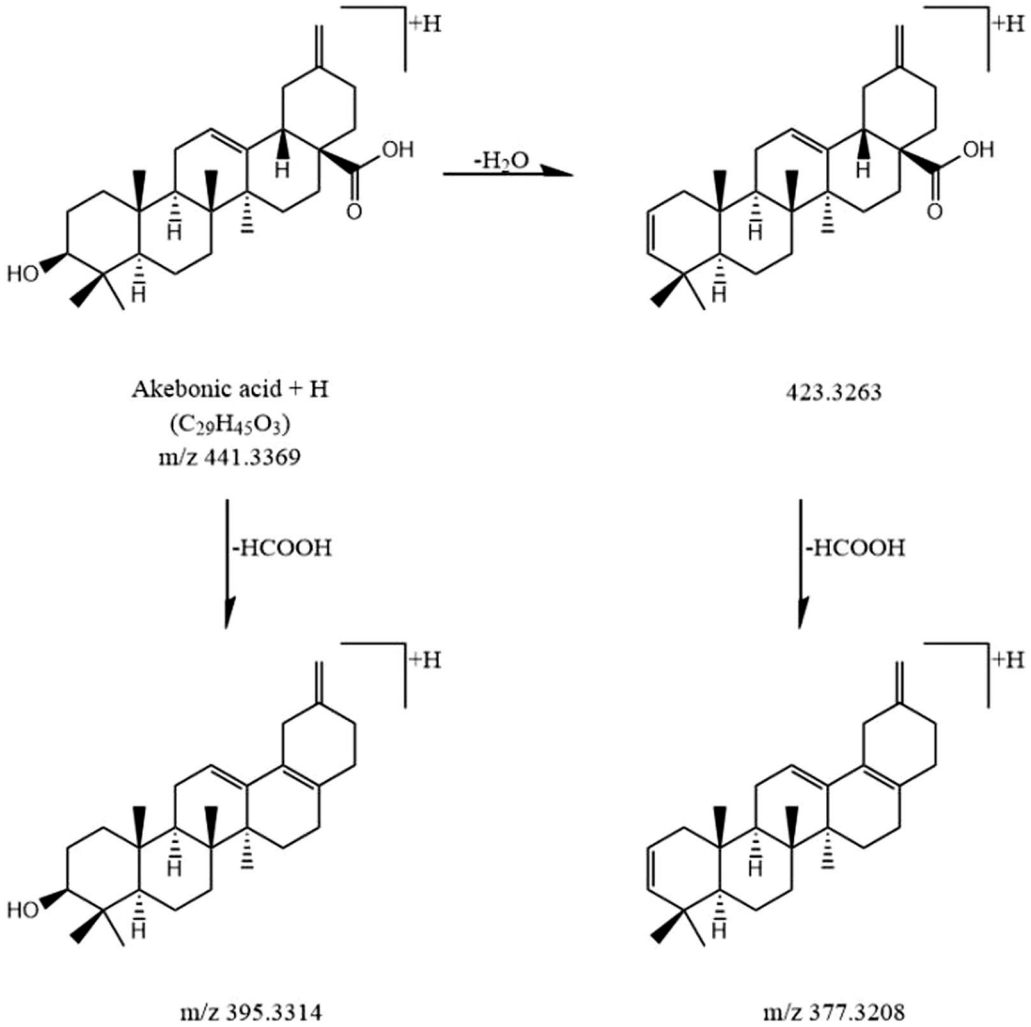


Fig. 4 ms/ms fragmentation pathway of akebonic acid aglycone in positive mode

and 473, showed the loss of 1 Ara, 1 acetyl-Rha, 1 Rha, 1 acetyl-Glc, and 1 Glc, respectively. As a result, **com pound 29** was determined as hederagenin 3-O-[α-L-arabinopyranosyl-(1→2)-4-O-acetyl-α-L-rhamnopyranoside], 28-O-[α-L-rhamnopyranosyl-(1→4)-6-O-acetyl-β-D-glucopyranosyl-(1→6)-β-D-glucopyranosyl] ester **or** heder agenin 3-O-[α-L-arabinopyranosyl-(1→2)-4-O-acetyl-α-L-rhamnopyranoside], 28-O-[α-L-rhamnopyranosyl-(1→4)-(6-O-acetyl-β-D-glucopyranosyl-(1→6))-β-D-glucopyranosyl] ester.

In the negative mode, at $T_R = 14.98$, **compound 30** showed the formula of $C_{59}H_{96}O_{25}$ ($[M\text{-}H]^-$ at m/z 1203.6178). Besides, **compound 30** had two fragment ions at m/z 733 and 455, corresponding to the loss of 2 Glc and 1 Rha at C-28, and a Rha-Ara sugar chain at C-3 of the oleanolic acid aglycone. Hence, **compound 30** was identified as Hederasaponin B.

The MS spectra of **compound 31** ($T_R = 15.55$ min) showed the formula of $C_{53}H_{86}O_{21}$ ($[M+HCOO]^-$ at m/z 1103.5666 and $[M\text{-}H]^-$ ion at m/z 1057.5604), and provided fragment ions at m/z 733, 587, and 455, corresponding to the loss of 2 Glc at C-28, and a Rha-Ara sugar chain at C-3 of the oleanolic acid aglycone. Hence, **compound 31** was oleanolic acid 3-O-[α-L-rhamnopyranosyl-(1→2)-α-L-arabinopyranoside], 28-O-[β-D-glucopyranosyl-(1→6)-β-D-glucopyranosyl] ester.

The MS spectra of **compound 32** ($T_R = 15.68$ min) yielded a parent ion $[M+HCOO]^-$ at m/z 1291.6340 and a deprotonated molecular ions $[M\text{-}H]^-$ at m/z 1245.6286 in the negative mode, primarily fragmented into ions at m/z 733 and 455, showed the loss of 1 Rha, 2 Glc and an acetyl group at C-28, the loss of 1 Rha and 1 Ara at C-3, and the aglycone corresponded

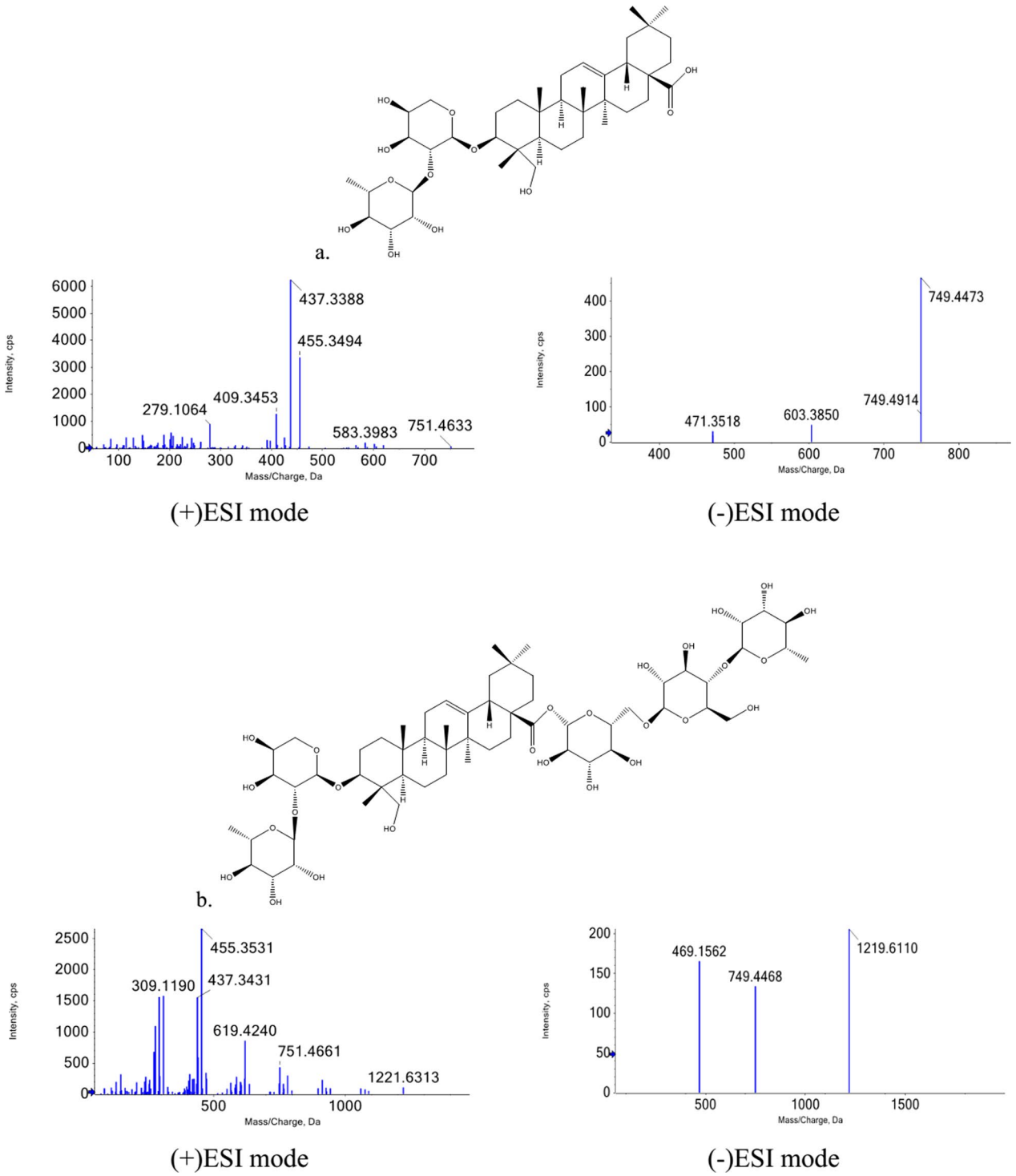
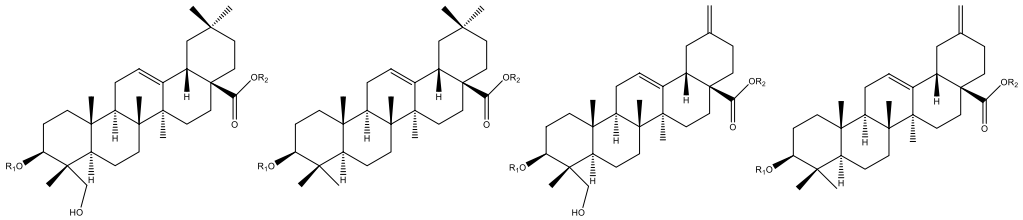


Fig. 5 Typical MS and MS/MS spectra in positive and negative electrospray ionization modes of a. α-hederin and b. Hederacoside C

to oleanolic acid. Besides, **compound 32** showed $[M+NH_4]^+$ ion at m/z 1264.6710, and daughter ions at m/z 1115, 969, 807, 603, and 457 because of the successive loss of Ara-Rha-Glc-(acetyl-Glc)-Rha. Therefore, **compound 32** was determined as oleanolic acid 3-O-[α-L-arabinopyranosyl-(1 → 2)-α-L-rhamnopyranoside], 28-O-[β-D-glucopyranosyl-(1 → 4)-6-O-acetyl-β-D-glucopyranosyl-(1 → 2)-α-L-rhamnopyranosyl] ester **or**



A-Hederagenin B-Oleanolic acid C-30-Norhederagenin D-Akebonic acid

Triterpene saponin No.	Name	Aglycone	R_1	R_2
15	Hederasaponin G	A	Glc-Ara-	Rha-Glc-Glc-
20	Hederagenin 3-O-[α-L-rhamnopyranosyl-(1→2)-β-D-glucopyranoside], 28-O-[α-L-rhamnopyranosyl-(1→4)-β-D-glucopyranosyl-(1→6)-β-D-glucopyranosyl] ester	A	Rha-Glc-	Rha-Glc-Glc
21	30-Norhederagenin 3-O-[α-L-arabinopyranosyl-(1→2)-α-L-rhamnopyranoside], 28-O-[β-D-glucopyranosyl-(1→6)-β-D-glucopyranosyl-(1→2)-α-L-rhamnopyranosyl] ester **or** 30-Norhederagenin 3-O-[α-L-arabinopyranosyl-(1→2)-α-L-rhamnopyranoside], 28-O-[β-D-glucopyranosyl-(1→2)-(β-D-glucopyranosyl-(1→4))-α-L-rhamnopyranosyl] ester	C	Ara-Rha-	Glc-Glc-Rha- Glc-Rha- | Glc
22	Hederagenin 3-O-α-L-rhamnopyranoside	A	Rha-	H-
	Hederagenin 28-O-α-L-rhamnopyranoside		H-	Rha-
23	Hederasaponin C	A	Rha-Ara-	Rha-Glc-Glc-
24	Hederagenin 3-O-[α-L-rhamnopyranosyl-(1→2)-α-L-arabinopyranoside], 28-O-[β-D-glucopyranosyl-(1→6)-β-D-glucopyranosyl] ester	A	Rha-Ara-	Glc-Glc-
25	Hederagenin 28-O-α-L-Rhamnopyranoside	A	H-	Rha-

Fig. 6 Chemical structures of identified triterpene saponins in *H. nepalensis*

	Hederagenin 3-O-α-L-Rhamnopyranoside		Rha-	H-
26	Ciwujianoside B	D	Rha-Ara-	Rha-Glc-Glc-
27	Hederagenin 3-O-[α-L-rhamnopyranosyl-(1→2)-α-L-arabinopyranoside], 28-O-[α-L-rhamnopyranosyl-(1→4)-6-O-acetyl-β-D-glucopyranosyl-(1→6)-β-D-glucopyranosyl] ester	A	Rha-Ara	Rha-acetyl Glc-Glc-
28	Momordin I	B	Ara-Glu-	H-
29	Hederagenin 3-O-[α-L-arabinopyranosyl-(1→2)-4-O-acetyl-α-L-rhamnopyranoside], 28-O-[α-L-rhamnopyranosyl-(1→4)-6-O-acetyl-β-D-glucopyranosyl-(1→6)-β-D-glucopyranosyl] ester or Hederagenin 3-O-[α-L-arabinopyranosyl-(1→2)-4-O-acetyl-α-L-rhamnopyranoside], 28-O-[α-L-rhamnopyranosyl-(1→4)-(6-O-acetyl-β-D-glucopyranosyl-(1→6))-β-D-glucopyranosyl] ester	A	Ara-acetyl Rha-	Rha-acetyl Glc-Glc- Rha-Glc- | acetyl Glc
30	Hederasaponin B	B	Rha-Ara-	Rha-Glc-Glc-
31	Oleanolic acid 3-O-[α-L-rhamnopyranosyl-(1→2)-α-L-arabinopyranoside], 28-O-[β-D-glucopyranosyl-(1→6)-β-D-glucopyranosyl] ester	B	Rha-Ara-	Glc-Glc-
32	Oleanolic acid 3-O-[α-L-arabinopyranosyl-(1→2)-α-L-rhamnopyranoside], 28-O-[β-D-glucopyranosyl-(1→4)-6-O-acetyl-β-D-glucopyranosyl-(1→2)-α-L-rhamnopyranosyl] ester or Oleanolic acid 3-O-[α-L-arabinopyranosyl-(1→2)-α-L-rhamnopyranoside], 28-O-[β-D-glucopyranosyl-(1→4)-(6-O-acetyl-β-D-glucopyranosyl-(1→2))-α-L-rhamnopyranosyl] ester	B	Ara-Rha-	Glc-acetyl Glc-Rha- Glc-Rha- | acetyl Glc
34	Oleanolic acid 3-O-[α-L-arabinopyranosyl-(1→2)-4-O-acetyl-α-L-rhamnopyranoside], 28-O-[4-O-acetyl-α-L-rhamnopyranosyl-(1→4)-β-D-glucopyranosyl-(1→6)-β-D-glucopyranosyl] ester	B	Ara-acetyl Rha-	acetyl Rha-Glc-Glc- acetyl Rha-G

Fig. 6 continued

	or Oleanolic acid 3-O-[α-L-arabinopyranosyl-(1→2)-4-O-acetyl-α-L-rhamnopyranoside], 28-O-[4-O-acetyl-α-L-rhamnopyranosyl-(1→4)-(β-D-glucopyranosyl-(1→6))-β-D-glucopyranosyl] ester			lc- | Glc
35	30-Norhederagenin 3-O-[α-L-rhamnopyranosyl-(1→2)-α-L-arabinopyranoside]	C	Rha- Ara-	H-
36	Hederagenin 3-O-β-D-glucoside	A	Glc-	H-
	Hederagenin 28-O-β-D-glucoside		H-	Glc-
37	Hederagenin 3-O-β-D-Glucuronopyranoside	A	Glu-	H-
	Hederagenin 28-O-β-D-Glucuronopyranoside		H-	Glu-
39	α-Hederin	A	Rha- Ara-	H-
40	Oleanolic acid 3-O-β-D-glucuronopyranoside	B	Glu-	H-
	Oleanolic acid 28-O-β-D-glucuronopyranoside		H-	Glu-
43	Oleanolic acid 3-O-α-L-arabinopyranoside, 28-O-α-L-rhamnopyranosyl ester	B	Ara-	Rha-
	β-Hederin		Rha- Ara-	H-

Fig. 6 continued

oleanolic acid 3-O-[α-L-arabinopyranosyl-(1→2)-α-L-rhamnopyranoside], 28-O-[β-D-glucopyranosyl-(1→4)-(6-O-acetyl-β-D-glucopyranosyl-(1→2))-α-L-rhamnopyranosyl] ester.

Compound 34 (T_R = 17.11 min) showed an animated ion $[M+NH_4]^+$ at m/z 1306.6813 and oleanolic acid aglycone ions at m/z 393, 411, 439, and 457 in the positive mode. The fragments were 1157, 969, 781, and 457, corresponding to the loss of 1 Ara, 1 acetyl-Rha, 1 acetyl-Rha, and 2 Glc, respectively. In addition, **compound 34** yielded a parent ion $[M+HCOO]^-$ at m/z 1333.6454 and an $[M-H]^-$ ion at m/z 1287.6387 in the negative mode, with fragment ions of 775 showed the elimination of 2 Glc, 1 Rha, and an acetyl group at C-28. Thus, **compound 34** was identified as oleanolic acid 3-O-[α-L-arabinopyranosyl-(1→2)-4-O-acetyl-α-L-rhamnopyranoside], 28-O-[4-O-acetyl-α-L-rhamnopyranosyl-(1→4)-β-D-glucopyranosyl-(1→6)-β-D-glucopyranosyl] ester **or** oleanolic acid 3-O-[α-L-arabinopyranosyl-(1→2)-4-O-acetyl-α-L-rhamnopyranoside], 28-O-[4-O-acetyl-α-L-rhamnopyranosyl-(1→4)-(β-D-glucopyranosyl-(1→6))-β-D-glucopyranosyl] ester.

At T_R = 18.40, **compound 35** yielded an $[M-H]^-$ ion at m/z 733.4182, showed the formula of $C_{40}H_{62}O_{12}$. The fragment ions at m/z 587 and 455 indicated the loss 1 Rha at C-28 and 1 Ara at C-3, or a Rha-Ara sugar chain at C-3. In the positive mode, **compound 35** showed an $[M+H]^+$ ion at m/z 735.4308, and fragmented to 589 and 457, proved the successive loss of Rha-Ara sugar chain at C-3 of the aglycone. Hence, **compound 35** was characterized as 3,23-Dihydroxy-30-nor-12,20(29)-oleanadien-28-oic acid; 3β-form, 3-O-[α-L-rhamnopyranosyl-(1→2)-α-L-arabinopyranoside] (30-norhederagenin 3-O-[α-L-rhamnopyranosyl-(1→2)-α-L-arabinopyranoside]).

In the negative mode, **compounds 36** (T_R = 19.42 min) produced a deprotonated molecular ion $[M-H]^-$ at m/z 633.4031, and fragmented into an ion at m/z 471, showed the elimination of a Glc at C-3 or C-28 sugar chain of hederagenin. Therefore, **compound 36** was indicated as hederagenin 3-O-β-D-glucoside **or** hederagenin 28-O-β-D-glucoside.

Compound 37 (T_R = 19.56 min) yielded an $[M-H]^-$ ion at m/z 647.3817 with a fragment ion at m/z 471 in the negative mode, indicated hederagenin aglycone lost a Glu

Table 1 Saponins of *Hedera nepalensis* characterized by UHPLC-Q-TOF-MS/MS

Peak No	T_R (min)	Formula	Chemical name	ESI mode	Error (ppm)	Exact mass	Found at mass	MS/MS
15	8.29	$C_{59}H_{96}O_{27}$	Hederasaponin G	+	2.31	1254.6482	1254.6511 $[M+NH_4]^+$	1237.6251 $[M+H]^+$ 1075.5465 $[M+H\text{-}C_6H_{10}O_5]^+$ 943.5270 $[M+H\text{-}C_{11}H_{18}O_9]^+$ 797.4641 $[M+H\text{-}C_{16}H_{24}O_{14}]^+$ 635.4247 $[M+H\text{-}C_{22}H_{34}O_{19}]^+$ 473.6549 $[M+H\text{-}C_{28}H_{44}O_{24}]^+$
20	11.09	$C_{60}H_{98}O_{27}$	Hederagenin 3-O-[α-L-Rhamnopyranosyl-(1 → 2)-β-D-glucopyranoside], 28-O-[α-L-rhamnopyranosyl-(1 → 4)-β-D-glucopyranosyl-(1 → 6)-β-D-glucopyranosyl] ester	–	2.88	1249.6217	1249.6253 $[M\text{-}H]^-$	779.4580 $[M\text{-}H\text{-}C_{18}H_{30}O_{14}]^-$ 469.1681 $[M\text{-}H\text{-}C_{30}H_{50}O_{23}]^-$
21	11.29	$C_{58}H_{92}O_{26}$	30-Norhederagenin 3-O-[α-L-arabinopyranosyl-(1 → 2)-α-L-rhamnopyranoside], 28-O-[β-D-glucopyranosyl-(1 → 6)-β-D-glucopyranosyl-(1 → 2)-α-L-rhamnopyranosyl] ester or 30-Norhederagenin 3-O-[α-L-arabinopyranosyl-(1 → 2)-α-L-rhamnopyranoside], 28-O-[β-D-glucopyranosyl-(1 → 2)-(β-D-glucopyranosyl-(1 → 4))-α-L-rhamnopyranosyl] ester	+	0.90	1222.6221	1222.6232 $[M+NH_4]^+$	1205.6032 $[M+H]^+$ 1073.5480 $[M+H\text{-}C_5H_8O_4]^+$ 927.4980 $[M+H\text{-}C_{11}H_{18}O_8]^+$ 765.4387 $[M+H\text{-}C_{16}H_{24}O_{14}]^+$ 603.3930 $[M+H\text{-}C_{22}H_{34}O_{19}]^+$ 457.3304 $[M+H\text{-}C_{28}H_{44}O_{23}]^+$ 439.3211 $[M+H\text{-}C_{28}H_{46}O_{24}]^+$
22	12.57	$C_{36}H_{58}O_8$	Hederagenin 3-O-α-L-Rhamnopyranoside or Hederagenin 28-O-α-L-Rhamnopyranoside	+	0.65	619.4210	619.4214 $[M+H]^+$	473.3622 $[M+H\text{-}C_6H_{10}O_4]^+$ 455.3503 $[M+H\text{-}C_6H_{12}O_5]^+$ 437.3398 $[M+H\text{-}C_6H_{14}O_6]^+$ 391.3355 $[M+H\text{-}C_7H_{16}O_8]^+$
23	12.64	$C_{59}H_{96}O_{26}$	Hederasaponin C	–	0.82	1219.6112	1219.6122 $[M\text{-}H]^-$	749.4516 $[M\text{-}H\text{-}C_{18}H_{30}O_{14}]^-$ 603.3934 $[M\text{-}H\text{-}C_{24}H_{40}O_{18}]^-$ 469.1586 $[M\text{-}H\text{-}C_{29}H_{50}O_{22}]^-$
24	13.27	$C_{53}H_{86}O_{22}$	Hederagenin 3-O-[α-L-Rhamnopyranosyl-(1 → 2)-α-L-arabinopyranoside], 28-O-[β-D-glucopyranosyl-(1 → 6)-β-D-glucopyranosyl] ester	–	0.63	1119.5587	1119.5594 $[M+HCOO]^-$	1073.5595 $[M\text{-}H]^-$ 749.4546 $[M\text{-}H\text{-}C_{12}H_{20}O_{10}]^-$ 603.3944 $[M\text{-}H\text{-}C_{18}H_{30}O_{14}]^-$ 469.1593 $[M\text{-}H\text{-}C_{23}H_{40}O_{18}]^-$
25	13.38	$C_{36}H_{58}O_8$	Hederagenin 28-O-α-L-Rhamnopyranoside or Hederagenin 3-O-α-L-Rhamnopyranoside	+	− 0.32	619.4210	619.4208 $[M+H]^+$	473.3628 $[M+H\text{-}C_6H_{10}O_4]^+$ 455.3506 $[M+H\text{-}C_6H_{12}O_5]^+$ 437.3400 $[M+H\text{-}C_6H_{14}O_6]^+$ 391.3358 $[M+H\text{-}C_7H_{16}O_8]^+$
26	13.75	$C_{58}H_{92}O_{25}$	Ciwujianoside B	–	1.47	1187.5850	1187.5867 $[M\text{-}H]^-$	717.4183 $[M\text{-}H\text{-}C_{18}H_{30}O_{14}]^-$ 469.1551 $[M\text{-}H\text{-}C_{40}H_{62}O_{11}]^-$
27	13.85	$C_{61}H_{98}O_{27}$	Hederagenin 3-O-[α-L-Rhamnopyranosyl-(1 → 2)-α-L-arabinopyranoside], 28-O-[α-L-rhamnopyranosyl-(1 → 4)-6-O-acetyl-β-D-glucopyranosyl-(1 → 6)-β-D-glucopyranosyl] ester	+	0.32	1263.6374	1263.6378 $[M+H]^+$	1117.5852 $[M+H\text{-}C_6H_{10}O_4]^+$ 985.5379 $[M+H\text{-}C_{11}H_{18}O_8]^+$ 839.4808 $[M+H\text{-}C_{17}H_{28}O_{12}]^+$ 473.3631 $[M+H\text{-}C_{31}H_{50}O_{23}]^+$ 455.3498 $[M+H\text{-}C_{31}H_{52}O_{24}]^+$ 437.3405 $[M+H\text{-}C_{31}H_{54}O_{25}]^+$ 409.3453 $[M+H\text{-}C_{32}H_{54}O_{26}]^+$
28	14.33	$C_{41}H_{64}O_{13}$	Momordin I	–	4.36	763.4269	763.4302 $[M\text{-}H]^-$	631.3833 $[M\text{-}H\text{-}C_5H_8O_4]^-$ 455.3520 $[M\text{-}H\text{-}C_{11}H_{16}O_{10}]^-$
29	14.57	$C_{63}H_{100}O_{28}$	Hederagenin 3-O-[α-L-arabinopyranosyl-(1 → 2)-4-O-acetyl-α-L-rhamnopyranoside], 28-O-[α-L-rhamnopyranosyl-(1 → 4)-6-O-acetyl-β-D-glucopyranosyl-(1 → 6)-β-D-glucopyranosyl] ester or Hederagenin 3-O-[α-L-arabinopyranosyl-(1 → 2)-4-O-acetyl-α-L-rhamnopyranoside], 28-O-[α-L-rhamnopyranosyl-(1 → 4)-(6-O-acetyl-β-D-glucopyranosyl-(1 → 6))-β-D-glucopyranosyl] ester	+	0.54	1305.6479	1305.6486 $[M+H]^+$	1173.6058 $[M+H\text{-}C_5H_8O_4]^+$ 985.5483 $[M+H\text{-}C_{13}H_{20}O_9]^+$ 839.4754 $[M+H\text{-}C_{19}H_{30}O_{13}]^+$ 635.4241 $[M+H\text{-}C_{27}H_{42}O_{19}]^+$ 473.3641 $[M+H\text{-}C_{33}H_{52}O_{24}]^+$ 455.3576 $[M+H\text{-}C_{33}H_{54}O_{25}]^+$ 437.3468 $[M+H\text{-}C_{33}H_{56}O_{26}]^+$ 409.3480 $[M+H\text{-}C_{34}H_{56}O_{27}]^+$

Table 1 (continued)

Peak No	T_R (min)	Formula	Chemical name	ESI mode	Error (ppm)	Exact mass	Found at mass	MS/MS
30	14.98	$C_{59}H_{96}O_{25}$	Hederasaponin B	–	1.29	1203.6163	1203.6178 $[M-H]^-$	733.4534 $[M-H-C_{18}H_{30}O_{14}]^-$ 455.3608 $[M-H-C_{29}H_{48}O_{22}]^-$
31	15.55	$C_{53}H_{86}O_{21}$	Oleanolic acid 3-O-[α-L-Rhamnopyranosyl-(1 → 2)-α-L-arabinopyranoside], 28-O-[β-D-glucopyranosyl-(1 → 6)-β-D-glucopyranosyl] ester	–	2.54	1103.5638	1103.5666 $[M+HCOO]^-$	1057.5611 $[M-H]^-$ 733.4489 $[M-H-C_{12}H_{20}O_{10}]^-$ 587.4014 $[M-H-C_{18}H_{30}O_{14}]^-$ 455.3503 $[M-H-C_{23}H_{38}O_{18}]^-$
32	15.68	$C_{61}H_{98}O_{26}$	Oleanolic acid 3-O-[α-L-arabinopyranosyl-(1 → 2)-α-L-rhamnopyranoside], 28-O-[β-D-glucopyranosyl-(1 → 4)-6-O-acetyl-β-D-glucopyranosyl-(1 → 2)-α-L-rhamnopyranosyl] ester or Oleanolic acid 3-O-[α-L-arabinopyranosyl-(1 → 2)-α-L-rhamnopyranoside], 28-O-[β-D-glucopyranosyl-(1 → 4)-(6-O-acetyl-β-D-glucopyranosyl-(1 → 2))-α-L-rhamnopyranosyl] ester	+	1.50	1264.6691	1264.6710 $[M+NH_4]^+$	1247.6405 $[M+H]^+$ 1115.5701 $[M+H-C_5H_8O_4]^+$ 969.5428 $[M+H-C_{11}H_{18}O_8]^+$ 807.4700 $[M+H-C_{16}H_{24}O_{14}]^+$ 603.4191 $[M+H-C_{24}H_{36}O_{20}]^+$ 457.3599 $[M+H-C_{30}H_{46}O_{24}]^+$ 439.3587 $[M+H-C_{30}H_{48}O_{25}]^+$
34	17.11	$C_{63}H_{100}O_{27}$	Oleanolic acid 3-O-[α-L-arabinopyranosyl-(1 → 2)-4-O-acetyl-α-L-rhamnopyranoside], 28-O-[4-O-acetyl-α-L-rhamnopyranosyl-(1 → 4)-β-D-glucopyranosyl-(1 → 6)-β-D-glucopyranosyl] ester **or** Oleanolic acid 3-O-[α-L-arabinopyranosyl-(1 → 2)-4-O-acetyl-α-L-rhamnopyranoside], 28-O-[4-O-acetyl-α-L-rhamnopyranosyl-(1 → 4)-(β-D-glucopyranosyl-(1 → 6))-β-D-glucopyranosyl] ester	+	1.30	1306.6796	1306.6813 $[M+NH_4]^+$	1289.6569 $[M+H]^+$ 1157.5950 $[M+H-C_5H_8O_4]^+$ 781.2318 $[M+H-C_{21}H_{32}O_{14}]^+$ 457.3660 $[M+H-C_{33}H_{52}O_{24}]^+$ 439.3566 $[M+H-C_{33}H_{54}O_{25}]^+$ 393.1386 $[M+H-C_{34}H_{56}O_{27}]^+$
35	18.40	$C_{40}H_{62}O_{12}$	30-Norhederagenin 3-O-[α-L-rhamnopyranosyl-(1 → 2)-α-L-arabinopyranoside]	–	2.59	733.4163	733.4182 $[M-H]^-$	587.3593 $[M-H-C_6H_{10}O_4]^-$ 455.3200 $[M-H-C_{11}H_{18}O_8]^-$
36	19.42	$C_{36}H_{58}O_9$	Hederagenin 3-O-β-D-glucoside **or** Hederagenin 28-O-β-D-glucoside	–	4.48	633.4003	633.4031 $[M-H]^-$	471.3386 $[M-H-C_6H_{10}O_5]^-$
37	19.56	$C_{36}H_{56}O_{10}$	Hederagenin 3-O-β-D-Glucuronopyranoside **or** Hederagenin 28-O-β-D-Glucuronopyranoside	–	3.40	647.3795	647.3817 $[M-H]^-$	471.3496 $[M-H-C_6H_{10}O_5]^-$
39	20.52	$C_{41}H_{66}O_{12}$	α-Hederin	–	3.74	749.4476	749.4504 $[M-H]^-$	603.3910 $[M-H-C_6H_{10}O_4]^-$ 471.3516 $[M-H-C_{11}H_{18}O_8]^-$
40	23.12	$C_{36}H_{56}O_9$	Oleanolic acid 3-O-β-D-glucuronopyranoside **or** Oleanolic acid 28-O-β-D-glucuronopyranoside	–	2.85	631.3846	631.3864 $[M-H]^-$	455.3292 $[M-H-C_6H_8O_6]^-$
43	24.15	$C_{41}H_{66}O_{11}$	Oleanolic acid 3-O-α-L-arabinopyranoside, 28-O-α-L-rhamnopyranosyl ester **or** β-Hederin	–	2.32	733.4527	733.4544 $[M-H]^-$	587.4001 $[M-H-C_6H_{10}O_4]^-$ 455.3547 $[M-H-C_{11}H_{18}O_8]^-$

at C-3 or C-28. Hence, **compound 37** was identified as hederagenin 3-O-β-D-glucuronopyranoside **or** hederagenin 28-O-β-D-glucuronopyranoside.

At $T_R = 20.52$ min, **compound 39** showed $[M+H]^+$ ion at m/z 751.4618 $[M-H]^-$ ion at m/z 749.4504 in the positive and negative modes, respectively. Comparing the MS/MS information and retention time with the reference standards, **compound 39** was unambiguously determined as α-hederin.

The MS spectra of **compound 40** ($T_R = 23.12$ min) showed the formula of $C_{36}H_{56}O_9$ ($[M-H]^-$ at m/z 631.3864), and provided fragment ion at m/z 455, corresponding to the loss of 1 Glu at C-3 or C-28 of the oleanolic acid aglycone. Hence, **compound 40** was determined as oleanolic acid 3-O-β-D-glucuronopyranoside **or** oleanolic acid 28-O-β-D-glucuronopyranoside.

Compound 43 (T_R = 24.15 min) yielded an [M-H]$^-$ ion at m/z 733.4544 with fragment ions at m/z 587 and 455 in the negative mode, indicated oleanolic acid aglycone lost 1 Rha at C-28 and 1 Ara at C-3, or a Rha-Ara sugar chain at C-3. Hence, **compound 43** was identified as oleanolic acid 3-O-α-L-arabinopyranoside, 28-O-α-L-rhamnopyranosyl ester **or** oleanolic acid 3-O-[α-L-rhamnopyranosyl-(1 → 2)-α-L-arabinopyranoside] (β-Hederin).

In short, 8 saponin triterpene structures of **compounds 21, 29, 32, 34** have been reported for the first time.

Conclusion

Applying UHPLC-Q-TOF-MS/MS method, the characterization of triterpene saponins structural information in *H. nepalensis* was successfully analyzed in positive and negative modes. Forty-five phytochemicals including 21 triterpene saponins were characterized and eight structures have not been reported yet. This study could serve as an effective tool for rapid determination of phytochemicals, and provided a base for quality control of *H. nepalensis* raw materials in Northern Vietnam. The profile of saponin components and other compounds could contribute the information for development this plant in pharmaceutical and medicinal material further study.

Supplementary Information

The online version contains supplementary material available at https://doi.org/10.1186/s40543-023-00369-2.

Additional file 1. Figure S1a: Total ion chromatogram (TIC) of the sample in positive mode. **Figure S1b:** Total ion chromatogram (TIC) of the sample in negative mode. **Table S1.** The MS/MS data of standard compounds. **Table S2.** Non-saponin compounds of Hedera nepalensis characterized by UHPLC-Q-TOF-MS/MS

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Author contributions

PHN performed UHPLC-Q-TOF analysis, processed the experimental data, analyzed the data, and contributed to the writing of the manuscript. TCA performed the analytic calculations, analyzed the data, and contributed to the writing of the manuscript. NTH helped in material collection and prepared the sample. TPHN prepared the sample and analyzed MS data. LNH devised the project and the main conceptual ideas. NQT was involved in interpretation and exploring the MS data. BQM helped in interpretation of MS/MS data. PVT devised the project, the main conceptual ideas, and developed the theoretical framework. All authors have read and approved the final version of the manuscript.

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Availability of data and materials

The data used to support the results of this study are included within the article. Any further information is available from the authors upon request.

Declarations

Competing interests

The authors declare that there is no conflict of interest regarding the publication of this paper.

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References

Ackerfield J, Wen J. A morphometric analysis of *Hedera* L. (the ivy genus, Araliaceae) and its taxonomic implications. Adansonia. 2002;24(2):197–212.

Bashir A, Nida M, Shumaila B, Sadiq A, Ibrar K, Mohammad A. Biological screening of *Hedera nepalensis*. J Med Plants Res. 2012;6(39):5250–7.

Chua SL, Mohd-Amin AN, Hong-Neo CJ, Lee HT, Lee TC, Sarmidi RM, Aziz AR. LC–MS/MS-based metabolites of *Eurycoma longifolia* (Tongkat Ali) in Malaysia (Perak and Pahang). J Chromatogr B. 2011;879:3909–19.

Er-Fei Z, Yun L, Zi Y, Qing Z. Identification and structural characterisation of triterpene saponins. Nat Prod Res. 2018;32(8):918–23.

Haruhisa K, Shingo K, Fukio N, Tsuyoshi T, Tsuneo N. Studies on nepalese crude drugs. III. On the Saponins of *Hedera nepalensis* K. Koch. Chem Pharm Bull. 1985;33(8):3324–9.

Huang C, Xu Q, Chen C, Song C, Yu Y, Xiang Y, Feng Y, Ouyang H, Zhang Y, Jiang H. The rapid discovery and identification of physalins in the calyx of *Physalis alkekengi* L.*var.franchetii* (Mast.) Makino using ultra-high performance liquid chromatography-quadrupole time of flight tandem mass spectrometry together with a novel three-step data mining strategy. J Chromatogr A. 2014;1361:139–52.

Kumaria S, Elancheran R, Kotoky J, Devi R. Rapid screening and identification of phenolic antioxidants in *Hydrocotyle sibthorpioides* Lam. by UPLC-ESI-MS/MS. Food Chem. 2016;203:521–9.

Leonid AY and Vladimir IG. Chapter 11: Ivy and licorice triterpene glycosides: promising molecular containers for some drugs and biomolecules. Studies in natural products chemistry. 2017;55:351–83.

Miao-miao J, Wen-dan Z, Hong-hong J, Ying-feng D, Wei G, Liang C, Hui-jun X. UPLC-Q-TOF-MS/MS-guided dereplication of *Pulsatilla chinensis* to identify triterpenoid saponins. Phytochem Anal. 2018;29(5):516–27.

Qi Y, Qingwang L, Cunyong H, Xiaohua M, Xiaoli G, Amer T, Jianping Z. UPLC-Q-TOF/MS characterization, HPLC fingerprint analysis and species differentiation for quality control of *Nigella glandulifera* Freyn et Sint seeds and *Nigella sativa* L. seeds. Anal Methods. 2014;6:4845–52.

Saleem S, Jafri L, Haq IU, Chang LC, Calderwood D, Green BD, Mirza B. Plants *Fagonia cretica* L. and *Hedera nepalensis* K. Koch contain natural compounds with potent dipeptidyl peptidase-4 (DPP-4) inhibitory activity. J Ethnopharmacol. 2014;156:26–32.

Samia I, Rukhsana I, Ateeq-ur R, Fayyaz MC, Bushra M. Biological evaluation of some selected plant species of Pakistan. Pharm Biol. 2007;45(5):397–403.

Samia I, Paul DP, Hassan KO, Ata-ur R, Bushra M. Bioprospecting traditional Pakistani medicinal plants for potent antioxidants. Food Chem. 2012;132(1):222–9.

Simab K, Nazif U, Ihsan UH, Imran A, Bushra M. Antioxidant, antitumor activities and phytochemical investigation of *Hedera nepalensis* K. Koch, an important medicinal plant from Pakistan. Pak J Bot. 2011;43:85–9.

Sun TT, Liang XL, Zhu HY, Peng XL, Guo XJ, Zhao LS. Rapid separation and identification of 31 major saponins in *Shizhu ginseng* by ultra-high performance liquid chromatography–electron spray ionization–MS/MS. J Ginseng Res. 2016;40(3):220–8.

Yue-Wei G, Shu Z, Kayo Y, Katsuko K. MS/MS similarity networking accelerated target profiling of triterpene saponins in *Eleutherococcus senticosus* leaves. Food Chem. 2017;227:444–52.

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