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ORIGINAL ARTICLE

A systematic strategy for rapid identification of chlorogenic acids derivatives in *Duhaldea nervosa* using UHPLC-Q-Exactive Orbitrap mass spectrometry

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Abstract *Duhaldea nervosa* (Wallich ex Candolle) A. Anderberg was widely used for food spice and folk medicine. However, it is still insufficient in the constituent's characterization of *D. nervosa*. In this study, a systematic strategy for rapid detection and identification of constituents was proposed based on UHPLC-Q-Exactive Orbitrap mass spectrometry in parallel reaction monitoring mode combining anion exchange resin separation, expected compounds predicted and diagnosis fragmentation ions techniques. Finally, 149 chlorogenic acids derivatives were unanimously and tentatively characterized from *D. nervosa*, 102 of them were report for the first time. This results widely extended the chemical constituents of *D. nervosa*, which will facilitate understanding the effective substance and quality control. Meantime, it is possible for this strategy to exhibit a wide application for chemical's characterization in different sample.

1. Introduction

Duhaldea nervosa (Wallich ex Candolle) A. Anderberg (*D. nervosa*), commonly called Maoxiucai or Xiaoheiyao, belongs to the Asteraceae family (Xiao, 2004; Editorial Board, 2010). It has been widely used as food flavor and folk medicine especially in Dong minority for treating traumatic injury and relieving rheumatism (Xiao, 1997; Long, 2004). Previous investigations had shown that *D. nervosa* contained steroid, terpenes, polysaccharide, and chlorogenic acids derivatives

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(CGAs) (Yan et al., 2011; Guan et al., 2017), especially CGAs, which has multiple biological activities, including promoting cell proliferation and differentiation, anti-inflammatory (Naveed et al., 2018; Zhang et al., 2014). However, it is still insufficient in the constituent's characterization of D. nervosa, which is very helpful for understanding the material basis and quality control. Therefore, it is necessary to develop a systematic strategy for rapid detection and identification of constituents in *D. nervosa*.

In the past few decades, Liquid Chromatography-Mass Spectrometry (LC-MS), especially Ultra-High performance liquid chromatography-high resolution mass spectrometry (UHPLC-HRMS) has become the most powerful and reliable analytical instruments in detection and characterization of constituents from traditional Chinese medicine, drug, or biological samples (Wang et al., 2019; Cai et al., 2017; Koley et al., 2020). However, numerous mass spectrometric data acquired by HRMS will be a new challenge for structure identification. Therefore, several algorithms including metabolic reaction network-based recursive (Shen et al., 2019) and mass spectral trees similarity filter (MTSF) were proposed to solve this problem. In our previous work, MTSF was established and applied in the detection and identification of CGAs in *D. nervosa* (Liu et al., 2018). Generally, the parent ion of constituents (MS^1) and subsequent fragments (MS^n) were used for structural elucidation and also for the construct of the mass spectral trees data. However, the parent ion of trace constituents especially when they co-eluted with higher content constituents could not be acquired, and the subsequent fragments could not be trigged due to the relatively lower content in the mass analyzer, which result in the insufficient of CGAs in *D. nervosa*. In order to obtain the fragments of relatively lower content, the parallel reaction monitoring (PRM) mode (Xiang et al., 2017) was adapted in this experiment. Hence, a systematic strategy was proposed for rapid detection and identification of CGAs in *D. nervosa* using UHPLC-Q-Exactive Orbitrap mass spectrometry in PRM mode based on expected compounds predicted and diagnosis fragmentation ions techniques.

In this study, anion exchange resin column was used to enrich the trace amount of CGAs at first. Then, the sample was performed on UHPLC-Q-Exactive Orbitrap MS in negative mode to gain the high resolution mass spectrum, which was processed by Compound Discover version 3.0 using high resolution extracted ion chromatography and expected compounds predicted. The MS^2 data of expected compounds was obtained by PRM mode. Finally, the diagnosis fragmentation ions were established and used to rapidly identify 149 CGAs from *D. nervosa*, 102 of them were report for the first time.

2. Materials and methods

2.1. Materials and chemicals

Reference standards trans-3-caffeoylquinic acid (trans-3-CQA, neochlorogenic acid, X-014–170309), trans-4-caffeoylquinic acid (trans-4-CQA, cryptochlorogenic acid, Y-067-180320), trans-5-caffeoylquinic acid (trans-5-CQA, chlorogenic acid, L-007-171216), 3,5-dicaffeoylquinic acid (3,5-DiCQA, isochlorogenic acid A, Y-068-170903), 3,4-dicaffeoylquinic acid (3,4-DiCQA, isochlorogenic acid B, Y-069-180105), 4,5-dicaffeoylquinic acid (4,5-DiCQA, isochlorogenic acid C, Y-070-170515) were provided by Chengdu Herbpurify CO., LTD (Chengdu, China). Anion exchange resin column (WondaSep MAX, 500 mg/6mL) was purchased from Shimadzu Corporation. HPLC grade of water, methanol, acetonitrile, and formic acid were from Fisher scientific (New jersey, USA). Other reagents were of analytical grade.

D. nervosa was purchased from Yunyao company (Yunan, China). The voucher specimen was deposited at School of Pharmaceutical Sciences, Hunan university of medicine.

2.2. Standard and sample preparation

Each reference standard was accurately weighted and dissolved in methanol.

The dried powder of *D. nervosa* (10 g) was reflux-extracted in 50 mL 70% aqueous ethanol for 1 h, and then the extracted solution was filtrated and dried under reduce pressure to yield the brown residues, which was dissolved in water with 2% formic acid then subjected to anion exchange resign column (WondaSep MAX, 500 mg/6mL), eluting with water and methanol with 2% formic acid, successively. The eluted was evaporated under nitrogen at room temperature. The residue was re-dissolved in 1 mL methanol/water (1:1) and centrifuged at 13000 rpm for 30 min. A volume of 2 μL was injected into UHPLC-Q-Exactive Orbitrap MS for analysis.

2.3. Instrument and condition

All LC-MS analysis were performed on a Q-Exactive Focus Orbitrap MS (Thermo Electron, Bremen, Germany) connected to the Thermo Scientific Dionex Ultimate 3000 RS (Thermo Fisher Scientific, California, USA) via an ESI source. An HYPERSIL GOLD C18 column (100 × 2.1 mm, 1.9 μm) was used for chromatographic separation at 35 °C. The mobile phase consisted of 0.1% formic acid (A) and Acetonitrile (B) at a flow rate of 0.3 mL/min in the following gradient: 0 min, 5% B; 2 min, 8% B; 5 min, 10% B; 20 min, 40% B; 22 min, 95% B; 23 min, 95% B; 23.1 min, 5% B; 25 min, 5% B.

All Sample were analyzed in the negative mode as the following tune method. The nitrogen (purity ≥ 99.99%) served as sheath gas and auxiliary gas at the flow rate of 30 and 10 (arbitrary unit), respectively; the capillary temperature is 320 °C; the auxiliary gas heater temperature is 350 °C; spray voltage is 3.2 KV. High resolution mass spectrum was acquired at full scan in a mass range of m/z 100–1200 at a resolution of 70,000 detected by Orbitrap analyzer. The MS^2 data at a resolution of 35,000 was obtained by parallel reaction monitoring mode triggered by inclusion ions list, which was built by molecule predicted. The nitrogen (purity ≥ 99.999%) served as collision gas to generate the fragment ions and the energy was set as normalized collision energy 30%.

2.4. Expected compounds prediction

It is well known that constituents in plant including traditional Chinese medicine could be classified into several families and the chemical constituents in the same family usually share the same carbon skeleton for the similar biosynthetic pathways. For example, CGAs analogues are a large family of esters formed between quinic acid or shikimic acid and one

to four or three special residues, most commonly *p*-coumaric acid, caffeic acid, sinapic acid and ferulic acid. Therefore, the CGAs analogues can be predicted. In this method, shikimic acid ($C_7H_{10}O_5$), and quinic acid ($C_7H_{12}O_6$) were set as the carbon skeleton, and substituents was summarized according to published paper, including methyl (CH_2), ethyl (C_2H_4), *p*-coumaroyl ($C_9H_6O_2$), caffeoyl ($C_9H_6O_3$), sinapoyl ($C_{11}H_{10}O_4$), feruloyl ($C_{10}H_8O_3$), and glucoside ($C_6H_{10}O_5$), xyloside ($C_5H_8O_4$), rhamnoside ($C_6H_{10}O_4$). Expected compounds prediction and high resolution extracted ions chromatography (HREIC) were performed by Compound Discover version 3.0 and Xcalibur version 4.1 (Thermo Fisher Scientific, California, USA).

2.5. The establishment of diagnosis fragmentation ions

It is easily understood that CGAs analogues with the same carbon skeletons will generate the similar fragmentations, which can be define as diagnosis fragmentation ions for the screening and characterization of CGAs analogues. The fragmentation patterns of 6 reference standards were investigated by UHPLC-Q-Exactive Orbitrap MS in negative mode to establish the diagnosis fragmentation ions, such as 191.056 ($C_7H_{11}O_6$), 173.045 ($C_7H_{10}O_5$) generated from quinic acid moiety, 179.034 ($C_9H_7O_3$), 135.045 ($C_8H_7O_2$) yielded by caffeic acid moiety.

3. Result and discussion

3.1. Analytical strategy

In order to detect and identify CGAs analogues fully, a strategy based on UHPLC-Q-Exactive Orbitrap MS was proposed in this study. First, anion exchange resin column was used to enrich the trace amount of CGAs because CGAs as a weak acid is destined to enrich by anion exchange resin column. Second, the sample contained CGAs was injected into UHPLC-Q-Exactive Orbitrap MS to gain the high resolution mass data acquired by full MS scanning. Third, metabolism workflow of Compound Discover was modified to predict the molecule of CGAs by setting the parameter as followed: the drug was set as shikimic acid, and quinic acid. The transformations were set as the substituents list mentioned above. The molecule of CGAs was confirmed by data processing including compound discover and high resolution extracted ion chromatography (HREIC) to generated an inclusion ions list. Fourth, the fragmentation ions were acquired using UHPLC-Q-Exactive Orbitrap MS by parallel reaction monitoring mode triggered by inclusion ions list built above. Finally, The CGAs candidates were identified based on diagnosis fragmentation ions, retention time, and bibliography.

3.2. Optimization of UHPLC-Q-Exactive Orbitrap MS condition

In order to obtain satisfactory separation for all the CGAs analogues, the UHPLC parameter were optimized based on single factor experiment including the kind of mobile phase (acetonitrile/water, and methanol/water), the kind and content of acid (formic acid and acetic acid, 0.05, 0.1, and 0.2%), column (HYPERSIL GOLD C18 column, 100 × 2.1 mm, 1.9 μm and Waters ACQUITY BEH C18 column, 100 × 2.1 mm, 1.7 μm), flow rate of mobile phase (0.2, 0.3, and 0.4 mL/min), compartment temperature (25, 30, 35, 40 °C) and the mobile phase gradient. The MS parameters including the flow rate of sheath gas and auxiliary, the temperature of capillary and auxiliary, spray voltage, et al were examined. In the optimization condition of UHPLC-Q-Exactive Orbitrap MS, most of the CGAs analogues have shown good separation, quasi-molecular ions and fragmentation ions.

3.3. Structure elucidation of CGAs analogous

A total of 149 CGAs analogous was tentatively characterization in *D. nervosa* by UHPLC-Q-Exactive Orbitrap MS, 102 of them were report for the first time. The chromatographic and mass data of those detected constituents are summarized in Table 1 and table 1S, and the HREICs are shown in Fig. 1.

3.3.1. Identification of monoacyl-quinic acids or monoacyl-shikimic acids

Compounds 59, 60, 64, 66, 69, and 70 were eluted at 7.18, 7.26, 8.08, 8.23, 8.69, and 9.35 min, with the same quasi-molecular ions $[M-H]^-$ at *m/z* 335.076 ($C_{16}H_{15}O_8$), which could be caffeoylquinic acid lactones (CQL) or caffeoylshikimic acids (CSA). Quinic acid lactones are prone to generate ion at *m/z* 161.023 by the loss of the lactone and H_2O moiety, which can be used as the distinguished ions between CQLs and CSAs (Jaiswal et al., 2010, 2012, 2014a, 2014b, 2014c). Therefore, they were tentatively identified as 5-CSA, 4-CSA, 3-CSA, 3-CQL, 1-CQL, and 4-CQL, respectively. Compounds 38, 49, 52, and 56 generated the same deprotonated molecular ion *m/z* 497.129 ($C_{22}H_{25}O_{13}$), 162 Da ($C_6H_{10}O_5$) more than that of CSA or CQL, suggesting they were the hexoside of CQL or CSA, which were further confirmed by the present of *m/z* 335.076 ($C_{16}H_{15}O_8$), 179.033 ($C_9H_7O_4$), 135.043 ($C_8H_7O_2$) in MS^2 data. The ion at *m/z* 161.023 ($C_9H_5O_3$) in MS^2 of compound 52 possessed a higher intensity than *m/z* 179.033 ($C_9H_7O_4$), indicated that compound 52 was CQL-hexoside. The others (38, 49, and 56) were tentatively inferred as CSA-hexoside.

Compounds 26, 30, 47, 51, 61, and 62 eluted at 4.50, 4.69, 6.00, 6.38, 7.40, and 7.69 min and showed a deprotonated molecular ion $[M-H]^-$ at *m/z* 337.09232 (−1.69 ppm, $C_{16}H_{17}O_8$), 337.09225 (−1.90 ppm, $C_{16}H_{17}O_8$), 337.09229 (−1.78 ppm, $C_{16}H_{17}O_8$), 337.09229 (−1.78 ppm, $C_{16}H_{17}O_8$), 337.09222 (−1.99 ppm, $C_{16}H_{17}O_8$), and 337.09210 (−2.35 ppm, $C_{16}H_{17}O_8$), respectively. According to the published paper (Xiang et al., 2017; Clifford et al., 2003), they were tentatively assigned as Tran-3-O-*p*-coumaroylquinic acid (*p*CoQA), Cis-3-*p*CoQA, Tran-5-*p*CoQA, Cis-4-*p*CoQA, Tran-4-*p*CoQA, and Cis-5-*p*CoQA based on the different base peak ion in MS^2 spectrum. Compounds 16, 19, 24, 32, 36, and 46 was eluted at 3.35, 3.72, 4.21, 4.69, 4.83, and 5.61 min, with the deprotonated ions $[M-H]^-$ at *m/z* 499.14459 (−2.25 ppm, $C_{22}H_{27}O_{13}$), 499.14536 (−0.71 ppm, $C_{22}H_{27}O_{13}$), 499.14407 (−3.29 ppm, $C_{22}H_{27}O_{13}$), 499.14447 (−2.49 ppm, $C_{22}H_{27}O_{13}$), 499.14526 (−0.91 ppm, $C_{22}H_{27}O_{13}$), and 499.14488 (−1.67 ppm, $C_{22}H_{27}O_{13}$), respectively, 162 Da ($C_6H_{10}O_5$) and 146 Da ($C_6H_{10}O_4$) more than *p*CoQA ($C_{16}H_{17}O_8$) and CQA ($C_{16}H_{17}O_9$), respectively. The fragment

Table 1 The retention time and mass spectrometric data of CGAs in *D. nervosa*.

Peak	t_R	Theoretical Mass *m/z*	Experimental Mass *m/z*	Error (ppm)	Formula [M−H]	Identification	Peak	t_R	Theoretical Mass *m/z*	Experimental Mass *m/z*	Error (ppm)	Formula [M−H]	Identification
1	1.64	353.10894	353.10833	−1.71	$C_{13}H_{21}O_{11}$	QA-hexoside	76	10.58	677.17232	677.16992	−3.55	$C_{31}H_{33}O_{17}$	DiCQA-hexoside
2	1.64	677.19345	677.19128	−3.20	$C_{28}H_{37}O_{19}$	CQA-Dihexoside	77	10.89	677.17232	677.16980	−3.71	$C_{31}H_{33}O_{17}$	DiCQA-hexoside
3	1.81	353.10894	353.10812	−2.31	$C_{13}H_{21}O_{11}$	QA-hexoside	78	11.05	677.17232	677.16913	−4.72	$C_{31}H_{33}O_{17}$	DiCQA-hexoside
4	1.93	353.10894	353.10845	−1.37	$C_{13}H_{21}O_{11}$	QA-hexoside	79	11.09	559.14571	559.14508	−1.13	$C_{27}H_{27}O_{13}$	SCQA
5	2.06	677.19345	677.19312	−0.49	$C_{28}H_{37}O_{19}$	CQA-Dihexoside	80	11.20	559.14571	559.14429	−2.55	$C_{27}H_{27}O_{13}$	SCQA
6	2.10	353.10894	353.10825	−1.94	$C_{13}H_{21}O_{11}$	QA-hexoside	81	11.22	677.17232	677.16962	−3.99	$C_{31}H_{33}O_{17}$	DiCQA-hexoside
7	2.30	353.10894	353.10848	−1.29	$C_{13}H_{21}O_{11}$	QA-hexoside	82	11.47	677.17232	677.16974	−3.81	$C_{31}H_{33}O_{17}$	DiCQA-hexoside
8	2.31	677.19345	677.19269	−1.12	$C_{28}H_{37}O_{19}$	CQA-Dihexoside	83	11.56	515.11950	515.11835	−2.23	$C_{25}H_{23}O_{12}$	1,4-DiCQA
9	2.40	353.10894	353.10822	−2.02	$C_{13}H_{21}O_{11}$	QA-hexoside	84	11.83	515.11950	515.11829	−2.35	$C_{25}H_{23}O_{12}$	3,4-DiCQA
10	2.65	353.08781	353.08707	−2.08	$C_{16}H_{17}O_9$	Cis-3-CQA	85	11.89	677.17232	677.17023	−3.09	$C_{31}H_{33}O_{17}$	DiCQA-hexoside
11	2.99	677.19345	677.19263	−1.21	$C_{28}H_{37}O_{19}$	CQA-Dihexoside	86	11.91	559.14571	559.14441	−2.33	$C_{27}H_{27}O_{13}$	SCQA
12	3.04	529.15628	529.15509	−2.25	$C_{23}H_{29}O_{14}$	3-FQA-hexoside	87	11.99	515.11950	515.11792	−3.07	$C_{25}H_{23}O_{12}$	3,5-DiCQA
13	3.08	677.19345	677.19180	−2.44	$C_{28}H_{37}O_{19}$	CQA-Dihexoside	88	12.09	677.17232	677.17346	1.68	$C_{31}H_{33}O_{17}$	DiCQA-hexoside
14	3.21	515.14063	515.13934	−2.50	$C_{22}H_{27}O_{14}$	CQA-4′-hexoside	89	12.21	559.14571	559.14463	−1.94	$C_{27}H_{27}O_{13}$	SCQA
15	3.27	353.08781	353.08682	−2.79	$C_{16}H_{17}O_9$	trans-3-CQA	90	12.25	677.15119	677.14978	−2.09	$C_{34}H_{29}O_{15}$	Cis-TriCQA
16	3.35	499.14571	499.14459	−2.25	$C_{22}H_{27}O_{13}$	4-*p*CoQA-hexoside	91	12.35	559.14571	559.14441	−2.33	$C_{27}H_{27}O_{13}$	SCQA
17	3.49	341.08781	341.08701	−2.33	$C_{15}H_{17}O_9$	CA-hexoside	92	12.47	515.11950	515.11853	−1.88	$C_{25}H_{23}O_{12}$	1,5-DiCQA
18	3.64	341.08781	341.08691	−2.63	$C_{15}H_{17}O_9$	CA-hexoside	93	12.65	559.14571	559.14392	−3.02	$C_{27}H_{27}O_{13}$	SCQA
19	3.72	499.14571	499.14536	−0.71	$C_{22}H_{27}O_{13}$	4-*p*CoQA-hexoside	94	12.84	677.15119	677.15101	−0.27	$C_{34}H_{29}O_{15}$	Cis-TriCQA
20	3.80	515.14063	515.13959	−2.02	$C_{22}H_{27}O_{14}$	CQA-3′-hexoside	95	12.94	515.11950	515.11804	−2.83	$C_{25}H_{23}O_{12}$	4,5-DiCQA
21	4.14	529.15628	529.15521	−2.02	$C_{23}H_{29}O_{14}$	4-FQA-hexoside	96	13.07	499.12458	499.12323	−2.71	$C_{25}H_{23}O_{11}$	Cis-3-*p*Co, 5CQA
22	4.19	515.14063	515.13954	−2.11	$C_{22}H_{27}O_{14}$	CQA-4′-hexoside	97	13.24	721.17741	721.17786	0.63	$C_{36}H_{33}O_{16}$	DiCSQA
23	4.20	341.08781	341.08734	−1.36	$C_{15}H_{17}O_9$	CA-hexoside	98	13.29	499.12458	499.12363	−1.91	$C_{25}H_{23}O_{11}$	3-*p*Co, 5CQA
24	4.21	499.14571	499.14407	−3.29	$C_{22}H_{27}O_{13}$	5-*p*CoQA-hexoside	99	13.35	529.13515	529.13409	−2.00	$C_{26}H_{25}O_{12}$	3F,4CQA
25	4.48	353.08781	353.08701	−2.25	$C_{16}H_{17}O_9$	Cis-4-CQA	100	13.43	499.12458	499.12363	−1.91	$C_{25}H_{23}O_{11}$	3C, 5-*p*CoQA
26	4.50	337.09289	337.09232	−1.69	$C_{16}H_{17}O_8$	Tran-3-*p*CoQA	101	13.45	721.17741	721.17828	1.97	$C_{36}H_{33}O_{16}$	DiCSQA
27	4.50	397.11402	397.11374	−0.70	$C_{18}H_{21}O_{10}$	3-SQA	102	13.61	529.13515	529.13428	−1.64	$C_{26}H_{25}O_{12}$	3C,4FQA
28	4.51	515.14063	515.13947	−2.25	$C_{22}H_{27}O_{14}$	CQA-3′-hexoside	103	13.66	499.12458	499.12341	−2.35	$C_{25}H_{23}O_{11}$	4-*p*Co, 5CQA
29	4.68	341.08781	341.08688	−2.71	$C_{15}H_{17}O_9$	CA-hexoside	104	13.78	529.13515	529.13391	−2.34	$C_{26}H_{25}O_{12}$	3F,5CQA
30	4.69	337.09289	337.09225	−1.90	$C_{16}H_{17}O_8$	Cis-3-*p*CoQA	105	13.78	677.15119	677.14990	−1.91	$C_{34}H_{29}O_{15}$	1,3,5-TriCQA
31	4.69	397.11402	397.11395	−0.18	$C_{18}H_{21}O_{10}$	4-SQA	106	13.81	497.10893	497.10910	0.33	$C_{25}H_{21}O_{11}$	DiCQL
32	4.69	499.14571	499.14447	−2.49	$C_{22}H_{27}O_{13}$	CQA-pentoside	107	13.92	529.13515	529.13416	−1.87	$C_{26}H_{25}O_{12}$	3C,5FQA
33	4.74	397.11402	397.11322	−1.99	$C_{18}H_{21}O_{10}$	5-SQA	108	13.93	497.10893	497.10834	−1.20	$C_{25}H_{21}O_{11}$	DiCSA
34	4.75	529.15628	529.15594	−0.64	$C_{23}H_{29}O_{14}$	4-FQA-hexoside	109	14.07	721.17741	721.17737	−0.05	$C_{36}H_{33}O_{16}$	DiCSQA
35	4.80	515.14063	515.13916	−2.85	$C_{22}H_{27}O_{14}$	CQA-3′-hexoside	110	14.15	497.10893	497.10867	−0.53	$C_{25}H_{21}O_{11}$	DiCQL
36	4.83	499.14571	499.14526	−0.91	$C_{22}H_{27}O_{13}$	CQA-pentoside	111	14.17	677.15119	677.14978	−2.09	$C_{34}H_{29}O_{15}$	1,3,4-TriCQA

Table 1 (*continued*)

Peak	t_R	Theoretical Mass *m/z*	Experimental Mass *m/z*	Error (ppm)	Formula [M−H]	Identification	Peak	t_R	Theoretical Mass *m/z*	Experimental Mass *m/z*	Error (ppm)	Formula [M−H]	Identification
37	4.84	341.08781	341.08685	−2.80	$C_{15}H_{17}O_9$	CA-hexoside	112	14.28	499.12458	499.12402	−1.13	$C_{25}H_{23}O_{11}$	Cis-4-*p*Co, 5CQA
38	4.95	497.13006	497.12930	−1.54	$C_{22}H_{25}O_{13}$	CSA-hexoside	113	14.29	721.17741	721.18115	5.95	$C_{36}H_{33}O_{16}$	DiCSQA
39	5.10	353.08781	353.08682	−2.79	$C_{16}H_{17}O_9$	Trans-5-CQA	114	14.41	515.11950	515.11847	−2.00	$C_{25}H_{23}O_{12}$	Tran-4-Cis-5-DiCQA
40	5.11	515.14063	515.13910	−2.97	$C_{22}H_{27}O_{14}$	CQA-4′-hexoside	115	14.53	529.13515	529.13422	−1.76	$C_{26}H_{25}O_{12}$	4F,5CQA
41	5.25	341.08781	341.08710	−2.07	$C_{15}H_{17}O_9$	CA-hexoside	116	14.55	499.12458	499.12378	−1.61	$C_{25}H_{23}O_{11}$	4C, 5-*p*CoQA
42	5.39	839.22515	839.22614	1.18	$C_{37}H_{43}O_{22}$	DiCQA-Dihexoside	117	14.58	497.10893	497.10822	−1.44	$C_{25}H_{21}O_{11}$	DiCSA
43	5.42	367.10346	367.10273	−1.98	$C_{17}H_{19}O_9$	Tran-3-FQA	118	14.68	721.17741	721.18005	4.42	$C_{36}H_{33}O_{16}$	DiCSQA
44	5.43	353.08781	353.08682	−2.79	$C_{16}H_{17}O_9$	Trans-4-CQA	119	14.72	677.15119	677.14984	−2.00	$C_{34}H_{29}O_{15}$	1,4,5-TriCQA
45	5.51	515.14063	515.13995	−1.32	$C_{22}H_{27}O_{14}$	CQA-4′-hexoside	120	14.73	529.13515	529.13446	−1.30	$C_{26}H_{25}O_{12}$	4C,5FQA
46	5.61	499.14571	499.14488	−1.67	$C_{22}H_{27}O_{13}$	5-*p*CoQA-hexoside	121	14.78	497.10893	497.10852	−0.83	$C_{25}H_{21}O_{11}$	DiCQL
47	6.00	337.09289	337.09229	−1.78	$C_{16}H_{17}O_8$	Tran-5-*p*CoQA	122	14.91	721.17741	721.18073	5.37	$C_{36}H_{33}O_{16}$	DiCSQA
48	6.08	529.15628	529.15546	−1.55	$C_{23}H_{29}O_{14}$	4-FQA-hexoside	123	15.10	661.15628	661.15533	−1.44	$C_{34}H_{29}O_{14}$	*p*CoDiCQA
49	6.31	497.13006	497.12994	−0.25	$C_{22}H_{25}O_{13}$	CSA-hexoside	124	15.12	691.16684	691.16681	−0.05	$C_{35}H_{31}O_{15}$	DiCFQA
50	6.31	839.22515	839.22431	−1.00	$C_{37}H_{43}O_{22}$	DiCQA-Dihexoside	125	15.12	721.17741	721.17316	−5.13	$C_{36}H_{33}O_{16}$	DiCSQA
51	6.38	337.09289	337.09229	−1.78	$C_{16}H_{17}O_8$	Cis-4-*p*CoQA	126	15.23	691.16684	691.16638	−0.67	$C_{35}H_{31}O_{15}$	DiCFQA
52	6.51	497.13006	497.12903	−2.08	$C_{22}H_{25}O_{13}$	CQL-hexoside	127	15.28	661.15628	661.15533	−1.44	$C_{34}H_{29}O_{14}$	*p*CoDiCQA
53	6.55	367.10346	367.10239	−2.90	$C_{17}H_{19}O_9$	Cis-3-FQA	128	15.40	661.15628	661.15485	−2.16	$C_{34}H_{29}O_{14}$	*p*CoDiCQA
54	6.65	529.15628	529.15540	−1.66	$C_{23}H_{29}O_{14}$	5-FQA-hexoside	129	15.54	497.10893	497.10815	−1.58	$C_{25}H_{21}O_{11}$	DiCQL
55	6.76	839.22515	839.22369	−1.73	$C_{37}H_{43}O_{22}$	DiCQA-Dihexoside	130	15.58	721.17741	721.17554	−2.59	$C_{36}H_{33}O_{16}$	DiCSQA
56	6.80	497.13006	497.12918	−1.78	$C_{22}H_{25}O_{13}$	CSA-glycoside	131	15.62	661.15628	661.15473	−2.34	$C_{34}H_{29}O_{14}$	*p*CoDiCQA
57	6.85	529.15628	529.15594	−0.64	$C_{23}H_{29}O_{14}$	4-FQA-hexoside	132	15.62	691.16684	691.16608	−1.10	$C_{35}H_{31}O_{15}$	DiCFQA
58	6.90	353.08781	353.08694	−2.45	$C_{16}H_{17}O_9$	Cis-5-CQA	133	15.92	677.15119	677.14996	−1.82	$C_{34}H_{29}O_{15}$	3,4,5-TriCQA
59	7.18	335.07724	335.07678	−1.37	$C_{16}H_{15}O_8$	5-CSA	134	16.29	721.17741	721.17627	−1.58	$C_{36}H_{33}O_{16}$	DiCSQA
60	7.26	335.07724	335.07687	−1.11	$C_{16}H_{15}O_8$	4-CSA	135	16.35	497.10893	497.10785	−2.18	$C_{25}H_{21}O_{11}$	DiCSA
61	7.40	337.09289	337.09222	−1.99	$C_{16}H_{17}O_8$	Tran-4-*p*CoQA	136	16.99	661.15628	661.15466	−2.45	$C_{34}H_{29}O_{14}$	*p*CoDiCQA
62	7.69	337.09289	337.09210	−2.35	$C_{16}H_{17}O_8$	Cis-5-*p*CoQA	137	17.01	721.17741	721.17603	−1.15	$C_{36}H_{33}O_{16}$	DiCSQA
63	7.73	367.10346	367.10264	−2.20	$C_{17}H_{19}O_9$	Cis-4-FQA	138	17.13	661.15628	661.15479	−2.25	$C_{34}H_{29}O_{14}$	*p*CoDiCQA
64	8.08	335.07724	335.07654	−2.09	$C_{16}H_{15}O_8$	3-CSA	139	17.22	691.16684	691.16644	−0.58	$C_{35}H_{31}O_{15}$	DiCFQA
65	8.09	515.11950	515.11835	−2.23	$C_{25}H_{23}O_{12}$	1,3-DiCQA	140	17.31	661.15628	661.15453	−2.65	$C_{34}H_{29}O_{14}$	*p*CoDiCQA
66	8.23	335.07724	335.07663	−1.82	$C_{16}H_{15}O_8$	3-CQL	141	17.36	691.16684	691.16632	−0.76	$C_{35}H_{31}O_{15}$	DiCFQA
67	8.47	367.10346	367.10242	−2.82	$C_{17}H_{19}O_9$	Tran-4-FQA	142	17.36	721.17741	721.17743	0.03	$C_{36}H_{33}O_{16}$	DiCSQA
68	8.62	367.10346	367.10242	−2.82	$C_{17}H_{19}O_9$	Tran-5-FQA	143	17.49	691.16684	691.16628	−0.81	$C_{35}H_{31}O_{15}$	DiCFQA
69	8.69	335.07724	335.07645	−2.36	$C_{16}H_{15}O_8$	1-CQL	144	17.87	721.17741	721.17682	−0.06	$C_{36}H_{33}O_{16}$	DiCSQA
70	9.35	335.07724	335.07687	−1.11	$C_{16}H_{15}O_8$	4-CQL	145	18.34	691.16684	691.16608	−1.10	$C_{35}H_{31}O_{15}$	DiCFQA
71	9.80	367.10346	367.10257	−2.41	$C_{17}H_{19}O_9$	Cis-5-FQA	146	18.49	721.17741	721.17639	−1.41	$C_{36}H_{33}O_{16}$	DiCSQA
72	9.83	677.17232	677.17023	−3.09	$C_{31}H_{33}O_{17}$	DiCQA-hexoside	147	18.82	721.17741	721.17578	−1.50	$C_{36}H_{33}O_{16}$	DiCSQA
73	9.98	839.22515	839.22376	−1.65	$C_{37}H_{43}O_{22}$	DiCQA-Dihexoside	148	19.07	721.17741	721.17584	−2.17	$C_{36}H_{33}O_{16}$	DiCSQA

(*continued on next page*)

Table 1 (*continued*)

Peak	t_R	Theoretical Mass *m/z*	Experimental Mass *m/z*	Error (ppm)	Formula [M−H]	Identification	Peak	t_R	Theoretical Mass *m/z*	Experimental Mass *m/z*	Error (ppm)	Formula [M−H]	Identification
74	10.10	677.17232	677.17297	0.96	$C_{31}H_{33}O_{17}$	DiCQA-hexoside	149	19.17	721.17741	721.17761	1.04	$C_{36}H_{33}O_{16}$	DiCSQA
75	10.28	677.17232	677.16852	−5.62	$C_{31}H_{33}O_{17}$	DiCQA-hexoside							

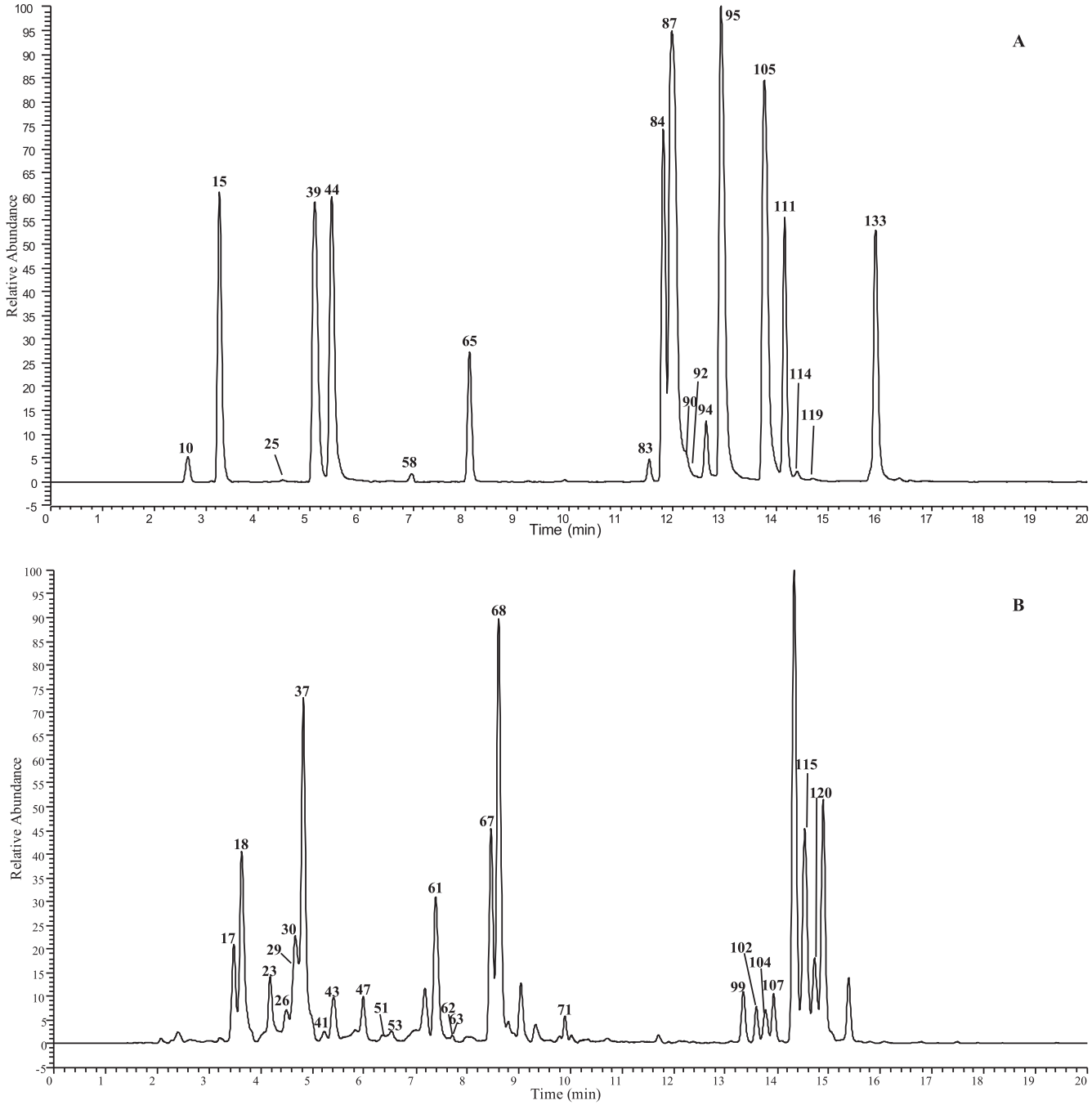

Fig. 1 The high-resolution extracted ion chromatogram (HREIC) in 5 ppm for the multiple compounds in *Duhaldea nervosa*. (A) *m/z* 353.08781, 515.11950, 677.15119; (B) *m/z* 337.09289, 341.08781, 367.10346, 529.13515; (C) *m/z* 335.07724, 353.10894, 497.10893, 499.12458, 515.14063, 529.15628; (D) *m/z* 499.14571, 559.14571, 661.15628, 677.17232, 691.16684; (E) *m/z* 397.11402, 677.19345, 721.17741, 839.22515.

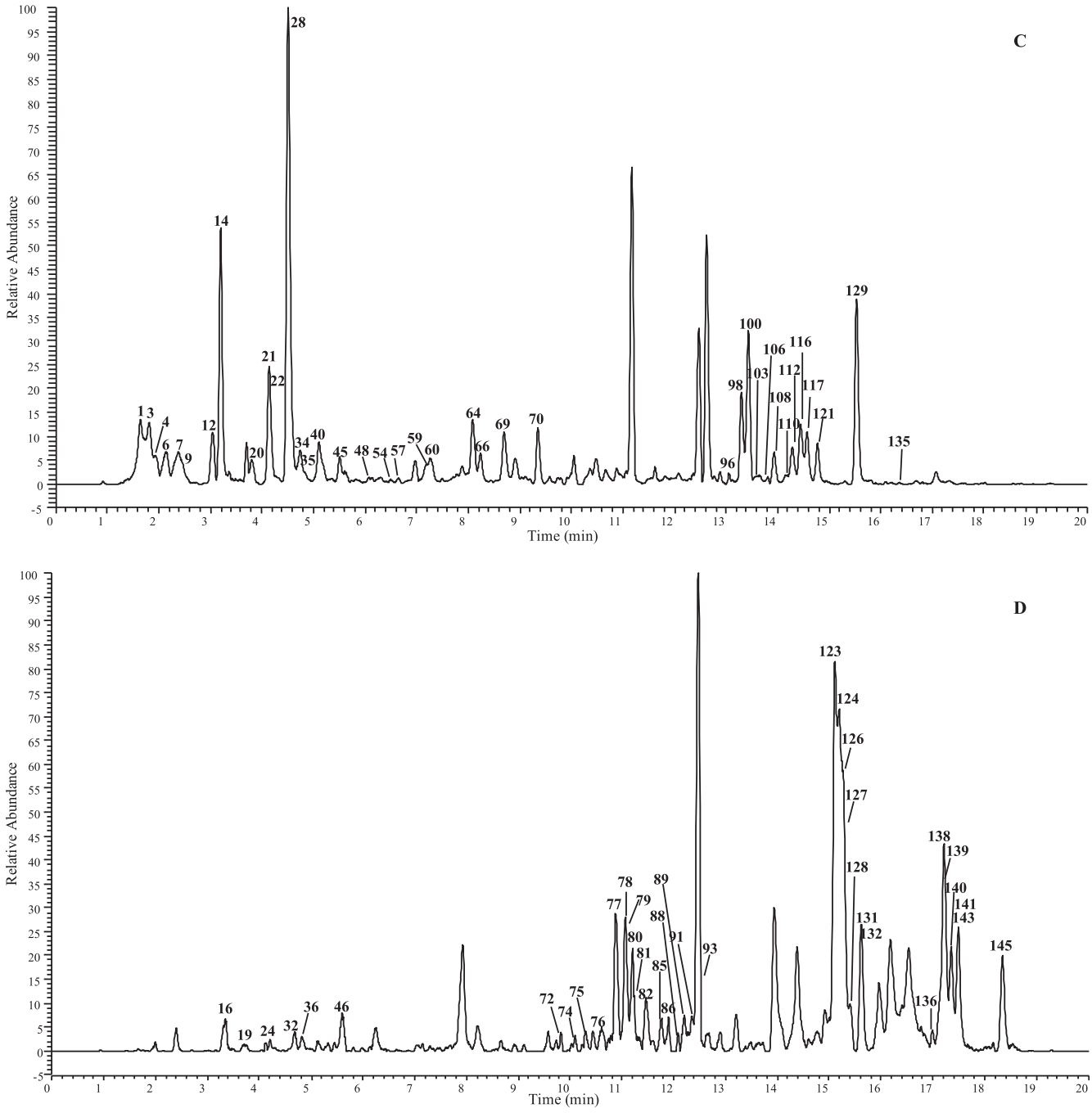

Fig. 1 (*continued*)

ion at m/z 337.09 ($C_{16}H_{17}O_8$) by loss the $C_6H_{10}O_5$ moiety was detected in MS^2 spectrum of compounds 16, 19, 24 and 46, suggesting they were hexoside of *p*CoQA. The base peak at m/z 173.044 and 191.054 was shown in the MS^2 spectrum of compounds 16, 19, and compounds 24, 46, respectively, indicated that compounds 16, 19, 24, and 46 were 4-*p*CoQA-hexoside, 4-*p*CoQA-hexoside, 5-*p*CoQA-hexoside, and 5-*p*-CoQA-hexoside, respectively (Jaiswal et al., 2014a, 2014b, 2014c). The fragment ions at m/z 179.033 ($C_9H_7O_4$) and 191.054($C_7H_{11}O_6$) of compounds 32, 36 were similar to the MS^2 spectrum of CQA. Therefore, compounds 32, 36 were tentatively identified as CQA-pentoside.

Compounds 10, 15, 25, 39, 44, and 58 with the same deprotonated ions $[M-H]^-$ at m/z 353.08 ($C_{16}H_{17}O_9$) were eluted at 2.65, 3.27, 4.48, 5.10, 5.43, and 6.90 min, of which compounds 15, 39, and 44 were accurately characterized as Tran-3-CQA, Tran-5-CQA, and Tran-4-CQA by comparing the retention time, MS data with those reference standards. Meantime, compounds 10, 25 and 58 were tentatively presumed as Cis-3-CQA, Cis-4-CQA and Cis-5-CQA, respectively (Clifford et al., 2008; Jaiswal et al., 2011). Compounds 14, 20, 22, 28, 35, 40, and 45 eluted at 3.21, 3.80, 4.19, 4.51, 4.80, 5.11, and 5.51 min, with the quasi-molecular ions $[M-H]^-$ at m/z 515.139 ($C_{22}H_{27}O_{14}$), 162 Da ($C_6H_{10}O_5$) more than CQA, suggesting

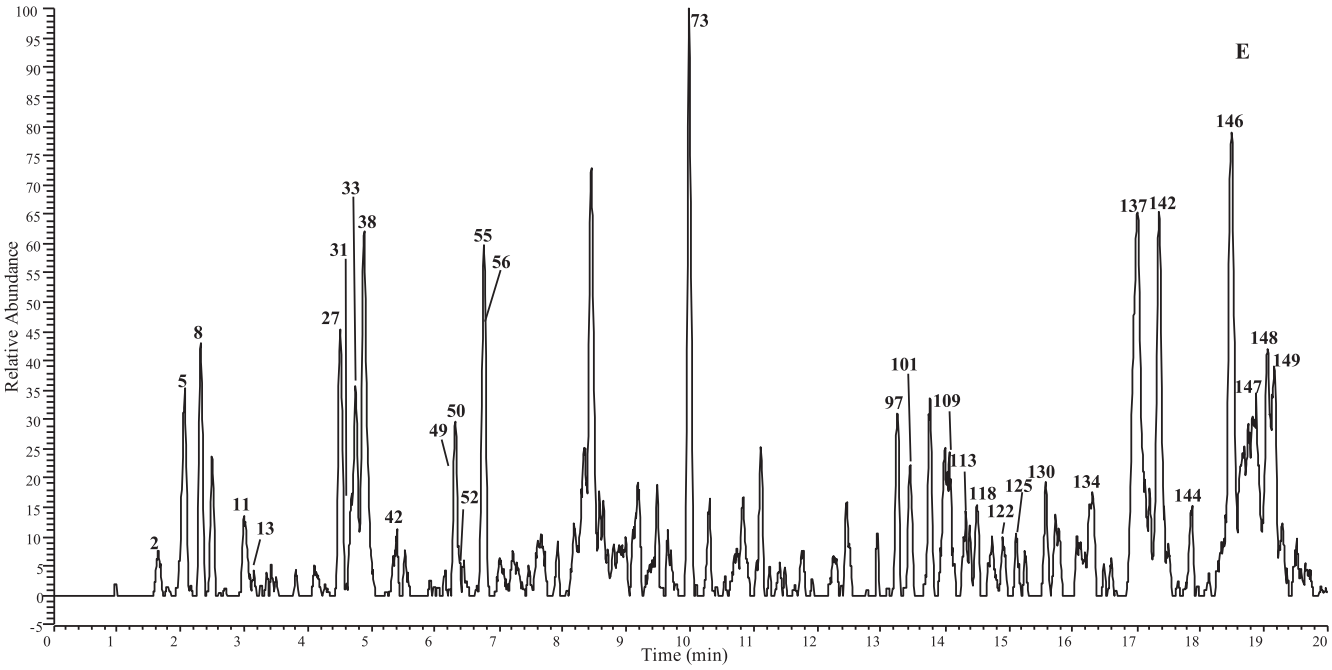

Fig. 1 (*continued*)

they were the hexoside of CQA. The presence of fragment ion at *m/z* 323.076 ($C_{15}H_{15}O_8$) was used to distinguish the position of hexoside (Clifford et al., 2007; Jaiswal et al., 2014a, 2014b, 2014c). Therefore, compounds 20, 28, and 35 were tentatively identified as CQA-3′-hexoside. The others (14, 22, 40, and 45) might be CQA-4′-hexoside. Compounds 2, 5, 8, 11, and 13 were detected 1.64, 2.06, 2.31, 2.99, 3.08 min and yielded a deprotonated ion $[M-H]^-$ at *m/z* 667.19128 (−3.20 ppm, $C_{28}H_{37}O_{19}$), 667.19312 (−0.49 ppm, $C_{28}H_{37}O_{19}$), 667.19269 (−1.12 ppm, $C_{28}H_{37}O_{19}$), 667.19263 (−1.21 ppm, $C_{28}H_{37}O_{19}$), and 667.19180 (−2.44 ppm, $C_{28}H_{37}O_{19}$), 162 Da ($C_6H_{10}O_5$) more than CQA-hexoside. Therefore, Compounds 2, 5, 8, 11, and 13 were tentatively characterized as CQA-Dihexoside.

Compounds 43, 53, 63, 67, 68, and 71, possessing a deprotonated ion $[M-H]^-$ at *m/z* 367.10273 (−1.98 ppm, $C_{17}H_{19}O_9$), 367.10239 (−2.90 ppm, $C_{17}H_{19}O_9$), 367.10264 (−2.20 ppm, $C_{17}H_{19}O_9$), 367.10242 (−2.82 ppm, $C_{17}H_{19}O_9$), 367.10242 (−2.82 ppm, $C_{17}H_{19}O_9$), and 367.10257 (−2.41 ppm, $C_{17}H_{19}O_9$), were detected at 5.42, 6.55, 7.73, 8.47, 8.62, and 9.80 min, suggesting that they might be feruloylquinic acid (FQA). The isomers were identified to be as follows: Cis-3-FQA and Tran-3-FQA produced the base peak ion at *m/z* 193.049 ($C_{10}H_9O_4$); Cis-4-FQA and Tran-4-FQA yielded the base peak ion at *m/z* 173.044 ($C_7H_9O_5$); Cis-5-FQA and Tran-5-FQA yielded the base peak ion at *m/z* 191.054 ($C_7H_{11}O_6$); and the configuration of Cis or Tran was judged by the intensity of those peaks that cis-compound show lower intensity for its instability (Clifford et al., 2003, 2008). Therefore, they were tentatively characterized as Tran-3-FQA, Cis-3-FQA, Cis-4-FQA, Tran-4-FQA Tran-5-FQA, and Cis-5-FQA, respectively. Compounds 12, 21, 34, 48, 54, and 57, possessed the same quasi-molecular ion $[M-H]^-$ at *m/z* 529.155($C_{26}H_{25}O_{12}$), 162 Da ($C_6H_{10}O_5$) more than FQA, suggesting they were the hexoside of FQA (Jaiswal et al., 2014a, 2014b, 2014c), which were further confirmed by the existence of fragment ions *m/z* 193.049, 173.044, and 367.102. The base peak (the second higher peak of compound 54) of *m/z* 529 can also be used to discriminate the submitted position as above. Therefore, compounds 12 and 54 were tentatively characterized as 3-FQA-hexoside, 5-FQA-hexoside, respectively. The others were 4-FQA-hexoside.

Compounds 27, 31, and 33 were detected at 4.50, 4.69, and 4.74 min, with a quasi-molecular ion $[M-H]^-$ at *m/z* 397.11374 (−0.70 ppm, $C_{18}H_{21}O_{10}$), 397.11395 (−0.18 ppm, $C_{18}H_{21}O_{10}$), and 397.11322 (−1.99 ppm, $C_{18}H_{21}O_{10}$), respectively. The present of *m/z* 173.044 ($C_7H_9O_5$), 191.054 ($C_7H_{11}O_6$), and 205.049 ($C_{11}H_9O_4$) in MS^2 spectrum of those compounds indicated that they were Sinapoylquinic acids (SQA). The positional isomers can be distinguished by the base peak of MS^2 data. The base peak at 191.0548 (−6.86 ppm, $C_7H_{11}O_6$), 173.0442 (−7.78 ppm, $C_7H_9O_5$), and 205.0496 (−5.03 ppm, $C_{11}H_9O_4$) were detected in MS^2 spectrum of those compound, respectively, indicated they were 3-SQA, 4-SQA, and 5-SQA by referring to the literature data (Zhang et al., 2016).

3.3.2. Identification of diacyl-quinic acids or diacyl-shikimic acids

Compounds 106, 108, 110, 117, 121, 129, and 135 were eluted at 13.81, 13.93, 14.15, 14.58, 14.78, 15.54, and 16.35 min and possessed a deprotonated ion $[M-H]^-$ *m/z* 497.10910 (0.33 ppm, $C_{25}H_{21}O_{11}$), 497.10867 (−0.53 ppm, $C_{25}H_{21}O_{11}$), 497.10834 (−1.20 ppm, $C_{25}H_{21}O_{11}$), 497.10867 (−1.44 ppm, $C_{25}H_{21}O_{11}$), 497.10852 (−0.83 ppm, $C_{25}H_{21}O_{11}$), 497.10815 (−1.58 ppm, $C_{25}H_{21}O_{11}$), and 497.10785 (−2.18 ppm, $C_{25}H_{21}O_{11}$), which could be Dicaffeoylquinic acid lactones (DiCQL) or Dicaffeoylshikimic acids (DiCSA). Compounds 106, 110, 121, and 129 afforded a same base peak at *m/z* 161.0230, which were formed by losing the lactone and H_2O

moiety from quinic acids lactone (Jaiswal et al., 2014a, 2014b, 2014c), thus, they were tentatively characterized as DiCQL. The others (108, 117, and 43) were assigned as DiCSA (Jaiswal et al., 2010).

Compounds 96, 98, 100, 103, 112, and 116 showed the deprotonated ion $[M-H]^-$ *m/z* 499.12323 (−2.71 ppm, $C_{25}H_{23}O_{11}$), 499.12363 (−1.91 ppm, $C_{25}H_{23}O_{11}$), 499.12363 (−1.91 ppm, $C_{25}H_{23}O_{11}$), 499.12341 (−2.35 ppm, $C_{25}H_{23}O_{11}$), 499.12402 (−1.13 ppm, $C_{25}H_{23}O_{11}$), and 499.12378 (−1.61 ppm, $C_{25}H_{23}O_{11}$), respectively. the appearance of fragment ions *m/z* 173.044 ($C_7H_9O_5$), 179.033 ($C_9H_7O_4$), and 191.054 ($C_7H_{11}O_6$) in MS^2 spectrum of those compounds indicated they were *p*-coumaroylcaffeoylquinic acids (*p*CoCQA). The absence of base peak at *m/z* 173.044 ($C_7H_9O_5$) of compounds 96, 98, and 100 are consistent with their being 3,5-*p*CoCQA. thus, compounds 96, 98, and 100 were tentatively identified as Cis-3-*p*Co,5CQA, 3-*p*Co,5CQA, and 3C, 5-*p*CoQA according the base peak and retention time (Jaiswal et al., 2010; Clifford et al., 2006). Likewise, compounds 103, 112, and 116 were tentatively characterized as 4-*p*Co,5CQA, Cis-4-*p*Co,5CQA, and 4C, 5-*p*CoQA, respectively.

Compounds 84, 87, and 95 possessed the same retention time, mass spectrum data with these reference standards 3,4-DiCQA, 3,5-DiCQA, and 4,5-DiCQA, respectively. Thus, they were unambiguously assigned as 3,4-DiCQA, 3,5-DiCQA, and 4,5-DiCQA. Compounds 65, 83, 92, and 114 generated the same deprotonated ion $[M-H]^-$ *m/z* 515.118 ($C_{25}H_{23}O_{12}$) and fragment ions *m/z* 173.044 ($C_7H_9O_5$), 179.033 ($C_9H_7O_4$), and 191.054 ($C_7H_{11}O_6$) with compounds above, suggesting they are isomers. The present of base peak *m/z* 173.044 ($C_7H_9O_5$) in MS^2 of 83 and 114 indicated they are n, 4-DiCQA. According the retention time (Liu et al., 2018; Clifford et al., 2005), compounds 65, 83, 92, and 114 were tentatively characterized as 1,3-DiCQA, 1,4-DiCQA, 1,5-DiCQA, and Tran-4-Cis-5-DiCQA, respectively. Compounds 74, 75, 76, 77, 78, 81, 82, 85, and 88 were eluted at 10.10, 10.28, 10.58, 10.89, 11.05, 11.22, 11.47, 11.89, and 12.09 min, with the same deprotonated ion $[M-H]^-$ *m/z* 677.169 ($C_{31}H_{33}O_{17}$), 162 Da ($C_6H_{10}O_5$) more than DiCQA, suggesting they were the hexoside of DiCQA, which were further confirmed by the presence of fragment ions *m/z* 515.117 ($C_{25}H_{23}O_{12}$), 173.044 ($C_7H_9O_5$), 179.033 ($C_9H_7O_4$), and 191.054 ($C_7H_{11}O_6$). Therefore, they were inferred as DiCQA-hexoside (Clifford et al., 2008; Jaiswal et al., 2014a, 2014b, 2014c). Compounds 42, 50, 55, and 73 generated a deprotonated ion $[M-H]^-$ *m/z* 839.22614 (1.18 ppm, $C_{37}H_{43}O_{22}$), 839.22431 (−1.00 ppm, $C_{37}H_{43}O_{22}$), 839.22369 (−1.73 ppm, $C_{37}H_{43}O_{22}$), and 839.22376 (−1.65 ppm, $C_{37}H_{43}O_{22}$), 162 Da ($C_6H_{10}O_5$) more than DiCQA-hexoside, indicated they were the dihexoside of DiCQA. Therefore, they were tentatively identified as DiCQA-dihexoside.

Compounds 99, 102, 104, 107, 115, and 120 yielded a quasi-molecular ion $[M-H]^-$ at *m/z* 529.13409 (−2.00 ppm, $C_{26}H_{25}O_{12}$), 529.13428 (−1.64 ppm, $C_{26}H_{25}O_{12}$), 529.13391 (−2.34 ppm, $C_{26}H_{25}O_{12}$), 529.13416 (−2.00 ppm, $C_{26}H_{25}O_{12}$), 529.13422 (−1.76 ppm, $C_{26}H_{25}O_{12}$), and 529.13444 (−1.30 ppm, $C_{26}H_{25}O_{12}$) and were eluted at 13.35, 13.61, 13.78, 13.92, 14.53, and 14.73 min, respectively. All of those compounds showed the fragment ions at *m/z* 173.044 ($C_7H_9O_5$), 193.049 ($C_{10}H_9O_4$) or 353.086 ($C_{16}H_{17}O_9$), 367 ($C_{17}H_{19}O_9$), which were consistent with caffeoylferuloylquinic acids (CFQA). According the retention time and diagnosis ions in bibliography (Liu et al., 2018; Clifford et al., 2003), Compounds 99, 102, 104, 107, 115, and 120 were tentatively identified as 3F, 4CQA, 3C,4FQA, 3F,5CQA, 3C,5FQA, 4F,5CQA, 4C,5FQA, respectively.

Compounds 79, 80, 86, 89, 91, and 93 were detected at 11.09, 11.20, 11.91, 12.21, 12.35, 12.65, and 15.10, with the same deprotonated ion $[M-H]^-$ *m/z* 559.144 ($C_{27}H_{27}O_{13}$). All of those compounds yield the fragment ions *m/z* 173.044 ($C_7H_9O_5$), 191.054 ($C_7H_{11}O_6$), 179.033 ($C_9H_7O_4$), and 397.112 ($C_{18}H_{21}O_{10}$), which were consistent with Caffeoylsinapoylquinic acids (CSQA) (Lin and Harnly, 2008).

3.3.3. Identification of triacyl-quinic acids or triacyl-shikimic acids

Compounds 123, 127, 128, 131, 136, 138, and 140 were eluted at 15.10, 15.28, 15.40, 15.62, 16.99, 17.13, and 17.31 min and yielded a deprotonated ion $[M-H]^-$ *m/z* 661.15533 (−1.44 ppm, $C_{34}H_{29}O_{14}$), 661.15533 (−1.44 ppm, $C_{34}H_{29}O_{14}$), 661.15485 (−2.16 ppm, $C_{34}H_{29}O_{14}$), 661.15473 (−2.34 ppm, $C_{34}H_{29}O_{14}$), 661.15466 (−2.45 ppm, $C_{34}H_{29}O_{14}$), 661.15479 (−2.25 ppm, $C_{34}H_{29}O_{14}$), and 661.15453 (−2.65 ppm, $C_{34}H_{29}O_{14}$), respectively. the appearance of ions *m/z* 173.044 ($C_7H_9O_5$), 179.033 ($C_9H_7O_4$), 337.092 ($C_{16}H_{17}O_8$), and 353.087 ($C_{16}H_{17}O_9$) indicated they are *p*-coumaroyl-dicaffeoylquinic acids (*p*CoDiCQA).

Compounds 90, 94, 105, 111, 119, and 133 generated a quasi-molecular ion $[M-H]^-$ at *m/z* 677.14978 (−2.09 ppm, $C_{34}H_{29}O_{15}$), 677.15101 (-0.27 ppm, $C_{34}H_{29}O_{15}$), 677.14990 (−1.91 ppm, $C_{34}H_{29}O_{15}$), 677.14978 (−2.09 ppm, $C_{34}H_{29}O_{15}$), 677.14984 (−2.00 ppm, $C_{34}H_{29}O_{15}$), and 677.14996 (−1.82 ppm, $C_{34}H_{29}O_{15}$), respectively. The existence of fragment ions *m/z* 353.0869 ($C_{16}H_{17}O_9$), *m/z* 515.1180 ($C_{25}H_{23}O_{12}$) and the absence of ions *m/z* 497.1070 ($C_{25}H_{21}O_{11}$) of compound 133 was consistent assignment as 3,4,5-TriCQA. Likewise, the others (92–96) were characterized as Cis-TriCQA, Cis-TriCQA, 1,3,5-TriCQA, 1,3,4-TriCQA, 1,4,5-TriCQA according the published paper (Liu et al., 2018).

Compounds 124, 126, 132, 139, 141, 143, and 145 eluted at 15.12, 15.23, 15.62, 17.22, 17.36, 17.49, and 18.34, with a quasi-molecular ion $[M-H]^-$ at *m/z* 691.166 ($C_{35}H_{31}O_{15}$). The fragment ions *m/z* 529.133 ($C_{26}H_{25}O_{12}$), 367.118 ($C_{21}H_{19}O_6$), 173.044($C_7H_9O_5$), 179.033 ($C_9H_7O_4$), and 179.033 ($C_9H_7O_4$) were detected in MS^2 data of those compound, suggesting that they were dicaffeoylferuloylquinic acids (DiCFQA). Likewise, compounds 97, 101, 109, 113, 118, 122, 125, 130, 134, 137, 142, 144, 146, 147, 148 and 149 were tentatively characterized as Dicaffeoylsinapoylquinic acids (DiCSQA).

3.3.4. Others

Compounds1, 3, 4, 6, 7, and 37 were detected between 1.64 and 2.40 min, possessing the quasi-molecular ion $[M-H]^-$ at *m/z* 353.10833 (−1.71 ppm, $C_{13}H_{21}O_{11}$), 353.10812 (−2.31 ppm, $C_{13}H_{21}O_{11}$), 353.10845 (−1.37 ppm, $C_{13}H_{21}O_{11}$), 353.10825 (−1.94 ppm, $C_{13}H_{21}O_{11}$), 353.10848 (-1.29 ppm, $C_{13}H_{21}O_{11}$), and 353.10822 (−2.02 ppm, $C_{13}H_{21}O_{11}$), respectively. all of those compounds yielded fragment ions at *m/z* 191.054 ($C_7H_{11}O_6$), 173.044 ($C_7H_9O_5$), 129.054 ($C_6H_9O_3$), and 101.059 ($C_5H_9O_2$), which is consisted to the fragment of quinic acid moiety (Zhang et al., 2016). Thus, they might be considered as hexoside of quinic acid (QA-hexoside).

Compounds 17, 18, 23, 29, 37, and 41 yielded a deprotonated ion $[M-H]^-$ m/z 341.08701 (−2.33 ppm, $C_{15}H_{17}O_9$), 341.08691 (−2.63 ppm, $C_{15}H_{17}O_9$), 341.08734 (−1.36 ppm, $C_{15}H_{17}O_9$), 341.08688 (−2.71 ppm, $C_{15}H_{17}O_9$), 341.08685 (−2.80 ppm, $C_{15}H_{17}O_9$), 341.08710 (−2.07 ppm, $C_{15}H_{17}O_9$), which show a fragment ion at m/z 179.033 ($C_9H_7O_4$) by losing the saccharide moiety 162 Da in the MS^2 experiment. The base peak of m/z 135.043 ($C_8H_7O_2$) and the fragment ion 179.033 ($C_9H_7O_4$) were consisted with the caffeic acids (Gavrilova et al., 2011) [28, therefore, they were tentatively identified as caffeoyl hexoside (CA-hexoside).

4. Conclusion

In this study, a systematic strategy was proposed for rapid detection and identification of CGAs by UHPLC-Q-Exactive Orbitrap mass spectrometry combining anion exchange resin separation, expected compounds predicted and diagnosis fragmentation ions techniques. Using this strategy, 149 CGAs were unanimously and tentatively characterized from *D. nervosa*, 102 of them were report for the first time. This results widely extended the chemical constituents of *D. nervosa*, which will facilitate understanding the effective substance and quality control. Meantime, it is possible for this strategy to exhibit a wide application for characterization and profile of compounds in other kinds of sample, such as, fruits, vegetable, beverage and so on.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Appendix A. Supplementary material

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