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Analysis of Flavonoids and Hydroxycinnamic Acid Derivatives in Feng Liao of Chinese Herbal Medicine Using UPLC-PDA-ESI(-)-HRMS^n Method

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ABSTRACT

Flavonoids and hydroxycinnamic acid derivatives were extracted from Feng Liao of Chinese herbal medicine with water and purified by Sephadex LH-20 column chromatography. An UPLC-PDA-ESI(-)-HRMS^n method for the extract has been set. Fifty two compounds were totally detected, which included 34 components of baicalein derivatives, isorhamnetin derivatives, kaempferol derivatives, myricetin derivatives, quercetin derivatives, flavanols and their oligomers and 9 hydroxycinnamic acid derivatives. Twenty six compounds have not been reported in Feng Liao before. This research provides an effective way in the identification of compounds in plant materials using LCMS-IT-TOF.

KEYWORDS

Feng Liao, Herbal medicine, LCMS-IT-TOF.

INTRODUCTION

Acute or chronic gastroenteritis, the digestive system disease, is common among the aged people. The illness is more with aged. However, the gastroenteritis mechanism of pathogenesis has not yet been completely understood at present and the treatment was by comprehensive method. Traditional Chinese medicine (TCM) possessed a unique system to diagnose and cure illness. Traditional Chinese medicine approach is fundamentally different from that of Western medicine. Based on traditional Chinese medicine, traditional Chinese herbal medicine contain multiple botanicals, which contains many compounds. These compounds may be relevant to the medicine's putative activity with synergistic effect [1]. Modern medical research found that Feng Liao of Chinese herbal medicine is rich in many active constituents and have greatly positive effect on gastroenteritis disease.

There were constituted of *Daphniphyllum calycinum* Benth. and *Polygonum hydropiper* Linn. In Feng Liao. The shrub *Daphniphyllum calycinum* Benth. (the genus of Daphniphyllum), mainly grown in southern China, contains many diverse Daphniphyllum alkaloids and flavonoids. It was used for the healing of wounds and as an anti-inflammatory remedy [2-4]. *Polygonum hydropiper* Linn. (family polygonaceae), has a long history of herbal use, including anti-inflammatory, carminative, astringent, diuretic, diaphoretic and stomachic [5,6]. This plant is also rich in flavonoids. Thus, it is necessary to identify the flavonoids in Feng Liao, in order to improve the scientific knowledge of cure mechanism, quality control and other research.

In this study, we established a systematic way in the confirmation of chemical structures in Feng Liao of Chinese Herbal Medicine using UPLC-PDA-ESI(-)-HRMS^n (LCMS-IT-TOF) method. This method is different from the others. It should offer as much as 10th MS^n fragmentation and the ratio of mass to charge for each ion is achieving high-resolution mass. The more MS^n fragmentation we get, the result should be more accurate. We successfully proved that LCMS-IT-TOF is a highly efficient and elaborate technique for chemical identification. Using this strategy, 43 flavonoids and hydroxycinnamic acid were characterized, most of them were not reported before in Feng Liao.

EXPERIMENTAL

All reagents were of analytical reagent (AR) grade. Methanol, formic acid and acetonitrile were of HPLC grade (Fisher Scientific, Fair Lawn, USA). Water was purified using a Pure Lab Ultra system (ELGA, HP14 3BY, UK). Sephadex LH-20 (dry particle size 25-100 µm) was purchased from GE Healthcare BioSciences AB (Uppsala, Sweden).

Preparation of extract: The materials *Daphniphyllum calycinum* Benth. (A) and *Polygonum hydropiper* Linn. (B) were purchased from local drug store in China. They were dried by freeze-drying machine and grounded into powders, then passed through 60 mesh sieves prior to extraction.

The powder (90 g) of Feng Liao (w/w, A:B = 2:1) was extracted with 10 volumes of waters in an ultrasonicator bath at room temperature for 1 h. The supernatant was collected after centrifugation (10000 rpm, 10 min), then concentrated up to dryness using a vacuum rotary evaporator at 80 °C. 10 g extract was dissolved in 2 volumes of water, filtered through 0.45 µm membrane.

The procedure of column chromatography was carried out according to the method described by Yang *et al.* with a few modifications [7]. The crude extract (3 mL) was loaded onto a 26 mm i.d. × 600 mm length glass column packed with Sephadex LH-20; the resin was swelled in H_2O for 24 h and equilibrated with H_2O before use. Finally, the column was washed with 10 % methanol (200 mL) first, then eluted with 50 % methanol (200 mL). The speed of flow was 1.0 mL min^{-1}. The elution was again concentrated till to dryness using a vacuum rotary evaporator at 50 °C and redissolved in 2 volumes of methanol, then filtered with teflon membrane and analyzed with UPLC-PDA-ESI(-)-HRMS^n.

UPLC-PDA-ESI(-)-HRMS^n analysis: The crude extract, was analyzed using an UPLC-PDA-ESI(-)-HRMS^n (LCMS-IT-TOF) instrument to determine the UV absorption characteristics, their exact molecular mass, calculated elemental composition and MS^n fragmentation patterns of the compounds. The data acquisition and processing was operated using LCMS solution version 3.0 software. A LCMS-IT-TOF (Shimadzu, Kyoto, JP) system coupled with ultra high pressure pumps, an autosampler, a photo-diode array detector (PDA), a column compartment (kept at 45 °C) and a high resolution IT-TOF mass spectrometry with an ESI negative mode was used. Separation was achieved on a HSS-T3 column (1.7 µm, 100 mm × 2.1 mm i.d., Waters) using a mobile phase composed by water/acetic acid (0.1 %, v/v) and CH_3CN (B) at a flow rate of 0.3 mL/min. The following gradient program was used: 85 % A (0 min), 50 % A (20 min), 10 % A (22 min), 5 % A (27-35 min) and 85 % A (35.10–41 min). The injection volume was 2 µL. The PDA was set to 254, 280 and 360 nm to record peaks and UV-visible spectra were recorded from 190 to 800 nm.

The *m/z* range of 100-400 and 400-1100 were analyzed separately. The acquisition of MS^n data was made in an auto MS^n mode. The conditions were as follows: Probe voltage, -3.5 Kv; nebulizer and drying gas (N_2) flow rate and pressure, 1.5 L/min and 100 kpa; Heat block temperature, 200 °C; CDL temperature, 250 °C; collision gas (Ar); IT area vacuum, 1.0 × 10^{-2} Pa; TOF area vacuum, 4 × 10^{-4} Pa; detector voltage, 1.80 kV; energy, 50 % (MS^2), 30 % (MS^3, MS^4); collision gas, 50 %; Q (frequency), 0.169 (30.0 kHz); loop time, 0.78 sec; ion accumulation time, 30 msec.

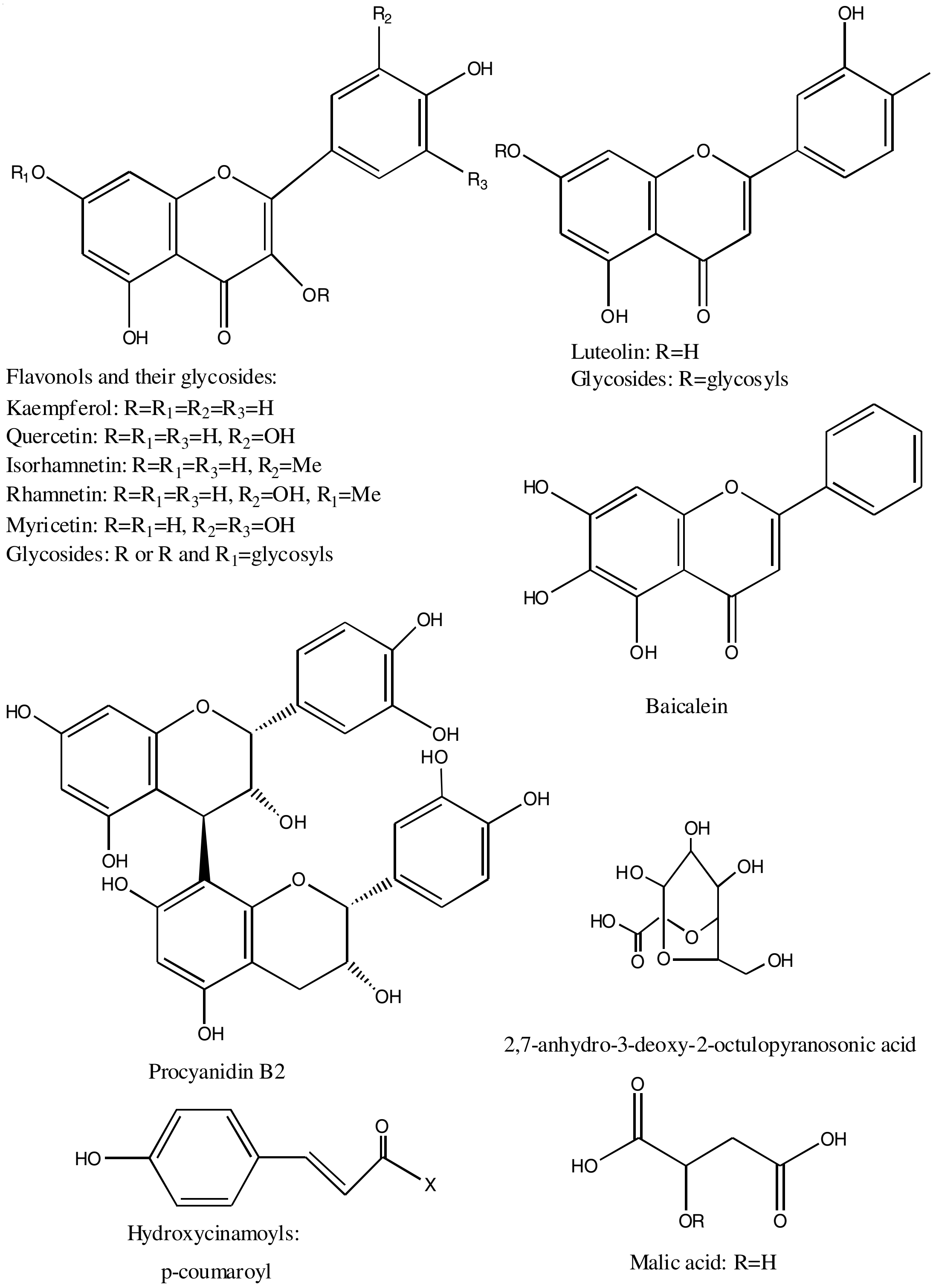
RESULTS AND DISCUSSION

Identification of flavonol glycosides: The structures of compounds identified in Feng Liao of Chinese herbal medicine are shown in Fig. 1. The UPLC-UV-visible (254 nm) profiles of flavonoids and hydroxycinnamic acid obtained from the extracts are presented in Fig. 2. Nineteen glucosylated flavonols were successfully identified according to their retention time, UV-visible wavelength maxima, MS^n ion characteristics, HRMS profiles and calculated elemental composition. The corresponding chemical information of the identified compounds is listed in Tables 1 and 1a.

On the basis of previous conclusions, the identified flavonol glycosides were analyzed according to their UV wavelength maxima (λ_{max}) and MS^n fragments. The UV spectra of flavonol glycosides showed λ_{max} at approximately 240-270 nm (band II) and 320-380 nm (band I), respectively [8-12].

During the formation of flavonol derivatives, glycosylation initially occurs at 3-OH and then at 7-OH. During MS^n analysis with negative ionization, the major MS^2 product ions resulting from the loss of 7-O-glycosides were first detected, followed by the loss of 3-O-glycosides. According to the size of the MS^n fragments, losses of rhamnosyl (146 Da), glucosyl (162 Da) and rutinosyl (308 Da) were detected [8]. The presence of rhamnosyl (146.0579 Da) or *p*-courmaryl (146.0368 Da) and glucosyl (162.0528 Da) or caffeoyl (162.0317 Da) can be obtained from HRMS analysis, which is in agreement with alkaline hydrolysis characteristics. HRMS is obviously more effective.

Conjugates of kaempferol, quercetin, baicalein, myricetin, luteolin and isorhamnetin were characterized according to the sugar moieties attached to their aglycones (at 285, 301, 269, 317, 285 and 315, respectively). Glucosylated kaempferol mainly shows MS^n fragments at *m/z* 285, 255, 227, 151, 107. glucosylated quercetin at *m/z* 301, 271, 255, 229, 179, 151, 107. glucosylated isorhamnetin at *m/z* 315, 300, 286, 285, 271, 243. glucosylated myricetin at *m/z* 317, 287, 271, 179, 151.



Flavonols and their glycosides:
Kaempferol: R=R_1=R_2=R_3=H
Quercetin: R=R_1=R_3=H, R_2=OH
Isorhamnetin: R=R_1=R_3=H, R_2=Me
Rhamnetin: R=R_1=R_3=H, R_2=OH, R_1=Me
Myricetin: R=R_1=H, R_2=R_3=OH
Glycosides: R or R and R_1=glycosyls

Luteolin: R=H
Glycosides: R=glycosyls

Baicalein

Procyanidin B2

2,7-anhydro-3-deoxy-2-octulopyranosonic acid

Hydroxycinamoyls:
p-coumaroyl

Malic acid: R=H

Fig. 1. Structure of compounds identified in Feng Liao of Chinese herbal medicine

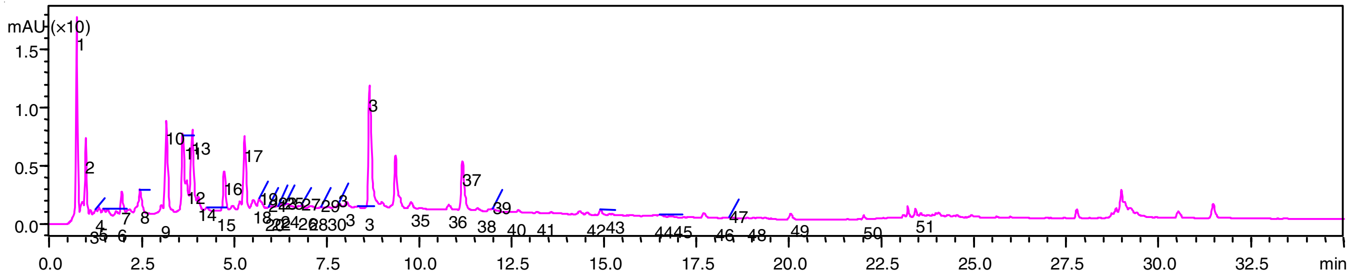


Fig. 2. LC chromatogram (254 nm) for the extract in Feng Liao of Chinese herbal medicine

TABLE-1
UPLC-PDA-ESI(-)-HRMS^n DATA AND PUTATIVE IDENTIFICATION OF FLAVONOIDS AND HYDROXYCINNAMIC ACID DERIVATIVES IN FENG LIAO OF CHINESE HERBAL MEDICINE

Peak	t_R (min)	$[M–H]^–$ (*m/z*)	Formula	Error (ppm)	DBE	MS^2 fragments (*m/z*) (%)
1	0.736	593.1468	$C_{27}H_{30}O_{15}$	-7.42	13	473 (19), 383 (29), 353 (56), 285 (100), 255 (32)
2	0.980	755.2012	$C_{33}H_{40}O_{20}$	-3.71	14	593 (100)
3	1.103	771.1959	$C_{33}H_{40}O_{21}$	-3.89	14	609 (100), 301 (54)
4	1.250	431.1009	$C_{21}H_{20}O_{10}$	7.19	7	269 (19), 251 (100), 225 (30), 207 (17), 165 (34), 147 (28)
5	1.337	577.1336	$C_{30}H_{26}O_{12}$	-2.77	18	451 (29), 425 (35), 407 (100), 299 (16), 289 (83), 245 (20)
6	1.462	755.2008	$C_{33}H_{40}O_{20}$	-4.24	14	593 (100), 285 (18)
7	1.951	421.1739	$C_{18}H_{30}O_{11}$	5.70	4	289 (100), 187 (68)
8	2.447	755.2021	$C_{33}H_{40}O_{20}$	-2.52	14	609 (7), 343 (14), 301 (42), 300 (100), 271 (43), 255 (21)
9	3.030	609.1448	$C_{27}H_{30}O_{16}$	-4.43	13	301 (30), 300 (100), 271 (41), 255 (23)
10	3.157	739.2082	$C_{33}H_{40}O_{19}$	-1.22	14	593 (9), 575 (54), 429 (7), 393 (20), 339 (14), 327 (47), 309 (19), 284 (98), 285 (70), 255 (100)
11	3.610	609.1461	$C_{27}H_{30}O_{16}$	4.92	13	301 (100), 300 (57), 271 (34), 255 (22)
12	3.712	463.0907	$C_{21}H_{20}O_{12}$	5.4	12	318 (13), 317 (60), 316 (100), 271 (31), 287 (9)
13	3.858	593.1535	$C_{27}H_{30}O_{15}$	4.05	13	429 (16), 327 (10), 309 (7), 285 (57), 284 (100), 255 (72)
14	4.021	463.0888	$C_{21}H_{20}O_{12}$	1.3	12	301 (100), 300 (49), 271 (18), 255 (15), 179 (8), 151 (6)
15	4.259	615.0967	$C_{28}H_{24}O_{16}$	-4.06	17	301 (100)
16	4.725	593.1587	$C_{27}H_{30}O_{15}$	12.64	13	285 (100), 284 (13), 255 (11)
17	5.277	447.0959	$C_{21}H_{20}O_{11}$	5.8	12	301 (100), 300 (53), 271 (16), 255 (13), 179 (16), 151 (9)
18	5.509	477.1037	$C_{22}H_{22}O_{12}$	0.21	11	315 (48), 314 (100), 300 (19), 299 (15), 286 (19), 285 (58), 271 (48), 270 (17), 243 (24)
19	5.666	517.2647	$C_{25}H_{42}O_{11}$	-1.35	5	371 (100), 209 (9)
20	5.857	609.1431	$C_{27}H_{30}O_{16}$	-5.25	13	301 (100)
21	5.939	447.0892	$C_{21}H_{20}O_{11}$	-9.17	12	285 (100), 284 (16), 257 (4), 151 (17)
22	6.022	677.4955	$C_{47}H_{66}O_3$	2.36	15	563 (80), 564 (40), 451 (50), 450 (48), 433 (26), 337 (78), 338 (84), 339 (21), 320 (27), 225 (100), 224 (28), 207 (6)
23	6.187	615.0959	$C_{28}H_{24}O_{16}$	-5.37	17	317 (100)
24	6.253	447.0901	$C_{21}H_{20}O_{11}$	-7.16	12	315 (28), 314 (94), 285 (100), 271 (37), 243 (16)
25	6.387	615.0977	$C_{28}H_{24}O_{16}$	-2.44	17	463 (18), 317 (100)
26	6.740	633.1674	$C_{33}H_{30}O_{13}$	9.48	19	487 (100), 469 (4), 307 (15)
27	6.835	677.4957	$C_{40}H_{70}O_8$	0.44	6	559 (2), 453 (7), 452 (39), 451 (100), 333 (10), 225 (79)
28	7.036	461.1061	$C_{22}H_{22}O_{11}$	6.07	11	315 (72), 314 (100), 285 (39), 271 (41), 243 (16)
29	7.350	435.0936	$C_{20}H_{20}O_{11}$	-3.22	11	315 (100), 281 (21), 153 (23), 152 (15)
30	7.535	489.1039	$C_{23}H_{22}O_{12}$	0.2	13	301 (42), 300 (100), 271 (27), 255 (18)
31	7.823	599.1031	$C_{28}H_{24}O_{15}$	-1.8	16	301 (100)
32	8.033	599.1027	$C_{28}H_{24}O_{15}$	-2.5	16	447 (25), 301 (100), 300 (15)
33	8.347	489.1036	$C_{23}H_{22}O_{12}$	-0.4	13	447 (11), 301 (61), 300 (100), 271 (23), 255 (17), 179 (8)
34a	8.658	285.0412	$C_{15}H_{10}O_6$	2.46	11	243 (57), 241 (53), 217 (39), 199 (57), 175 (100), 151 (21), 133 (9), 107 (8)
34b	8.658	301.0338	$C_{15}H_{10}O_7$	-5.32	11	179 (79), 151 (100)
35	9.776	315.0483	$C_{16}H_{12}O_7$	-8.57	11	300 (100)
36	10.795	269.0458	$C_{15}H_{10}O_5$	2.97	11	225 (68), 149 (100), 107 (10)
37	11.167	285.0427	$C_{15}H_{10}O_6$	7.72	11	257 (14), 229 (46), 165 (25), 151 (100), 107 (16)
38	11.577	779.2085	$C_{42}H_{36}O_{15}$	13.35	25	633 (69), 487 (100), 307 (8)
39	11.980	315.0537	$C_{16}H_{12}O_7$	8.57	11	300 (100), 151 (2)
40	12.378	265.1428	$C_{15}H_{22}O_4$	-6.41	5	221 (100), 191 (12)
41	13.189	821.2275	$C_{44}H_{38}O_{16}$	-2.80	26	675 (100), 653 (35), 633 (45), 529 (70), 487 (46), 453 (34)
42	14.541	925.2443	$C_{51}H_{42}O_{17}$	10.16	31	779 (67), 633 (100), 615 (19), 487 (28), 453 (23)
43	14.902	955.2516	$C_{52}H_{44}O_{18}$	6.39	31	809 (83), 663 (100), 453 (13)
44	16.358	237.1483	$C_{14}H_{22}O_3$	2.95	5	191 (100)
45	16.502	997.2710	$C_{54}H_{46}O_{19}$	14.94	32	851 (97), 809 (30), 705 (100), 663 (46), 527 (26), 453 (16), 323 (9)
46	18.008	$[504.1318]^{2-}$	nc	-0.40		863 (15), 717 (36), 676 (73), 675 (40), 530 (26), 529 (43), 467 (17), 307 (100), 235 (13), 229 (17)
47	18.395	$[519.1393]^{2-}$	nc	1.25		893 (15), 748 (28), 747 (63), 705 (78), 446 (21), 323 (61), 307 (24), 305 (21), 228 (33), 223 (18), 179 (100)
48	18.873	1069.2931	$C_{57}H_{50}O_{21}$	14.96	33	923 (100), 893 (45), 881 (58), 748 (32), 747 (96), 705 (56), 569 (13), 531 (12)
49	20.043	323.0915	$C_{19}H_{16}O_5$	-3.10	12	308 (12), 265 (5), 264 (100),
50	22.021	441.1155	$C_{30}H_{18}O_4$	5.21	22	426 (31), 359 (99), 344 (100)
51	23.422	467.3156	$C_{30}H_{44}O_4$	-2.35	9	423 (100)

TABLE-1a
UPLC-PDA-ESI(-)-HRMS^n DATA AND PUTATIVE IDENTIFICATION OF FLAVONOIDS AND HYDROXYCINNAMIC ACID DERIVATIVES IN FENG LIAO OF CHINESE HERBAL MEDICINE

Peak	MS^3 fragments (*m/z*) (%)	MS^4 fragments (*m/z*) (%)	λ_{max} (nm)	Tentative identification
1	284 (100), 255 (22)		273	Kaempferol-3-O-rhamnoside-7-O-glucoside[a]
2	447 (12), 429 (67), 327 (9), 285 (84), 284 (100), 257 (14), 255 (40), 227 (12)		280, 336	Kaempferol-3-O-rutinoside-7-O-glucoside[a]
3	343 (5), 301 (100), 271 (4), 255 (3)	301 (100), 251 (3), 179 (4)	265, 349	Quercetin-3-O-rutinoside-7-O-glucoside[a]
4	233 (20), 191 (100), 147 (80)		270	Baicalein-O-glucoside[a]
5			268	Procyanidin B2[a]
6	285 (100)		278, 350	Kaempferol-3-O-rutinoside-7-O-glucoside[a]
7	187 (100)	169 (7), 143 (77), 125 (55), 113 (100), 99 (70), 73 (63)	270	Unknown
8	271 (100), 255 (62)		266, 336	Quercetin-3-O-rutinoside-7-O-rhamnoside[a]
9	271 (100), 255 (72), 179 (4), 151 (3)		268, 344	Quercetin-O-rutinoside
10	227 (68), 213 (35), 211 (100), 199 (19)		286, 355	Kaempferol-3-O-rutinoside-7-O-rhamnoside[a]
11	271 (50), 255 (31), 179 (84), 151 (100)	107 (100)	256, 349	Quercetin-O-rutinoside
12	287 (40), 271 (100), 270 (17), 242 (15), 179 (15), 151 (7)		263, 344	Myricetin-3-O-rhamnoside[a]
13	255 (100), 227 (8), 151 (4)		268, 350	Kaempferol-O-rutinoside
14	271 (50), 255 (27), 229 (12), 179 (60), 151 (100), 107 (10)		279, 353	Quercetin-3-O-glucoside
15	271 (7), 229 (6), 179 (80), 151 (100)		269, 350	Quercetin-3-O-(O-galloyl)glucoside
16	286 (100), 257 (42), 255 (21), 241 (18), 229 (42), 227 (9), 213 (17), 211 (19), 199 (15)		268, 350	Kaempferol-O-rutinoside
17	300 (100), 271 (50), 255 (32), 179 (81), 151 (90)		254, 345	Quercetin-3-O-rhamnoside
18	300 (46), 299 (8), 286 (15), 285 (75), 272 (12), 271 (100)	243 (100)	268, 350	Isorhamnetin-3-O-glucoside
19	209 (53), 161 (100), 159 (85)		270	Unknown
20	271 (10), 229 (8), 179 (85), 151 (100)	151 (100)	272, 325	Quercetin-O-rutinoside
21	284 (100), 257 (82), 151 (39)		273	Kaempferol-3-O-glucoside
22	224 (63), 207 (100), 208 (21), 137 (13), 129 (30), 130 (4), 112 (7), 95 (7)		224, 272	Unknown
23	316 (17), 179 (100), 151 (57)	179 (100), 151 (47)	268, 353	Myricetin-O-(O-galloyl)rhamnoside[a]
24	270 (100), 241 (16), 175 (24), 151 (8)		278, 350	Isorhamnetin-O-pentoside[a]
25			268, 353	Myricetin-O-(O-galloyl)rhamnoside[a]
26	307 (100), 265 (30), 235 (36), 163 (36)	145 (100)	273	Benzoyl-*p*-dicoumaryl-2, 7-anhydro-3-deoxy-2-octulopyranosonic acid[a]
27	433 (12), 333 (12), 226 (9), 225 (100)	225 (100), 207 (15), 143 (4), 83 (63)	225, 275	Unknown
28	300 (100), 285 (87), 271 (90), 243 (28)		278, 350	Isorhamnetin-3-O-rhamnoside
29	153 (100), 152 (20)	152 (100), 109 (28)	227, 275	Putative Isorhamnetin derivative
30	271 (100), 255 (71), 227 (11), 179 (4), 151 (33)		228, 275	Quercetin-3-O-acetylrhamnoside[a]
31	229 (8), 179 (100), 151 (90)	151 (100)	269	Quercetin-3-O-(O-galloyl) rhamnoside[a]
32	300 (17), 257 (10), 255 (4), 229 (8), 179 (51), 151 (100)	107 (100)	273	Quercetin-3-O-(O-galloyl) rhamnoside[a]
33	271 (100), 255 (66), 179 (14), 151 (26)		228, 275	Quercetin-3-O-acetylrhamnoside[a]
34a			254	Luteolin
34b	107 (100)		226	Quercetin
35			226, 268	Isorhamnetin
36			270, 318	Baicalein[a]
37	107 (100), 83 (99)		231, 268, 364	Kaempferol
38	341 (11), 307 (100), 265 (19), 235 (70), 217 (23), 163 (6)	277 (17), 235 (28), 205 (21), 145 (30), 163 (28), 119 (14), 113 (17)	232, 278, 312	Benzoyl-*p*-tricoumaryl-2, 7-anhydro-3-deoxy-2-octulopyranosonic acid[a]
39			232, 274	Rhamnetin
40	221 (100), 203 (48), 193 (67), 177 (10)			Unknown
41	633 (14), 529 (100), 487 (43), 469 (17), 453 (17)	487 (70), 307 (40)	278, 325	Tetracoumaryl-2, 7-anhydro-3-deoxy-2-octulopyranosonic acid[a]
42	487 (5), 469 (19), 453 (2), 307 (12), 235 (7)	487 (100), 307 (4), 235 (2), 163 (7)	310	Benzoyl-*p*-tetracoumaryl-2, 7-anhydro-3-deoxy-2-octulopyranosonic acid[a]
43	517 (48), 499 (21), 485 (100), 323 (59), 281 (38), 251 (31)		313	Octulopyranosonic acid derivative[a]
44				Unknown
45	663 (100), 645 (20), 527 (38), 485 (27), 323 (19)		311	Octulopyranosonic acid derivative[a]
46				Octulopyranosonic acid derivative[a]
47				Octulopyranosonic acid derivative[a]
48				Octulopyranosonic acid derivative[a]
49				Unknown
50				Unknown
51				Unknown

[a]Compound identified for the first in Feng Liao of Chinese herb medicine.

In summary, 19 glucosylated flavonols were successfully identified, including baicalein-O-glucoside, isorhamnetin-3-O-glucoside, isorhamnetin-3-O-rhamnoside, kaempferol-3-O-glucoside, kaempferol-3-O-rhamnoside-7-O-glucoside, kaempferol-O-rutinoside, kaempferol-3-O-rutinoside-7-O-glucoside, myricetin-3-O-rhamnoside, quercetin-3-O-glucoside, quercetin-3-O-rhamnoside, quercetin-3-O-rutinoside and quercetin-3-O-rutinoside-7-O-glucoside. They were consisted of 1 glycosylated baicalein, 3 glycosylated isorhamnetin, 7 glycosylated kaempferol, 1 glycosylated myricetin and 7 glycosylated quercetin [8-13].

LCMS-IT-TOF (Fig. 3) is the hybrid mass spectrometry of Ion Trap and Time of Flight, which could be given at most 10th fragmentation of high resolution mass. The structure of compounds was effectively confirmed by formula predict software. Chemical formula, for produced ion, was calculated based on high resolution mass and for molecular ion, based on high resolution mass, isotopic peak and formula of fragments. The more MS^n fragmentation, the result should be more accurate.

Obviously, peak 10 (λ_{max} = 286, 355 nm) was identified as glucosylated flavonol. $C_{33}H_{40}O_{19}$ was considered as the most accurate formula, according to DBE, C/H, C/O, score and isotope score (Table-2).

Peak 10 (t_R = 3.157 min) was identified as kaempferol-3-O-rutinoside-7-O-rhamnoside based on the $[M-H]^-$ (739.2082 Da, $C_{33}H_{40}O_{19}$, error -1.22 ppm), $[M-H-rhamnosyl]^-$ (593.1505 Da, $C_{27}H_{30}O_{15}$, error -1.88 ppm, from loss of 146.0579 Da, $C_6H_{10}O_4$ for rhamnosyl, instead of loss of 146.0368 Da, $C_9H_6O_2$ for *p*-courmaryl), $[M-H-rhamnosyl-rutinosyl]^-$ (285.0379 Da, $C_{15}H_{10}O_6$, error -9.12 ppm) and $[M-H-rhamnosyl-rutinosyl-H]^-$ (284.0290 Da, $C_{15}H_9O_6$, error -12.67 ppm), respectively. Other major $MS^{2,3}$ fragments at *m/z* 575, 429, 393, 327, 309, 255, 227, 213, 211 and 199 highly supported the confirmation of this compound, which had been revealed, but not in these plants. In this way, the structure for the compound can be figured out easily (Table-3).

Peak 1 (t_R = 0.736 min, $[M-H]^-$ = 593.1468 Da, $C_{27}H_{30}O_{15}$, error -7.42 ppm) provided the largest peaks in chromatograms of Feng Liao extract and had an MS^2 fragment at *m/z* 473 $[M-H-C_4H_8O_4]^-$, 383 $[M-H-120-C_3H_6O_3]^-$, 353 $[M-H-120-90-CH_2O]^-$, 285 $[M-H-glucosyl-rhamnosyl]^-$, 284 $[M-H-162-146-H]^-$ and 255 $[M-H-162-146-CH_2O]^-$. Considering with elution time, it was putatively identified as kaempferol-3-O-rhamnoside-7-O-glucoside, which was previously reported [14-16].

Peak 2, 6 with different retention times (t_R = 0.980 and 1.462 min) had high-resolution masses close to 755.2040 Da ($C_{33}H_{40}O_{20}$, error less than 4.24 ppm) and both had similar base fragments at *m/z* 593 $[M-H-glucosyl]^-$, 285 $[M-H-162-rutinosyl]^-$, or 284 $[M-H-162-308-H]^-$. They had UV band II maximum absorbance at 278-280 nm and band I 336-350 nm, suggesting kaempferol-3-O-rutinoside-7-O-glucoside. Peak 2 also shows an MS^3 fragments at *m/z* 447 $[M-H-162-rhamnosyl]^-$, 429 $[M-H-162-146-H_2O]^-$, 327 $[M-H-162-146-18-C_4H_6O_3]^-$, 285, 284, 257, 255 and 227, respectively. This compound marks the first report in Feng Liao.

One flavonoid of peak 3 (t_R = 1.103 min, $[M-H]^-$ = 771.1959 Da, $C_{33}H_{40}O_{21}$, error -3.89 ppm) had a base MS^2 fragment at *m/z* 609 (calculated as 609.1443 Da, with error of 2.95 ppm for $C_{27}H_{29}O_{16}$, from loss of 162.0528 Da, $C_6H_{10}O_5$ for glucosyl, instead of loss of 162.0317 Da, $C_9H_6O_3$, caffeoyl) and a base MS^3 fragment at *m/z* 301 $[M-H-162-rutinosyl]^-$ with λ_{max} at 265 and 349 nm, which suggest it was characterized as quercetin-3-O-rutinoside-7-O-glucoside. Altogether, its major MS^3

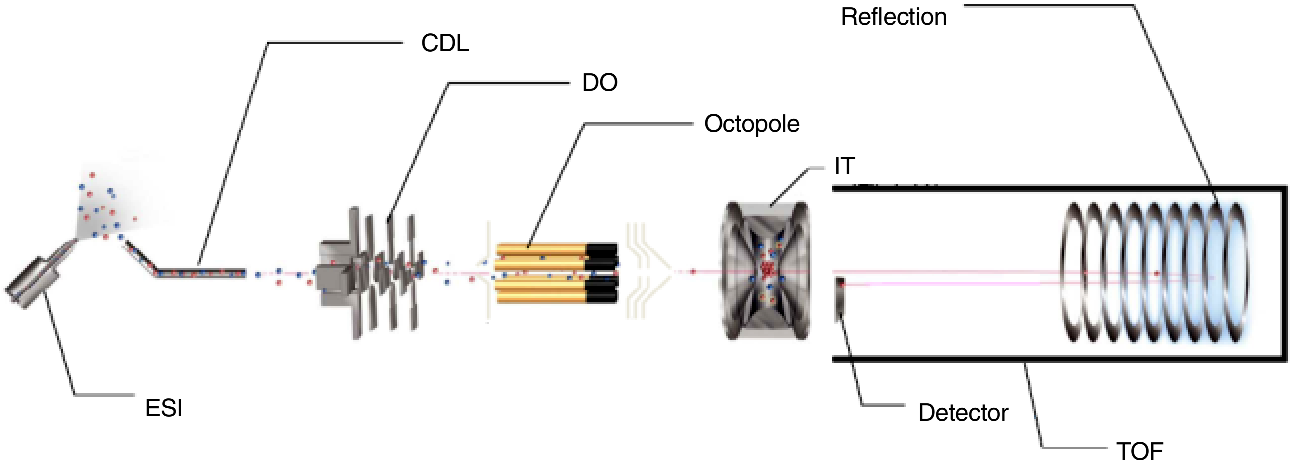


Fig. 3. LCMS-IT-TOF system

TABLE-2
CALCULATED CHEMICAL FORMULA OF MS^1 FOR PEAK 10

Rank	Score	Formula (M)	Ion	Meas. (*m/z*)	Pred. (*m/z*)	Error (ppm)	Iso score	DBE
1	40.71	$C_{33}H_{40}O_{19}$	$[M-H]^-$	739.2082	739.2091	-1.22	40.93	14.0
2	35.56	$C_{40}H_{36}O_{14}$	$[M-H]^-$	739.2082	739.2032	6.76	49.12	23.0
3	27.46	$C_{51}H_{32}O_6$	$[M-H]^-$	739.2082	739.2126	-5.95	34.11	36.0
4	17.62	$C_{22}H_{44}O_{27}$	$[M-H]^-$	739.2082	739.1997	11.50	48.95	1.0
5	15.45	$C_{26}H_{44}O_{24}$	$[M-H]^-$	739.2082	739.2150	-9.20	32.19	5.0
6	7.95	$C_{44}H_{36}O_{11}$	$[M-H]^-$	739.2082	739.2185	-13.93	26.92	27.0

TABLE-3
CALCULATED CHEMICAL FORMULA OF MS^n FRAGMENTS FOR PEAK 10

No.	Formula (M)	Ion	Meas. (*m/z*)	Pred. (*m/z*)	Error (ppm)	DBE
1	$C_{33}H_{40}O_{19}$	$[M-H]^-$	739.2082	739.2091	-1.22	14
2	$C_{27}H_{30}O_{15}$	739-146 (-rhamnosyl)	593.1505	593.1512	-1.18	13
3	$C_{27}H_{28}O_{14}$	593-18 (-H_2O)	575.1400	575.1406	-1.04	14
4	$C_{21}H1_8O_{10}$	575-146 (-rhamnosyl)	429.0861	429.0827	7.92	13
5	$C_{21}H_{14}O_8$	429-36 (-2H_2O)	393.0588	393.0616	-7.12	15
6	$C_{17}H_{12}O_7$	393-66 (-C_4H_2O)	327.0490	327.0510	-6.12	12
7	$C_{17}H_{10}O_6$	327-18 (-H_2O)	309.0401	309.0405	1.29	13
8	$C_{15}H_{10}O_6$	593-308 (-rutinosyl)	285.0379	285.0405	-9.12	11
9	$C_{15}H_9O_6$	593-309 (-rutinosyl-H)	284.0290	284.0326	-12.67	11.5
10	$C_{14}H_8O_5$	285-30 (-CH_2O)	255.0275	255.0299	-9.41	11
11	$C_{13}H_8O_4$	255-28 (-CO)	227.0350	227.0350	0.00	10
12	$C_{12}H_6O_4$	227-14 (-CH_2)	213.0184	213.0193	-4.22	10
13	$C_{13}H_8O_3$	255-44 (-CO_2)	211.0386	211.0401	-7.11	10
14	$C_{12}H_8O_3$	227-28 (-CO)	199.0410	199.0401	4.52	9

fragments at *m/z* 343 $[M-H-162-C_{10}H_{18}O_8]^-$, 301, 271 (from loss of methanol at *m/z* 301), 255 (from loss of CH_2O_2 at *m/z* 301) and MS^4 fragment at *m/z* 179 (from loss of $C_{10}H_{18}O_8$ at *m/z* 301) also suggested the aglycone was quercetin [8].

Peak 4 exhibited $[M-H]^-$ ion at *m/z* 431.1009 (t_R = 1.250 min, $C_{21}H_{20}O_{10}$, error 7.19 ppm) and produced ion at *m/z* 269, due to the loss of glucosyl. Further MS^{2,3} fragmentation produced characteristic ions of baicalein: at *m/z* 251 $[M-H-162-H_2O]^-$, 225 $[M-H-162-CO_2]^-$, 207 $[M-H-162-44-H_2O]^-$, 165 $[M-H-162-44-18-C_2H_2O]^-$, 233 $[M-H-162-2H_2O]^-$, 191 $[M-H-162-2H_2O-C_2H_2O]^-$, 147 $[M-H-162-H_2O-C_7H_4O]^-$, which is in agreement with the literature data. Thus peak 4 was characterized as baicalein-O-glucoside [8,16].

Peak 8 (t_R = 2.447 min, $[M-H]^-$ = 755.2021 Da, $C_{33}H_{40}O_{20}$, error -2.52 ppm) was plausibly identified as quercetin-3-O-rutinoside-7-O-rhamnoside, which characteristic UV spectrum with λ_{max} was at 266 and 336 nm. Further fragmentation showed an MS^2 fragments at *m/z* 609 (from loss of 146 Da, rhamnosyl), 343 $[M-H\text{-rhamnosyl-}C_{10}H_{18}O_8]^-$, 301 $[M-H-146\text{-rutinosyl}]^-$, 300 $[M-H-146-308-H]^-$ and an MS^3 fragments at *m/z* 271 $[M-H-146-308-1-CHO]^-$, 255 $[M-H-146-308-1-CHO_2]^-$. This fragmentation pattern was similar to that previous report [17]. Similary, peak 9, 11, 20 (t_R = 3.030, 3.610 and 5.857 min, $C_{27}H_{30}O_{16}$, error less than 5.25 ppm), with close λ_{max} at 256-268 and 344-349 nm, had a base MS^2 fragment at *m/z* 301(from loss of 308 Da, rutinosyl), a sequential MS^3 fragments at *m/z* 271 $[M-H-308-CH_2O]^-$, 255 $[M-H-308-CH_2O_2]^-$, 179 $[M-H-308-C_7H_6O_2]^-$, 151 $[M-H-308-122-CO]^-$ and a base MS^4 fragment at *m/z* 107 $[M-H-308-122-28-CO_2]^-$. These suggest them to be isomers of quercetin-O-rutinoside. This compound has been previously characterized in rorippa indica [12].

Peak 12 (t_R=3.712 min, $[M-H]^-$ = 463.0907 Da) had a deprotonated molecular ion of $C_{21}H_{20}O_{12}$ (error 5.4 ppm). It showed a typical MS^2 fragments at *m/z* 317 $[M-H\text{-rhamnosyl}]^-$, 316 $[M-H-146-H]^-$, 287 $[M-H-146-1-CHO]^-$, 271 $[M-H-146-1-CHO_2]^-$ and an MS^3 fragments at *m/z* 242 $[M-H-146-1-45-CHO]^-$, 179 $[M-H-146-1-C_7H_5O_3]^-$, 151 $[M-H-146-1-137-CO]^-$. With its λ_{max} at 263, 344 nm, this compound was putatively identified as myricetin-3-O-rhamnoside [18,19].

Two minor peaks (13, 16) had different retention times (t_R = 3.858, 4.725 min), the same UV maxima absorbance at 268, 350 nm and a similar mass ($[M-H]^-$ = 593.1535-593.1587 Da) corresponding to a formula of $C_{27}H_{30}O_{15}$ (errors less than 12.64 ppm). Both suffered the loss of 308 and 309 to produce the ion at *m/z* 285 and 284. Further MS^3 fragmentation produced typical ions of kaempferol at *m/z* 255, 227 and 151 (Table-3). Thus, they were plausibly classified as kaempferol-O-rutinoside [19].

Peak 14 (t_R= 4.021 min, $[M-H]^-$ = 463.0888 Da, $C_{21}H_{20}O_{12}$, error 1.3 ppm) with λ_{max} at 279, 353 nm, displayed a sequential characteristic production ion at *m/z* 301 $[M-H\text{-glucosyl}]^-$, 300 $[M-H-162-H]^-$, 271 $[M-H-162-CH_2O]^-$, 255 $[M-H-162-CH_2O_2]^-$, 179 $[M-H-162-C_7H_6O_2]^-$, 151 $[M-H-162-122-CO]^-$ and 107 $[M-H-162-122-28-CO_2]^-$. This compound was named as quercetin-3-O-glucoside [20].

Peak 17 (t_R = 5.277 min, λ_{max} = 254, 345 nm) displayed $[M-H]^-$ ion at 447.0959 (calculated as $C_{21}H_{20}O_{11}$, error 5.8 ppm). A sequential fragments at *m/z* 301(from loss of 146 Da, rhamnosyl), 300, 271, 255, 179 and 151 were observed. This fragmentation pattern was similar to peak 14. Thus, it was named as quercetin-3-O-rhamnoside [9,21].

Peak 18 (t_R = 5.509 min, $[M-H]^-$ = 477.1037 Da, $C_{22}H_{22}O_{12}$, error 0.21 ppm) with λ_{max} at 268 and 350 nm, was assigned as isorhamnetin-3-O-glucoside [7,16]. This structure was supported by an MS^2 fragments at *m/z* 315 (from loss of 162 Da, glucosyl), 314 $[M-H -162-H]^-$, an MS^3 fragments at *m/z* 300 $[M-H-162-1-CH_2]^-$, 285 $[M-H-162-1-CHO]^-$, 271 $[M-H-162-1-C_2H_3O]^-$ and an MS^4 fragment at *m/z* 243 $[M-H-162-1-43-CO]^-$.

Peak 21 (t_R = 5.939 min, $[M-H]^-$ = 447.0892 Da, $C_{21}H_{20}O_{11}$, error of -9.17 ppm) produced an MS^2 fragments at *m/z* 285 (from loss of 162 Da, glucosyl), 284 $[M-H-162-H]^-$ and an MS^3 fragments at *m/z* 257 $[M-H-162-CO]^-$, 151 $[M-H-162-C_8H_6O_2]^-$. These were consistent with those reported for kaempferol-3-O-glucoside [8,12].

Peak 24 (t_R = 6.253 min) showed $[M-H]^-$ ion at *m/z* 447.0901 Da ($C_{21}H_{20}O_{11}$, error -7.16 ppm) and a sequential characteristic fragments at *m/z* 315 (loss of 132, pentose), 314, 285, 271, 243 and 151. With its λ_{max} at 278 and 350 nm, it was identified as isorhamnetin-O-pentoside, being observed in myrtle berry seed.

Peak 28 (t_R = 7.036 min) exhibited its $[M-H]^-$ ion at *m/z* 461.1061 ($C_{22}H_{22}O_{11}$, error of 6.07 ppm) and λ_{max} at 278, 350 nm. It also showed fragments at *m/z* 315 (from loss of 146 Da, rhamnosyl), 314, 300, 285, 271 and 243, displayed the identical fragmentation pattern to peak 18. This compound was characterized as isorhamnetin-3-O-rhamnoside [8,13,22,23].

Identification of flavanol and its oligomers: In the present study, five flavanols and one polymer were obtained. Their UV maximum absorbances was shown at 226-274 nm. Neutral loss of 290 or 288 Da are always suffered during the fragmentation of these polymers described in the literature [8,24].

Peak 5 (t_R =1.337 min, [M-H]$^-$ = 577.1336 Da, $C_{30}H_{26}O_{12}$, error -2.77 ppm) displayed a typical product ions at *m/z* 451 [M-H-$C_6H_6O_3$]$^-$, 425 [M-H-126-C_2H_2]$^-$, 407 [M-H-126-26-H_2O]$^-$, 299 [M-H-126-26-18-$C_6H_4O_2$]$^-$, 289 [M-H-$C_{15}H_{12}O_6$]$^-$, 245 [M-H-288-C_2H_4O]$^-$ and showed its λ_{max} at 268 nm. This compound, procyanidin B2, has been reported in rape seeds by an HPLC-PDA-ESI(-)-MS^n/HRMS method [8].

Peak 34b (t_R = 8.658 min, λ_{max} = 226 nm) exhibited an [M-H]$^-$ ion at *m/z* 301.0338 (calculated as $C_{15}H_{10}O_7$, error -5.32 ppm). It also showed a typical MS^2 fragments at *m/z* 179 [M-H-$C_7H_6O_2$]$^-$, 151 [M-H-122-CO]$^-$ and a base MS^3 fragment at *m/z* 107 [M-H-122-28-CO_2]$^-$. All of the above information indicated that it was quercetin.

Peak 36 (t_R = 10.795 min, λ_{max} = 270, 318 nm) exhibited an [M-H]$^-$ ion at *m/z* 269.0458 ($C_{15}H_{10}O_5$, error of 2.97 ppm). This compound suffered a characteristic MS^2 fragmentation behaviour to baicalein at *m/z* 225 [M-H-CO_2]$^-$, 149 [M-H-$C_7H_4O_2$]$^-$ and 107 [M-H-120-C_2H_2O]$^-$.

Two pairs of isomers, peak 34a and 37 (t_R = 8.658 and 11.167 min, [M-H]$^-$ = 285.0412-285.0427 Da, calculated as $C_{15}H_{10}O_6$, error less than 7.72 ppm) were confirmed as luteolin and kaempferol, separately. They exhibited an MS^{2,3} fragments at *m/z* 243 [M-H-C_2H_2O]$^-$, 241 [M-H-CO_2]$^-$, 217 [M-H-42-C_2H_2]$^-$, 199 [M-H-42-26-H_2O]$^-$, 175 [M-H-42-26-C_2H_2O]$^-$, 151 [M-H-$C_8H_6O_2$]$^-$, 133 [M-H-134-H_2O]$^-$, 107 [M-H-134-CO_2]$^-$ for leuteon and 257 [M-H-CO]$^-$, 229 [M-H-28-CO]$^-$, 165 [M-H-$C_7H_4O_2$]$^-$, 151, 107 and 83 [M-H-134-44-C_2]$^-$ for kaempferol. Peak 35 and 39 (t_R = 9.776 and 11.980 min, [M-H]$^-$ = 315.0483-315.0537 Da, calculated as $C_{16}H_{12}O_7$, error less than 8.57 ppm) were characterized as isorhamnetin and rhamnetin. They displayed a characteristic MS^2 fragments at *m/z* 300 [M-H-CH_3]$^-$, 271 [M-H-CHO]$^-$ and 151 [M-H-$C_9H_8O_3$]$^-$.The isomers have different retention time due to molecular polarity. They were also confirmed with standard.

Identification of acylated flavonol glycosides: In this study, we identified seven acylated flavonol glycosides. Acyl groups on flavonol glycosides were mainly observed in galloyl and acetyl [24-26]. However, they were all minor components in the extract, which provided insufficient UV data or MS^n fragments to elucidate the isomeric structure. Six derivatives of quercetin acylated flavonol glycosides were observed.

Peak 15 (t_R = 4.259 min, [M-H]$^-$ = 615.0967 Da, $C_{28}H_{24}O_{16}$, error -4.06 ppm) with λ_{max} at 269 and 350 nm was identified as quercetin-3-O-(O-galloyl)glucoside, which exhibited a base MS^2 fragments at *m/z* 301 [M-H-$C_{13}H_{14}O_9$]$^-$ and an MS^3 fragments at *m/z* 271 [M-H-314-CH_2O]$^-$, 179 [M-H-314-$C_7H_6O_3$]$^-$ and 151 [M-H-314-122-CO]$^-$.

Peak 23, 25 (t_R = 6.187 and 6.387 min, [M-H]$^-$ = 615.0959-615.0977 Da, $C_{28}H_{24}O_{16}$, error less than -5.37 ppm), with λ_{max} at 268 and 353 nm, were confirmed as myricetin-O-(O-galloyl) rhamnoside, being reported for the first time in this plant. They displayed an MS^2 fragments at *m/z* 463 [M-H-$C_7H_4O_4$]$^-$, 317 [M-H-$C_{13}H_{14}O_8$]$^-$ and an MS^3 fragments at *m/z* 179 [M-H-298-$C_7H_6O_3$]$^-$ and 151 [M-H-298-138-CO]$^-$.

Peak 30, 33 (t_R = 7.535 and 8.347 min, [M-H]$^-$ = 489.1036-489.1039 Da, $C_{23}H_{22}O_{12}$, error less than -0.4 ppm) were identified as quercetin-3-O-acetylrhamnoside. Further characteristic fragments at *m/z* 447 [M-H-C_2H_2O]$^-$, 301 [M-H-42-rhamnosyl]$^-$, 300 [M-H-42-146-H]$^-$ and an MS^3 fragments at *m/z* 271 [M-H-42-146-1-CHO]$^-$, 255 [M-H-42-146-1-CHO_2]$^-$, 179 [M-H-42-146-1-$C_7H_5O_2$]$^-$ and 151 [M-H-42-146-1-121-CO]$^-$ supported the structure. This compound has been previously characterized in *Callistemon lanceolatus* flowers and leaves.

Peak 31, 32 (t_R = 7.823 and 8.033 min, [M-H]$^-$ = 599.1027-599.1031 Da, $C_{28}H_{24}O_{15}$, error less than -2.5 ppm), with λ_{max} 269 and 273 nm, were assigned as quercetin-3-O-(O-galloyl) rhamnoside, being reported for the first time in this plant. The main MS^2 fragments at *m/z* 447 [M-H-$C_7H_4O_4$]$^-$, 301 [M-H-$C_{13}H_{14}O_8$]$^-$, 300 [M-H-298-H]$^-$ and MS^3 fragments at *m/z* 271 [M-H-298-CH_2O]$^-$, 257 [M-H-298-CO_2]$^-$, 255 [M-H-298-CH_2O_2]$^-$, 229 [257-CO]$^-$, 179 [M-H-298-$C_7H_6O_2$]$^-$, 151 [M-H-298-122-CO]$^-$ and 107 [M-H-298-122-28-CO_2]$^-$ were observed.

Identification of hydroxycinnamic acid and unknown: To our best of knowledge, nine octulopyranosonic acid derivatives were reported in the extract of Feng Liao for the first time. They suffered the typical neutral loss of 146 Da. The presence of coumaryl group instead of rhamnosyl unit attached to the molecule was consistent with high-resolution mass and the longer retention time.

Peak 26 (t_R = 6.740 min, [M-H]$^-$ = 633.1674 Da, $C_{33}H_{30}O_{13}$, error 9.48 ppm), with λ_{max} at 273 nm, exhibited an MS^2 fragments at *m/z* 487 [M-H-courmaryl]$^-$, 341 [M-H-2courmaryl]$^-$, 469 [M-H-146-H_2O]$^-$ and an MS^3 fragments at *m/z* 307 [M-H-2 × 146-CH_6O]$^-$, 265 [M-H-2 × 146-C_6H_4]$^-$, 235 [M-H-2 × 146-C_7H_6O]$^-$. This behaviour was similar to that reported for benzoyl-*p*-dicoumaryl-2,7-anhydro-3-deoxy-2-octulopyranosonic acid [8,23,26,27].

Peak 38 (t_R = 11.577 min, [M-H]$^-$ = 779.2085 Da, $C_{42}H_{36}O_{15}$, error 13.35 ppm) with λ_{max} at 232, 278 and 312 nm was benzoyl-*p*-tricoumaryl-2,7-anhydro-3-deoxy-2-octulopyranosonic acid. An MS^2 fragments at *m/z* 633 [M-H-courmaryl]$^-$, 487 [M-H-2courmaryl]$^-$ and an MS^3 fragments at *m/z* 341 [M-H-2 × 146-courmaryl]$^-$, 307 [M-H-3 × 146-CH_6O]$^-$, 265 [M-H-3 × 146-C_6H_4]$^-$, 235 [M-H-3 × 146-C_7H_6O]$^-$ and 217 [M-H-3 × 146-106-H_2O]$^-$ were observed.

Peak 41 (t_R = 13.189 min, [M-H]$^-$ = 821.2275 Da, $C_{44}H_{38}O_{16}$, error -2.80 ppm) with λ_{max} at 232, 278 and 325 nm was named as tetracoumaryl-2,7-anhydro-3-deoxy-2-octulopyranosonic acid. This compound displayed a typical MS^2 fragments at *m/z* 675 [M-H-courmaryl]$^-$, 529 [M-H-2courmaryl]$^-$, an MS^3 fragments at *m/z* 653 [M-H-146-H_2O]$^-$, 633 [M-H-146-C_2H_2O]$^-$, 487 [M-H-2 × 146-C_2H_2O]$^-$, 469 [M-H-2 × 146-42-H_2O]$^-$ and 453 [M-H-2 × 146-42-CH_6O]$^-$ and an MS^4 fragment at *m/z* 307 [M-H-2 × 146-$C_{12}H_{14}O_4$]$^-$.

Peak 42 (t_R = 14.541 min, [M-H]$^-$ = 925.2443 Da, $C_{51}H_{42}O_{17}$, error 10.16 ppm) with λ_{max} at 310 nm might be benzoyl-*p*-

tetracoumaryl-2,7-anhydro-3-deoxy-2-octulopyranosonic acid. An MS^2 fragments at *m/z* 779 [M-H-courmaryl]$^-$, 633 [M-H-2courmaryl]$^-$, 615 [M-H-2 × 146-H_2O]$^-$, 487 [M-H-3courmaryl]$^-$ and an MS^3 fragments at *m/z* 469 [M-H-3 × 146-H_2O]$^-$, 307 and an MS^4 fragment at *m/z* 235 were highly supported this structure elucidation.

Five octulopyranosonic acid derivatives (peaks 43, 45, 46, 47 and 48) produced a neutral loss of 146 Da (courmaryl), which was consistent with their longer retention time.

Peak 29 (t_R = 7.350 min), with [M-H]$^-$ion at *m/z* 435.0936 Da ($C_{20}H_{20}O_{11}$, error -3.22 ppm), exhibited an MS^2 fragment at *m/z* 315. Without further information, it was identified as isorhamnetin derivative.

Other peaks (7, 19, 22, 27, 40, 44, 49, 50 and 51) were detected, but due to the insufficient information of UV and MS^n data, their structures were not well elucidated.

Conclusion

In conclusion, this is the first study to characterize highly glycosylated and acylated quercetin, myricetin, kaemperol, isorhamnetin, its oligomers and hydroxycinnamic acids in Feng Liao of Chinese Herbal Medicine using UPLC-PDA-ESI(-)-HRMS^n method. Twenty six compounds have not been reported before in Feng Liao. We successfully proved that LCMS-IT-TOF is a highly efficient and elaborate technique for chemical identification.

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