ACS OMEGA

http://pubs.acs.org/journal/acsodf

Article

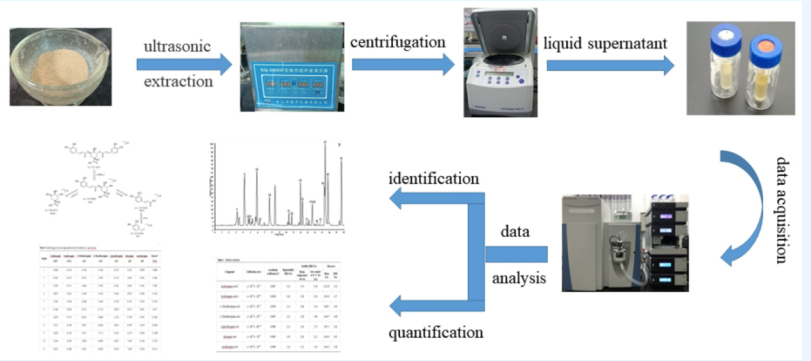
Identification and Quantification of Chlorogenic Acids from the Root Bark of *Acanthopanax gracilistylus* by UHPLC-Q-Exactive Orbitrap Mass Spectrometry

Jianbo Yang,[§] Lingwen Yao,[§] Kaiyan Gong, Kailin Li, Lei Sun,* and Wei Cai*

Cite This: *ACS Omega* 2022, 7, 25675−25685

Read Online

ACCESS | Metrics & More | Article Recommendations



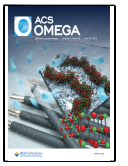
ABSTRACT: The purpose of this study is to identify and quantify the chlorogenic acids (CGAs) from the root bark of *Acanthopanax gracilistylus*, which is conventionally regarded as a tonic in folk Chinese Traditional medicine. The effective methods for identification and quantification analysis of CGAs were developed based on ultra high performance liquid chromatography-Q-exactive orbitrap mass spectrometry (UHPLC-Q-Orbitrap MS) in parallel reaction monitoring (PRM) and selected reaction monitoring (SIM), which showed high sensitivity and resolution for screening and quantifying compounds. The root bark of *A. gracilistylus* was extracted under ultrasonication with 70% methanol. Ultimately, a for total of 70 CGAs, 64 of these were tentatively identified for the first time. Moreover, a methodological study of seven kinds of CGAs was carried out. The proposed procedure was optimized and validated in terms of selectivity, linearity of analytical curves (r^2 > 0.990), accuracy (recovery range from 96.7 to 105%), and repeatability (relative standard deviation <5%). Then it was applied to determine the content of the CGAs in *A. gracilistylus* roots from 66 of different batches. The total CGAs was quantified in a range between 2.150 and 33.51 mg/g, which could be considered as excellent source of natural bioactive compound. The result was extremely useful for understanding the bioactive substance and quality control of *A. gracilistylus* in depth.

1. INTRODUCTION

Acanthopanax gracilistylus W. W. Smith (AGS), belonging to the genus Araliaceae, is generally distributed in the Hubei and Anhui provinces of China as a tonic and folk medicine that plays a crucial role in treating paralysis, bone pains, arthritis, rheumatism, and liver disease.[1−3] In addition, *A. gracilistylus* combined with several other kinds of traditional Chinese medicines (TCMs) have been made into Wujiapi liquor, which is a famous Chinese medicinal liquor that has been as a sort of TCM health food product for hundreds of years. It not only is not only a potable spirit that has a mellow taste and a long aftertaste but also has the functions of promoting blood circulation and enhancing human immunity as an ingredient of Chinese herba preparations.[4−6]

In addition, previous phytochemical investigations on the root bark of *A. gracilistylus* indicated that volatile oils, terpenoids, and phenolic acids are the primary chemical components that are responsible for its biological and pharmacological activities of anti-inflammatory, antifatigue, antiaging, and antidiabetic.[7] In general, it is recorded that the biological and pharmacological functions of herbs are extremely dependent on the composition of active ingredients, offering powerful assistance on reducing the probability of many chronic diseases.[8,9] In particular, chlorogenic acids (CGAs) play a vital role in the total dietary intake of phenols

Received: May 10, 2022
Accepted: June 21, 2022
Published: July 11, 2022



https://doi.org/10.1021/acsomega.2c02899
ACS Omega 2022, 7, 25675−25685

in the daily human diet and have been classified into a family of polyphenolic compound and esters formed between cinnamic acid derivatives (such as caffeic, ferulic, and coumaric acid) and quinic acid are also a kind of plant defense that has been shown to reduce the definite risk of type 2 diabetes, obesity, Alzheimer's disease, eclampsia, and stroke and shown to possess the active effects of promoting cell proliferation and differentiation as well as anti-inflammatory, antineoplastic, and antioxidant properties.[10−13] To our knowledge, a majority of reports draw strong attention to the pharmacological activities of *A. gracilistylus*, but there is no adequate relative literature that systematically illustrates the effective constituents and content of *A. gracilistylus*, especially CGAs. In addition, it is essential to thoroughly investigate the content difference of CGAs.[14] Thus, it is necessary to develop a sensitive, effective, and rapid method for identification and quantification of CGAs from the root bark of *A. gracilistylus*.

A few methods have been applied to identify the structure of CGAs with the goal of discovering important information that sufficiently explains or takes advantage of available plants. With the recent progress of mass spectrometry and separation methods, liquid chromatography coupled with tandem mass spectrometry, particularly, UHPLC-Q-exactive Orbitrap mass spectrometry, has acquired considerable attraction for the qualitative and quantitative analysis of phenolic acid compounds, which reveals its remarkable high resolution and separation capability in chemical characterization and affords accurate mass measurement (<5 ppm) for providing evidence in trace analytes in complex matrices and detailed mass spectral information. It also shows higher sensitivity in full scan mode and a higher intensity range than triple quadrupole mass spectrometry and time-of-flight mass spectrometry (TOF MS).[15−18] Finally, a rapid and sensitive UHPLC−MS method was considered as one of the most available detection techniques for determination of CGAs.

The aim of this study is to develop an effective UHPLC-Q-Exactive Orbitrap MS method for simultaneous determination of CGAs from the root bark of *A. gracilistylus*.

2. RESULTS AND DISCUSSION

2.1. Identification of Chemical Compositions. A total of 70 CGAs were explicitly identified using UHPLC-Q-exactive Orbitrap MS based on comparison of retention time and detailed mass spectrometric data in Table 1. The proposed fragmentation pathways for CGAs in the root bark of *A. gracilistylus* have been speculated, taking isochlorogenic acid A as an example in Figure 1. High-resolution extracted ion chromatography in negative mode is shown in Figure 2.

2.1.1. Identification of Chlorogenic Acid Moieties. Compound 19 with a precursor ion $[M - H]^-$ at m/z 179.0349 ($C_9H_7O_4$) was identified as caffeic acid, which yielded a product ion at m/z 135.044 [caffeic acid−H−CO_2]$^-$ corresponding to the public data.[19] Compound 1 was identified as quinic acid, which gave an ion at m/z 191.0561 ($C_7H_{11}O_6$) and produced fragment ions at m/z 111.007, 85.028, and 87.007 in keeping with ref 20. Compound 70 showed an $[M-H]^-$ ion at m/z 193.0506 ($C_{10}H_9O_4$) and yielded a product ion at m/z 178.026 $[M-H-CH_3]^{-\cdot}$ by the loss of a methyl radical and then loss of a CO_2 to obtain an ion at m/z 134.036 $[M-H-CH_3-CO_2]^{-\cdot}$ in line with the literature,[21] so it was identified as ferulic acid. Compound 13 with a precursor ion at m/z 341.0878 ($C_{15}H_{17}O_9$) showed a product ion at m/z 179.034 ($C_9H_7O_4$) [caffeic acid−H]$^-$, which indicated subsequent loss of the hexose, so it was annotated as CA-hexoside.

2.1.2. Identification of Caffeoylquinic Acids. Compounds 5, 8, 15, and 18 with retention times (t_R) of 2.75, 3.37, 5.22, 5.67 min gave the identical $[M-H]^-$ ion at m/z 353.0878 ($C_{16}H_{17}O_9$) [caffeoyquinic acid−H]$^-$ and a similar MS^2 product ion m/z 191.055 ($C_7H_{11}O_6$) [quinic acid−H]$^-$. Compound 8 was identified as 3-CQA by the presence of the distinctive ion with a peak at m/z 135.043 ($C_8H_7O_2$) [caffeic acid−H−CO_2]$^-$ in the MS^2 spectra of the targeted ion, distinguished with compound 15 as 5-CQA. Compound 18 was identified as 4-CQA possessing extraordinary and intense ion at m/z 173.044 ($C_7H_9O_5$) [quinate-H_2O]$^-$, and compounds 8, 15, and 18 were matched with those of the authentic standards by comparing their chromatography retention times, accurate mass measurement, and fragment pattern with those data. Respectively, 1-CQA (peak 5) and 5-CQA have remarkably similar product ion so as to difficultly recognize them except standards comparison and the consideration of their chromatographic behaviors on C18 columns.[22,23]

Compounds 16 and 20 were attributed to hydroxydihydrocaffeoylquinic acid, as these produced fragment ions at m/z 191.055 ($C_7H_{11}O_6$) [quinate]$^-$ and 173.044 ($C_7H_9O_5$) [quinate−H_2O]$^-$; compound 16 yielded a base peak product ion at m/z 191.055 ($C_7H_{11}O_6$) [quinate]$^-$, identified as 3-*O*-Hydroxydihydrocaffeoylquinic acid; and compound 20 yielded a base peak product ion at m/z 173.044 ($C_7H_9O_5$) [quinate−H_2O]$^-$, identified as 4-*O*-hydroxydihydrocaffeoylquinic acid.

Compounds 29, 45, 47, 49, and 54 were respectively identified as 1,3-DiCQA, 1,5-DiCQA, 3,4-DiCQA, 3,5-DiCQA, and 4,5-DiCQA based on comparison of the retention time and MS patterns with those reference standards.

Compounds 3, 4, 7, and 10 presented the same $[M-H]^-$ ion at m/z 515.1406 ($C_{22}H_{27}O_{14}$), their MS2 spectra gave the expected MS^2 ions at m/z 179.034 ($C_9H_7O_4$) [caffeic acid−H]$^-$, 191.055 ($C_7H_{11}O_6$) [quinate]$^-$, 173.044 ($C_7H_9O_5$) [quinate−H_2O]$^-$, and 353.0878 ($C_{16}H_{17}O_9$) [caffeoyquinic acid-H]$^-$, which indicated loss of a hexose. As far as we know, such compounds have not previously been characterized unequivocally; therefore, they were considered as CQA-hexoside isomers.[24]

Compounds 33, 37, 50, 60, 62, and 69 showed an MS base peak at m/z 677.1511 ($C_{34}H_{29}O_{15}$) [tricaffeoylquinic acid−H]$^-$ and MS^2 base peak at m/z 179.034 ($C_9H_7O_4$) [caffeic acid−H]$^-$ in addition to compound 69 an also yielded other major product ions at m/z 191.055 ($C_7H_{11}O_6$) [quinate]$^-$, 353.0878 ($C_{16}H_{17}O_9$) [caffeoyquinic acid−H]$^-$, and m/z 173.044 ($C_7H_9O_5$) [quinate−H_2O]$^-$; therefore, compounds 33, 37, 50, 60, and 62 were defined as TriCQA isomers, while compound 69 was identified as 3,4,5-TriCQA by a comparison of fragmentation pattern with those of 3,4,5-tri-*O*-caffeoylquinic acid presented in the published data.[25]

Compounds 21, 23, 25, 27, 32, 41, 42, and 43 all displayed a precursor ion at m/z 677.1723 ($C_{31}H_{33}O_{17}$) and produced MS^2 product ions characteristic of a quinic acid residue and a caffeic acid residue at m/z 179.034 ($C_9H_7O_4$) [caffeic acid−H]$^-$, 191.055 ($C_7H_{11}O_6$) [quinate]$^-$, 353.0878 ($C_{16}H_{17}O_9$) [caffeoyquinic acid−H]$^-$. As far as we know, such compounds have not previously been characterized definitely, and it is possible that these compounds were regarded as isomeric DiCQA-hexosides.[25]

https://doi.org/10.1021/acsomega.2c02899
ACS Omega 2022, 7, 25675−25685

Table 1. Retention Time and Mass Information of CGAs in *A. gracilistylus* W. W. Smith. by UHPLC Q Exactive Orbitrap MS

peak	t_R (min)	theoretical mass m/z	experimental mass m/z	error (ppm)	formula $[M-H]^-$	MS/MS fragment	identification	abbreviation
1	0.95	191.0561	191.0554	−3.61	$C_7H_{11}O_6$	MS^2[191]: 111.0076(100), 85.0282(48), 87.0074(38)	Quinic acid	QA
2	2.05	677.1935	677.1943	1.20	$C_{28}H_{37}O_{19}$	MS^2[677]: 191.0554(100)	Caffeoylquinic acid-dihexoside	CQA-Dihexoside
3	2.27	515.1406	515.1396	−2.02	$C_{22}H_{27}O_{14}$	MS^2[515]: 179.0343(100), 191.0560(12), 341.0879(6)	Caffeoylquinic acid-hexoside	CQA-hexoside
4	2.46	515.1406	515.1399	−1.43	$C_{22}H_{27}O_{14}$	MS^2[515]: 179.0343(100), 191.0554(68), 341.0876(37), 323.0779(23)	Caffeoylquinic acid-hexoside	CQA-hexoside
5	2.75	353.0878	353.0876	−0.72	$C_{16}H_{17}O_9$	MS^2 [353]: 191.0552 (100)	1-*O*-Caffeoylquinic acid	1-CQA
6	3.08	529.1563	529.1567	0.75	$C_{23}H_{29}O_{14}$	MS^2[529]: 193.0499(100)	Feruloylquinic acid-hexoside	FQA-hexoside
7	3.14	515.1406	515.1403	−0.72	$C_{22}H_{27}O_{14}$	MS^2 [515]: 191.0552(100), 179.0342(10), 173.0451(7), 353.0876(3)	Caffeoylquinic acid-hexoside	CQA-hexoside
8[a]	3.37	353.0878	353.0875	−0.98	$C_{16}H_{17}O_9$	MS^2 [353]: 191.0552 (100),179.0339 (68), 135.0438(24)	3-*O*-Caffeoylquinic acid	3-CQA
9	4.10	529.1563	529.1567	0.75	$C_{23}H_{29}O_{14}$	MS^2[529]: 173.0447(100), 191.0554(23), 193.0499(17)	Feruloylquinic acid-hexoside	FQA-hexoside
10	4.55	515.1406	515.1403	−0.72	$C_{22}H_{27}O_{14}$	MS^2 [515]: 191.0551(100), 323.0769(95), 161.0229(26)	Caffeoylquinic acid-hexoside	CQA-hexoside
11	4.65	337.0929	337.0928	−0.36	$C_{16}H_{17}O_8$	MS^2 [337]: 163.0388 (100), 119.0488(26), 191.0550(9)	3-*O*-*p*-coumaroylquinic acid	3-*p*CoQA
12	4.83	529.1563	529.1572	1.68	$C_{23}H_{29}O_{14}$	MS^2[529]: 173.0447(100), 193.0500(22)	Feruloylquinic acid-hexoside	FQA-hexoside
13	4.91	341.0878	341.0880	0.68	$C_{15}H_{17}O_9$	MS^2 [341]: 179.0342(100), 191.0554(33)	Caffeic acid-hexoside	CA-hexoside
14	5.09	367.1035	367.1030	−1.16	$C_{17}H_{19}O_9$	MS^2 [367]: 193.0497(100), 191.0550(65),173.0444(43), 134.0361(16)	1-*O*-Feruloylquinic acid	1-FQA
15[a]	5.22	353.0878	353.0873	−1.32	$C_{16}H_{17}O_9$	MS^2 [353]: 191.0552 (100), 179.0339 (2)	5-*O*-Caffeoylquinic acid	5-CQA
16	5.43	371.0984	371.0987	0.94	$C_{16}H_{19}O_{10}$	MS^2 [371]: 191.0554(100), 173.0449(20)	3-*O*-Hydroxydihydrocaffeoylquinic acid	3-*O*-HydroxydihydroCQA
17	5.62	367.1035	367.1029	−1.49	$C_{17}H_{19}O_9$	MS^2 [367]: 193.0496(100), 134.0360(20), 173.0444(5), 191.0554(4)	3-*O*-Feruloylquinic acid	3-FQA
18[a]	5.67	353.0878	353.0874	−1.06	$C_{16}H_{17}O_9$	MS^2 [353]: 173.0440(100), 191.0551(93), 179.0339(75), 135.0439(33)	4-*O*-Caffeoylquinic acid	4-CQA
19[a]	5.84	179.0350	179.0343	−3.64	$C_9H_7O_4$	MS^2[179]: 135.0441(100)	Caffeic acid	CA
20	5.90	371.0984	371.0987	0.86	$C_{16}H_{19}O_{10}$	MS^2 [371]: 173.0448(100)	4-*O*-Hydroxydihydrocaffeoylquinic acid	4-*O*-HydroxydihydroCQA
21	6.12	677.1723	677.1726	0.42	$C_{31}H_{33}O_{17}$	MS^2 [677]: 179.0342(100), 353.0883(44), 191.0550(43), 335.0780(13)	Dicaffeoylquinic acid-hexoside	DiCQA-hexoside
22	6.21	529.1563	529.1572	1.68	$C_{23}H_{29}O_{14}$	MS^2[529]: 173.0449(100), 367.1034(31), 193.0501(15)	Feruloylquinic acid-hexoside	FQA-hexoside
23	6.46	677.1723	677.1715	−1.20	$C_{31}H_{33}O_{17}$	MS^2 [677]: 179.0342(100), 353.0887(15)	Dicaffeoylquinic acid-hexoside	DiCQA-hexoside
24	7.42	691.1880	691.1888	1.16	$C_{32}H_{35}O_{17}$	MS^2 [691]: 179.0342(100), 191.0553(32), 353.0880(27), 135.0441(18), 335.0778(10)	Caffeoylferuloylquinic acid-hexoside	CFQA-hexoside
25	7.45	677.1723	677.1713	−1.47	$C_{31}H_{33}O_{17}$	MS^2 [677]: 179.0339(100), 341.0871(42), 191.0550(19), 515.1405(16), 323.0774(10), 353.0875(9)	Dicaffeoylquinic acid-hexoside	DiCQA-hexoside
26	7.60	337.0929	337.0927	−0.54	$C_{16}H_{17}O_8$	MS^2 [337]: 191.0551(100), 173.0444(11), 163.0388 (10)	5-*O*-*p*-coumaroylquinic acid	5-*p*CoQA
27	8.21	677.1723	677.1717	−0.93	$C_{31}H_{33}O_{17}$	MS^2 [677]: 179.0340(100), 191.0550(75), 353.0875(28), 323.0774(15), 161.0234(14), 135.0438(13), 341.0874(10)	Dicaffeoylquinic acid-hexoside	DiCQA-hexoside
28	8.37	335.0772	335.0765	−2.18	$C_{13}H_{21}O_{11}$	MS^2 [335]: 179.0341(100), 161.0235(52), 135.0440(40), 173.0446(12)	5-*O*-caffeoylshikimic acid	5-CSA
29[a]	8.37	515.1195	515.1188	−1.88	$C_{25}H_{23}O_{12}$	MS^2[515]: 191.0551(100), 179.0339(87), 353.0875(14), 135.0439(13)	1,3-*O*-Dicaffeoylquinic acid	1,3-DiCQA
30	8.79	367.1035	367.1031	−1.08	$C_{17}H_{19}O_9$	MS^2 [367]: 191.0555(100), 173.0448(18), 193.0499(8)	5-*O*-Feruloylquinic acid	5-FQA
31	9.20	691.1880	691.1893	1.96	$C_{32}H_{35}O_{17}$	MS^2 [691]: 179.0342(100), 341.0876(23), 335.0771(11)	Caffeoylferuloylquinic acid-hexoside	CFQA-hexoside
32	9.91	677.1723	677.1731	1.15	$C_{31}H_{33}O_{17}$	MS^2 [677]: 179.0343(100), 191.0555(70), 335.0773(13)	Dicaffeoylquinic acid-hexoside	DiCQA-hexoside
33	10.05	677.1512	677.1493	−2.81	$C_{34}H_{29}O_{15}$	MS^2 [677]: 179.0343(100), 191.0550(58), 135.0441(30)	Tricaffeoylquinic acid	TriCQA
34	10.14	529.1352	529.1348	−0.72	$C_{26}H_{25}O_{12}$	MS^2[529]: 173.0445(100), 179.0340(86), 203.0341(39), 191.0553(28), 135.0439(15), 353.0873(14)	3-*O*-feruloyl-4-*O*-caffeoylquinic acid	3F,4CQA
35	10.31	499.1246	499.1245	−0.21	$C_{25}H_{23}O_{11}$	MS^2[499]: 163.0389(100), 191.0551(6), 337.0930(5), 173.0445(5)	5-*O*-Caffeoyl-3-*O*-*p*-coumaroylquinic acid	5C,3*p*CoQA

https://doi.org/10.1021/acsomega.2c02899
ACS Omega 2022, 7, 25675−25685

Table 1. continued

peak	t_R (min)	theoretical mass m/z	experimental mass m/z	error (ppm)	formula $[M-H]^-$	MS/MS fragment	identification	abbreviation
36	10.44	529.1352	529.1348	−0.72	$C_{26}H_{25}O_{12}$	MS^2[529]: 173.0445(100), 193.0497(17), 367.1031(4)	3-*O*-caffeoyl-4-*O*-feruloylquinic acid	3C,4FQA
37	10.62	677.1512	677.1527	2.24	$C_{34}H_{29}O_{15}$	MS^2 [677]: 179.0343(100), 191.0555(81), 341.0672(23)	Tricaffeoylquinic acid	TriCQA
38	10.70	499.1246	499.1243	−0.63	$C_{25}H_{23}O_{11}$	MS^2[499]: 173.0447(100), 179.0342(93), 191.0551(40), 203.0345(25), 353.0884(20), 135.0442(18)	4-*O*-Caffeoyl-3-*O*-*p*-coumaroylquinic acid	4C,3*p*CoQA
39	10.76	691.1880	691.1905	3.64	$C_{32}H_{35}O_{17}$	MS^2 [691]: 193.0500(100), 173.0446(11)	Caffeoylferuloylquinic acid-hexoside	CFQA-hexoside
40	10.80	529.1352	529.1350	−0.26	$C_{26}H_{25}O_{12}$	MS^2[529]: 191.0552(100), 193.0496(89), 173.0444(72), 143.0341(20), 179.0341(15)	3-*O*-feruloyl-5-*O*-caffeoylquinic acid	3F,5CQA
41	10.98	677.1723	677.1078	−1.69	$C_{31}H_{33}O_{17}$	MS^2 [677]: 191.0555(100), 179.0342(73), 353.0881(32), 323.0769(22)	Dicaffeoylquinic acid-hexoside	DiCQA-hexoside
42	11.09	677.1723	677.1707	−2.37	$C_{31}H_{33}O_{17}$	MS^2 [677]: 191.0555(100), 179.0343(74), 323.0781(61), 161.0238(22)	Dicaffeoylquinic acid-hexoside	DiCQA-hexoside
43	11.19	677.1723	677.1718	−0.76	$C_{31}H_{33}O_{17}$	MS^2 [677]: 191.0552(100), 323.0769(94), 179.0342(23), 161.0229(10), 341.0864(9), 515.1417(7)	Dicaffeoylquinic acid-hexoside	DiCQA-hexoside
44	11.62	691.1880	691.1896	2.32	$C_{32}H_{35}O_{17}$	MS^2 [691]: 173.0447(100), 193.0499(16)	Caffeoylferuloylquinic acid-hexoside	CFQA-hexoside
45[a]	11.71	515.1195	515.1190	−1.05	$C_{25}H_{23}O_{12}$	MS^2[515]: 173.0444(100), 179.0339(80), 191.0552(30), 353.0874(18), 135.0440(13)	1,5-*O*-Dicaffeoylquinic acid	1,5-DiCQA
46	11.91	543.1508	543.1510	0.36	$C_{27}H_{27}O_{12}$	MS^2[543]: 193.0500(100), 134.0363(13), 173.0448(10)	Caffeoyl-*O*-dimethoxycinnamoylquinic acid	C,dimethoxyCiQA
47	12.01	515.1195	515.1192	−0.58	$C_{25}H_{23}O_{12}$	MS^2[515]: 173.0445(100), 179.0339(92), 191.0552(78), 353.0872(17), 161.0232(16), 135.0439(15), 335.0777(10)	3,4-*O*-Dicaffeoylquinic acid (Isochlorogenic acid B)	3,4-DiCQA
48	12.20	335.0772	335.0771	−0.45	$C_{13}H_{21}O_{11}$	MS^2 [335]: 179.0342(100), 173.0448(54), 135.0442(37), 161.0100(28)	4-*O*-Caffeoylshikimic acid	4-CSA
49[a]	12.20	515.1195	515.1190	−1.05	$C_{25}H_{23}O_{12}$	MS^2[515]: 191.0552(100), 179.0340(68), 353.0880(14), 135.0440(8)	3,5-*O*-Dicaffeoylquinic acid (Isochlorogenic acid A)	3,5-DiCQA
50	12.21	677.1512	677.1521	1.34	$C_{34}H_{29}O_{15}$	MS^2 [677]: 191.0555(100), 335.0772(20), 179.0341(19)	Tricaffeoylquinic acid	TriCQA
51	12.23	543.1508	543.1518	1.82	$C_{27}H_{27}O_{12}$	MS^2[543]: 193.0500(100), 134.0364(8), 173.0448(8)	Caffeoyl-*O*-dimethoxycinnamoylquinic acid	C,dimethoxyCiQA
52	12.76	691.1880	691.1895	2.13	$C_{32}H_{35}O_{17}$	MS^2 [691]: 179.0341(100), 323.0769(57), 191.0554(50), 173.0446(42), 335.0771(22)	Caffeoylferuloylquinic acid-hexoside	CFQA-hexoside
53	12.95	499.1246	499.1253	1.51	$C_{25}H_{23}O_{11}$	MS^2[499]: 173.0448(100), 163.0391(14)	3-*O*-Caffeoyl-4-*O*-*p*-coumaroylquinic acid	3C,4*p*CoQA
54[a]	13.09	515.1195	515.1190	−0.93	$C_{25}H_{23}O_{12}$	MS^2[515]: 173.0444(100), 179.0339(72), 191.0552(28), 353.0875(22), 135.0439(11)	4,5-*O*-Dicaffeoylquinic acid (Isochlorogenic acid C)	4,5-DiCQA
55	13.30	559.1457	559.1460	0.51	$C_{27}H_{27}O_{13}$	MS^2[559]: 223.0607(100), 173.0447(73), 179.0342(55), 161.0235(24), 335.0776(17)	Caffeoylsinapoylquinic acids	SCQA
56	13.37	529.1352	529.1348	−0.72	$C_{26}H_{25}O_{12}$	MS^2[529]: 191.0552(100), 173.0445(14)	3-*O*-Caffeoyl-5-*O*-feruloylquinic acid	3C,5FQA
57	13.57	337.0929	337.0931	0.74	$C_{16}H_{17}O_8$	MS^2 [337]: 191.0555(100), 173.0447(13)	1-*O*-*p*-coumaroylquinic acid	1-*p*CoQA
58	13.57	499.1246	499.1244	−0.33	$C_{25}H_{23}O_{11}$	MS^2[499]: 191.0551(100), 179.0340(16), 173.0445(10), 337.0940(4)	3-*O*-Caffeoyl-5-*O*-p-coumaroylquinic acid	3C,5*p*CoQA
59	13.96	529.1352	529.1346	−1.08	$C_{26}H_{25}O_{12}$	MS^2[529]: 173.0445(100), 193.0495(18), 367.1033(5)	4-*O*-feruloyl-5-*O*-caffeoylquinic acid	4F,5CQA
60	13.96	677.1512	677.1509	−0.37	$C_{34}H_{29}O_{15}$	MS^2 [677]: 191.0551(100), 353.0877(91), 179.0339(72),335.0769(29), 161.0231(18), 135.0439(10)	Tricaffeoylquinic acid	TriCQA
61	14.07	529.1352	529.1348	−0.60	$C_{26}H_{25}O_{12}$	MS^2[529]: 173.0445(100), 179.0340(46), 191.0553(36), 353.0874(9),135.0444(8)	4-*O*-Caffeoyl-5-*O*-feruloylquinic acid	4C,5FQA
62	14.30	677.1512	677.1509	−0.46	$C_{34}H_{29}O_{15}$	MS^2 [677]: 179.0340(100), 173.0445(91), 353.0876(73), 161.0233(58), 191.0553(27), 255.0657(27),335.0766(22)	Tricaffeoylquinic acid	TriCQA

https://doi.org/10.1021/acsomega.2c02899
ACS Omega 2022, 7, 25675−25685

Table 1. continued

peak	t_R (min)	theoretical mass m/z	experimental mass m/z	error (ppm)	formula $[M-H]^-$	MS/MS fragment	identification	abbreviation
63	14.39	559.1457	559.1462	0.83	$C_{27}H_{27}O_{13}$	MS^2[559]: 173.0446(100), 223.0605(17)	Caffeoylsinapoylquinic acids	SCQA
64	14.41	499.1246	499.1248	0.41	$C_{25}H_{23}O_{11}$	MS^2[499]: 173.0448(100), 163.0393(12)	5-*O*-Caffeoyl-4-*O*-*p*-coumaroylquinic acid	5C,4*p*CoQA
65	14.56	499.1246	499.1238	−1.61	$C_{25}H_{23}O_{11}$	MS^2[499]: 173.0449(100), 179.0343(72), 191.0554(40), 353.0881(28), 135.0442(9)	4-*O*-Caffeoyl-5-*O*-*p*-coumaroylquinic acid	4C,5*p*CoQA
66	15.26	691.1668	691.1675	1.01	$C_{35}H_{31}O_{15}$	MS^2 [691]: 179.0341(100), 191.0341(83), 353.0877(67), 335.0770(16)	Dicaffeoylferuloylquinic acids	DiCFQA
67	15.35	691.1668	691.1676	1.09	$C_{35}H_{31}O_{15}$	MS^2 [691]: 179.0342(100), 161.0235(67), 353.0880(55), 193.0499(49)	Dicaffeoylferuloylquinic acids	DiCFQA
68	15.75	691.1668	691.1676	1.09	$C_{35}H_{31}O_{15}$	MS^2 [691]: 173.0447(100), 179.0342(55)	Dicaffeoylferuloylquinic acids	DiCFQA
69	15.94	677.1512	677.1514	0.35	$C_{34}H_{29}O_{15}$	MS^2 [677]: 173.0445(100), 179.0340(89), 353.0876(83), 191.0554(25), 161.0235(23), 255.0657(14), 135.0440(10), 335.0781(5)	3,4,5-Tricaffeoylquinic acid	3,4,5-TriCQA
70	17.62	193.0506	193.0489	−8.86	$C_{10}H_9O_4$	MS^2[193]: 134.0362(100), 178.0263(99), 149.0598(34), 137.0233(21)	Ferulic acid	FA

[a]Identified by comparing with reference standards.

Compound 2 with the same precursor ion at m/z 677.1934 was identified as CQA-dihexoside base on he fragmentation pattern at m/z 191.055 ($C_7H_{11}O_6$) [caffeoyquinic acid−H−caffeoy−H_2O]$^-$, which indicated loss of two hexose.

2.1.3. Identification of Caffeoylshikimic Acids. Compounds 28 and 48 produced the identical precursor ion [M−H]$^-$ at m/z 335.0772 ($C_{13}H_{21}O_{11}$) and product ions at m/z 179.034 ($C_9H_7O_4$) [caffeic acid−H]$^-$ and 135.044 ($C_8H_7O_2$) [caffeic acid−H−CO_2]$^-$, respectively, identified as 5-CSA, 4-CSA according to the retention behavior of the C18 columns and the literature.[12]

2.1.4. Identification of Coumaroylquinic Acids. Three compounds 11, 26, and 57 with the same precursor ion at m/z 337.0928 ($C_{16}H_{17}O_8$) were, respectively, identified as 3-*p*-coumaroylquinic acid (3-*p*CoQA), 5-*p*-coumaroylquinic acid (5-*p*CoQA), and 1-*p*-coumaroylquinic acid (1-*p*CoQA). It is an essential distinction based on the different base peak ion in MS^2 spectrum to distinguish the compounds 11 and 26 that MS^2 base peak of 3-*p*CoQA at m/z 163.038 ($C_9H_7O_3$) [coumaric acid−H]$^-$ while 5-*p*CoQA at m/z 191.055 ($C_7H_{11}O_6$) [quinic acid−H]$^-$. According to the chromatographic behavior of the eluted sequence on C18 columns, compound 57 was identified as 1-*p*-coumaroylquinic acid (1-*p*CoQA).[26,27]

2.1.5. Identification of Feruloylquinic Acids. Compounds 14, 17, and 30 were eluted at 5.09, 5.62, and 8.79 min, and all displayed precursor ion with peaks at m/z 367.1034 ($C_{17}H_{19}O_9$) [feruloylquinic acid−H]$^-$. Their MS^2 spectra gave common ions at m/z 193.049 ($C_{10}H_9O_4$) [ferulic acid−H]$^-$, 191.055 ($C_7H_{11}O_6$) [quinic acid−H]$^-$, and 173.044 ($C_7H_9O_5$) [quinate−H_2O]$^-$. In MS^2 spectra, compound 30 was identified as 5-FQA and produced a strong base ion at m/z 191.055 ($C_7H_{11}O_6$) [quinate]$^-$, whereas 5-FQA (peak 10) yield a characterized MS^2 product ion at m/z 193.049 ($C_{10}H_9O_4$) [ferulate]$^-$. Additionally, 1-FQA (peak 14) displayed spectroscopic data similar to those of 3-FQA, which were distinguished by the relative intensity of the secondary ion at m/z 191.055 ($C_7H_{11}O_6$).[28,29]

Compounds 6, 9, 12, and 22 with a precursor ion [M−H]$^-$ at m/z 529.1562 ($C_{23}H_{29}O_{14}$) were observed. They were annotated as FQA-hexoside as these compounds fragmented to produce product ions at m/z 367.103 [feruloylquinic acid−H]$^-$, which indicated loss of a hexose and m/z 193.049 ($C_{10}H_9O_4$) [ferulic acid−H]$^-$, 173.044 ($C_7H_9O_5$) [quinate−H_2O]$^-$, and 191.055 ($C_7H_{11}O_6$) [quinate]$^-$.[12]

2.1.6. Identification of Caffeoyl-O-p-coumaroylquinic Acids. Compounds 35, 38, 53, 58, 64, and 65 were regarded as six caffeoyl-*p*-coumaroylquinic acid isomers and presented the same [M−H]$^-$ ion at m/z 499.1245 ($C_{25}H_{23}O_{11}$), respectively, as 5-caffeoyl-3-*p*-coumaroylquinic acid, 4-caffeoyl-3-p-coumaroylquinic acid, 3-caffeoyl-4-*p*-coumaroylquinic acid, 3-caffeoyl-5-*p*-coumaroylquinic acid, 5-caffeoyl-4-*p*-coumaroylquinic acid, and 4-caffeoyl-5-*p*-coumaroylquinic acid. Compound 35 produced the MS^2 base peak at m/z 163.038 ($C_9H_7O_3$) [*p*-hydroxycinnamic acid−H]$^-$ and also lost a caffeoyl residue at m/z 337.093 ($C_{16}H_{17}O_8$) [*p*-coumaroylquinic acid−H]$^-$. Compound 38 yielded the MS^2 base peak at m/z 173.044 ($C_7H_9O_5$) [quinate−H_2O]$^-$, which were similar to compounds 53, 64, and 65, respectively; compound 53 was given as m/z 163.039 ($C_9H_7O_3$) [*p*-hydroxycinnamic acid−H]$^-$, identified as 3C,4*p*CoQA the same as compound 64 (5C,4*p*CoQA) according to the published data.[30] Compound 65 produced the MS^2 product ions at m/z 179.034 ($C_9H_7O_4$)

https://doi.org/10.1021/acsomega.2c02899
ACS Omega 2022, 7, 25675−25685

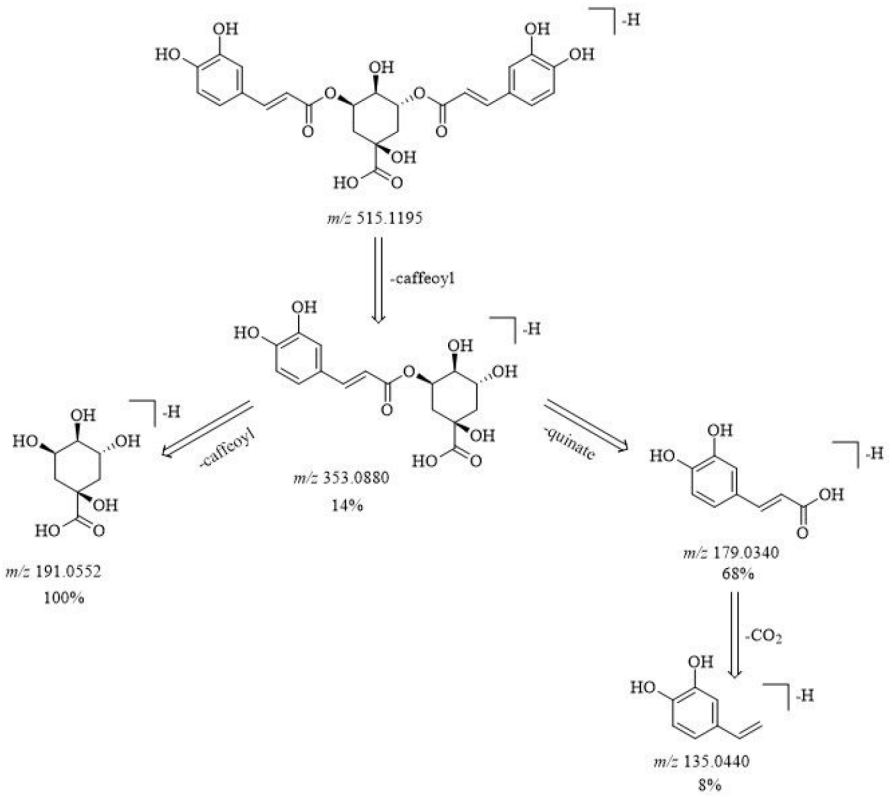


Figure 1. Proposed fragmentation patterns of the main fragment ions in negative-ion mode for isochlorogenic acid A in the root bark of *A. gracilistylus*.

[caffeic acid−H]$^-$, 191.055 ($C_7H_{11}O_6$) [quinate]$^-$, 353.088 ($C_{16}H_{17}O_9$) [caffeoyquinic acid−H]$^-$, 135.044 ($C_8H_7O_2$) [caffeic acid−H]$^-$ while compound 58 yielded the MS^2 base peak ion at *m/z* 191.055 ($C_7H_{11}O_6$) [quinate]$^-$, identified as 3C,5*p*CoQA.[12]

2.1.7. Identification of Feruloylcaffeoylquinic Acids. Compounds 34, 36, 40, 56, 59, and 61 all showed the precursor ion with a peak at *m/z* 529.1351 ($C_{26}H_{25}O_{12}$) [caffeoyl−feruloylquinic acids−H]$^-$; compound 34 was identified as 3F,4CQA due to the MS^2 base peak at *m/z* 173.044 ($C_7H_9O_5$) [M−H−2feruloyl]$^-$, the secondary product ion at *m/z* 179.034 ($C_9H_7O_4$) [caffeic acid−H]$^-$, and other ion at *m/z* 353.087 ($C_{16}H_{17}O_9$) [M−H−feruloyl−]$^-$; while 3C,4FQA (compound 36) showed as the MS^2 base peak of the product ion at *m/z* 173.044 ($C_7H_9O_5$) [M−H−2feruloyl]$^-$ and the secondary product ion at *m/z* 193.049 ($C_{10}H_9O_4$) [ferulic acid−H]$^-$ and spectra of the characteristic ions with peak at *m/z* 367.103 ($C_{17}H_{19}O_9$) [M−H−caffeoyl]$^-$ based on the literature;[26] compound 40 was characterized as 3F,5CQA that yielded a MS^2 base peak at *m/z* 191.055 ($C_7H_{11}O_6$) [M−H−2caffeoyl]$^-$, and the secondary product ion at *m/z* 193.049 ($C_{10}H_9O_4$) [ferulic acid−H]$^-$ distinguished from 3C,5FQA (compound 56) produced a secondary product ion at *m/z* 173.044 ($C_7H_9O_5$) [M−H−-2feruloyl]$^-$; 4F,5CQA (compound 59) generated a base peak product ion at *m/z* 173.044 ($C_7H_9O_5$) [M−H−2feruloyl]$^-$, and the secondary product ion at *m/z* 193.049 ($C_{10}H_9O_4$) [ferulic acid−H]$^-$ differentiated from the 4C,5FQA (compound 61) by the presence of the MS^2 secondary product ion at *m/z* 179.034 ($C_9H_7O_4$) [caffeic acid−H]$^-$ according to the public data.[25,28,31]

Compounds 66, 67, and 68 with a precursor ion [M−H]$^-$ at *m/z* 691.1668 were attributed to DiCFQA, based on the product ion at *m/z* 179.034 ($C_9H_7O_4$) [caffeic acid−H]$^-$, 173.044 ($C_7H_9O_5$) [quinate−H_2O]$^-$.[12]

Compounds 24, 31, 39, 44, and 52 were followed in the identification of CFQA-glycoside which were identified by their precursor ion [M−H]$^-$ at *m/z* 691.1879 and based on their product ions at *m/z* 179.034 ($C_9H_7O_4$) [caffeic acid−H]$^-$, 191.055 ($C_7H_{11}O_6$) [quinate]$^-$, 193.050 ($C_{10}H_9O_4$) [ferulic acid−H]$^-$.

2.1.8. Identification of Caffeoyl-O-dimethoxycinnamoylquinic Acids. Compounds 46 and 51 with the same precursor ion at *m/z* 543.1507 ($C_{27}H_{27}O_{12}$) as these produced product ions at *m/z* 193.050 ($C_{10}H_9O_4$) [ferulic acid−H]$^-$ and 173.044 ($C_7H_9O_5$) [quinate−H_2O]$^-$ and were annotated as caffeoyl-*O*-dimethoxycinnamoylquinic acids.[32]

2.1.9. Identification of Caffeoylsinapoylquinic Acids. Compounds 55 and 63 were identified as SCQA, which yielded a precursor ion [M−H]$^-$ at *m/z* 559.1457 ($C_{27}H_{27}O_{13}$), and based on their fragmentation patterns at *m/z* 173.044 ($C_7H_9O_5$) [quinate−H_2O]$^-$, 179.034 ($C_9H_7O_4$) [caffeic acid−H]$^-$.[12]

2.2. Method Performance. In this study, quantification of individual compounds was carried out by an external calibration method, and the CGA concentration of 66 batches was calculated by plotting the area response versus the analytes concentration using 1/*x* weighted calibration curves. Seven kinds of standard working mixture solutions of CGAs analogue were completely separated using the delicate gradient program by UHPLC-Q-Orbitrap-MS. Linearity equations were obtained by plotting corresponding peak areas versus different concentrations. All the linearity equations exhibited excellent

https://doi.org/10.1021/acsomega.2c02899
ACS Omega 2022, 7, 25675−25685

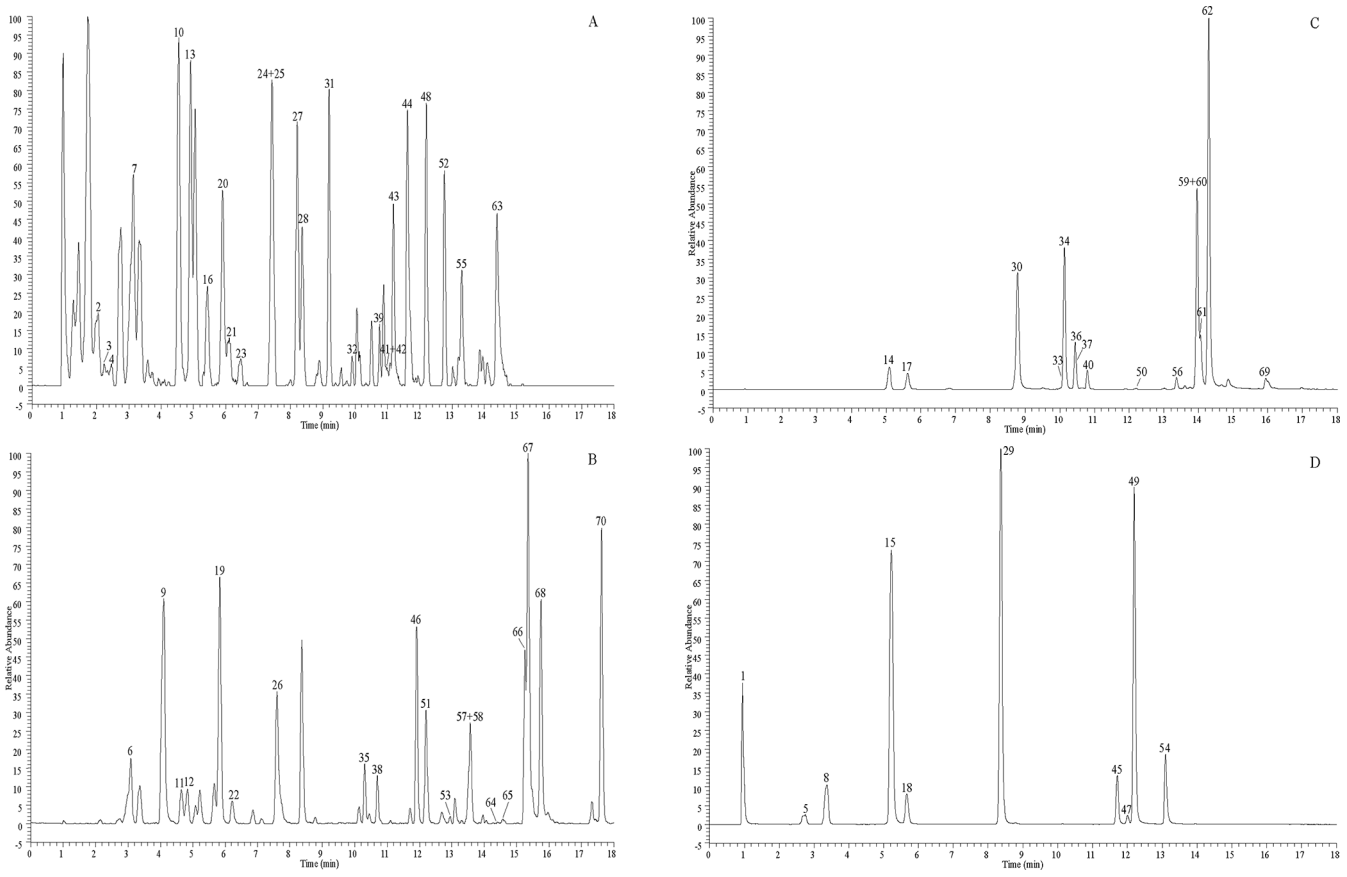


Figure 2. High-resolution extracted ion chromatogram (HREIC) for multiple compounds in *A. gracilistylus* W. W. Smith. (A) *m/z* 335.0772, 341.0878, 371.0983, 515.1406, 559.1457, 677.1723, 677.1934, 691.1879; (B) *m/z* 179.0349, 193.0506, 337.0928, 499.1245, 529.1562, 543.1507, 691.1668; (C) *m/z* 367.1034, 529.1351, 677.1511; (D) *m/z* 191.0561, 353.087, 515.1195.

Table 2. Method Validation

					stability RSD (%)		recovery	
compd	analytical curve	range (μg/mL)	linearity (r^2)	repeatability RSD (%)	rt for 4 h	autosampler at 10 °C for 24 h	mean (%)	RSD (%)
Isochlorogenic acid C	$y = 2E^{+07}x - 1E^{+07}$	0.428−42.8	0.9987	3.32	3.24	3.56	103.39	4.23
Isochlorogenic acid A	$y = 3E^{+07}x - 3E^{+06}$	0.224−22.4	0.9926	2.62	2.38	2.94	103.44	3.27
1,5-Dicaffeoylquinic acid	$y = 2E^{+07}x - 3E^{+07}$	1.08−108	0.9958	3.14	2.86	3.18	96.92	2.94
1,3-Dicaffeoylquinic acid	$y = 2E^{+07}x - 2E^{+07}$	1.14−114	0.9987	2.21	2.28	2.96	104.97	4.09
Cryptochlorogenic acid	$y = 2E^{+07}x - 6E^{+06}$	0.236−23.6	0.9988	2.13	2.36	2.74	96.74	3.83
Chlorogenic acid	$y = 1E^{+07}x - 2E^{+07}$	1.47−147	0.9983	3.04	2.84	3.12	104.43	4.03
Neochlorogenic acid	$y = 3E^{+07}x - 1E^{+07}$	0.246−24.6	0.9999	2.10	2.32	2.56	104.45	2.98

linearity, and the values of linear ranges (r^2) of CGAs calculated from analytical curves both were >0.990 and linearity equations were listed in Table 2. RSDs of the repeatability test of seven kinds of CGA ranged from 2.10% to 3.32%. In addition, the accuracy of the proposed method was assessed in which 0.25 g of the *A. gracilistylus* roots powder was mixed with a known amount of seven CGAs reference substances and then extracted by the "4.2 Sample preparation" method. Ultimately, the results indicated that the UHPLC-Q-orbitrap method possessed good accuracy with recoveries ranging from 96.7% to 105%, while all of the RSDs were less than 5% (Table 2).

2.3. Quantification of the CGAs from the Root Bark of *A. gracilistylus* from Different Batches. Sixty-six different batches of *A. gracilistylus* root were extracted by the "4.2" method, and four batches were parallel. The optimized and validated UHPLC-Q-Orbitrap method was used for analysis. The contents and total contents of seven kinds of CGA_S in different batches of the root bark of *A. gracilistylus* are shown in Table 3. The results are expressed as the average content, which range from 2.150 to 33.51 mg/g.

3. CONCLUSIONS

In conclusion, the qualitative and quantitative methods using UHPLC-Q-Exactive Orbitrap MS combined with PRM mode and SIM mode were successfully established in this study. Finally, a total of 70 CGAs (64 of them for the first time) and 7 CGAs were identified and quantified from the root bark of *A. gracilistylus*, which suggested that *A. gracilistylus* is an excellent

https://doi.org/10.1021/acsomega.2c02899
ACS Omega 2022, 7, 25675−25685

Table 3. Content (mg/g) of Seven Compounds in 66 Batches of *A. gracilistylus*

sample	isochlorogenic acid C	isochlorogenic acid A	1,5-dicaffeoylquinic acid	1,3-dicaffeoylquinic acid	cryptochlorogenic acid	chlorogenic acid	neochlorogenic acid	total of 7 CGAs
1	0.4394	0.1156	1.2204	1.2144	0.1335	2.4537	0.0928	5.6698
2	0.3302	0.0723	0.7922	1.5293	0.1713	2.4025	0.1181	5.4160
3	0.4783	0.1211	1.0640	2.2070	0.2161	2.9812	0.1385	7.2062
4	0.8879	0.1831	2.1499	3.4529	0.3040	4.9937	0.1909	12.1625
5	1.7865	0.3828	4.2989	5.4546	0.5336	8.5707	0.2919	21.3191
6	0.1348	0.0439	0.3245	0.3728	0.0850	0.8272	0.0655	1.8537
7	2.6670	0.3573	6.8101	8.8480	1.5234	12.5397	0.7607	33.5061
8	1.5031	0.2500	2.9831	6.0364	0.8261	8.9139	0.4276	20.9404
9	1.0828	0.2200	2.3473	3.7173	0.2765	4.5276	0.1694	12.3408
10	1.2616	0.2490	2.9858	4.9309	0.4935	7.0510	0.2784	17.2503
11	0.9332	0.2488	2.3801	4.6309	0.5019	7.3602	0.2976	16.3527
12	0.3664	0.0680	0.9773	1.7046	0.1973	2.5470	0.1190	5.9797
13	0.2234	0.0526	0.6306	1.2095	0.1781	2.2868	0.1087	4.6897
14	0.6201	0.0841	1.4992	4.3618	0.5675	5.3342	0.2909	12.7578
15	0.3553	0.1088	0.8689	1.0065	0.1315	1.8223	0.0942	4.3876
16	0.4121	0.0625	0.2259	0.1524	1.1007	6.7135	0.5096	9.1767
17	1.1915	0.0927	0.2257	0.1526	1.6890	14.9070	0.7693	19.0279
18	0.5806	0.0731	0.2257	0.1526	1.2095	7.8349	0.5514	10.6277
19	0.7340	0.0827	0.2259	0.1523	1.0969	9.4249	0.4960	12.2126
20	0.8554	0.0827	0.2255	0.1513	1.1499	9.1684	0.4255	12.0586
21	1.1278	0.2224	2.7329	5.6454	0.6323	7.5803	0.3197	18.2609
22	0.4302	0.0916	1.3544	2.4938	0.2246	3.3550	0.1297	8.0792
23	1.0061	0.1402	3.2950	6.6471	0.7794	8.8358	0.4052	21.1088
24	0.6241	0.0762	0.2278	0.1769	0.8244	8.9469	0.3895	11.2657
25	0.2393	0.0612	0.5896	0.8275	0.1180	1.2718	0.0847	3.1921
26	0.4483	0.1396	0.9919	1.4076	0.1474	2.3734	0.1060	5.6143
27	0.8515	0.1768	1.4544	3.4910	0.3786	4.3833	0.1974	10.9331
28	0.7188	0.1479	2.2285	3.6455	0.3429	4.4520	0.1780	11.7136
29	1.1125	0.2819	2.4977	4.0027	0.3419	5.1007	0.1967	13.5340
30	1.1743	0.1437	2.8828	6.4459	0.6489	8.2812	0.3792	19.9560
31	1.3846	0.1471	4.6142	7.3481	0.8489	10.0207	0.4282	24.7919
32	0.2127	0.0636	0.6896	1.3044	0.1212	1.8306	0.0844	4.3066
33	0.3465	0.0671	0.8200	2.2290	0.2445	3.6460	0.1385	7.4915
34	0.3144	0.0621	0.7011	1.1032	0.1548	2.0547	0.0989	4.4893
35	0.1374	0.0457	0.3529	0.4788	0.0917	0.9693	0.0696	2.1454
36	1.7910	0.2386	4.8744	7.6217	1.1431	10.5136	0.6422	26.8246
37	0.4089	0.0911	0.6533	0.9748	0.1576	1.6972	0.0986	4.0815
38	0.5105	0.1255	0.9811	2.3708	0.2258	2.6069	0.1374	6.9579
39	0.5768	0.1840	1.3572	1.9262	0.1943	2.9179	0.1252	7.2816
40	0.4451	0.1234	1.0176	2.2658	0.2048	2.6311	0.1284	6.8161
41	0.6479	0.1544	1.6070	2.6093	0.3415	4.8076	0.2038	10.3715
42	0.3011	0.0647	0.5976	0.8859	0.1568	2.0606	0.1052	4.1718
43	0.6872	0.1326	1.3026	2.8249	0.3069	3.5877	0.1679	9.0099
44	1.5833	0.2408	3.0702	5.0738	0.4881	5.8988	0.2702	16.6251
45	1.3846	0.2267	3.7062	6.1550	0.8085	8.3969	0.4244	21.1023
46	1.0668	0.2360	3.3036	5.2315	0.4652	7.1653	0.2811	17.7495
47	0.6570	0.1411	2.3286	3.6160	0.3659	6.2115	0.2039	13.5239
48	1.1735	0.1799	4.1459	6.5087	0.7401	8.6935	0.3826	21.8242
49	0.6935	0.0982	2.0010	4.2946	0.4486	6.3842	0.2603	14.1804
50	0.3733	0.3548	1.9426	2.1792	0.1693	7.3236	0.1119	12.4548
51	0.4652	0.1078	1.1403	2.5015	0.2238	3.2971	0.1443	7.8799
52	0.9932	0.1649	2.0831	4.0205	0.4474	5.4517	0.2260	13.3868
53	0.8968	0.1742	3.1494	5.6577	0.6011	8.3693	0.3357	19.1842
54	0.3330	0.0742	0.9370	1.7401	0.1698	2.3906	0.1079	5.7525
55	2.4113	0.3664	5.9124	7.9553	1.3870	12.2521	0.6290	30.9134
56	1.5661	0.3768	7.0236	6.1277	0.4254	10.8771	0.2593	26.6560
57	0.3396	0.0626	1.1852	2.2231	0.3003	4.1459	0.1656	8.4223
58	1.2344	0.2578	3.7820	5.4153	0.7176	8.7960	0.4072	20.6103
59	0.2732	0.0791	0.6962	1.2328	0.1721	2.1886	0.1102	4.7522
60	0.9107	0.2500	2.1171	3.1238	0.2688	4.9800	0.1682	11.8187

https://doi.org/10.1021/acsomega.2c02899
ACS Omega 2022, 7, 25675−25685

Table 3. continued

sample	isochlorogenic acid C	isochlorogenic acid A	1,5-dicaffeoylquinic acid	1,3-dicaffeoylquinic acid	cryptochlorogenic acid	chlorogenic acid	neochlorogenic acid	total of 7 CGAs
61	1.2000	0.2217	2.4788	5.3140	0.5450	6.1195	0.2213	16.1002
62	0.8603	0.2128	1.2014	2.1413	0.2124	2.1439	0.1318	6.9039
63	0.3572	0.0623	0.8102	1.2717	0.1470	1.4440	0.0929	4.1854
64	0.7848	0.1841	1.4553	2.7977	0.3560	4.0524	0.2156	9.8459
65	0.8741	0.2677	2.0449	2.8329	0.2643	5.0967	0.1666	11.5472
66	0.3533	0.0876	0.9504	1.4404	0.1500	2.3428	0.1118	5.4364

source of CGAs. Meantime, this result is very useful for the further investigation of *A. gracilistylus* including bioactive chemical and quality control.

4. MATERIALS AND METHODS

4.1. Materials and Reagents. *A. gracilistylus* sample was authenticated in line with the Chinese Pharmacopoeia (edition 2020, volume 1) by Associate Professor Jian-Bo Yang. The root sample of *A. gracilistylus* has been deposited at the Research and Inspection Center of Traditional Chinese Medicine and Ethnomedicine, National Institutes for Food and Drug Control, State Food and Drug Administration, Beijing, China. Reference standards of *trans*-3-caffeoylquinic acid (*trans*-3-CQA, nechlorogenic acid, ≥98%, L-007-171216), *trans*-4-caffeoylquinic acid (*trans*-4-CQA, cryptochlorogenic acid, ≥98%, Y-067-180320), *trans*-5-caffeoylquinic acid (*trans*-5-CQA, chlorogenic acid, ≥98%, X-014-170309), 3,5-dicaffeoylquinic acid (3,5-DiCQA, isochlorogenic acid A, ≥98%, Y-068-170903), 4,5-dicaffeoylquinic acid (4,5-DiCQA, isochlorogenic acid C, ≥98%, Y-070-170515) were provided by Chengdu Herbpurify Co., Ltd. (Chengdu, China); 1,3-dicaffeoylquinic acid (1,3-DiCQA, ≥98%, MUST-16022610) and 1,5-dicaffeoylquinic acid (1,5-DiCQA, ≥98%, MUST-15080115) were provided by Chengdu Must Biological Technology Co., Ltd. (Chengdu China); caffeic acid (≥98%, C108306) was purchased by Shanghai Aladdin Biochemical Technology Co., Ltd. (Shanghai China). HPLC-grade acetonitrile and methanol were obtained from Fisher scientific (New Jersey), LC−MS grade formic acid was supplied by Thermo Fisher Scientific China, water used as the LC mobile phase, and aqueous solvents were prepared by watsons water. Other reagents were of analytical grade.

4.2. Sample Preparation. A stock standard solution of each standard at 1 mg/mL was prepared by accurately weighing solid standards and being dissolved in methanol. The individual solutions of 7 reference standards were mixed and diluted in methanol at 10 μg/mL to prepare standard working mixture solutions.

The root bark of *A. gracilistylus* samples was ground into powder, accurately weighed to 0.5 g, and extracted under ultrasonication with about 5 and 75 mL of 70% methanol for 1 h, respectively; afterward, the extracted solution was filtered by a 0.45 μm microfiltration membrane. All working solutions were stored at (4 °C) until qualitative and quantitative analysis.

4.3. Instruments and Conditions. *4.3.1. Identification of CGAs in A. gracilistylus.* Chromatographic analysis was performed on a Thermo Scientific Dionex Ultimate 3000 RS (Thermo Fisher Scientific, CA) composed of an online degasser, pump, autoinjector, column heater, and UV detector. Sample separations were carried out on a HYPERSIL GOLD C18 column (100 × 2.1 mm, 1.9 μm) from (Thermo Scientific) using gradient elution at 45 °C. The mobile phases were made up of solvent A (0.1% formic acid water, v/v) and solvent B (100% acetonitrile). at a flow rate of 0.3 mL/min. The gradient conditions of the mobile phases were optimized as follows: 0−2 min, 95−92% A; 2−5 min, 92−90% A; 5−20 min, 90−60% A; 20−24 min, 60−5% A; 24−26 min, 5% A; 26−27 min, 5−95% A; 27−30 min, 95% A. The injection volume was 2 μL.

MS analysis was performed on a Thermo Scientific Q-Exactive Focus Orbitrap MS (Thermo Electron, Bremen, Germany) operated with a heated electrospray ionization (HESI) in negative ion mode. The mass spectra were acquired with full MS mode in a mass range from m/z 100−1200 at a resolution of 70000, combined with the data dependent scan (dd-MS^2) at a resolution of 35000 and isolation window at m/z 3.0. Other Q-Exactive general parameters were nebulizer pressure at 10 arb, sheath gas and auxiliary gas at the flow rate of 30 arb, capillary temperature at 320 °C, auxiliary gas heater temperature at 350 °C, spray voltage at 3.2 kV, and S-lens level at 50.

4.3.2. Quantification of CGAs in A. gracilistylus. LC−MS analysis was performed on the same machine as described in section 4.3.1. Agilent-XDB-C18 (100 mm × 2.1 mm, 1.8 μm) was applied for chromatographic separation with a column temperature of 40 °C. The mobile phase consisted of solvent A (0.1% formic acid water, v/v) and solvent B (100% acetonitrile). The gradient elution condition was as follows: 0−2 min, 95−89% A; 2−3 min, 89−78% A; 3−4 min, 78−80% A; 4−4.5 min, 80−88% A; 4−4.5 min, 80−88% A; 4.5−5.5 min, 80−50% A; 5.5−6.5 min, 50−75% A; 6.5−7.0 min, 75−20% A; 7.0−8.0 min, 20−20% A; 8−8.1 min, 20−95% A; and 8.1−11 min, 95−95% A. The samples were injected in 1 μL with constant flow rates of 0.28 mL/min. The MS scan mode was detected in selected reaction monitoring (SIM) mode at a resolution of 35000. The major MS parameters used were identical with the condition of identification.

4.4. Method Validation. The method for quantitative analysis of CGAs was validated with regard to its selectivity, linearity, sensibility, accuracy, and precision following the 2020 edition of Chinese Pharmacopoeia guidance document on analytical quality control and method validation procedures. The selectivity of the method was ascertained by analyzing the standards of seven kinds of CGAs and the samples. The peaks for the studied compounds in the samples were confirmed by comparing the retention times of the peaks with those of standards as well as by recognizing both the full MS precursor and product ions MS^2 with an mass error below 5 ppm. The linearity of the methods of isochlorogenic acid C, isochlorogenic acid A, 1,5-dicaffeoylquinic acid, 1,3-dicaffeoylquinic acid, cryptochlorogenic acid, neochlorogenic acid, and chlorogenic acid were assessed using six concentration ranges, respectively. Repeatability is a measure of repeatability of the

https://doi.org/10.1021/acsomega.2c02899
ACS Omega 2022, 7, 25675−25685

analytical method in the normal operating conditions and expressed as the percentage relative standard deviation (% RSD). The accuracy is based on recovery studies in the present work. Stability studies of the method including short-term stability (room temperature, 4 h), and postpreparative stability (storage in the autosampler, 10 °C, 24 h) were achieved by the test of sample with six replicates.

4.5. Data Processing and Analysis. Xcalibur 4.1 (Thermo Scientific, CA) was applied in the acquisition of raw data in full-scan/dd-MS^2 mode. Compound Discoverer version 3.0 (Thermo Scientific, CA) was used to dispose the data which passed the workflow templates to predict some expected compounds.

The data were input into Excel for statistical analysis. Seven kinds of chlorogenic acids were determined from 66 different producing areas of the root bark of *A. gracilistylus*. All analyses were conducted in triplicate. The data is presented as a mean.

AUTHOR INFORMATION

Corresponding Authors

Lei Sun − *Institute for Control of Chinese Traditional Medicine and Ethnic Medicine & National Institutes for Food and Drug Control, Beijing 100050, P.R. China*; Email: dasunlei@sina.com

Wei Cai − *School of Pharmaceutical Sciences & Hunan University of Medicine, Huaihua 418000, P.R. China*; orcid.org/0000-0002-8849-0280; Email: 20120941161@bucm.edu.cn

Authors

Jianbo Yang − *Institute for Control of Chinese Traditional Medicine and Ethnic Medicine & National Institutes for Food and Drug Control, Beijing 100050, P.R. China*

Lingwen Yao − *Institute for Control of Chinese Traditional Medicine and Ethnic Medicine & National Institutes for Food and Drug Control, Beijing 100050, P.R. China*

Kaiyan Gong − *School of Pharmaceutical Sciences & Hunan University of Medicine, Huaihua 418000, P.R. China*

Kailin Li − *School of Pharmaceutical Sciences & Hunan University of Medicine, Huaihua 418000, P.R. China*

Complete contact information is available at: https://pubs.acs.org/10.1021/acsomega.2c02899

Author Contributions

§J.Y. and L.Y. contributed equally to the manuscript.

Notes

The authors declare no competing financial interest.

ACKNOWLEDGMENTS

This work was financially supported by the Scientific Research Fund of Hunan Provincial Education Department (no. 19A353).

ABBREVIATIONS

CGAs	chlorogenic acids
UHPLC-Q-Orbitrap MS	ultra high performance liquid chromatography-Q-exactive orbitrap mass spectrometry
PRM	parallel reaction monitoring
SIM	selected reaction monitoring
TOF-MS	time-of-flight mass spectrometry
HESI	heated electrospray ionization
dd-MS^2	data-dependent MS^2 scan
QA	quinic acid
CQA-dihexoside	caffeoylquinic acid-dihexoside
CQA-hexoside	caffeoylquinic acid-hexoside
FQA-hexoside	feruloylquinic acid-hexoside
3-CQA	3-*O*-caffeoylquinic acid
3-*p*CoQA	3-*O*-p-coumaroylquinic acid
CA-hexoside	caffeic acid-hexoside
1-FQA	1-*O*-feruloylquinic acid
5-CQA	5-*O*-caffeoylquinic acid
3-*O*-hydroxydihydroCQA	3-*O*-hydroxydihydrocaffeoylquinic acid
3-FQA	3-*O*-feruloylquinic acid
4-CQA	4-*O*-faffeoylquinic acid
CA	caffeic acid
4-*O*-hydroxydihydroCQA	4-*O*-hydroxydihydrocaffeoylquinic acid
DiCQA-hexoside	dicaffeoylquinic acid-hexoside
CFQA-hexoside	caffeoylferuloylquinic acid-hexoside
5-*p*CoQA	5-*O*-*p*-coumaroylquinic acid
1,3-DiCQA	1,3-*O*-dicaffeoylquinic acid
5-FQA	5-*O*-feruloylquinic acid
CFQA-hexoside	caffeoylferuloylquinic acid-hexoside
TriCQA	tricaffeoylquinic acid
3F,4CQA	3-*O*-feruloyl-4-*O*-caffeoylquinic acid
5C,3*p*CoQA	5-*O*-caffeoyl-3-*O*-*p*-coumaroyl-quinic acid
3C,4FQA	3-*O*-caffeoyl-4-*O*-feruloylquinic acid
4C,3*p*CoQA	4-*O*-caffeoyl-3-*O*-*p*-coumaroyl-quinic acid
3F,5CQA	3-*O*-feruloyl-5-*O*-caffeoylquinic acid
CFQA-hexoside	caffeoylferuloylquinic acid-hexoside
1,5-DiCQA	1,5-*O*-dicaffeoylquinic acid
C,DimethoxyCiQA	caffeoyl-*O*-dimethoxycinnamoyl-quinic acid
3,4-DiCQA	3,4-*O*-dicaffeoylquinic acid (iso-chlorogenic acid B)
4-CSA	4-*O*-caffeoylshikimic acid
3,5-DiCQA	3,5-*O*-dicaffeoylquinic acid (iso-chlorogenic acid A)
3C,4*p*CoQA	3-*O*-caffeoyl-4-*O*-*p*-coumaroyl-quinic acid
4,5-DiCQA	4,5-*O*-dicaffeoylquinic acid (iso-chlorogenic acid C)
SCQA	caffeoylsinapoylquinic acids
3C,5FQA	3-*O*-caffeoyl-5-*O*-feruloylquinic acid
1-*p*CoQA	1-*O*-*p*-coumaroylquinic acid
3C,5*p*CoQA	3-*O*-caffeoyl-5-*O*-*p*-coumaroyl-quinic acid
4F,5CQA	4-*O*-feruloyl-5-*O*-caffeoylquinic acid
4C,5FQA	4-*O*-caffeoyl-5-*O*-feruloylquinic acid
5C,4*p*CoQA	5-*O*-caffeoyl-4-*O*-*p*-coumaroyl-quinic acid
4C,5*p*CoQA	4-*O*-caffeoyl-5-*O*-*p*-coumaroyl-quinic acid
DiCFQA	dicaffeoylferuloylquinic acids
3,4,5-TriCQA	3,4,5-tricaffeoylquinic acid

https://doi.org/10.1021/acsomega.2c02899
ACS Omega 2022, 7, 25675−25685

FA ferulic acid

REFERENCES

(1) Wu, Z. Y.; Zhang, Y. B.; Zhu, K. K.; Luo, C.; Zhang, J. X.; Cheng, C. R. Anti-inflammatory diterpenoids from the root bark of *Acanthopanax gracilistylus*. *J. Nat. Prod.* **2014**, *77*, 2342−2351.

(2) Xu, H. B.; Yang, T. H.; Xie, P.; Tang, Z. S.; Song, X.; Xu, H. L. LC-MS guided isolation of gracilistones A and B, a pair of diastereomeric sesquiterpenoids with an unusual tetrahydrofuran-fused tricyclic skeleton from *Acanthopanax gracilistylus* and their potential anti-inflammatory activities. *Fitoterapia.* **2018**, *130*, 265−271.

(3) Zou, Q. P.; Liu, X. Q.; Huang, J. J.; Yook, C. S.; Whang, W. K.; Lee, H. K. Inhibitory effects of lupane-type triterpenoid saponins from the leaves of *Acanthopanax gracilistylus* on lipopolysaccharide-induced TNF-α, IL-1$\beta$and high-mobility group box 1 release in macrophages. *Mol. Med. Rep.* **2017**, *16*, 9149−9156.

(4) Ma, L.; Gao, W.; Chen, F.; Meng, Q. HS-SPME and SDE combined with GC-MS and GC-O for characterization of flavor compounds in Zhizhonghe Wujiapi medicinal liquor. *Food Res. Int.* **2020**, *137*, 109590.

(5) Ma, L. H.; Chen, F.; Meng, Q. R. Characterization of Volatile Components in Zhizhonghe Wujiapi Medicinal Liquor by HS-SPME/GC-MS/GC-O. *Liquor-making Sci. Technol.* **2021**, *01*, 92−101.

(6) Wang, J. G. Formula, preparation method, efficacy and historical origin of *Acanthopanax senticosus* wine in different periods. *Chin Brewing.* **2008**, *18*, 103−104.

(7) Yang, J. B.; Cai, W.; Li, M. H. Review on the chemical constituents and pharmacological activities of *Acanthopanax alba*. *Chin Mod. Chin Mater. Med.* **2020**, *22*, 652−662.

(8) Lepiniec, L.; Debeaujon, I.; Routaboul, J. M.; Baudry, A.; Pourcel, L.; Nesi, N.; Caboche, M. Genetics and biochemistry of seed flavonoids. *Annu. Rev. Plant Biol.* **2006**, *57*, 405−430.

(9) Zhang, H.; Tsao, R. Dietary polyphenols, oxidative stress and antioxidant and anti-inflammatory effects. *Curr. Opin. Food Sci.* **2016**, *8*, 33−42.

(10) Stalmach, A.; Steiling, H.; Williamson, G.; Crozier, A. Bioavailability of chlorogenic acids following acute ingestion of coffee by humans with an ileostomy. *Arch. Biochem. Biophys.* **2010**, *501*, 98−105.

(11) Zhao, Y.; Wang, J.; Ballevre, O.; Luo, H.; Zhang, W. Antihypertensive effects and mechanisms of chlorogenic acids. *Hypertens Res.* **2012**, *35*, 370−374.

(12) Cai, W.; Li, K. L.; Xiong, P.; Gong, K. Y.; Zhu, L.; Yang, J. B.; Wu, W. H. A systematic strategy for rapid identification of chlorogenic acids derivatives in *Duhaldea nervosa* using UHPLC-Q-Exactive Orbitrap mass spectrometry. *Arab J. Chem.* **2020**, *13*, 3751−3761.

(13) Gismondi, A.; Serio, M.; Canuti, L.; Canini, A. Biochemical, antioxidant and antineoplastic properties of Italian saffron (*Crocus sativus* L.). *Am. J. Plant Sci.* **2012**, *3*, 1573.

(14) Bajpai, V.; Kumar, S.; Singh, A.; Bano, N.; Patha, M.; Kumar, N.; Kuma, B. Metabolic fingerprinting of dioecious *Tinospora cordifolia* (Thunb) Miers stem using DART TOF MS and differential pharmacological efficacy of its male and female plants. *Ind. Crops Prod.* **2017**, *101*, 46−53.

(15) Bai, Y. L.; Hong, Z. D.; Zhang, T. Y.; Cai, B. D.; Zhang, Y. Z.; Feng, Y. Q. A Method for Simultaneous Determination of 14 Carbonyl-Steroid Hormones in Human Serum by Ultra High Performance Liquid Chromatography-Tandem Mass Spectrometry. *J. Anal Test.* **2020**, *4*, 1−12.

(16) Hogenboom, A. C.; Van, L. J. A.; De, V. P. Accurate mass screening and identification of emerging contaminants in environmental samples by liquid chromatography-hybrid linear ion trap Orbitrap mass spectrometry. *J. Chromatogr A* **2009**, *1216*, 510−519.

(17) Shi, T.; Yao, Z.; Qin, Z.; Ding, B.; Dai, Y.; Yao, X. Identification of absorbed constituents and metabolites in rat plasma after oral administration of Shen-Song-Yang-Xin using ultra-performance liquid chromatography combined with quadrupole time-of-flight mass spectrometry. *Biomed Chromatogr.* **2015**, *29*, 1440−1452.

(18) Sun, Z.; Zuo, L.; Sun, T.; Tang, J.; Ding, D.; Zhou, L.; Zhang, X. Chemical profiling and quantification of XueBiJing injection, a systematic quality control strategy using UHPLC-Q Exactive hybrid quadrupole-orbitrap high-resolution mass spectrometry. *Sci. Rep.* **2017**, *7*, 1−15.

(19) Wang, X.; Li, W.; Ma, X.; Chu, Y.; Li, S.; Guo, J.; Liu, C. Simultaneous determination of caffeic acid and its major pharmacologically active metabolites in rat plasma by LC-MS/MS and its application in pharmacokinetic study. *Biomed Chromatogr.* **2015**, *29*, 552−559.

(20) Joo, Y. H.; Nam, M. H.; Chung, N.; Lee, Y. K. UPLC-QTOF-MS/MS screening and identification of bioactive compounds in fresh, aged, and browned *Magnolia denudata* flower extracts. *Food Res. Int.* **2020**, *133*, 109192.

(21) Khallouki, F.; Ricarte, I.; Breuer, A.; Owen, R. W. Characterization of phenolic compounds in mature Moroccan Medjool date palm fruits (*Phoenix dactylifera*) by HPLC-DAD-ESI-MS. *J. Food Compost Anal.* **2018**, *70*, 63−71.

(22) Chen, J.; Mangelinckx, S.; Lü, H.; Wang, Z. T.; Li, W. L.; De, K. N. Profiling and elucidation of the phenolic compounds in the aerial parts of *Gynura bicolor* and *G. divaricata* collected from different Chinese origins. *Chem. Biodivers.* **2015**, *12*, 96−115.

(23) Sun, Y.; Li, H.; Hu, J.; Li, J.; Fan, Y. W.; Liu, X. R.; Deng, Z. Y. Qualitative and quantitative analysis of phenolics in *Tetrastigma hemsleyanum* and their antioxidant and antiproliferative activities. *J. Agric. Food Chem.* **2013**, *61*, 10507−10515.

(24) Zhong, J.; Chen, N.; Huang, S.; Fan, X.; Zhang, Y.; Ren, D.; Yi, L. Chemical profiling and discrimination of green tea and Pu-erh raw tea based on UPLC-Q-Orbitrap-MS/MS and chemometrics. *Food Chem.* **2020**, *326*, 126760.

(25) Zhang, J. Y.; Zhang, Q.; Li, N.; Wang, Z. J.; Lu, J. Q.; Qiao, Y. J. Diagnostic fragment-ion-based and extension strategy coupled to DFIs intensity analysis for identification of chlorogenic acids isomers in *Flos Lonicerae Japonicae* by HPLC-ESI-MSn. *Talanta.* **2013**, *104*, 1−9.

(26) Clifford, M. N.; Johnston, K. L.; Knight, S.; Kuhnert, N. Hierarchical scheme for LC-MS^n identification of chlorogenic acids. *J. Agric. Food Chem.* **2003**, *51*, 2900−2911.

(27) Zhao, T.; He, J.; Wang, X.; Ma, B.; Wang, X.; Zhang, L.; Zhang, X. Rapid detection and characterization of major phenolic compounds in Radix *Actinidia chinensis* Planch by ultra-performance liquid chromatography tandem mass spectrometry. *J. Pharm. Biomed Anal.* **2014**, *98*, 311−320.

(28) Rodrigues, N. P.; Bragagnolo, N. Identification and quantification of bioactive compounds in coffee brews by HPLC-DAD-MS^n. *J. Food Compost Anal.* **2013**, *32*, 105−115.

(29) Parveen, I.; Threadgil, M. D.; Hauck, B.; Donnison, I.; Winters, A. Isolation, identification and quantitation of hydroxycinnamic acid conjugates, potential platform chemicals, in the leaves and stems of *Miscanthus* × *giganteus* using LC-ESI-MS^n. *Phytochemistry.* **2011**, *72*, 2376−2384.

(30) Jaiswal, R.; Deshpande, S.; Kuhnert, N. Profiling the chlorogenic acids of *Rudbeckia hirta*, *Helianthus tuberosus*, *Carlina acaulis* and *Symphyotrichum novae-angliae* leaves by LC-MS^n. *Phytochem Anal.* **2011**, *22*, 432−441.

(31) Zhang, J. Y.; Cai, W.; Li, Y.; Liu, R.; Wang, Z.; Qiao, Y. Rapid characterization of chlorogenic acids analogues in *Artemisia younghusbandii* using HPLC/LTQ-Orbitrap MS^n coupled with MDF data mining technology. *J. Chin Mass Spectrom Soc.* **2015**, *36*, 321−327.

(32) Jaiswal, R.; Matei, M. F.; Golon, A.; Witt, M.; Kuhnert, N. Understanding the fate of chlorogenic acids in coffee roasting using mass spectrometry based targeted and non-targeted analytical strategies. *Food Funct.* **2012**, *3*, 976−984.

https://doi.org/10.1021/acsomega.2c02899
ACS Omega 2022, 7, 25675−25685