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Identification of Components in Citri Sarcodactylis Fructus from Different Origins via UPLC-Q-Exactive Orbitrap/MS

Kanghui Wang,[§] Jingyuan Tian,[§] Yueshan Li, Mengshi Liu, Yingxin Chao, Yi Cai, Guodong Zheng,* and Yi Fang*

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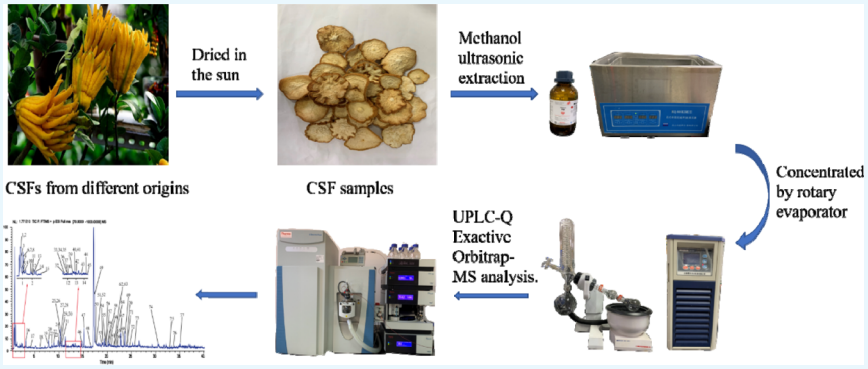

ABSTRACT: To systematically analyze the chemical constituents of Citri Sarcodactylis Fructus (CSF) from different origins, an efficient approach based on ultraperformance liquid chromatography plus Q-Exactive Orbitrap tandem mass spectrometry (UPLC-Q-Exactive Orbitrap/MS) detection for the discrimination of chemical components from of 15 batches of CSF from four main origins was used in this research. Through parent peaks, fragment peaks, fragmentation characteristics, and comparative analysis with the literature and reference standards, a total of 77 components from the methanol extracts including 18 coumarins, 24 flavonoids, seven organic acids, three limonoids, and 25 other compounds were detected and identified. Among them, 15 components have not been reported previously in the CSF. Notably, the stachydrine peak initially showed a higher content in the total ion current chromatogram. Overall, CSF produced in the Zhejiang province contained a richer variety of chemical compositions. These observations provided a theoretical basis for the further quality assessment and application of CSF.

1. INTRODUCTION

Citri Sarcodactylis Fructus (CSF, Foshou in China), the dried fruit of *Citrus medica* L. var. sarcodactylis Swingle, belongs to Citrus botany in Rutaceae.[1] CSF has a long cultivation history in China, which is widely distributed in Guangdong, Guangxi, Zhejiang, Sichuan, and Yunnan provinces.[2] As a medicinal and edible plant, CSF shows various pharmacological effects of antitumor, neuroprotection, antioxidation, anti-inflammatory, antimicrobial, antiblood pressure, lipid-lowering, and antianxiety.[3−11]

Due to the complex compositions and diverse types of natural medicinal plants, it is difficult to separate and identify their ingredients. With a high separation efficiency, a fast scanning speed, a high throughput, a high resolution, and high sensitivity, Q-Exactive Orbitrap tandem mass spectrometry (UPLC-Q-Exactive Orbitrap/MS) technology combines the separation capabilities of chromatography and the qualitative functions of mass spectrometry are widely used in the component analysis of complex systems of traditional Chinese medicine.[2,12,13] It is especially suitable for the qualitative identification of complex natural plant systems and the discovery of new compounds lacking reference substances.[14]

At present, research on CSF mainly concentrated on its pharmacological activity,[6,7] and the components mostly focused on were essential oil,[6,7] and coumarins[15,16] such as 5,7-dimethoxycoumarin, 7-hydroxycoumarin, scopoletin, and bergapten, as well as flavonoids such as hesperidin,[17] however, few research systematically reported on the chemical components of CSF. In addition, CSF has a wide range of production areas, and different growth environment and geographical locations may result in intermingled quality and

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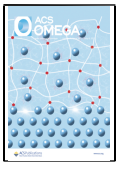

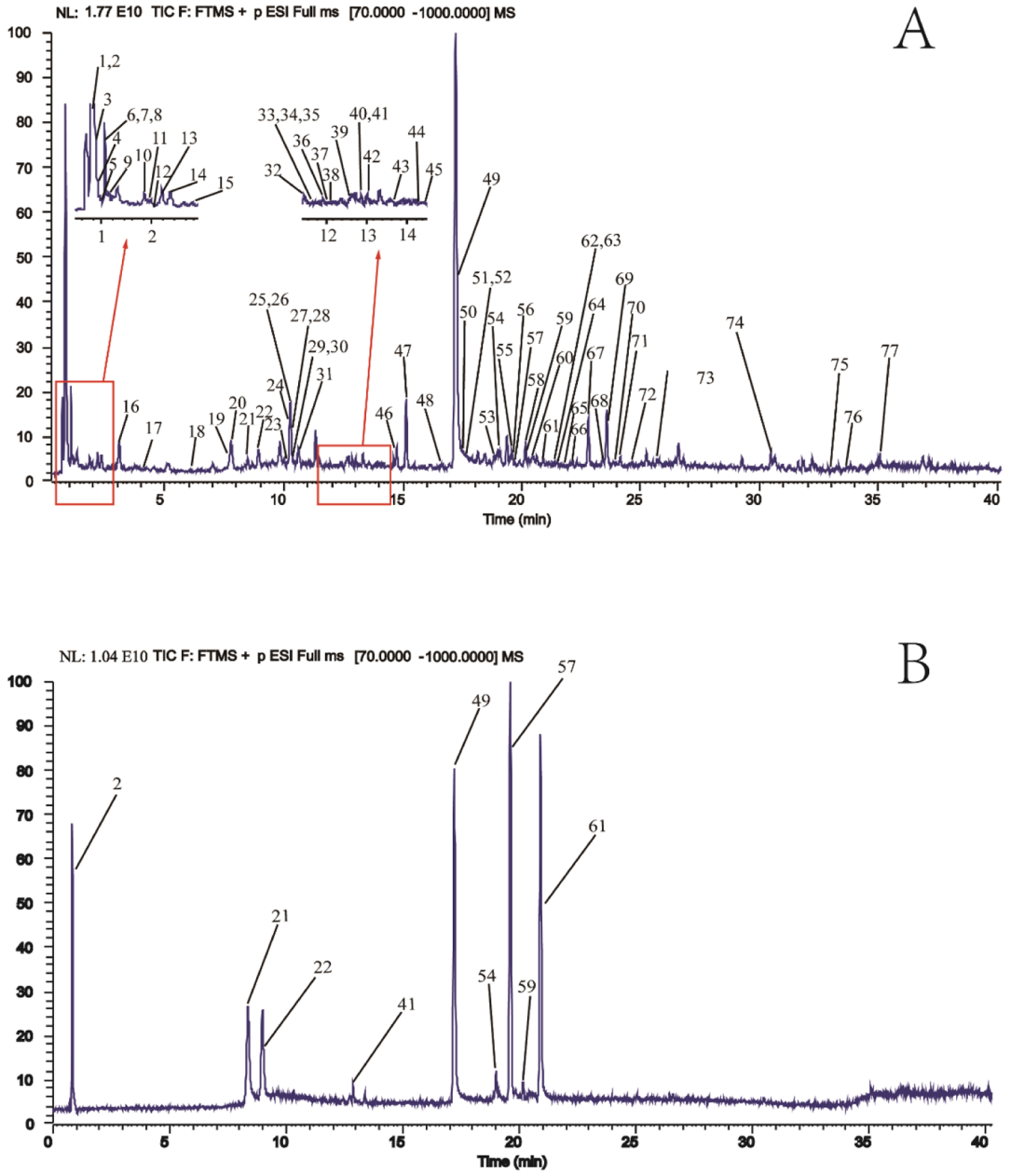

Figure 1. Total ion chromatograms of CSF (A) and the mixed reference standards (B).

differences in compositions. For a better comprehensive comparison, an analysis of the compositions from different origins was provided as a reference for evaluating the quality of CSF.

2. RESULTS AND DISCUSSION

In this work, more than 77 peaks were efficiently separated and detected within 40 min in the total ion current (TIC) chromatogram in positive ion modes via a UPLC-Q Exactive Orbitrap/MS system. The CSF extract samples were able to be isolated in the positive-ion mode, where the detection signal and resolution were both better than those in the negative-ion one. The TIC of extract samples and mixed reference standards is shown in Figure 1. According to the peak time, standards, and relevant literature, 77 compounds were separated and identified within 40 min, and are shown in detail in Table 1, including 18 coumarins, 24 flavonoids, seven organic acids, three limonoids, and 26 other compounds. Among them, 15 components were detected in the CSF for the first time: five flavonoids including eriocitrin, eriodictyol, astragalin, isotrifoliin, and glabrone; two coumarins including isopimpinellin and isofraxidin; one organic acid, *p*-hydroxycinnamic acid; and seven other compounds including oleamide, erucamide, stachydrine, coniferin, *o*-veratraldehyde, 6-hydroxyindole, and linderalactone. Chemical structures of 77 compounds that were tentatively identified are shown in detail in Figure 2. This discovery provides a new direction for follow-up research on the quantitative analysis, component separation, and pharmacological activity of CSF.

2.1. Identification of Coumarins. Coumarins are a kind of natural compounds with a benzo-α-pyranone parent nucleus,[18] and are the primary constituents in the CSF extracts.[15,17] Twenty coumarins were identified in the CSF extracts. In the positive mode, these kinds of compounds may

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Table 1. Summary of Compounds Identified in CSF by UPLC-Q Exactive Orbitrap/MS

no	t_R (min)	$[M + H]^+$ (m/z)	major secondary fragment ions (MS/MS)	compound formulas	identification
			Coumarins		
13	2.24	325.0914	293.0921, 219.0570, 182.0581, 163.0388, 150.7622, 135.0440, 107.0494, 91.0547, 85.0290	$C_{15}H_{16}O_8$	skimmin
14	2.33	209.0443	194.0210, 181.0497, 177.0524, 167.0703, 165.0549, 163.0389, 155.0702, 153.0547, 149.0234, 135.0441, 121.0286, 107.0495, 91.0546, 79.0550, 65.0392	$C_{10}H_8O_5$	fraxetin
15	2.83	193.0498	178.0260, 165.0545, 150.0312, 137.0596, 133.0285, 122.0364, 117.0338, 107.9602, 105.0701, 91.0549, 77.0392, 56.9655	$C_{10}H_8O_4$	isoscopoletin
16	3.08	355.1023	235.5171, 193.0497, 178.0261, 165.0546, 149.0597, 137.0598, 133.0285, 122.0364, 89.0390, 73.0291	$C_{16}H_{18}O_9$	scopolin
19	7.80	163.0391	149.0450, 139.9821, 135.0441, 119.0493, 107.0494, 95.0495, 91.0547, 84.9604, 79.0548, 68.9979, 61.6360, 53.0395	$C_9H_6O_3$	7-hydroxycoumarin
21	8.40	193.0498	178.0258, 165.0545, 150.0309, 137.0595, 133.0283, 122.0362, 117.0334, 107.9600, 94.0415, 81.0339, 66.0473	$C_{10}H_8O_4$	scopoletin
37	12.14	223.0604	208.0365, 195.0176, 190.0260, 179.0338, 167.0703, 162.0311, 151.0392, 134.0363, 110.0366, 106.0416, 95.0495, 78.0470, 73.0290, 56.9656	$C_{11}H_{10}O_5$	fraxinol
45	14.43	177.0547	162.0309, 149.0599, 135.1169, 133.0649, 131.0854, 121.0650, 118.0416, 103.0546, 91.0548, 79.0549, 69.0705, 57.7384, 53.0394	$C_{10}H_8O_3$	7-methoxycoumarin
46	14.76	305.1022	263.1425, 203.0339, 175.0393, 159.0441, 147.0441, 131.0492, 119.0494, 91.0547, 67.0548, 59.0500, 57.0707	$C_{16}H_{16}O_6$	oxypeucedan hydrate
47	15.15	317.102	299.0910, 273.0755, 245.0446, 233.0445, 231.0288, 218.0219, 217.0130, 203.0339, 188.0104, 175.0391, 160.0155, 119.0494, 91.0548, 89.0390, 85.0654, 67.0550, 65.0393	$C_{17}H_{16}O_6$	byakangelicol
49	17.23	207.0654	192.0417, 179.0702, 164.0467, 163.0753, 151.0754, 149.0233, 148.0518, 139.0753, 121.0650, 103.0545, 91.0547, 79.0548, 65.0393	$C_{11}H_{10}O_4$	5,7-dimethoxycoumarin
51	17.74	217.0495	202.0260, 178.0259, 174.0312, 173.0598, 161.0595, 146.0363, 131.0492, 118.0416, 115.0545, 91.0545, 73.2902, 53.6608	$C_{12}H_8O_4$	bergapten
52	17.75	247.0596	232.0363, 217.0130, 207.0661, 189.0180, 161.0232, 133.0287, 106.0868, 95.0131, 81.0839, 57.0706	$C_{13}H_{10}O_5$	isopimpinellin
55	19.51	287.0913	257.6765, 240.9097, 203.0338, 175.0391, 159.0441, 147.0440, 131.0491, 119.0492, 91.0547, 85.0653, 67.0549, 59.0499	$C_{16}H_{14}O_2$	oxypeucedanin
64	21.43	223.0599	208.0367, 193.0132, 180.0418, 179.0337, 167.0702, 165.0183, 163.0338, 152.0465, 149.0594, 139.0750, 137.0228, 135.0443, 134.0363, 119.0492, 106.0416, 91.0547, 79.0547, 67.0549	$C_{11}H_{10}O_5$	isofraxidin
68	23.52	271.0961	249.1167, 227.1049, 216.7790, 203.0339, 175.0389, 159.0441, 147.0441, 131.0492, 119.0494, 119.0494, 103.0546, 91.0548, 81.0702, 69.0706, 65.0392	$C_{16}H_{14}O_4$	isoimperatorin
69	23.59	193.0497	178.0260, 165.0549, 149.0598, 137.0598, 134.0363, 121.0650, 109.0612, 105.0338, 91.0546, 79.0548, 67.0186, 53.0392	$C_{10}H_8O_4$	5,7-dihydroxy-4-methylcoumarin
70	23.93	245.117	229.0861, 215.0704, 201.0547, 187.0389, 175.0393, 159.0440, 133.0645, 121.0650, 91.0547, 79.0547, 69.0706, 55.0549	$C_{15}H_{16}O_3$	suberosin
			Polymethoxy Flavones		
53	18.86	361.0920	346.0683, 345.0606, 331.0449, 330.0365, 318.0731, 315.0507, 303.0500, 301.0347, 285.0392, 257.0449, 229.0495, 201.0546, 169.0132, 121.0286, 95.0498, 68.9976	$C_{18}H_{16}O_8$	5,7,3′-trihydroxy-6,4′,5′-trimethoxyflavone
57	19.61	403.1388	388.1151, 373.0916, 358.0680, 327.0859, 301.0704, 258.0523, 229.0338, 211.0236, 183.0288, 165.0546, 127.0389, 99.0445, 68.9976	$C_{21}H_{22}O_8$	nobliletin
60	20.49	331.0813	316.0579, 315.0500, 301.0343, 288.0628, 285.0397, 273.0394, 257.0442, 245.0444, 229.0499, 199.0391, 169.0135, 148.0521, 135.0441, 121.0650, 91.0547, 68.9978	$C_{17}H_{14}O_7$	jaceosidin
61	20.93	373.1286	358.1048, 343.0813, 328.0576, 297.0758, 271.0599, 254.0572, 229.0318, 211.0238, 193.0134, 183.0289, 168.0050, 135.0441, 99.0442, 69.0342	$C_{20}H_{20}O_7$	tangeretin
65	21.68	389.1220	374.0986, 359.0753, 341.0649, 316.0571, 285.0761, 285.0781, 260.0668, 227.054548, 215.0182, 178.1149, 169.0128, 148.0518, 133.0857, 113.0232, 89.0601, 81.0700	$C_{20}H_{20}O_8$	demethylnobiletin
66	22.00	389.1227	372.2306, 359.0759, 348.0127, 331.0607, 257.0809, 215.0699, 177.1121, 165.0546, 153.0181, 133.0859, 107.0699, 89.0602, 69.0700	$C_{21}H_{22}O_{11}$	arteMitin
			Other Flavonoids		
23	10.14	433.1127	416.2572, 397.0919, 367.0613, 323.0906, 313.0705, 283.0600, 256.0731, 217.0493, 187.0394, 177.1121, 145.0283, 133.0860, 121.0285, 89.0602, 81.0341	$C_{21}H_{20}O_{10}$	vitexin
25	10.32	465.1026	345.0661, 315.0609, 303.0500, 285.0395, 257.0446, 229.0406, 201.0547, 153.0184, 97.0288, 86.0290, 69.0342	$C_{21}H_{20}O_{12}$	isotrifoliin
26	10.33	433.1127	415.1025, 397.0919, 367.0612, 337.0704, 313.0705, 283.0600, 267.0652, 229.0495, 195.0290, 165.0182, 149.0233, 121.0285, 109.0290, 89.0602, 81.0340	$C_{21}H_{20}O_{10}$	isovitexin
27	10.35	303.0496	285.0395, 257.0443, 247.0608, 229.0495, 219.0657, 201.0545, 183.0288, 165.0183, 153.0182, 137.0234, 121.0286, 95.0496, 68.9978	$C_{15}H_{10}O_7$	quercetin

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Table 1. continued

no	t_R (min)	$[M + H]^+$ (m/z)	major secondary fragment ions (MS/MS)	compound formulas	identification
			Other Flavonoids		
28	10.35	661.1603	465.1027, 345.0584, 315.0499, 303.0498, 285.0395, 229.0494, 201.0544, 195.0305, 165.0183, 161.0592, 153.0181, 137.0233, 129.0545, 97.0288, 85.0289, 71.0498	$C_{27}H_{30}O_{16}$	rutin
29	10.47	597.1812	435.1286, 399.1075, 331.0811, 301.0703, 289.0705, 263.0548, 245.0443, 219.0287, 195.0288, 171.0288, 163.0389, 153.0182, 145.0284, 135.0441, 129.0547, 111.0444, 85.0290	$C_{27}H_{32}O_{15}$	eriocitrin
30	10.47	289.0702	271.0600, 247.0599, 229.1275, 205.0492, 179.0338, 171.0286, 163.0388, 153.0181, 135.0440, 117.0336, 89.0390, 67.0185	$C_{15}H_{12}O_6$	eriodictyol
33	11.69	449.1074	425.0141, 361.0229, 326.0648, 299.0546, 287.0550, 258.0518, 213.0548, 153.0183, 97.0288, 85.0290, 69.0342	$C_{21}H_{20}O_{11}$	astragalin
34	11.71	287.0547	258.0520, 231.0650, 213.0546, 183.0287, 165.0184, 153.0182, 137.0233, 121.0286, 111.0080, 95.0494, 68.9978	$C_{15}H_{10}O_6$	kaempherol
35	11.71	595.1652	507.1806, 461.1209, 449.1077, 386.5186, 327.1189, 287.0549, 269.0442, 241.0488, 189.0538, 153.0182, 117.0338, 85.0289	$C_{27}H_{30}O_{15}$	kaempfeol-3-*O*-rutinoside
36	11.96	317.0652	302.0419, 285.0390, 257.0442, 229.0494, 217.0496, 200.0464, 165.0181, 153.0181, 139.0389, 121.0285, 92.0261, 65.0393	$C_{16}H_{12}O_7$	isorhamnetin
38	12.18	579.1702	433.1126, 313.0728, 283.0570, 271.0598, 247.0599, 225.0551, 171.0286, 153.0181, 85.0289, 71.0498	$C_{27}H_{30}O_{14}$	rhoifolin
39	12.75	609.1811	549.1959, 463.1232, 333.5535, 301.0704, 286.0470, 258.0520, 229.0482, 171.0282, 129.0546, 85.0289	$C_{28}H_{32}O_{15}$	diosmin
40	12.89	303.0860	285.0760, 261.0752, 219.0650, 201.0544, 183.0285, 179.0337, 177.0545, 171.0287, 163.0388, 151.0754, 153.0181, 145.0284, 137.0596, 135.0440, 117.0336, 111.0079, 89.0390, 83.0133, 67.0185	$C_{16}H_{14}O_6$	hesperetin
41	12.89	611.1964	537.9714, 449.1439, 413.1228, 345.0965, 303.0860, 263.0547, 245.0441, 219.0287, 195.0287, 191.0337, 179.0337, 177.0545, 163.0387, 153.0181, 149.0596, 129.0546, 85.0289	$C_{28}H_{34}O_{15}$	hesperiden
48	16.69	301.0703	286.0470, 269.0456, 258.0521, 229.0492, 205.1206, 177.0542, 153.0180, 153.0180, 133.0857, 105.0698, 89.0601, 69.0706	$C_{16}H_{22}O_8$	diosmetin
58	20.08	331.0812	316.0578, 301.0340, 285.0393, 273.0394, 257.0444, 242.0573, 214.0629, 186.0159, 148.0519, 121.0649, 91.0547, 68.9977	$C_{17}H_{14}O_7$	iristectorigenina
71	24.04	337.1064	319.0962, 307.0595, 293.0799, 278.0779, 248.0627, 219.0646, 201.0544, 191.0334, 163.0390, 145.0646, 121.0285, 105.0702, 91.0546, 68.9978	$C_{20}H_{16}O_5$	glabrone
			Limonoids		
54	19.03	471.2013	453.1900, 425.1958, 409.2017, 383.1849, 367.1902, 339.1950, 305.1546, 279.1390, 251.1073, 213.0912, 187.0753, 161.0597, 133.0649, 105.0702, 95.0132, 79.0548	$C_{26}H_{30}O_8$	limonin
59	20.18	515.2272	469.2223, 455.2069, 437.1965, 411.2167, 393.2060, 341.2108, 289.1222, 249.1271, 215.1068, 205.0497, 187.0758, 161.0598, 133.0649, 105.0703, 79.0549	$C_{28}H_{34}O_9$	nomilin
62	21.32	455.2064	437.1962, 409.2010, 391.1902, 349.1439, 315.1381, 303.1379, 235.1117, 209.0964, 175.0755, 161.0598, 133.0649, 105.0702, 95.0132, 81.0341	$C_{26}H_{30}O_7$	obacunone
			Organic Acids		
4	0.93	124.0394	140.0345, 112.0394, 97.8558, 96.0448, 90.0200, 82.9286, 80.0501, 78.0343, 64.9278, 53.0393	$C_6H_5O_2N$	nicotinic acid
10	1.80	169.0493	162.5643, 154.0257, 151.0389, 141.0548, 128.0191, 125.0597, 121.1012, 111.0443, 105.0701, 93.0339, 81.0704, 67.0549, 65.0393, 55.0549	$C_8H_8O_4$	vanillin acid
17	4.06	225.0756	214.1057, 207.0652, 192.0416, 179.0701, 175.0390, 164.0469, 155.0705, 147.0441, 132.0206, 119.0494, 107.0494, 95.0497, 91.0547, 79.0548, 65.0393	$C_{11}H_{12}O_5$	sinapic acid
22	9.07	195.0650	186.0550, 177.0547, 171.9590, 163.0390, 153.9485, 149.0599, 145.0285, 135.0442, 121.0648, 117.0338, 109.9590, 106.0418, 95.0496, 89.0392, 79.0547	$C_{10}H_{10}O_4$	ferulic acid
42	13.03	165.0544	152.5574, 147.0439, 141.9648, 123.0442, 119.0493, 111.0441, 103.0546, 95.0495, 91.0547, 82.9287, 82.9287, 65.0392, 56.9655	$C_9H_8O_3$	*p*-hydroxycinnamic acid
43	13.63	195.0650	186.0548, 177.0544, 171.9590, 163.0387, 153.0547, 149.0596, 145.0283, 135.0440, 121.0648, 117.0336, 109.9588, 93.0703, 89.0390, 86.9435, 79.0548, 63.0232	$C_{10}H_{10}O_4$	isoferulic acid
73	25.65	279.2315	261.2214, 243.2107, 233.1689, 209.1531, 209.1531, 187.1477, 173.1326, 149.1326, 137.1324, 123.1170, 109.1014, 95.0860, 93.0704, 81.0705, 83.0861, 79.0548, 69.0706, 67.0550	$C_{18}H_{30}O_2$	α-linolenic acid
			Other Compounds		
1	0.82	118.0652	109.6228, 72.0814, 70.0658, 59.0737, 58.0659, 56.7322, 52.8238	$C_5H_{11}NO_2$	valine
2	0.83	144.1018	102.0553, 92.4927, 84.0813, 72.0816, 60.8924, 58.0659, 54.08577	$C_7H_{13}O_2N$	stachydrine
3	0.87	182.0811	165.0546, 154.0863, 147.0441, 140.0713, 136.0757, 123.0442, 119.0493, 107.0492, 98.0603, 95.0496, 91.0547, 87.0445, 70.0657, 53.0393	$C_9H_{11}O_3N$	tyrosine
5	1.01	116.0706	96.0080, 92.9993, 81.0338, 74.0968, 70.0657, 68.0499, 60.0527, 56.9655, 53.0030	$C_5H_9NO_2$	proline

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Table 1. continued

no	t_R (min)	$[M + H]^+$ (m/z)	major secondary fragment ions (MS/MS)	compound formulas	identification
			Other Compounds		
6	1.06	268.1035	229.84142, 203.7506, 165.9773, 136.0618, 129.8893, 119.0353, 94.0403, 73.0292, 57.0343	$C_{10}H_{13}O_4N_5$	adenosine
7	1.07	132.1016	86.0986, 78.0406, 73.0653, 69.0705, 67.0546, 58.0658	$C_6H_{13}NO_2$	leucine
8	1.07	127.039	115.5870, 109.0287, 99.0445, 97.0288, 93.0208, 83.0497, 81.0341, 79.0547, 71.0499, 69.0342, 60.4606, 57.0343, 53.0394	$C_6H_6O_3$	5-hydroxymethylfurfural
9	1.09	123.0544	118.3506, 111.5361, 100.0247, 106.0290, 97.0288, 96.0448, 95.0495, 88.0236, 81.0704, 80.0501, 79.0184, 78.0344, 76.5157, 70.0130, 67.0549, 56.9655	$C_6H_6ON_2$	nicotinamide
11	2.00	205.0970	188.0705, 183.0775, 170.0599, 159.0916, 149.0233, 146.0599, 142.0650, 132.0807, 127.0582, 118.0653, 109.0287, 91.0546, 74.0243, 60.0452	$C_{11}H_{12}O_2N_2$	tryptophan
12	2.15	360.1649	292.1393, 246.0113, 193.0497, 181.0497, 163.0755, 145.0497, 131.0493, 109.0290, 103.0547, 85.0290, 69.0343	$C_{16}H_{22}O_8$	coniferin
18	6.13	153.0547	148.0277, 135.1168, 129.9789, 125.0598, 120.0327, 111.0443, 110.0365, 107.0859, 95.0496, 93.0339, 88.9529, 81.0705, 70.9425, 65.0393, 56.9656	$C_8H_8O_3$	vanillin
20	7.81	137.0597	130.9721, 122.0367, 109.0651, 105.0339, 95.0496, 94.0418, 91.0546, 81.0705, 79.0549, 77.0392, 66.0472, 53.0393	$C_8H_8O_2$	anisic aldehyde
24	10.31	167.0700	155.0701, 152.0467, 137.0596, 124.0516, 109.0650, 105.0337, 94.0416, 91.0545, 81.0705, 79.0548, 69.0341, 66.0471, 53.0392	$C_9H_{10}O_3$	*o*-veratraldehyde
31	10.60	245.1170	227.1065, 217.1223, 212.1222, 203.1066, 199.1117, 188.0830, 184.0883, 171.1167, 158.0934, 143.0855, 141.0696, 135.0805, 131.1011, 123.0442, 107.0858, 97.0652, 91.0547, 69.0342, 55.188	$C_{15}H_{16}O_3$	linderalactone
32	11.59	233.1539	227.0593, 215.1431, 205.1583, 191.1065, 187.1481, 177.0909, 173.1324, 163.0754, 159.1169, 157.1009, 147.1168, 145.1012, 133.1013, 131.0856, 121.1014, 119.0857, 109.1014, 105.0702, 93.0704, 81.0705, 67.0550	$C_{15}H_{20}O_2$	2-atractylenolide
44	14.31	134.0600	117.0571, 112.7435, 107.0495, 100.7975, 92.0500, 79.0548, 65.0393, 56.9655	C_8H_7NO	6-hydroxyindole
50	17.59	151.1117	146.0297, 136.0883, 128.0203, 123.1170, 119.0179, 105.0702, 95.0860, 89.0600, 81.0705, 79.0548, 69.0706, 67.0550, 56.9656	$C_{10}H_{14}O$	perillen
56	19.57	331.0813	316.0577, 301.0335, 285.0392, 273.0392, 257.0442, 245.0443, 217.0487, 183.0287, 161.0599, 135.0440, 112.0157, 68.9977	$C_{17}H_{14}O_7$	aurantio-obtusin
63	21.32	133.0648	131.0492, 115.0545, 107.0493, 105.0702, 103.0546, 95.0496, 89.0601, 79.0615, 72.0615, 61.0040, 55.0106	C_9H_8O	cinnamaldehyde
67	22.60	191.1068	173.0961, 163.1116, 149.9528, 145.1012, 135.0442, 130.0777, 121.0649, 117.0701, 105.9633, 95.0496, 91.0547, 83.0497, 79.0548, 71.0499, 67.0550, 55.0550	$C_{12}H_{14}O_2$	ligustilide
72	24.61	219.1746	201.1636, 177.0642, 173.0234, 163.0389, 159.1166, 145.1010, 135.0441, 131.0856, 117.0700, 107.0867, 97.0651, 93.0703, 79.0548, 67.0549, 55.0560	$C_{15}H_{22}O$	germacrone
74	30.47	282.2787	265.2528, 247.2421, 240.2688, 212.2006, 177.1641, 163.1483, 149.1325, 167.1430, 163.1479, 149.1323, 139.1116, 135.1170, 121.1014, 111.1170, 107.0857, 100.0762, 97.1016, 95.0859, 83.0862, 81.0704, 69.0706, 55.0551	$C_{18}H_{35}NO$	oleamide
75	33.01	307.2634	261.2212, 243.2106, 223.4683, 219.2107, 191.1434, 173.1324, 145.1008, 137.1324, 121.1014, 119.0857, 123.1169, 109.1015, 107.0859, 105.0702, 95.0859, 83.0862, 81.0704, 79.0548, 67.0550, 65.0393	$C_{20}H_{34}O_2$	linolenic acid ethyl ester
76	33.66	295.2638	277.2165, 263.2367, 245.2262, 221.2261, 207.1744, 193.1586, 189.1638, 179.1428, 175.1482, 165.1274, 163.1476, 151.1116, 147.1167, 135.1166, 133.1012, 121.1014, 109.1014, 95.0860, 69.0706, 55.0186	$C_{19}H_{34}O_2$	methyl linoleate
77	35.05	338.3418	321.3150, 303.3045, 282.2791, 226.2171, 212.2006, 177.1637, 163.1476, 156.1384, 149.1324, 142.1226, 135.1167, 114.0913, 109.1015, 100.0760, 97.1016, 95.0860, 93.0702, 86.0605, 85.1017, 83.0861, 69.0706	$C_{22}H_{43}NO$	erucamide

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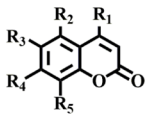
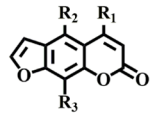
Coumarins



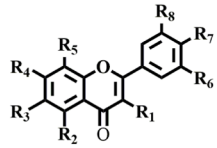
	R_1	R_2	R_3	R_4	R_5
13.Skimmin	H	H	H	$C_6H_{11}O_6$	H
14. Fraxetin	H	H	OCH_3	OH	OH
15.Isoscopoletin	H	H	OH	OCH_3	H
16.Scopolin	H	H	OCH_3	$C_6H_{11}O_6$	H
19.7-Hydroxycoumarin	H	H	H	OH	H
21.Scopoletin	H	H	OCH_3	OH	H
37.Fraxinol	H	OCH_3	OH	OCH_3	H
45.7-Methoxycoumarin	H	H	H	OCH_3	H
49.5,7-Dimethoxycoumarin	H	OCH_3	H	OCH_3	H
64.Isofraxidin	H	H	OCH_3	OH	OCH_3
69.5,7-Dihydroxy-4-methylcoumarin	CH_3	OH	H	OH	H
70.Suberosin	H	H	CH_2CH-$C(CH_3)_2$	OCH_3	H



	R_1	R_2	R_3
46.Oxypeucedan hydrate	H	$OCH_2CH(OH)$-$CH(CH_2)_2OH$	H
47.Byakangelicol	H	OCH_3	OCH_2CHO-$C(CH_3)_2$
51.Bergapten	H	OCH_3	H
52.Isopimpinellin	H	OCH_3	OCH_3
55.Oxypeucedanin	H	OCH_2CHO-$C(CH_3)_2$	H
68.Isoimperatorin	H	OCH_2CH-$C(CH_3)$	H

Flavonoids

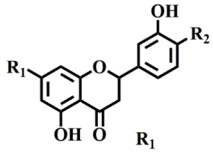
PMFs



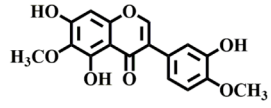
	R_1	R_2	R_3	R_4	R_5	R_6	R_7	R_8
53.5,7,3'-Trihydroxy-6,4',5'-trimethoxyflavone	H	OH	OCH_3	OH	H	OCH_3	OCH_3	OH
57.Nobliletin	H	OCH_3	OCH_3	OCH_3	OCH_3	OCH_3	OCH_3	H
60.Jaceosidin	H	OH	OCH_3	OH	H	OCH_3	OH	H
61.Tangeretin	H	OCH_3	OCH_3	OCH_3	OCH_3	H	OCH_3	H
65.Demethylnobiletin	H	OH	OCH_3	OCH_3	OCH_3	OCH_3	OCH_3	H
66.ArteMitin	OCH_3	OH	OCH_3	OCH_3	H	OCH_3	OCH_3	H

Other flavonoids

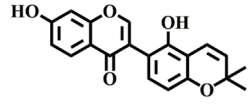
	R_1	R_2	R_3	R_4	R_5	R_6	R_7	R_8
23. Vitexin	H	OH	H	OH	$C_6H_{12}O_5$	H	OH	H
25. Isotrifoliin	O-glu	OH	H	OH	H	H	OH	OH
26. Isovitexin	H	OH	$C_6H_{12}O_5$	OH	H	H	OH	H
27.Quercetin	OH	OH	H	OH	H	H	OH	OH
28.Rutin	O-rutin	OH	H	OH	H	H	OH	OH
33.Astragalin	O-glu	OH	H	OH	H	H	OH	H
34.Kaempherol	OH	OH	H	OH	H	H	OH	H
35. Kaempfeol-3-O-rutinoside	O-rutin	OH	H	OH	H	H	OH	H
36.Isorhamnetin	OH	OH	H	OH	H	OCH_3	OH	H
38.Rhoifolin	H	OH	H	O-neohesperid	H	H	OH	H
39.Diosmin	H	OH	H	O-rutin	H	H	OCH_3	OH
48.Diosmetin	H	OH	H	OH	OH	H	OCH_3	OH



	R_1	R_2
29.Eriocitrin	O-rutin	OH
30.Eriodictyol	OH	OH
40.Hesperetin	OCH_3	OCH_3
41.Hesperiden	O-glu-rha	OCH_3

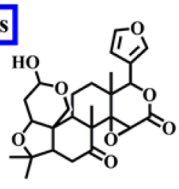


58.Iristectorigenina

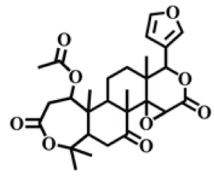


71.Glabrone

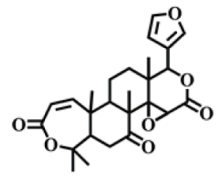
Limonoids



54. Limonin



59.Nomilin



62. Obacunon

Figure 2. continued

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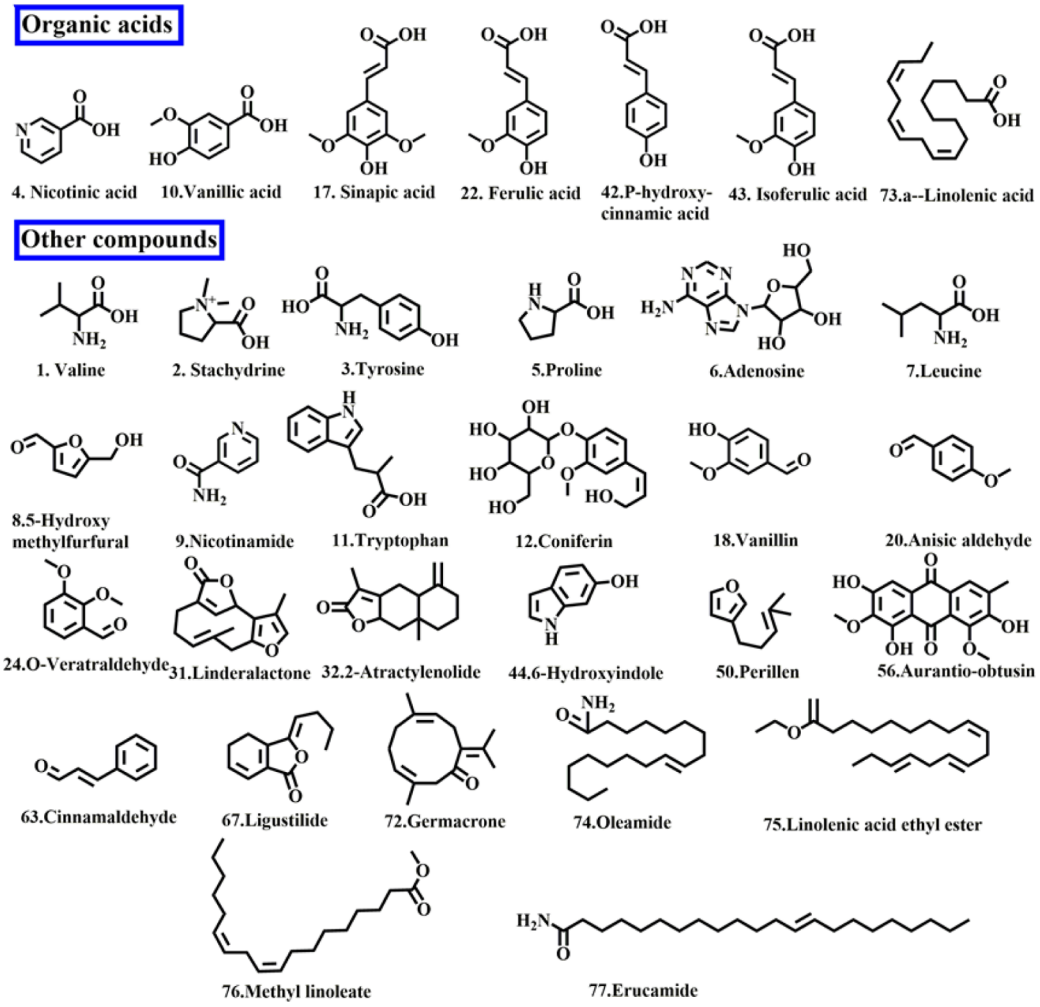

Figure 2. Chemical structures of the 77 components that were tentatively identified.

exhibit the characteristic ions by losing of 28, 44, 56, 72, and 84 Da by removal of neutral small molecules of CO, CO_2, 2CO, (CO + CO_2), and 3CO.[15]

The molecular ions of compounds 15, 21, and 69 were both at m/z 193.0498 $[M + H]^+$, with similar second-order fragment ions at m/z 178.0258 $[M + H - CH_3]^+$, 165.0545 $[M + H - CO]^+$, 150.0309 $[M + H - CH_3 - CO]^+$, and 149.0598 $[M + H - CO_2]^+$; accordingly, they were found to be isomers. The retention times of compound 15, compound 21, and compound 69 were, respectively, 8.40 min, 11.59 min, and 23.59 min. In accordance with the reference standard, relevant literature,[2,19,20] and the Orbitrap Traditional Chinese Medicine Library (OTCML) database as well as the above information, compound 15 was finally identified as isoscopoletin, compound 21 was determined as scopoletin (fragmentation pattern shown in Figure 3b), and compound 69 was identified as 5,7-dihydroxy-4-methylcoumarin.

Furanocoumarins can be divided into linear furanocoumarin and angular furanocoumarin.[21] Due to the construction features of furanocoumarins, it is easy for them to continuously lose CO (28 Da) and CO_2 (44 Da) groups, so the fragment ions of these kinds of compounds in the second MS were characterized by m/z 203 and m/z 158.[22] Compounds 46, 47, 51, 52, 55, and 68 belong to the linear furanocoumarins. Take compounds 46 and 55 for example, which exhibited a protonated molecular ion at m/z 305.1022 $[M + H]^+$ and m/z 287.0913 $[M + H]^+$. Compound 46 exhibited diagnostic fragments at m/z 203.0339 $[M + H - C_5H_{10}O_2]^+$, m/z 159.0441 $[M + H - C_5H_{10}O_2 - CO_2]^+$, 147.0441 $[M + H - C_5H_{10}O_2 - 2CO]^+$, 131.0492 $[M + H - C_5H_{10}O_2 - CO_2 - CO]^+$, and 91.0547 $[M + H - C_5H_{10}O_2 - 4CO]^+$. Compound 55 exhibited MS2 fragments at m/z 203.0336 $[M + H - C_5H_8O]^+$, m/z 159.0441 $[M + H - C_5H_8O - CO_2]^+$, 147.0440 $[M + H - C_5H_8O - 2CO]^+$, 131.0491 $[M + H - C_5H_8O - CO_2 - CO]^+$, and 91.0547 $[M + H - C_5H_8O - 4CO]^+$. Both of these compounds conform to the fragmentation pattern of furanocoumarins. Combined with relevant literature[23] and information above, compound 46 was confirmed as oxypeucedanin hydrate and compound 55, as oxypeucedanin. Similarly, in the MS^2 spectrogram, compound 68 exhibited a fragment ion at m/z 159.0441 by the loss of CO_2, as well as at m/z 175.0389 and at m/z 147.0441 by the continuous removal of CO groups. Consequently, compound 68 was determined as isoimperatorin.[23]

Furthermore, compound 51 exhibited a molecular ion at m/z 217.0495 $[M + H]^+$ in the positive mode. The second order spectrum shows fragment ions at m/z 202.0261 and m/z 173.0598, which correspond to the loss of CH_3 and CO of the parent ion, respectively. Therefore, compound 51 was identified as bergapten[15] (the fragmentation pattern is shown in Figure 3f). In addition, compound 49 was confirmed as 5,7-dimethoxycoumarin, which was known as a common

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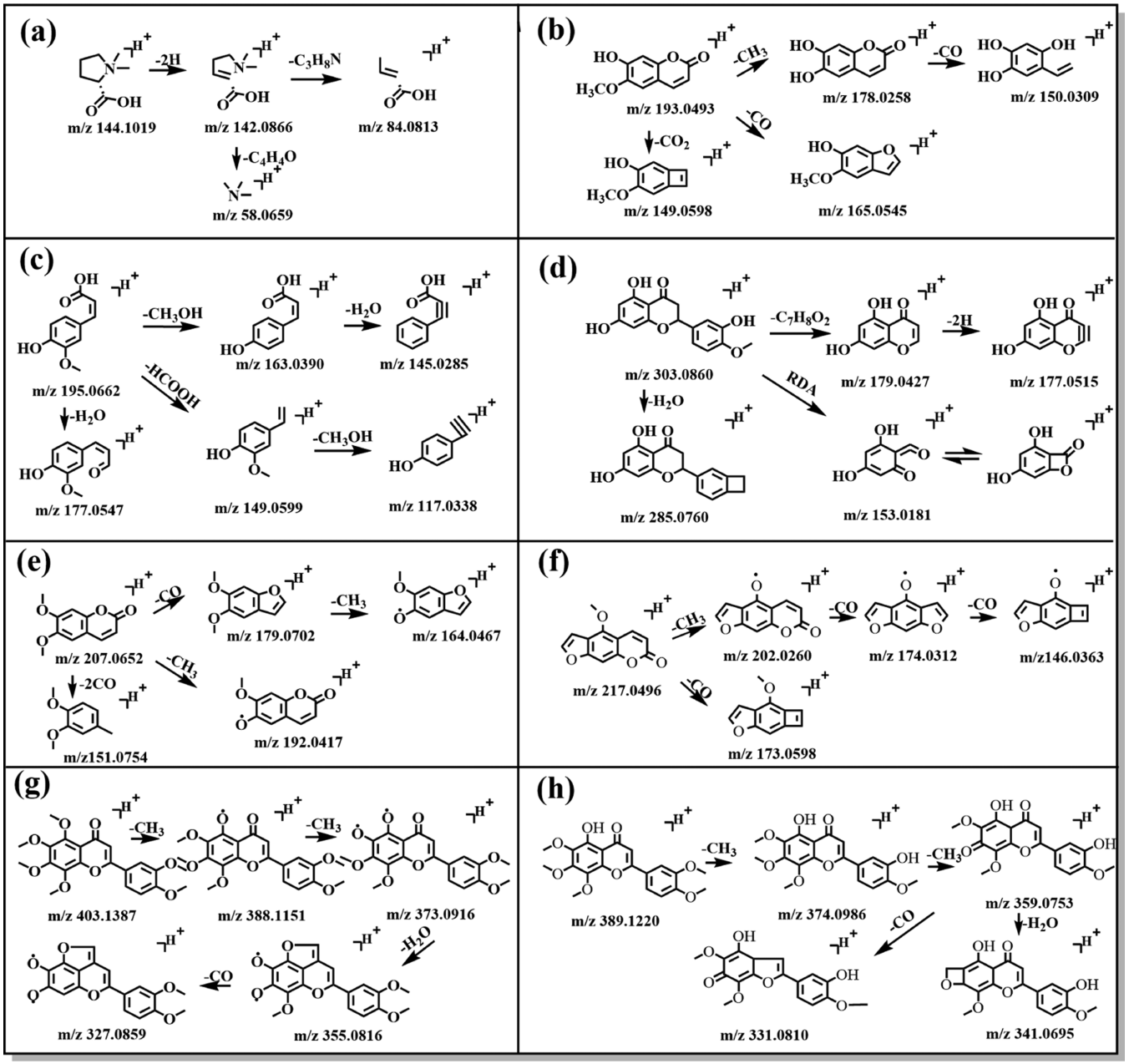

Figure 3. Detailed fragmentation patterns of the main fragment ions in positive ion mode for (a) stachydrine; (b) scopoletin; (c) ferulic acid; (d) hesperetin; (e) 5,7-dimethoxycoumarin; (f) pergapten; (g) nobliletin; and (h) 5-demethylnobiletin.

component in CSF in accordance with the reference standards (the fragmentation pattern is shown in Figure 3e).

2.2. Identification of Flavonoids. *2.2.1. Identification of Polymethoxyflavones.* Polymethoxyflavones (PMFs) are known to possess amounts of substituted $-OCH_3$ on the flavonoid parent nucleus that is rich in *Citrus* genus.[24] In general, according to the literature, such compounds in the positive mode exhibited characteristic fragment ions by the loss of nCH_3, consequently m/z $[M + H - CH_3]^+$ and m/z $[M + H - nCH_3]^+$ were formed.[22] High-resolution mass spectrometry data showed that compound 65 exhibited a molecular ion at m/z 389.1220 $[M + H]^+$, 14 Da (CH_2) less than that of nobiletin, and its MS^2 peaks were at m/z 374.0986 $[M + H - CH_3]^+$ and m/z 359.0753 $[M + H - 2CH_3]^+$, which is in line with the fragmentation pattern of PMFs. According to fragmentation information given above, compound 65 was identified as 5-demethylnobiletin[25] (the fragmentation pattern is shown in Figure 3h).

Compounds 57 and 61 were, respectively, identified as nobiletin and tangeretin in the light of standard references. The comprehensive fragmentation pattern of compound 57 is shown in Figure 3g.

2.2.2. Identification of Other Flavonoids. Besides the above PMFs, other flavonoids were also identified in this work. Based on the literature, it is easy for flavonoids to lose some neutral fragments of CO, CO_2, H_2O, and CH_3 in the MS/MS, subsequently the retro-Diels–Alder (RDA) reaction occurred. Under the same collision energy, flavonoid glycosides easily lose their linked sugar molecules, afterward the RDA reaction occurs as well.[22,26]

Glycoside is composed of aglycone and sugar. Analogously, diosmin is composed of diosmetin, a rhamnose, and a glucose.

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Table 2. Chemical Component Differences Among CSF From Different Origins

peak no.	compounds	S1	S2	S3	S4	S5	S6	S7	S8	S9	S10	S11	S12	S13	S14	S15
	Coumarins															
13	skimmin		*		*	*	*	*	*	*	*					
14	fraxetin					*	*			*						
15	isoscopoletin	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*
16	scopolin	*	*	*	*	*	*	*	*	*	*	*	*	*		*
19	7-hydroxycoumarin								*			*				
21	scopoletin	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*
37	fraxinol		*	*	*	*	*		*	*	*	*	*	*	*	*
45	7-methoxycoumarin	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*
46	oxypeucedanin hydrate	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*
47	byakangelicol	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*
49	5,7-dimethoxycoumarin	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*
51	bergapten	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*
52	isopimpinellin						*			*						
55	oxypeucedanin	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*
64	isofraxidin						*	*	*	*	*	*	*	*		*
68	isoimperatorin	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*
69	5,7-dihydroxy-4-methylcoumarin	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*
70	suberosin		*											*	*	*
	Polymethoxyflavones															
53	5,7,3′-trihydroxy-6,4′,5′-trimethoxyflavone	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*
57	nobliletin	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*
60	jaceosidin	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*
61	tangeretin[a]	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*
65	demethylnobiletin	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*
66	arteMitin	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*
	Other Flavonoids															
23	vitexin	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*
25	isotrifoliin						*			*	*			*	*	*
26	isovitexin	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*
27	quercetin			*	*	*	*	*	*	*	*		*	*	*	*
28	rutin			*	*	*	*	*	*	*	*		*	*	*	*
29	eriocitrin			*	*	*										
30	eriodictyol			*	*	*										
33	astragalin						*				*					
34	kaempherol			*		*	*	*	*	*	*	*	*	*	*	*
35	kaempfeol-3-*O*-rutinoside			*		*	*	*	*	*	*	*	*	*	*	*
36	isorhamnetin			*	*		*	*	*	*	*	*	*	*	*	*
38	rhoifolin			*	*	*	*	*	*	*	*	*	*	*	*	*
39	diosmin	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*
40	hesperetin	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*
41	hesperiden	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*
48	diosmetin	*		*		*		*	*	*		*	*	*	*	*
58	iristectorigenina	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*
71	glabrone				*	*								*		
	Limonoids															
54	limonoid	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*
59	nomilin	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*
62	obacunone	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*
	Organic Acids															
4	nicotinic acid	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*
10	vanillin acid		*			*	*	*		*	*		*	*	*	*
17	sinapic acid	*	*	*	*	*	*	*		*	*		*		*	*
22	ferulic acid	*	*	*	*	*	*	*	*	*	*		*	*	*	*
42	*p*-hydroxycinnamic acid	*	*	*	*	*	*	*	*	*	*	*				
43	isoferulic acid	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*
73	α-linolenic acid	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*
	Other Compounds															
1	valine					*	*	*	*	*		*	*			
2	stachydrine	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*
3	tyrosine	*		*	*	*	*	*	*	*	*	*	*	*	*	*

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Table 2. continued

peak no.	compounds	S1	S2	S3	S4	S5	S6	S7	S8	S9	S10	S11	S12	S13	S14	S15
							Other Compounds									
5	proline	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*
6	adenosine	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*
7	leucine	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*
8	5-hydroxymethylfurfural	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*
9	nicotinamide	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*
11	tryptophan	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*
12	coniferin	*	*	*				*			*		*	*	*	*
18	vanillin	*	*		*	*	*	*	*	*	*	*	*	*	*	*
20	anisic aldehyde	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*
24	*o*-veratraldehyde	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*
31	linderalactone	*	*	*	*		*	*	*	*	*			*	*	*
32	2-atractylenolide			*											*	*
44	6-hydroxyindole												*			
50	perillen		*	*	*		*	*	*	*	*	*	*	*		*
56	aurantio-obtusin	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*
63	cinnamaldehyde	*	*		*	*		*	*	*	*		*			
67	ligustilide		*													
72	germacrone	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*
74	oleamide	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*
75	linolenic acid ethyl ester	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*
76	methyl linoleate	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*
77	erucamide	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*

Compound 39 exhibited a $[M + H]^+$ peak at m/z 609.1811; its MS^2 fragment ions m/z 463.1232 and m/z 301.0704 were due to the loss of a neutral fragment of rhamnose (146 Da) and a consequent neutral fragment of glucose (162 Da). Precisely, Compound 48 exhibited a molecular ion at m/z 301.1070 $[M + H]^+$, subsequently lost a CH_3, CO, and COH, and the corresponding fragment ions at m/z 286.0470 $[M + H - CH_3]^+$, m/z 258.0520 $[M + H - CH_3 - CO]^+$, and m/z 229.0492$[M + H - CH_3 - CO - COH]^+$ were formed. In addition, the fragment ion at m/z 153.0180 was attributed to the RDA reaction of the fragment ion m/z 301.1070. Confirmed by the related literature,[26] compound 39 was identified as diosmin and compound 48 was identified as diosmetin.

In the positive mode, compounds 40 and 41 produced quasimolecular ions at m/z 303.0860 $[M + H]^+$ and m/z 611.1960 $[M + H]^+$. The same secondary fragment ions at m/z 285.0760, 179.0341, 177.0545, 153.0181, 147.0440, and 137.0599 were formed for the both compounds. Among them, m/z 285.0760 was generated by the removal of a H_2O molecule from the parent ion, furthermore, the parent ion lost its B ring and m/z 177.0545 was obtained. Fragment ions at m/z 153.0181, 147.0390, and 137.0752 were attributed to the RDA reaction of the flavonoid skeleton, which was the typical fragmentation method of the dihydroflavonoids. In accordance with reference standards and second MS fragmentation information above, compound 40 was finally determined as hesperetin and compound 41 was determined as hesperidin. The comprehensive fragmentation pattern of compound 40 is shown in Figure 3d.

2.3. Identification of Limonoids. Combined with earlier literature, limonoids are rich in citrus and show antitumor, anti-inflammatory, antibacterial, antiviral, antioxidant, and liver protection effects.[2,4,9,17,22] Three limonoids were detected from the CSF extracts: limonin, nomilin, and obacunone. Compound 62 exhibited a quasimolecular ion at m/z 455.2058 $[M + H]^+$. In the second MS, under high-energy collision, the parent ion of compound 62 can lose a H_2O molecule or a CO_2 or a HCOOH to obtain fragments 437.1962, 411.2162, and 409.2010, respectively, so that compound 62 was identified as obacunone.[27] Compounds 54 and 59 were detected as limonin and nomilin by comparing the reference substances and peak times.

2.4. Identification of Organic Acid. Seven organic acids, including vanillin acid, sinapic acid, ferulic acid, isoferulic acid, *p*-hydroxycinnamic acid, and α-linolenic acid, were identified in this study under positive-ion full MS scanning. These kinds of compounds are known to remove some neutral molecules of H_2O, HCOOH, and CO_2 in the positive mode.[28] For example, compounds 22 and 43 both have a protonated molecular ion at m/z 195.0547 $[M + H]^+$. The secondary fragment ions at m/z 177.0547 $[M + H - H_2O]^+$, m/z 163.0390 $[M + H - CH_3OH]^+$, m/z 149.0599 $[M + H - HCOOH]^+$, m/z 145.0285 $[M + H - CH_3OH - H_2O]^+$, and m/z 117.0338 $[M + H - HCOOH - CH_3OH]^+$ in the mass spectrum of compounds 22 and 43 are similar, only the ion peak response values are slightly different. Compound 22 was determined as ferulic acid and compound 43 was determined as isoferulic acid. The comprehensive fragmentation pattern of compound 22 is shown in Figure 3c.

2.5. Identification of Other Compounds. In the positive ion mode, five amino acids were identified from CSF, and their $[M + H]^+$ peaks were at m/z 118.0652, 182.0811, 116.0706, 132.1016, and 205.0970, respectively. It is easy to produce secondary characteristic fragment ions HCOOH (46 Da) and NH_3 (17 Da) in the mass spectrometry during the cleavage process, which result from the unique neutral fragment loss of amino acids.[29] By comparing the secondary fragment ion peaks under the full MS scanning, combined with the literature and the OTCML database, the compounds 1, 3, 5, 7, and 11 were decided as valine, tyrosine, proline, leucine, and tryptophan, respectively.

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Four aldehydes were detected in the CSF, where compounds 8, 20, 21, and 63 were identified as 5-hydroxymethylfurfural, anisic aldehyde, *o*-veratraldehyde, and cinnamaldehyde. Among them, *o*-veratraldehyde was reported in the CSF for the first time. Compound 8 exhibited a protonated molecular ion at *m/z* 127.0389 $[M + H]^+$, and the characteristic fragmentation peaks were observed at *m/z* 109.0651 $[M + H - H_2O]$+, 81.0705 $[M + H - H_2O - CO]^+$, and 53.0393 $[M + H - H_2O - 2CO]^+$. Overall, based on a previous study, compound 8 was identified as 5-hydroxymethylfurfural.[30]

Notably, compound 2 had a fairly high peak in the TIC chromatogram, and was detected as stachydrine. The carbon atom connected to the carboxyl group and the nitrogen atom in the stachydrine molecule had high reactivity, and was prone to electron transfer and dehydrogenation to produce a double bond. Then, the single bond between the methylene group and the nitrogen atom was broken to eventually form fragment ions at *m/z* 84.0813 and *m/z* 58.0659. The comprehensive fragmentation pattern of compound 2 is shown in Figure 3a.

In addition, in combination with the literature[31] and structural information provided by MS, compounds 74 and 77 were identified as oleamide and erucamide, which were rarely reported in the form of compounds in natural products.

2.6. Discussion. In general, all origins contained mostly the same chemical components, such as coumarins. On the whole, the coumarins extracted from different origins of CSF were abundant. According to this study and combined with relevant literature,[15] the coumarins are the main chemical components of CSF, thus the coumarins were well preserved by means of using the extraction method in this work. The chemical component differences among CSF from different origins are shown in Table 2. As for the flavonoids, other CSFs were more abundant than that of CSF-Guang. In addition, eriocitrin, eriodictyol, and glabrone were only detected in the Guangxi province, while none of the other origins were detected, which needs a further verification. Among them, eriocitrin and eriodictyol are common in citrus, while they have not been reported previously in the CSF. Overall, CSF produced in the Zhejiang province contained a richer variety of chemical components. More noticeably, according to previous studies, the research on the compositions of CSF mostly focused on its coumarins, whereas few reported on the polymethoxyflavonoids.[15] In this work, a total of seven polymethoxyflavonoids have been found, including nobliletin, tangeretin, 5-demethylnobiletin, etc., which have shown different pharmacological activities such as antitumor, antibacterial, antineuritis, antioxidant, cardiovascular protection, and antihyperlipidemic effects.[2,10,12,22,32]

3. CONCLUSIONS

In this paper, UPLC-Q-Exactive Orbitrap/MS technology was used for the first time to establish a qualitative analysis method for the chemical composition of different origins of CSF. A total of 77 chemical components in the CSF extracts were successfully isolated and identified, 15 of which were first detected. In the four main origins, CSF-Jin contained a richer variety of chemical components. In summary, this research provided an efficient and rapid method for qualitative analysis and quality control of chemical components in CSF.

4. MATERIALS AND METHODS

4.1. Chemicals and Materials. Different origins of CSF samples from 15 batches certified by Prof. Guodong Zheng were dried and appropriately stored at the Laboratory of Pharmacognosy, Guangzhou Medical University, Guangdong Province, China (Table 3).

Table 3. Information on CSF Samples From 15 Origins

no.	origins	sample source	collection time
S1	CSF-Guang[a]	Lecheng town, Zhaoqing city, Guangdong province	2019/10/25
S2		Lecheng town, Zhaoqing city, Guangdong province	2019/10/25
S3		Yongfu county, Guilin city, Guangxi province	2019/10/28
S4		Yongfu county, Guilin city, Guangxi province	2019/10/29
S5		Longjiang town, Guilin city, Guangxi province	2019/11/04
S6	CSF-Jin[b]	Jinhua city, Zhejiang province	2019/10/28
S7		Longquan city, Zhejiang province	2019/11/07
S8		Luodian town, Jinhua city, Zhejiang province	2019/11/07
S9		Chisong town, Jinhua city, Zhejiang province	2019/11/15
S10	CSF-Chuan[c]	Huidong county, Liangshan prefecture, Sichuan province	2019/10/21
S11		Peng' an county, Nanchong city, Sichuan province	2019/11/17
S12		Shawan town, Leshan city, Sichuan province	2019/11/12
S13		Baiyang town, Wanzhou district, Chongqing province	2019/10/28
S14	CSF-Yun[d]	Qujing city, Yunnan province	2019/10/23
S15		Huaning county, Yuxi city, Yunnan province	2019/10/28

[a]CSF-Guang was CSF from the Guangdong and Guangxi provinces. [b]CSF-Jin was CSF from the Zhejiang province. [c]CSF-Chuan was CSF from the Sichuan and Chongqing provinces. [d]CSF-Yun was CSF from the Yunnan province.

Reference standards of stachydrine, scopoletin, 5,7-dimethoxycoumarin, and nomilin were obtained from Sichuan Weikeqi Biotechnology (China), and hesperiden, ferulic acid, nobliletin, limonin, and tangeretin were purchased from Chengdu Mansite Biotechnology (China). All references were of above 98.5% purity. The chromatographic grade formic acid and acetonitrile were purchased from Thermo Fisher Scientific (China) and Honeywell (USA), respectively. The analytical grade methanol was obtained from Guangdong Guanghua Science Technology Company (China).

4.2. Experimental Instrumentation. The SB25-12DTD ultrasonic cleaners were obtained from Qunshan Machinery Equipment (China), and the Hk-04b swing crusher and the ME-104 electronic analytical balance were purchased from Xuyang Machinery Equipment (China) and Mettler Toledo (China), respectively. The XHRE-2000C rotary evaporator connected with a XHDL-200 low-temperature circulating pump was obtained from Shanghai Xiaohan Industrial Development Company (China). The ZORBAX Rclipse Plus C_{18} column (2.1 mm × 50 mm, 1.8 μm) was purchased from Agilent Technologies (USA). The UPLC-Q-Exactive Orbitrap/MS system equipped with a Dionex Ultimate 3000 UPLC system (Thermo Scientific, USA) consisted of an autosampler,

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an online degasser, a quaternary pump, and a column temperature compartment, and it was combined with a Q Exactive Orbitrap tandem mass spectrometer (Thermo Scientific, USA) by means of an electrospray ionization interface.

4.3. Preparation of Samples and Standards. *4.3.1. Preparation of Samples.* Approximately 0.5 g of 15 batches of CSF were, respectively, weighed, which were passed through the 40-mesh screen, added to 25 mL of methanol, and subjected to ultrasonic extraction (320 W, 40 kHz) for 30 min. Different batches of the CSF filtrate were, respectively, concentrated to 1 mL using a rotary evaporator and then transferred to an automatic injection bottle after passing through a 0.22 μm PTFE membrane for UPLC-Q Exactive Orbitrap-MS analysis.

4.3.2. Preparation of Standards. The nine standard compounds were accurately weighed, added to methanol and jointly dissolved into a volumetric flask, and diluted. The mixed standard solution was stored in a refrigerator at 4 °C, diluted with methanol to a proper concentration before use, and then passed through a 0.22 μm PTFE membrane for UPLC-Q Exactive Orbitrap-MS analysis.

4.4. UPLC-Q Exactive Orbitrap-MS System Conditions. The CSF extracts of different origins were separated on the C_{18} column at 35 °C at a flow velocity of 0.30 mL/min with a l μL sample size. The mobile phase consisted of 0.05% formic acid solution (A phase) and acetonitrile (B phase). The gradient elution procedure was as follows: 0−5 min, 10−10% B; 5−10 min, 10−20% B; 10−20 min, 20−50% B; 20−30 min, 50−85% B; and 30−40 min, 85−100% B.

High-resolution MS source parameters include a 3.5 kV spray voltage, a 320 °C capillary temperature, a 30 unit sheath gas, a 10 unit auxiliary gas, a 300 °C auxiliary gas heater temperature, and a 5 unit sweep gas, and were used in positive ion mode. Data acquired from 70−1000 Da in full MS scan mode were processed via Metworks software. All operations above were controlled by Xcalibur software.

AUTHOR INFORMATION

Corresponding Authors

Guodong Zheng − *Key Laboratory of Molecular Target & Clinical Pharmacology and the State Key Laboratory of Respiratory Disease, School of Pharmaceutical Sciences & The Fifth Affiliated Hospital, Guangzhou Medical University, Guangzhou 511436, PR China*; orcid.org/0000-0003-3708-1193; Email: gd200237@126.com

Yi Fang − *Key Laboratory of Molecular Target & Clinical Pharmacology and the State Key Laboratory of Respiratory Disease, School of Pharmaceutical Sciences & The Fifth Affiliated Hospital, Guangzhou Medical University, Guangzhou 511436, PR China*; Email: phaseistudy@163.com

Authors

Kanghui Wang − *Key Laboratory of Molecular Target & Clinical Pharmacology and the State Key Laboratory of Respiratory Disease, School of Pharmaceutical Sciences & The Fifth Affiliated Hospital, Guangzhou Medical University, Guangzhou 511436, PR China*

Jingyuan Tian − *Key Laboratory of Molecular Target & Clinical Pharmacology and the State Key Laboratory of Respiratory Disease, School of Pharmaceutical Sciences & The Fifth Affiliated Hospital, Guangzhou Medical University, Guangzhou 511436, PR China*

Yueshan Li − *Department of Pharmacy, Xinjiang Second Medical College, Xinjiang 834099, China*

Mengshi Liu − *Key Laboratory of Molecular Target & Clinical Pharmacology and the State Key Laboratory of Respiratory Disease, School of Pharmaceutical Sciences & The Fifth Affiliated Hospital, Guangzhou Medical University, Guangzhou 511436, PR China*

Yingxin Chao − *Key Laboratory of Molecular Target & Clinical Pharmacology and the State Key Laboratory of Respiratory Disease, School of Pharmaceutical Sciences & The Fifth Affiliated Hospital, Guangzhou Medical University, Guangzhou 511436, PR China*

Yi Cai − *Key Laboratory of Molecular Target & Clinical Pharmacology and the State Key Laboratory of Respiratory Disease, School of Pharmaceutical Sciences & The Fifth Affiliated Hospital, Guangzhou Medical University, Guangzhou 511436, PR China*

Complete contact information is available at: https://pubs.acs.org/10.1021/acsomega.1c02124

Author Contributions

[§]K.W. and J.T. contributed equally to the manuscript.

Notes

The authors declare no competing financial interest.

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REFERENCES

(1) Committee of National Pharmacopoeia. *The Pharmacopoeia of the People's Republic of China*; China Medical Science and Technology Press: Beijing, 2020; pp 185−186.

(2) Fu, C.; Liu, M.; Li, Y.; Wang, K.; Yang, B.; Deng, L.; Tian, J.; Yang, G.; Zheng, G. UPLC-Q-Exactive Orbitrap MS Analysis for Identification of Lipophilic Components in Citri Sarcodactylis Fructus from Different Origins in China Using Supercritical CO_2 Fluid Extraction Method. *ACS Omega* **2020**, *5*, 11013−11023.

(3) Bajerová, P.; Adam, M.; Bajer, T.; Ventura, K. Comparison of various techniques for the extraction and determination of ntioxidants in plants. *J. Sep. Sci.* **2014**, *37*, 835−844.

(4) Jayaprakasha, G. K.; Dandekar, D. V.; Tichy, S. E.; Patil, B. S. Simultaneous separation and identification of limonoids from citrus using liquid chromatography-collision-induced dissociation mass spectra. *J. Sep. Sci.* **2011**, *34*, 2−10.

(5) Adam, M.; Dobiás, P.; Eisner, A.; Ventura, K. Extraction of antioxidants from plants using ultrasonic methods and their antioxidant capacity. *J. Sep. Sci.* **2009**, *32*, 288−294.

(6) Kim, K.-N.; Ko, Y.-J.; Yang, H.-M.; Ham, Y.-M.; Roh, S. W.; Jeon, Y.-J.; Ahn, G.; Kang, M.-C.; Yoon, W.-J.; Kim, D.; Oda, T. Anti-inflammatory effect of essential oil and its constituents from fingered citron (Citrus medica L. var. sarcodactylis) through blocking JNK, ERK and NF-KB signaling pathways in LPS-activated RAW 264.7 cells. *Food Chem. Toxicol.* **2013**, *57*, 126−131.

(7) Li, Z.-H.; Cai, M.; Liu, Y.-S.; Sun, P.-L.; Luo, S.-L. Antibacterial Activity and Mechanisms of Essential Oil from Citrus medica L. var. sarcodactylis. *Molecules* **2019**, *24*, 1577−1587.

(8) Haggag, Y. A.; El-Ashmawy, N. E.; Okasha, K. M. Is hesperidin essential for prophylaxis and treatment of COVID-19 Infection? *Med. Hypotheses* **2020**, *144*, 109957−109966.
(9) Hassan, N. A.; Bassossy, H. M. E.; Fahmy, A.; Mahmoud, M. F. Limonin alleviates macro- and micro-vascular complications of metabolic syndrome in rats: A comparative study with azelnidipine. *Phytomedicine* **2018**, *43*, 92−102.
(10) Wang, Z.; Li, S.; Ferguson, S.; Goodnow, R.; Ho, C.-T. Validated reversed phase LC method for quantitative analysis of polymethoxyflavones in citrus peel extracts. *J. Sep. Sci.* **2008**, *31*, 30−37.
(11) Wang, S.-N.; Yao, Z.-W.; Zhao, C.-B.; Ding, Y.-S.; Jing-Luo, J.; Bian, L.-H.; Li, Q.-Y.; Wang, X.-M.; Shi, J.-L.; Guo, J.-Y.; Wang, C.-G. Discovery and proteomics analysis of effective compounds in Valeriana jatamansi jones for the treatment of anxiety. *J. Ethnopharmacol.* **2021**, *265*, 113452.
(12) Zheng, G.; Yang, X.; Chen, B.; Chao, Y.; Hu, P.; Cai, Y.; Wu, B.; Wei, M. Identification and determination of chemical constituents of Citrus reticulata semen through ultra high performance liquid chromatography combined with Q Exactive Orbitrap tandem mass spectrometry. *J. Sep. Sci.* **2020**, *43*, 438−451.
(13) Li, X.; He, Q.; Hou, H.; Zhang, S.; Zhang, X.; Zhang, Y.; Wang, X.; Han, L.; Liu, K. Targeted lipidomics profiling of marine phospholipids from different resources by UPLC-Q-Exactive Orbitrap/MS approach. *J. Chromatogr. B: Anal. Technol. Biomed. Life Sci.* **2018**, *1096*, 107−112.
(14) Li, L.; Wang, Y.; Xiu, Y.; Liu, S. Chemical Differentiation and Quantitative Analysis of Different Types of Panax Genus Stem-Leaf Based on a UPLC-Q-Exactive Orbitrap/MS Combined with Multivariate Statistical Analysis Approach. *J. Anal. Methods Chem.* **2018**, *2018*, 9598672−9598688.
(15) Yanmei, Z.; Yifan, F.; Wu, X.; Guo, J. Rapid identification of coumarins from Fructus citri Sarcodactylis by UPLC/Q-TOF-MS. *Nat. Prod. Res.* **2015**, *29*, 53−58.
(16) Dugrand, A.; Olry, A.; Duval, T.; Hehn, A.; Froelicher, Y.; Bourgaud, F. Coumarin and furanocoumarin quantitation in citrus peel via ultraperformance liquid chromatography coupled with mass spectrometry (UPLC-MS). *J. Agric. Food Chem.* **2013**, *61*, 10677−10684.
(17) Chu, J.; Li, S.-L.; Yin, Z.-Q.; Ye, W.-C.; Zhang, Q.-W. Simultaneous quantification of coumarins, flavonoids and limonoids in Fructus Citri Sarcodactylis by high performance liquid chromatography coupled with diode array detector. *J. Pharm. Biomed. Anal.* **2012**, *66*, 170−175.
(18) Leonart, L.; Gasparetto, J.; Pontes, F.; Cerqueira, L.; de Francisco, T.; Pontarolo, R. New Metabolites of Coumarin Detected in Human Urine Using Ultra Performance Liquid Chromatography/Quadrupole-Time-of-Flight Tandem Mass Spectrometry. *Mol* **2017**, *22*, 2031−2043.
(19) Zeng, Y.; Li, S.; Wang, X.; Gong, T.; Sun, X.; Zhang, Z. Validated LC-MS/MS Method for the Determination of Scopoletin in Rat Plasma and Its Application to Pharmacokinetic Studies. *Mol* **2015**, *20*, 18988−19001.
(20) Zhao, M.; Ding, W.; Wang, S.; Wang, C.; Du, Y.; Xu, H.; Wang, Q.; Jin, S. Simultaneous determination of nine coumarins in rat plasma by HPLC−MS/MS for pharmacokinetics studies following oral administration of Fraxini Cortex extract. *J. Chromatogr. B: Anal. Technol. Biomed. Life Sci.* **2016**, *1025*, 25−32.
(21) Sumorek-Wiadro, J.; Zając, A.; Maciejczyk, A.; Jakubowicz-Gil, J. Furanocoumarins in anticancer therapy - For and against. *Fitoterapis* **2020**, *142*, 104492−104500.
(22) Zheng, G.; Liu, M.; Chao, Y.; Yang, Y.; Zhang, D.; Tao, Y.; Zhang, J.; Zeng, C.; Wei, M. Identification of lipophilic components in Citri Reticulatae Pericarpium cultivars by supercritical CO_2 fluid extraction with ultra-high-performance liquid chromatography-Q Exactive Orbitrap tandem mass spectrometry. *J. Sep. Sci.* **2020**, *43*, 3421−3440.
(23) Park, A. Y.; Park, S.-Y.; Lee, J.; Jung, M.; Kim, J.; Kang, S. S.; Youm, J.-R.; Han, S. B. Simultaneous determination of five coumarins in Angelicae dahuricae Radix by HPLC/UV and LC-ESI-MS/MS. *Biomed. Chromatogr.* **2009**, *23*, 1034−1043.
(24) Long, T.; Lv, X.; Xu, Y.; Yang, G.; Xu, L.-Y.; Li, S. Supercritical fluid CO_2 extraction of three polymethoxyflavones from Citri reticulatae pericarpium and subsequent preparative separation by continuous high-speed counter-current chromatography. *J. Chromatogr. B Analyt. Technol. Biomed. Life Sci.* **2019**, *1124*, 284−289.
(25) Zheng, J.; Song, M.; Dong, P.; Qiu, P.; Guo, S.; Zhong, Z.; Li, S.; Ho, C.-T.; Xiao, H. Identification of novel bioactive metabolites of 5-demethylnobiletin in mice. *Mol. Nutr. Food Res.* **2013**, *57*, 1999−2007.
(26) Chen, X.; Xu, L.; Guo, S.; Wang, Z.; Jiang, L.; Wang, F.; Zhang, J.; Liu, B. Profiling and comparison of the metabolites of diosmetin and diosmin in rat urine, plasma and feces using UHPLC-LTQ-Orbitrap MS^n. *J. Chromatogr. B Analyt. Technol. Biomed. Life Sci.* **2019**, *1124*, 58−71.
(27) Ren, W.; Li, Y.; Zuo, R.; Wang, H.-J.; Si, N.; Zhao, H.-Y.; Han, L.-Y.; Yang, J.; Bian, B.-L. Species-related difference between limonin and obacunone among five liver microsomes and zebrafish using ultra-high-performance liquid chromatography coupled with a LTQ-Orbitrap mass spectrometer. *Rapid Commun. Mass Spectrom.* **2014**, *28*, 2292−2300.
(28) Sun, D.; Dong, L.; Guo, P.; Yan, W.; Wang, C.; Zhang, Z. Simultaneous determination of four flavonoids and one phenolic acid in rat plasma by LC-MS/MS and its application to a pharmacokinetic study after oral administration of the Herba Desmodii Styracifolii extract. *J. Chromatogr. B Analyt. Technol. Biomed. Life Sci.* **2013**, *932*, 66−73.
(29) Luan, H.; Yang, L.; Ji, F.; Cai, Z. PCI-GC-MS-MS approach for identification of non-amino organic acid and amino acid profiles. *J. Chromatogr. B Analyt. Technol. Biomed. Life Sci.* **2017**, *1047*, 180−184.
(30) Serra-Cayuela, A.; Castellari, M.; Bosch-Fusté, J.; Riu-Aumatell, M.; Buxaderas, S.; López-Tamames, E. Identification of 5-hydroxymethyl-2-furfural (5-HMF) in Cava sparkling wines by LC-DAD-MS/MS and NMR spectrometry. *Food Chem.* **2013**, *141*, 3373−3380.
(31) Dabur, R.; Mittal, A. Detection and qualitative analysis of fatty acid amides in the urine of alcoholics using HPLC-QTOF-MS. *Alcohol* **2016**, *52*, 71−78.
(32) Wang, M.; Meng, D.; Zhang, P.; Wang, X.; Du, G.; Brennan, C.; Li, S.; Ho, C.-T.; Zhao, H. Antioxidant Protection of Nobiletin, 5-Demethylnobiletin, Tangeretin, and 5-Demethyltangeretin from Citrus Peel in Saccharomyces cerevisiae. *J. Agric. Food Chem.* **2018**, *66*, 3155−3160.

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