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Characterization of Chemical Constituents of *Oxytropis microphylla* (Pall.) DC. by Ultra-High-Performance Liquid Chromatography Coupled with Quadrupole-Time-of Flight Tandem Mass Spectrometry

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Abstract: *Oxytropis microphylla* (Pall.) DC. is a traditional Tibetan medicine used as an external preparation for clearing heat and detoxification, healing sore muscles, astringent vein hemostasis, defecation, and treating plague, constipation, anthrax, and swollen and painful furuncles. It remains a challenge to comprehensively analyze and identify the chemical constituents of *Oxytropis microphylla* (Pall.) DC. In this study, a new analytical method using a combination of ultra-high-performance liquid chromatography–mass spectrometry (UHPLC-MS) and effective data mining techniques was established to identify the chemical constituents of *Oxytropis microphylla*. A total of 127 compounds were identified in *O. microphylla* extract, including 92 flavonoids, 15 indole alkaloids, and 20 others. After the oral administration of the extract to rats, 22 metabolites were identified in the plasma. The primary in vivo metabolic reactions that occurred after the administration of *O. microphylla* extract were glucuronidation and sulfation. Therefore, we successfully devised a high-efficiency method to distinguish compounds and used it as a source of post-study to identify the active biological components of *O. microphylla* extract.

Keywords: *Oxytropis microphylla* (Pall.) DC.; HPLC-TOF-MS; data mining strategy; chemical profiling; metabolites

1. Introduction

More than 140 species and 3 varieties of *Oxytropis* (Family: Leguminosae) are found in China. *Oxytropis* plants were used extensively in the west owing to their outstanding efficiency [1–3]. *Oxytropis microphylla* (Pall.) DC. (OMDC) is a perennial herb, locally identified as the "king of herbs" on account of its analgesic and outstanding anti-inflammatory effects. It is found in the valleys, hillsides, and meadows of the Qinghai–Tibet plateau at an altitude of 2700–4300 m [4–6]. In ancient books, OMDC has been recorded for its efficiency as an analgesic and for anti-inflammatory, detoxification, promotion of blood circulation, and heat-clearing effects [7,8]. Several bioactive compounds have been separated from *Oxytropis* using phytochemical methods. Flavones are the major active ingredients of *Oxytropis* that are known to exert anti-inflammatory, analgesic, ultraviolet damage–protective, and antitumor effects, and to enhance immune cofunction [9,10]. Currently, only a few reports exist

in the literature on the systematic characterization of the chemical components of this plant, which do not effectively explain its active components [11]. Therefore, there is an urgent requirement to establish a scientific method to identify the chemical constituents of OMDC.

To better understand the characteristics of OMDC, it is necessary to establish a robust identification method to analyze the chemical constituents of the plant [12,13]. The current technology used to analyze the potential active components of traditional Chinese medicine is UHPLC-QTOF-MS, which can predict the elemental composition of the tested compound and accurately determine its quality [13–16]. Its high resolution, efficiency, and structure recognition ability can detect hundreds or thousands of chemical components in complex samples and rapidly characterize the extracts used in traditional Chinese medicine [17–20]. Therefore, UHPLC-QTOF-MS is widely used in the qualitative analysis of medicinal materials.

To the best of our knowledge, only a few reports have used UHPLC-QTOF-MS to identify compounds in the crude extract of OMDC, and there is no comprehensive or systematic explanation to analyze their chemical components. Therefore, there is an urgent requirement to establish a reliable method to identify the chemical components of OMDC at micro-concentration levels and to summarize MS fragmentation behaviors. To address this problem, we established a UHPLC-QTOF-MS method for the identification, classification, and systematic investigation of the chemical components of OMDC.

2. Materials and Methods

2.1. Chemicals and Reagents

Acetonitrile and formic acid used for sample processing were sourced from Fisher Scientific (Fair Lawn, NJ, USA) and Sigma Aldrich (Sigma Aldrich, St. Louis, MO, USA), respectively. Pure distilled water was obtained using a Millipore Alpha-Q water purification system. All 6 reference standards having a purity >98% (2′,4′-dihydroxychalcone, 7-hydroxyflavanone, pinocembrin, quercetin, apigenin, luteolin) were purchased from Sichuan Weikeqi Biological Technology Co., Ltd. (Chengdu, China).

2.2. Animal Experiments

Three male Sprague-Dawley rats (weighing 180 ± 20 g) were obtained from the Hunan Laike Jingda Experimental Animal Co. Ltd., Changsha, China. All rats were housed in a room (a temperature of 20 °C and humidity of 50%) and were provided access to food and water ad libitum. OMDC extract was suspended in 0.5% CMC-Na and administered orally to rats at a dose of 150 mg/kg body weight. After drug administration, blood was collected from the inner canthus vein at fixed intervals (1, 2, 4, 6, 12 h). Blood samples were centrifuged at 5000 rpm for 10 min to obtain the serum. The animal experiments were approved by the Laboratory Animal Ethics Committee of Jiangxi University of Traditional Chinese Medicine (approval No. SYXK2017-0004).

2.3. Sample Preparation

OMDC was obtained from Tibet Province in 2020. The sample was identified by Professor Guo-yue Zhong and Ming Yao (Jiangxi University of Traditional Chinese Medicine). The crude extract of OMDC was prepared using the medicinal powder. Briefly, 1 g of OMDC powder was mixed with 50 mL methanol and extracted for 30 min using ultrasonication in a water bath maintained at room temperature (20–30 °C). Extracts were filtered through a 0.22-μM microporous membrane filter and 3.0 μL of the filtered sample was used for UHPLC-QTOF-MS.

All reference materials were dissolved in methanol to yield the respective stock solutions, which were stored at 4 °C. After mixing all the reference stock solutions, they were diluted with methanol to a concentration of 10.0 μg/mL, and 3.0 μL of the solution was used for UHPLC-QTOF-MS.

The plasma of the 3 rats from each time point was mixed. Plasma samples were added to 3 times their volume of methanol, vortexed for 2 min, and centrifuged at 12,000× *g* rpm

for 10 min. This supernatant was dried under a nitrogen stream at 35 °C. The residue was redissolved in 100 µL of 50% methanol and vortexed for 2 min. The solution was centrifuged at 12,000× *g* rpm for 10 min, and 5 µL of the supernatant was injected for UHPLC-QTOF-MS.

2.4. UHPLC-QTOF-MS/MS

A Shimadzu system (Kyoto, Japan) was used for separation. The other systems included a CTO-30AC column oven, a DGU-20A3 degasser, an LC-3AD solvent delivery system, a CBM-20A controller, and a SIL-30ACXR auto-sampler. A Welch UHPLC AQ-C18 (100 mm × 2.1 mm, 1.8 µm) was used at a temperature of 40 °C. The mobile phase was composed of 0.1% formic acid in water (solvent A) and acetonitrile (solvent B) and the flow rate was 0.3 mL/min. The elution conditions were as follows: 0.1–2.0 min, 5–10% B; 2.0–10.0 min, 10–14% B; 10.0–13.0 min, 14–20% B; 13.0–30.0 min, 20–40% B; 30.0–37.0 min, 40–50% B; 37.0–45.0 min, 50–65% B; 45.0–50.0 min, 65–95% B; 50.0–53.0 min, 95% B; 53.0–58.0 min, 5% B.

UHPLC-QTOF-MS/MS analyses were performed in the negative electrospray ionization mode using a Triple TOF™ 5600+ system with a Duo Spray source (AB SCIEX, Foster City, CA, USA). The mass spectrometry conditions were as follows: ion spray voltage, −4500 V; ion source temperature, 550 °C; curtaingas, 25 psi; nebulizer gas (GS1), 55 psi; heater gas (GS2), 55 psi; and decluster potential (DP), −100 V. The mass ranges of TOF-MS and TOF MS/MS experiments were all set at 50–1250 Da.

3. Results and Discussion

3.1. Screening and Identification of Chalcone

In the mass spectrometry of protonated chalcones, phCO+ and ph'CH=CHCO+ were observed as the major fragments. Compound **82** was obtained as a quasi-molecular ion at *m*/*z* 239.0713, which was in accordance with the chemical formula of $C_{15}H_{12}O_3$ (Figures 1–3; Table 1). The characteristic ions at *m*/*z* 135.0090 [M-H-C_8H_8]$^-$, 197.0600 [M-H-C_2H_2O]$^-$, 148.0169 [M-H-C_7H_7]$^-$, and 109.0308 [M-H-C_9H_6O]$^-$ were yielded by C-C bond. Thus, the compound was identified as 2′,4′-dihydroxychalcone by retention time of the standard. Compound **58** was inferred by sifting using the DPI acquired at *m*/*z* 239.0712 and the NL at 162 Da ($C_6H_{10}O_5$). The chemical formula was computed as $C_{21}H_{22}O_8$ based on the exact mass precursor ion. This compound was determined to be 2′,4′-dihydroxychalcone-glucoside. Compound **43** has two more OH radicals than 2′,4′-dihydroxychalcone. The molecular fragment had *m*/*z* 253.0507 [M-H-H_2O]$^-$, 151.0038 [M-H-C_8H_8O]$^-$, 125.0244 [M-H-$C_9H_6O_2$]$^-$, 119.0508 [M-H-$C_7H_4O_4$]$^-$, 93.0354 [M-H-$C_9H_6O_4$]$^-$ and it was structurally similar naringenin chalcone that is reported in the literature [21]. Compound **80** had a mass of 2 Da (2H) more than 2′,4′-dihydroxychalcone and was identified as 2′,4′-dihydroxydihydrochalcone [22]. The deprotonation molecular ion of compound **59** ($C_{15}H_{12}O_4$) was discovered at *m*/*z* 256.0735. The fragment ions (*m*/*z* 237.056 [M-H-H_2O]$^-$, 135.0093 [M-H-C_8H_8O]$^-$, 119.0512 [M-H-$C_7H_4O_3$]$^-$, and 91.0204 [M-H-H_2O-$C_9H_7O_2$]$^-$) were consistent with isoliquiritigenin reported in the literature [23]. Compounds **81** had a mass of 28 Da (C_2H_2), more than isoliquiritigenin. This generated fragment ions at *m*/*z* 225.0916[M-H-CO_2]$^-$, 148.0151[M-H-CH_3-C_7H_6O]$^-$, 119.0507 [M-H-$C_8H_6O_3$]$^-$, which was substantially identical with those reported previously, and was tentatively identified as 2′,4-dihydroxy-4′-methoxy chalcone (Figure 2).

3.2. Screening and Identification of Flavone

The fragmentation of $A^{1,3-}$ and $B^{1,4-}$ was formed by Retro-Diels-Alder (RDA) reaction on the C ring of flavone (Figure 4) and flavonol aglycones. Furthermore, isoflavone aglycones appeared specific fragmentations of $B^{0,3-}$ and $A^{1,3-}$, which derived from the C ring's breakage (Figure 4). The reason may be that the various conjugated systems belong to flavone and isoflavone aglycones influenced the fragmentation behavior of C

ring. They also tend to lose some small neutral molecules, for example CO, CHO, C_3O_2, CO_2, C_2H_2O, etc. The ion fragments of some compounds are listed in this work.

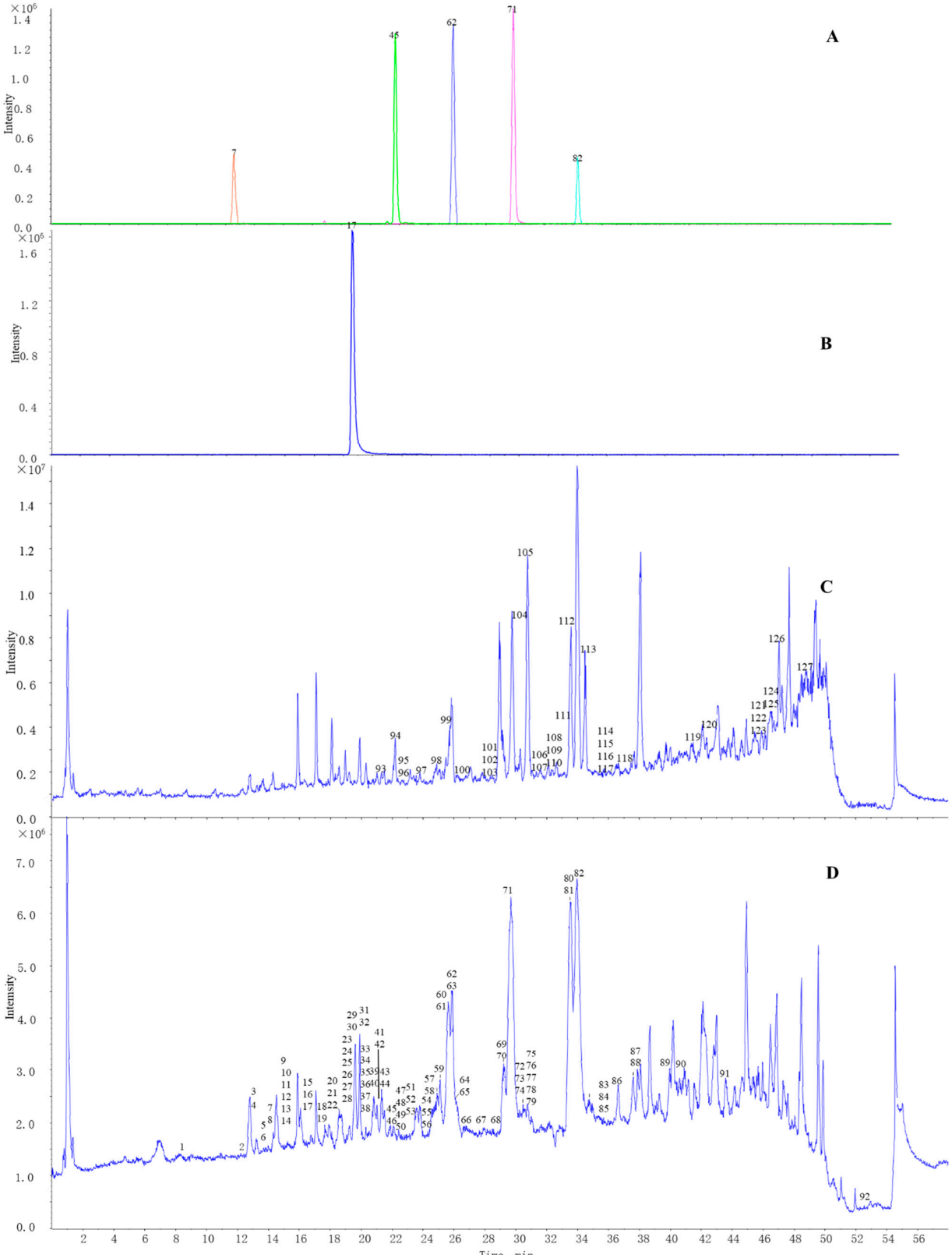
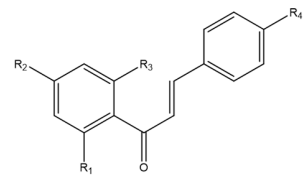
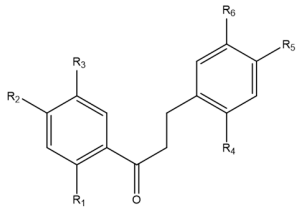


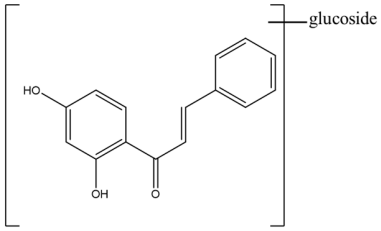
Figure 1. Representative chromatograms from the analysis of OMDC extracts. (**A**,**B**): extracted ion chromatogram (EIC) of reference standards; (**C**): positive ion mode; (**D**): negative ion mode) total ion chromatogram (TIC) of OMDC extract.



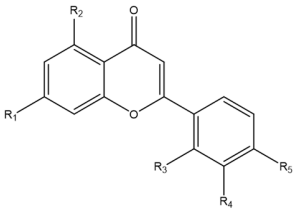
43 R_1,R_2,R_3,R_4=OH
59 R_1,R_2,R_4=OH, R_3=H
63,81 R_1,R_4=OH, R_2=OCH_3, R_3=H
82 R_1,R_2=OH, R_3,R_4=H



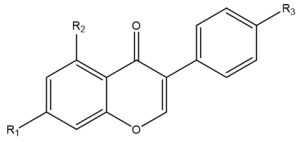
14 R_1,R_3,R_4,R_6=OH, R_2,R_5=H
52 R_1,R_2,R_5=OH, R_3,R_4,R_6 =H
58 R_1,R_2=OH, R_3,R_4 R_5,R_6 =H
85 R_1,R_2,R_3 =H, , R_4=OCH_3, R_5,R_6=OH



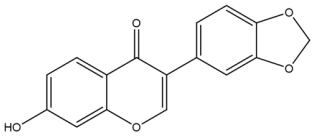
43 naringenin chalcone



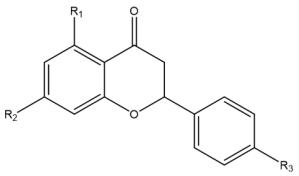
17 R_1,R_2,R_4,R_5=OH, R_3=H
32 R_1,R_4,R_5=OH, R_5= OCH_3, R_6 =H
45 R_1,R_2, R_5 =OH, R_3,R_4 =H
51 R_1,R_2,R_3=OH, R_5= OCH_3, R_6 =H
53 R_1=OH, R_3,R_4,R_5 =H
69 R_1,R_2=OH, , R_3, R_4,R_5=H



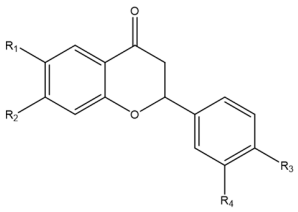
22,24,27,41,44 R_1,R_2=OH, R_3= OCH_3
61 R_1=OH, R_3= OCH_3, R_2 =H



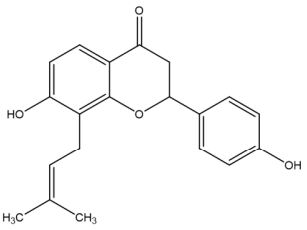
57 pseudobaptigenin



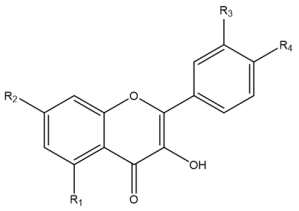
18 R_1=H, R_2,R_3=OH
31 R_1=H, R_2=OH, R_3= OCH_3
71 R_1,R_2=OH, R_3=H
84 R_1=OH, R_2= OCH_3, R_3 =H



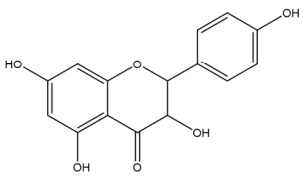
49 R_1,R_2 = OCH_3, R_3,R_4=OH
90 R_1R_2 = OCH_3, R_2=H, R_3,R_4=OH



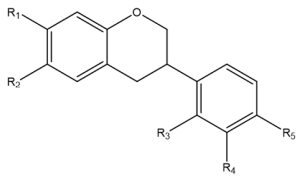
89 isobavachin



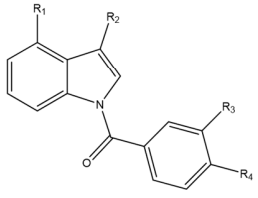
7 R_1,R_2,R_3,R_4 =OH
79 R_1,R_4=OH, R_2= OCH_3, R_3=H



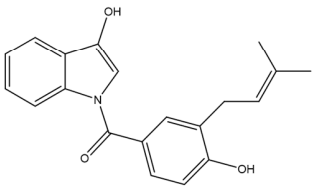
9 dihydrokaempferol



67 R_1,R_4=OH, R_3,R_5 = OCH_3, R_2=H
39 R_1,R_3, ,R_4= OH, R_5=H, R_2= OCH_3



93 R_2,R_3=OH, R_1,R_4,R_5 =H
99 R_2,R_4=OH, R_1,R_3,R_5 =H
96 R_1,R_4=OH, R_2= OCH_3, R_3 =H
109 R_2= OCH_3, R_4 =OH, R_1,R_3 ,R_5 =H
113 R_2, =OH, R_1,R_3,R_4,R_5 =H



119 3- hydroxy-N - (3- isopentenyl -4-hydroxybenzoyl) indoles

Figure 2. Structures of compounds identified from OMDC.

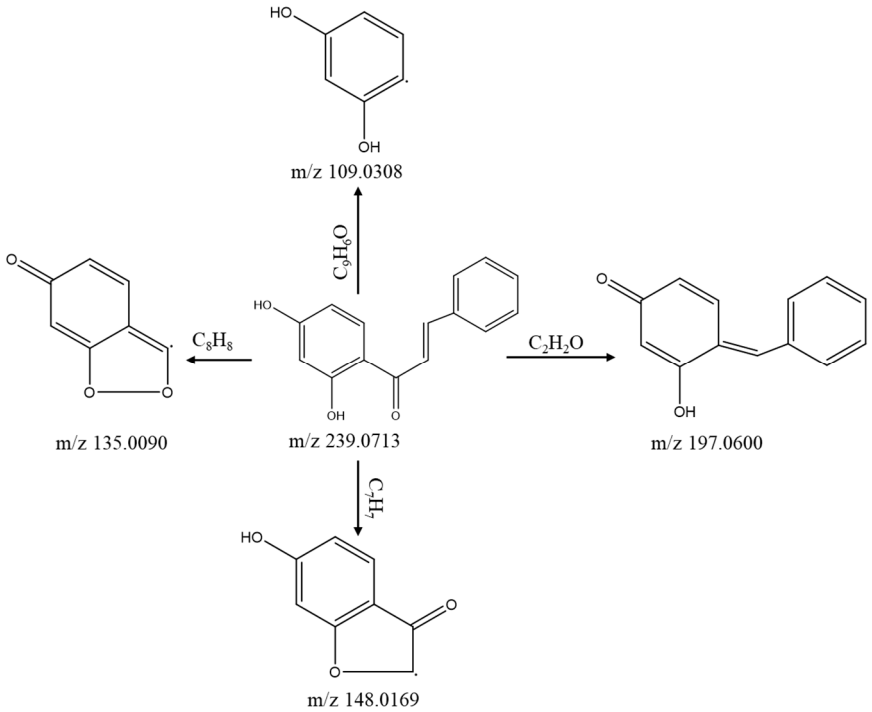


Figure 3. Proposed fragmentation pathways of 2′,4′-dihydroxychalcone.

Table 1. Chromatographic and mass spectrometric data (negative ion) of compounds identified from OMDC using UHPLC-QTOF-MS/MS.

No.	Formula	$[M-H]^-$	Error (ppm)	tR (min)	MS/MS (Characteristic Product Ions)	Identity	Intensity
1	$C_{15}H_{12}O_5$	271.0612	−0.9	8.17	243.0656, 227.0728, 185.0579, 164.0089, 136.0160, 109.0292, 91.0215, 65.0417	2′,3,4,4′-Tetrahydroxychalcone	7488
2	$C_{15}H_{12}O_6$	287.0561	−0.7	12.52	269.0428, 259.0615, 243.0683, 203.0330, 173.0608, 151.0031, 125.0255	(−)-dihydrokaempferol	3122
3	$C_{15}H_{14}O_5$	273.0769	−4	12.87	255.0650, 243.0622, 109.0287,	2,4,2′,5′ -Tetrahydrodihydrochalcone	1984
4	$C_{15}H_{12}O_5$	271.0612	−0.7	12.94	253.0493, 243.0659, 227.0566, 13.0819, 164.0107, 136.0168, 109.0303, 91.0195, 67.0190	2′,3,5,4′-Tetrahydroxychalcone	21,430
5	$C_{15}H_{10}O_5$	269.0456	−0.9	13.75	241.0515, 225.0545, 197.0611, 181.0653, 135.0096, 91.0205	2′,5,7-Trihydroxyflavone	5705
6	$C_{21}H_{22}O_{10}$	433.11402	3.2	13.84	271.0590, 165.0192	Naringenin-glucose	1139
7	$C_{15}H_{10}O_7$	301.0354	−0.6	14.08	283.0248, 255.0286, 215.0340, 151.0003, 145.9294	Quercetin; 2-(3,4-Dihydroxyphenyl)-3,5,7-trihydroxy-4H-chromen-4-one	3244
8	$C_{15}H_{14}O_5$	273.0769	−1.4	14.12	227.0768, 167.0349, 149.0244, 137.0246, 123.0453, 109.0302	2′,4′,6′,4-Tetrahydroxydihydrochalcone	3608
9	$C_{15}H_{12}O_6$	287.0561	−0.5	14.79	269.0435, 259.0605, 243.0667, 201.0534, 177.0558, 151.0031, 125.0242, 83.0133, 63.0250	Dihydrokaempferol; 3,5,7-trihydroxy-2-(4-hydroxyphenyl)-2,3-dihydrochromen-4-one	6907
10	$C_{17}H_{16}O_6$	315.08741	−1.2	14.83	300.0646, 257.0497, 178.9552, 149.0271	3′,5-Dihydroxy-4′,7-Dimethoxyflavanone	1362

Table 1. *Cont.*

No.	Formula	[M-H]$^-$	Error (ppm)	tR (min)	MS/MS (Characteristic Product Ions)	Identity	Intensity
11	$C_{16}H_{14}O_6$	301.07176	−0.7	15.1	286.0473, 283.0595, 271.0616, 255.0702, 191.0359, 179.0344, 176.0093, 164.9257, 151.0394, 135.0081, 121.0295	3′,5,7-Trihydroxy-4′-Methoxyflavanone	4211
12	$C_{15}H_{10}O_5$	269.0456	−0.7	15.26	251.0317, 241.0492, 225.0556, 181.0643, 149.0225, 135.0082, 91.0232	7,8,3′-Trihydroxyflavone	8460
13	$C_{21}H_{22}O_{10}$	433.11402	0.7	15.42	271.0601, 165.0179	Naringenin-glucose	1470
14	$C_{15}H_{14}O_5$	273.0769	−0.9	15.62	255.0670, 227.0683, 167.0347, 148.0172, 137.0131, 123.0455, 109.0286	2,4,2′,5′-Tetrahydrodihydrochalcone	5233
15	$C_{17}H_{16}O_6$	315.08741	−0.8	16.23	300.0618, 257.0438, 178.9567, 149.0228, 121.0309	4′,6-Dihydroxy-5,7-Dimethoxyflavanone	1794
16	$C_{16}H_{14}O_6$	301.07176	−0.9	16.39	283.0612, 271.0613, 225.0554, 179.0352, 151.0418, 136.0136, 121.0305, 93.0353	7-methoxy-3′,4′,5-trihydroxyflavanone	4732
17	$C_{15}H_{10}O_6$	285.0405	−0.8	16.9	257.0449, 217.0506, 199.0390, 175.0396, 149.0229, 133.0292, 83.0140, 65.0201	Luteolin; 3′,4′,5,7-Tetrahydroxyflavone	42,838
18	$C_{15}H_{12}O_4$	255.0663	−1.4	17.65	135.0088, 119.0504, 91.0198	Liquiritigenin; 4′, 7-dihydroxyflavanone	114,322
19	$C_{15}H_{10}O_6$	285.0405	−0.8	17.71	257.0504, 271.0504, 199.0396, 175.0391, 149.0239, 133.0293, 83.0159, 65.0047	2′,4′,5,7-Tetrahydroxyisoflavone	56,144
20	$C_{16}H_{14}O_6$	301.07176	−1.3	18.06	191.0353, 176.0103, 164.9285, 148.0148, 109.0297, 67.0208	3′,4′,7-Trihydroxy-5-methoxyflavanone	2937
21	$C_9H_8O_2$	147.04515	−0.8	18.3	103.0556, 77.0391, 61.9902	Cinnamic acid	8302
22	$C_{16}H_{12}O_5$	283.0612	−0.5	18.42	268.0377, 239.0341, 215.0326, 211.0400, 195.0450, 184.0531, 147.0451, 112.9849, 61.9902	6,7-dihydroxy-5-methoxyflavone	9831
23	$C_{15}H_{12}O_4$	255.0663	−1.3	18.64	237.0560, 209.0608, 199.0761, 167.0861, 149.0247, 135.0090, 109.0301, 91.0206	6,7-dihydroxyflavanone	225,821
24	$C_{16}H_{12}O_5$	283.0612	−1	18.73	268.0376, 239.0345, 211.0394, 184.0519, 148.0168, 135.0089	5,7-dihydroxy-4′-Methoxy isoflavones	91,281
25	$C_{16}H_{16}O_5$	287.0925	−1.4	18.91	272.0683, 257.0439, 163.0405, 150.0321, 135.0439, 121.0306, 109.0280, 91.0580	7,2′,3′-Trihydroxy-4′-methoxyisoflavane	4783
26	$C_{21}H_{22}O_9$	417.1191	0.6	18.96	255.0659, 177.0147, 151.0057	Pinocembrin-glucoside	4369
27	$C_{16}H_{12}O_5$	283.0612	−0.4	19.22	268.0375, 239.0344, 211.0399, 184.0526, 148.0168, 135.0089	4′,7-Dihydroxy-2′-methoxyisoflavon	131,630
28	$C_{21}H_{22}O_9$	417.1191	0.4	19.47	255.0669, 177.0191, 151.0029	Pinocembrin-glucoside	4277
29	$C_{16}H_{16}O_5$	287.0925	−0.4	19.48	272.0690, 257.0435, 163.0397, 150.0317, 135.0463, 121.0292, 109.0279, 91.0615	7,2′,3′-Trihydroxy-6-Methoxy isoflavane	5798
30	$C_{15}H_{12}O_4$	255.0663	−1.1	19.52	237.0610, 209.0610, 199.0765, 165.0709, 135.0089, 109.0302, 91.0202	3′, 6-dihydroxyflavanone	279,373
31	$C_{16}H_{14}O_4$	269.0819	−1	19.75	253.0522, 163.0401, 148.0178, 135.0084, 119.0499, 109.0299, 91.0224	7-hydroxy-4′-methoxy dihydroflavone	36,522
32	$C_{16}H_{12}O_6$	299.0561	−1	19.96	109.0297, 65.0433	7,3′,4′-trihydroxy-5-methoxy flavone	13,371
33	$C_{16}H_{14}O_4$	269.0819	−0.6	20.26	253.0499, 225.0541, 163.0397, 148.0158, 135.0085, 119.0508, 109.0294, 91.0188	2′,4′-Dihydroxy-2-methoxychalcone	46,597

Table 1. *Cont.*

No.	Formula	[M-H]$^-$	Error (ppm)	tR (min)	MS/MS (Characteristic Product Ions)	Identity	Intensity
34	$C_{21}H_{20}O_{10}$	431.09837	0.1	20.32	269.0456, 226.9665, 158.9784, 140.9788	Apigenin-glucoside	1697
35	$C_{21}H_{22}O_9$	417.1191	0.7	20.33	255.0658, 145.0644	Pinocembrin-glucoside	9326
36	$C_{16}H_{12}O_6$	299.0561	−0.4	20.45	109.0299, 65.0411	2′,4′,5-Trihydroxy-7-methoxyisoflavone	14,610
37	$C_{21}H_{20}O_{10}$	431.09837	1.5	20.62	269.0439, 223.0971, 140.9806	Apigenin-glucoside	1657
38	$C_{21}H_{22}O_9$	417.1191	0.9	20.76	255.0659, 211.0790, 171.0418, 151.0031	Pinocembrin-glucoside	10,565
39	$C_{16}H_{16}O_5$	287.0925	−0.7	20.83	257.0818, 239.0698, 224.0470, 136.0171, 109.0297, 91.0210	7,2′,3′-Trihydroxy-4′-methoxyisoflavane	11,412
40	$C_{15}H_{12}O_5$	271.0612	−0.4	20.84	253.0506, 225.0542, 215.0712, 197.0600, 177.0190, 161.0607, 151.0033, 119.0504, 107.0144, 93.0352	Naringenin; 4′,5,7-trihydroxyflavanone	196,476
41	$C_{16}H_{12}O_5$	283.0612	−0.8	21.16	268.0372, 239.0360, 211.0406, 184.0529, 146.9650, 135.0069, 61.9916	3′-methoxy-5,7-dihydroxyflavone	9696
42	$C_{16}H_{16}O_5$	287.0925	−0.7	21.26	257.0820, 239.0712, 224.0484, 136.0170, 109.0298, 91.0204	7,2′,3′-Trihydroxy-4′-methoxyisoflavane	13,087
43	$C_{15}H_{12}O_5$	271.0612	−0.7	21.32	253.0507, 225.0548, 215.0706, 197.0598, 185.0599, 177.0191, 161.0605, 151.0038, 119.0508, 107.0143, 93.0354, 63.0268	Naringenin chalcone; 2′,4,4′,6′-tetrahydroxychalcone	300,571
44	$C_{16}H_{12}O_5$	283.0612	−0.7	21.53	268.0375, 239.0348, 211.0406, 195.0449, 184.0515, 146.9653, 135.0073, 61.9892	6,4′-dihydroxy-7-methoxyflavone	11,665
45	$C_{15}H_{10}O_5$	269.0456	−1	21.83	225.0554, 201.0550, 181.0660, 151.0031, 149.0242, 117.0347, 107.0154, 87.0472	Apigenin; 4′,5,7-Trihydroxyflavone	48,246
46	$C_{17}H_{16}O_6$	315.08741	−0.2	21.84	297.0389, 269.0453, 109.0294, 65.0404	5,4′-dihydroxy-7,3′-dimethoxy-flavanone	6428
47	$C_{16}H_{12}O_7$	315.051	0.2	22.08	297.0397, 269.0450, 254.0218, 226.0249, 165.0199, 109.0293, 65.0390	Rhamnetin	4246
48	$C_{15}H_{12}O_5$	271.0612	−0.8	22.1	253.0502, 227.0706, 185.0606, 151.0034, 143.0499, 107.0138, 83.0146, 65.0044	7,3′,5′-trihydroxyflavanone	46,118
49	$C_{17}H_{16}O_6$	315.08741	−0.6	22.14	297.0401, 269.0448, 254.0230, 226.0184, 198.0315, 165.0202, 109.0293, 65.0433	3′,4′-Dihydroxy-6,7-methoxyflavanone	6592
50	$C_{15}H_{12}O_5$	271.0612	−0.8	22.43	253.0500, 227.0706, 185.0601, 151.0030, 143.0495, 107.0143, 83.0157, 65.0053	3,7,4′-Trihydroxyflavanone	55,627
51	$C_{16}H_{12}O_6$	299.0561	−0.5	23.26	284.0323, 255.0261, 227.0338, 211.0398, 148.0180, 91.0200	5,7,2′-trihydroxy-4′-methoxy flavone	8908
52	$C_{15}H_{14}O_4$	257.0819	−1.1	23.56	163.0400, 151.0405, 135.0088, 107.0511, 93.0358, 65.0414	2′,4′,4-Trihydroxydihydrochalcone	218,604
53	$C_{15}H_{10}O_3$	237.0557	−1.2	23.69	208.0531, 193.0658, 180.0573, 165.0721, 132.0213, 91.0209	7-Hydroxyflavone	52,893
54	$C_{16}H_{12}O_4$	267.0663	−0.3	24.19	252.0418, 223.0387, 196.0520, 168.0583, 135.0085, 117.0344, 91.0192	7-methoxy-4′-hydroxyisoflavone	26,052
55	$C_{15}H_{10}O_3$	237.0557	−2.4	24.54	208.0515, 193.0641, 180.0579, 135.0094, 117.0348, 91.0268	6-Hydroxyflavone	10,396
56	$C_{16}H_{14}O_5$	285.07685	−1	24.56	267.0644, 163.0393, 135.0452, 121.0306, 109.03222, 91.0673	Vestitone; 2′,7-dihydroxy-4′-methoxyisoflavanone	5450

Table 1. *Cont.*

No.	Formula	[M-H]$^-$	Error (ppm)	tR (min)	MS/MS (Characteristic Product Ions)	Identity	Intensity
57	$C_{16}H_{10}O_5$	281.0456	−0.9	24.73	253.0504, 224.0477, 209.0602, 195.0446, 167.0493, 135.0088, 117.0337, 91.0201	Pseudobaptigenin	158,719
58	$C_{21}H_{22}O_8$	401.1242	0.8	24.76	239.0712, 197.0607, 135.0087, 112.9851	2′,4′-Dihydroxychalcone-glucose	13,190
59	$C_{15}H_{12}O_4$	255.0663	−1.2	25.06	135.0093, 119.0512, 91.0204	Isoliquiritigenin; 2′,4,4′-Trihydroxychalcone	518,486
60	$C_{16}H_{12}O_6$	299.0561	−0.9	25.39	284.0315, 256.0351, 165.0191, 149.9952, 121.0293, 65.0262	3′-Methoxy-4′,5,7-trihydroxyflavone	39,790
61	$C_{16}H_{12}O_4$	267.0663	−1.2	25.45	252.0424, 223.0391, 208.0522, 195.0442, 167.0485, 132.0214, 91.0181	Formononetin; 7-Hydroxy-4′-methoxyisoflavone	101,086
62	$C_{15}H_{12}O_3$	239.0714	−2.3	25.85	197.0603, 169.0658, 148.0164, 135.0090, 109.0302, 91.0207	7-Hydroxyflavanone	1,785,720
63	$C_{16}H_{14}O_4$	269.0819	−0.8	25.9	225.0899, 148.0184, 119.0510	4,4′-dihydroxy-2′-methoxychalcone	20,639
64	$C_{15}H_{12}O_4$	255.0663	−1.1	26.12	237.0560, 209.0605, 193.0657, 169.0661, 145.0294, 135.0088, 119.0503, 109.0302, 91.0201	(2R)-Pinocembrin; (2R)-5,7-dihydroxy-2-phenyl-2,3-dihydrochromen-4-one	202,511
65	$C_{16}H_{12}O_6$	299.0561	−0.7	26.31	284.0326, 255.03040, 227.0347, 211.0384, 148.0152,	3′,4′,7-Trihydroxy-5-methoxyflavone	12,184
66	$C_{15}H_{10}O_4$	253.0506	−0.8	26.78	223.0384, 208.0526, 195.0450, 180.0582, 152.0633, 132.0422, 116.9286, 92.0318	4′,7-Dihydroxyisoflavone	26,456
67	$C_{17}H_{18}O_5$	301.1082	−0.9	27.07	286.0853, 271.0602, 179.0728, 164.0482, 149.0234, 135.0451, 109.0293	7,3 ‘-dihydroxy-2′, 4 ‘-dimethoxy isoflavane	5886
68	$C_{16}H_8O_6$	295.02481	−0.8	27.88	267.0292, 266.0218, 239.0352, 211.0407, 195.0285, 158.9768, 114.9876	Medicagol	2425
69	$C_{15}H_{10}O_4$	253.0506	−0.9	29.23	225.0561, 209.0610, 181.0659, 143.0504, 119.0504, 107.0147, 63.0270	Chrysin; 5,7-Dihydroxyflavone	872,015
70	$C_{16}H_{14}O_5$	285.07685	−0.1	29.5	269.0472, 165.0191, 119.0505, 97.0290, 89.0041, 65.0129	Sakuranetin, 4′,5-Dihydroxy-7-methoxyflavanone	52,887
71	$C_{15}H_{12}O_4$	255.0663	−1.3	29.68	227.0708, 213.0549, 211.0762, 185.0603, 171.0447, 169.0655, 151.0035, 145.0659, 107.0146, 83.0160, 65.0068	Pinocembrin; 5,7-Dihydroxyflavanone	2,838,626
72	$C_{15}H_{12}O_5$	271.0612	−2	29.72	253.0504, 243.0659, 227.0706, 185.0603, 173.0606, 152.0107, 124.0162, 95.0139, 65.0050	7,3′,4′-Trihydroxyflavanone	45,968
73	$C_{15}H_{10}O_3$	237.0557	−0.3	29.79	208.0519, 193.0671, 180.0560, 165.0724, 135.0106, 107.0156, 91.0193, 65.0103	5-Hydroxyflavone	13,845
74	$C_{15}H_{12}O_3$	239.0714	−1.7	30.26	211.0796, 197.0601, 195.0805, 169.0653, 148.0164, 135.0089, 109.0305, 91.0203, 65.0057	2′,5′-Dihydroxychalcone	74,066
75	$C_{15}H_{10}O_5$	269.0456	−0.1	30.44	252.0426, 239.0369, 224.0454, 200.8814, 169.0643,	3′,4′,5-Trihydroxyflavon	7107
76	$C_{16}H_{14}O_5$	285.07685	−1.9	30.57	267.0656, 145.0293, 139.0398, 124.0168, 96.0229	3′,4′-Dihydroxy-5′-methoxyflavanone	63,776
77	$C_{15}H_{14}O_4$	257.0819	−2.2	30.73	239.0721, 213.0925, 195.0814, 173.0610, 151.0039, 122.0377, 107.0153, 81.0367, 65.0043	2′,4′,6′-Trihydroxydihydrochalcone	127,231

Table 1. *Cont.*

No.	Formula	[M-H]$^-$	Error (ppm)	tR (min)	MS/MS (Characteristic Product Ions)	Identity	Intensity
78	$C_{16}H_8O_6$	295.02481	−0.8	30.79	267.0293, 266.0216, 237.0185, 211.0393, 135.0100	Medicagol; 7-Hydroxy-11,12-methylenedioxycoumestan	23,276
79	$C_{16}H_{12}O_6$	299.0561	−0.6	30.99	284.0320, 271.0610, 256.0367, 240.0415, 178.0270, 165.0190, 151.0033, 122.0014, 93.0355, 65.0054	Rhamnocitrin; 3,4′,5-Trihydroxy-7-methoxyflavone	64,943
80	$C_{15}H_{14}O_3$	241.087	−1.2	33.52	223.0762, 197.0970, 150.0321, 135.0091, 122.0380, 109.0307, 91.0209, 65.0436	2′,4′-Dihydroxydihydrochalcone	2,684,260
81	$C_{16}H_{14}O_4$	269.0819	−1.2	33.69	225.0916, 148.0151, 119.0507, 93.0337	2′,4-dihydroxy-4′-Methoxy chalcone	108,605
82	$C_{15}H_{12}O_3$	239.0714	−1.4	33.95	211.0761, 197.0600, 195.0812, 169.0662, 148.0169, 135.0090, 109.0308, 91.0210, 65.0074	2′,4′-Dihydroxychalcone	3,251,005
83	$C_{15}H_{10}O_5$	269.0456	−0.5	35.22	241.0498, 225.0544, 197.0596, 181.0645	3′,4′,7-Trihydroxyflavone	9165
84	$C_{16}H_{14}O_4$	269.0819	−1.2	35.86	254.0574, 226.0620, 198.0652, 165.0185, 149.9948, 122.0016, 65.0080	5-hydroxy-7-methoxy dihydroflavone	5383
85	$C_{16}H_{16}O_4$	271.09758	−0.2	36.11	256.0734, 165.0207, 152.0110, 139.0396, 124.0166	4,5-Dihydroxy-2-Methoxy dihydrochalcone	7714
86	$C_{16}H_{16}O_4$	271.09758	−1.4	36.66	256.0752, 253.0872, 165.0202, 151.0116, 139.0392, 124.0158	4,5-Dihydroxy-2-Methoxy dihydrochalcone	7147
87	$C_{16}H_{14}O_4$	269.0819	−0.9	38.02	254.0580, 226.0628, 177.0184, 165.0196, 149.9946, 122.0012, 65.0046	5-hydroxy-7-methoxy dihydroflavone	40,090
88	$C_{16}H_{14}O_5$	285.07685	−0.9	38.02	270.0547, 165.0202, 145.0286, 139.0408, 93.0353	3′,4′-Dihydroxy-5′-methoxyflavanone	3772
89	$C_{20}H_{20}O_4$	323.1289	−0.7	39.75	255.0667, 203.0706, 159.0805, 119.0506, 93.0361	isobavachin; 4′,7-dihydroxy-8-prenylflavanone	25,579
90	$C_{16}H_{14}O_5$	285.07685	−0.9	40.66	267.0642, 241.0865, 176.0101, 148.0137, 109.0191	3′,4′-Dihydroxy-6-methoxyflavanone	3395
91	$C_{20}H_{20}O_4$	323.1289	−0.4	43.34	305.1204, 277.1659, 255.0628, 219.0698, 186.9312	4,2′,4′-trihydroxy-3′-isopentenyl chalcone	2040
92	$C_{16}H_{14}O_6$	301.07176	−0.8	52.85	283.0601, 257.0847, 192.0055, 173.0594, 164.0103	4′,5,7-trihydroxy-3′-methoxyflavanone	9203

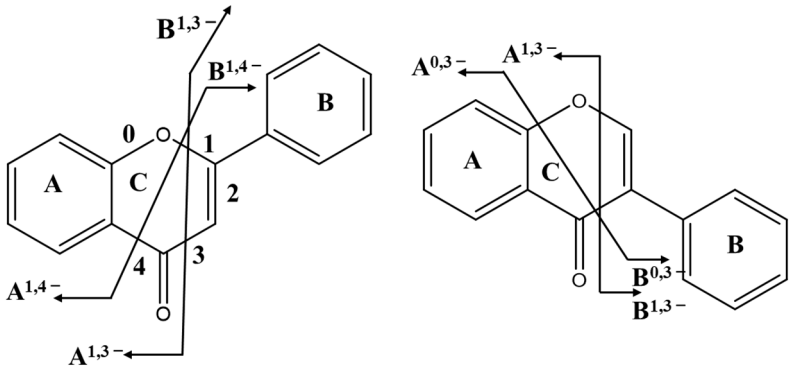


Figure 4. RDA collision of flavone and isoflavone aglycones at C ring (A, B, and C stand for different six-membered rings).

Compound **53** had a deprotonated molecular ion at m/z 238.0629 and its molecular formula was determined to be $C_{15}H_{10}O_3$. This compound generated fragment ions

at m/z 208.0531, 193.0658, 180.0573, 165.0721, and 91.0209, and was tentatively identified as 7-hydroxyflavone (Figure 3) [22]. The molecular formulae for **69, 45**, and **17** were $C_{15}H_{10}O_4$, $C_{15}H_{10}O_5$, and $C_{15}H_{10}O_6$, respectively, and the compounds were identified as chrysin, apigenin, and luteolin, respectively, by DPIs (chrysin: m/z 225.0561 $[M\text{-}H\text{-}CO]^-$, 209.0610 $[M\text{-}H\text{-}CO_2]^-$, 151.0034 $[M\text{-}H\text{-}C_8H_6]^-$, 143.0504 $[M\text{-}H\text{-}C_6H_6O_2]^-$, 107.0147 $[M\text{-}H\text{-}C_9H_6O_2]^-$; apigenin: m/z 225.0554 $[M\text{-}H\text{-}CO_2]^-$, 151.003 $[M\text{-}H\text{-}C_8H_6O]^-$, 117.0347 $[M\text{-}H\text{-}C_7H_4O_4]^-$, 107.0154 $[M\text{-}H\text{-}C_9H_6O_3]^-$; luteolin: m/z 257.0048 $[M\text{-}H\text{-}H_2O]^-$, 241.0504 $[M\text{-}H\text{-}CO_2]^-$, 217.0504 $[M\text{-}H\text{-}CO_2\text{-}C_2H_2]^-$, 149.0239 $[M\text{-}H\text{-}C_7H_4O_3]^-$, 133.0293 $[M\text{-}H\text{-}C_7H_4O_4]^-$) and NL [44 Da ($CO_2$) and 68 Da ($C_4H_4O$)], the results were consistent with those reported in the literature [24,25]. (Compound **61**) The quasi-molecular ions acquired at m/z 267.0670 were 14 Da (CH_2) more than chrysin and the compound was determined to be formononetin via the DPIs (252.0424 $[M\text{-}H\text{-}CH_3]^-$, 223.0391 $[M\text{-}H\text{-}CO_2]^-$, 195.0442 $[M\text{-}H\text{-}CO_2\text{-}CO]^-$, 132.0214 $[M\text{-}H\text{-}C_7H_3O_3]^-$, 91.0181 $[M\text{-}H\text{-}CH_3\text{-}C_9H_5O_3]^-$) [26]. Compound **24** was screened in OMDC extracts based on the precursor ions at m/z 283.0609 and the chemical formula was computed as $C_{16}H_{12}O_5$. The fragment ions (m/z 268.0375 $[M\text{-}H\text{-}CH_3]^-$, 239.0344 $[M\text{-}H\text{-}CO_2]^-$, 184.0526 $[M\text{-}H\text{-}C_4H_4O_3]^-$, 148.0526 $[M\text{-}H\text{-}C_7H_3O_3]^-$, 135.0089 $[M\text{-}H\text{-}C_9H_8O_2]^-$) of these compounds were the same as those reported in the literature [4,27]. Compound **24** was, therefore, tentatively deduced to be 5,7-dihydroxy-4′-methoxy isoflavones (Figures 3 and 5). The molecular formula for compound **51** was determined to be $C_{16}H_{12}O_6$ based on the accurate mass of the quasi-molecular ions at m/z 299.0558. This organic compound was confirmed to be 5,7,2′-trihydroxy-4′-methoxy flavone (Figure 3) by the DPI m/z 284.0323, 255.0261, 227.0338, 211.0398, 148.0180 [2]. Compound **57** was determined to be pseudobaptigenin based on the parent ions at m/z 281.0455, and its molecular formula was deduced as $C_{16}H_{10}O_5$. It generated fragment ions at m/z 253.0504 $[M\text{-}H\text{-}CO_2]^-$, 225.0557$[M\text{-}H\text{-}CO_2\text{-}CO]^-$, 135.0088$[M\text{-}H\text{-}C_9H_6O_2]^-$, and 91.0201$[M\text{-}H\text{-}C_{10}H_6O_4]^-$, and its structure was consistent with that reported in the literature [28,29].

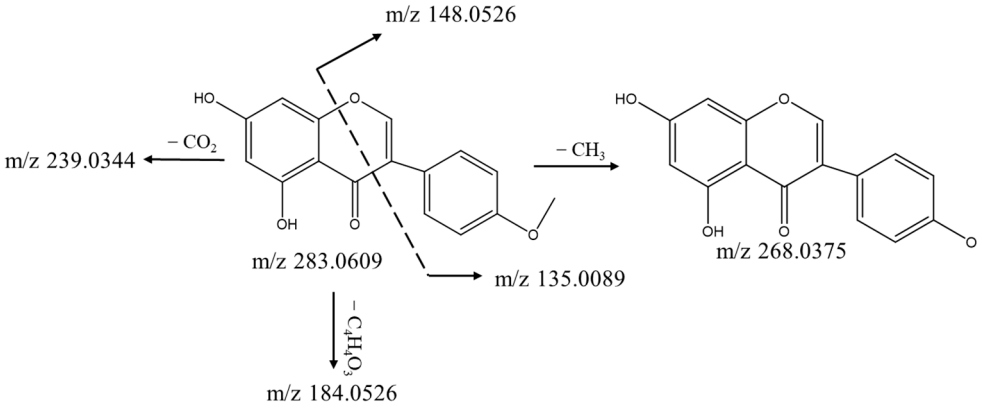


Figure 5. Proposed fragmentation pathways of 5,7-dihydroxy-4′-methoxy isoflavones.

Compounds **71** and **18** yielded parent ions at m/z 255.0656, corresponding to the formula $C_{15}H_{12}O_4$. Compound **71** was determined to be pinocembrin based on its chromatographic data and comparison with the fragment ions with standard pinocembrin. Compounds **26**, **28**, and **38** were determined to be pinocembrin derivatives based on their DPIs acquired at m/z 211.0790, 171.0418, and 151.0031. These compounds were tentatively classified as pinocembrin-glucoside due to the DPIs and NL 162 Da ($C_6H_{10}O_5$). Compound **18** and isoliquiritigenin were structural isomers. It found to be liquiritigenin by the DPIs acquired at m/z 135.0093$[M\text{-}H\text{-}C_8H_8O]^-$, 119.0512$[M\text{-}H\text{-}C_7H_4O_3]^-$, and 91.0204$[M\text{-}H\text{-}C_9H_8O_3]^-$. These compounds were similar, as reported in the literature [30]. Two compounds (**31** and **84**) had a deprotonated molecular ion at m/z 269.0815 in the MS data, which matched with the chemical formula $C_{16}H_{14}O_4$. Based on the DPIs (m/z 163.0397$[M\text{-}H\text{-}C_6H_5O]^-$, 148.0151 $[M\text{-}H\text{-}C_6H_3O_2]^-$, 135.0085 $[M\text{-}H\text{-}C_8H_8O]^-$, 119.0507 $[M\text{-}H\text{-}C_7H_4O_3]^-$, and 91.0188 $[M\text{-}H\text{-}C_9H_8O_3]^-$) of the compound **31** was identified as 7-hydroxy-4′-methoxy

dihydroflavone (Figure 6) [31], and compound **84** was identified as 5-hydroxy-7-methoxy dihydroflavone by DPIs (*m*/*z* 165.0196, 149.9946, 122.0012, and 65.0046) [32]. Compound **89** had a mass of 68 Da (C_5H_8), more than pinocembrin and its chemical formula was calculated to be $C_{20}H_{20}O_4$. This compound was provisionally designated as isobavachin based on DPIs (*m*/*z* 255.0667 [M-H-C_5H_8]$^-$, 203.0706 [M-H-$C_6H_3O_2$]$^-$, 159.0805 [M-H-$C_9H_8O_3$]$^-$, 119.0506 [M-H-C_5H_8-$C_7H_3O_3$]$^-$, and 93.0361 [M-H-$C_{14}H_{14}O_3$]$^-$) [33].

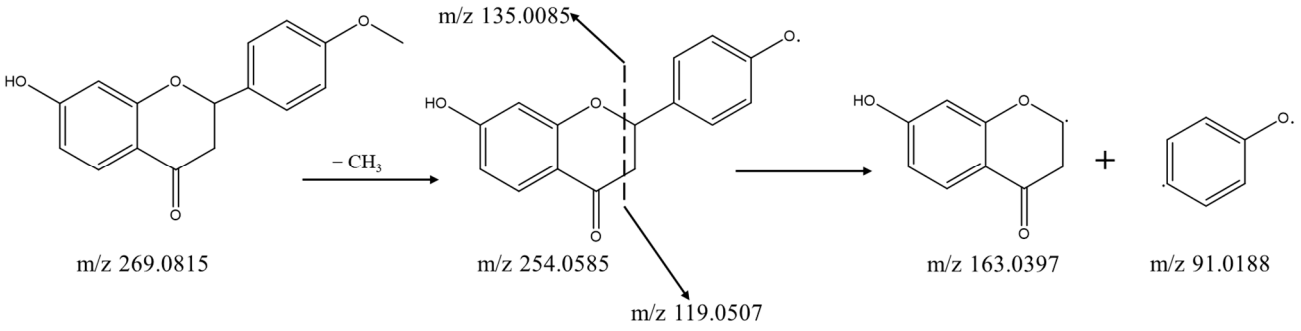


Figure 6. Proposed fragmentation pathways of 7-hydroxy-4′-methoxy dihydroflavone.

3.3. Screening and Identification of Flavonol

The molecular formula of compound **7** was calculated as $C_{15}H_{10}O_7$, and the ion fragments that were obtained were mainly *m*/*z* 283.0248[M-H-H_2O]$^-$, 255.0286[M-H-H_2O-CO]$^-$, and 151.0003 [M-H-$C_8H_6O_3$]$^-$. Based on the results that were consistent with those reported in the literature, the compound was identified as quercetin [34]. Compound **79** had a deprotonated molecular ion at *m*/*z* 299.0565 and its molecular formula was calculated as $C_{16}H_{12}O_6$. These fragments (*m*/*z* 284.0320 [M-H-CH_3]$^-$, 271.0610 [M-H-CO]$^-$, 165.0190 [M-H-$C_8H_6O_2$]$^-$, 151.0033 [M-H-CH_3-$C_8H_6O_2$]$^-$, and 93.0355 [M-H-$C_{10}H_6O_5$]$^-$) were determined to be those of rhamnocitrin (Figures 3 and 7), which was consistent with that reported in the literature [35]. Compound **9** was identified as dihydrokaempferol (Figure 8) by DPIs (*m*/*z* 259.0605 [M-H-CO]$^-$, 269.00435[M-H-H_2O]$^-$, 177.0558[M-H-H_2O-C_6H_4O]$^-$, and 151.0031[M-H-H_2O-C_8H_8O]$^-$) and its molecular formula was $C_{15}H_{12}O_6$.

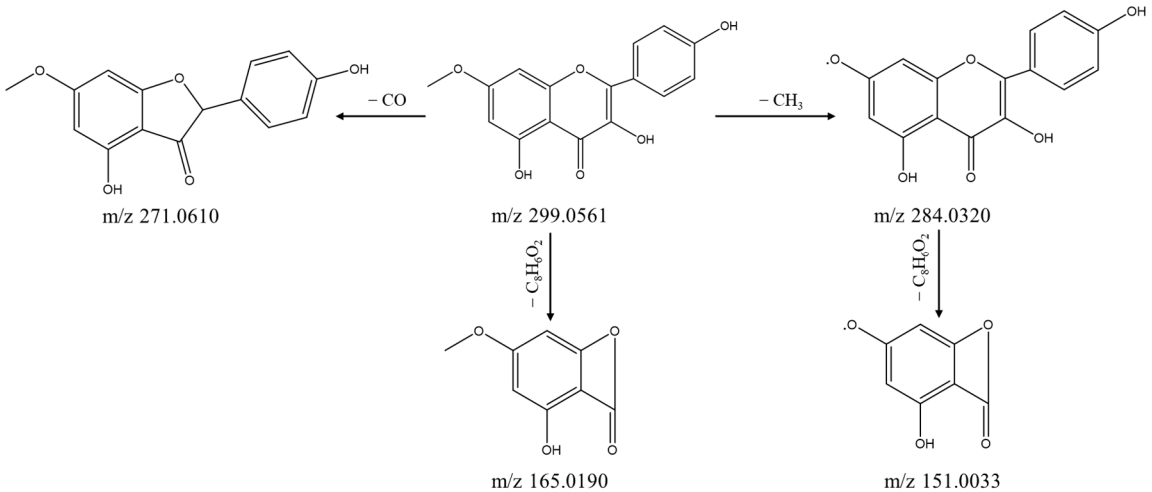


Figure 7. Proposed fragmentation pathways of rhamnocitrin.

3.4. Screening and Identification of Isoflavane

The molecular ion peak of compound **67** at *m*/*z* 301.1048 was determined based on quantitative data and its molecular formula was estimated to be $C_{17}H_{18}O_5$. The compound was confirmed as 5,7-dimethoxy-2′,4′-dihydroxy isoflavane (Figures 3 and 9) by the DPIs at 286.0853[M-H-CH_3]$^-$, 271.0602[M-H-CH_3-CH_3]$^-$, 135.0451[M-H-$C_9H_{10}O_3$]$^-$,

and 109.0293[M-H-$C_{11}H_{12}O_3$]$^-$ (Figure 2), and further confirmed by comparison with fracture modes reported in the literature [4,36]. The parent ion of Compound **39** was identified as *m*/*z* 287.0912 ($C_{16}H_{16}O_5$) and it had a mass of 14 Da (CH_2) less than that of compound **67**. The compound was provisionally identified as 7,2′,3′-trihydroxy-4′-methoxy isoflavane (Figure 3) by DPIs (*m*/*z* 257.0820 [M-H-CH_3O]$^-$, 239.0712 [M-H-CH_3O-H_2O]$^-$, 136.0170[M-H-$C_8H_7O_3$]$^-$, and 109.0298[M-H-$C_{10}H_{10}O_3$]$^-$) [36].

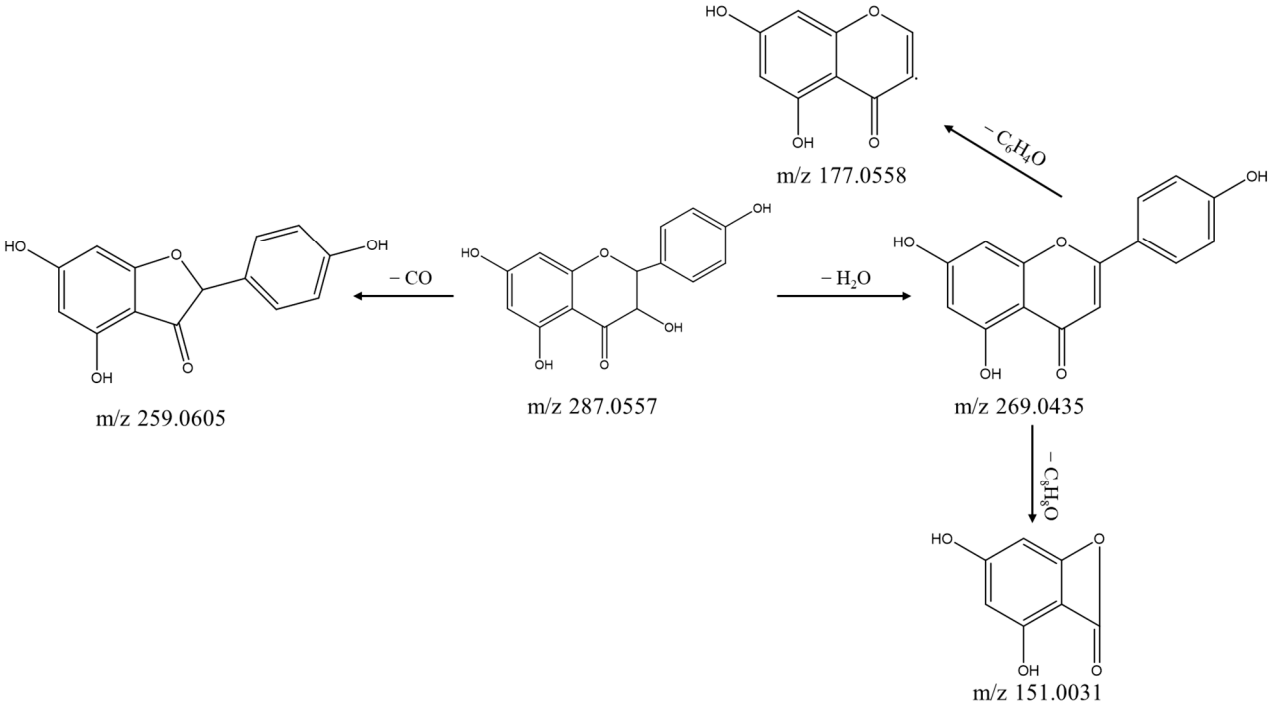


Figure 8. Proposed fragmentation pathways of dihydrokaempferol.

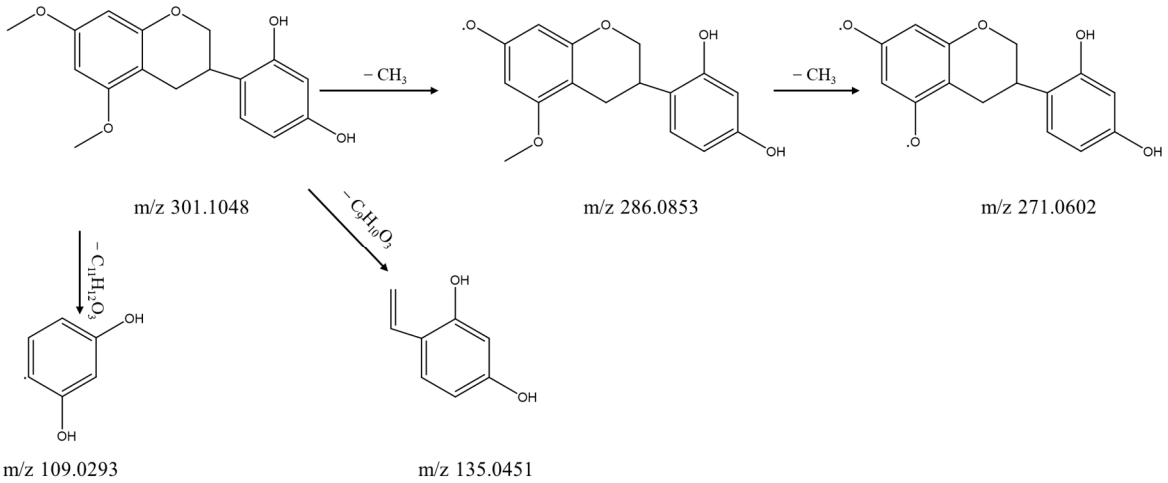


Figure 9. Proposed fragmentation pathways of 5,7-dimethoxy-2′,4′-dihydroxy isoflavane.

3.5. Screening and Identification of Indole Alkaloids

Compound **112** was an isomer with a quasi-molecular ion *m*/*z* 238.0867, which was consistent with the chemical formula of $C_{15}H_{11}NO_2$. It was determined to be 3-hydroxy-N-benzoyl indole based on the resulting DPIs (*m*/*z* 165.0705, 105.0342, 77.0397) [36]. The deprotonated molecular ions at *m*/*z* 254.0821 ($C_{15}H_{11}NO_3$) for two compounds (**93** and **98**) were 16 Da (O) more than that of 3-hydroxy-N-benzoyl indole. The structure of Compound 93 was deduced from the DPIs (165.0695, 121.0288, 107.0498) and speculated as 3-hydroxy-N-(3-hydroxybenzoyl) indole. Based on the DPIs at *m*/*z* 135.0449, Compound **98** was

tentatively identified as 3-hydroxy-N-(p-hydroxybenzoyl) indole [36]. Compounds **108** had a mass of 30 Da (CH_2O) more than that of 3-hydroxy-N-benzoyl indole and was identified as 3-methoxy-N-(p-hydroxybenzoyl) indole by the fragmentation ions (*m*/*z* 253.0742, 121.0291, 93.0347) [36]. The precursor ion of Compound **118** was identified as *m*/*z* 322.1456, and had a mass of 84 Da (C_5H_8O) more than that of 3-hydroxy-N-benzoyl indole. It was provisionally identified as 3-hydroxy-N-(3-isopentenyl-4-hydroxybenzoyl) (Figure 10 and Table 2) indole on the basis of DPIs (*m*/*z* 266.0825 $[M+H-C_4H_7]^+$, 238.0865 $[M+H-C_4H_8-CO]^+$, 189.0913, 133.0289).

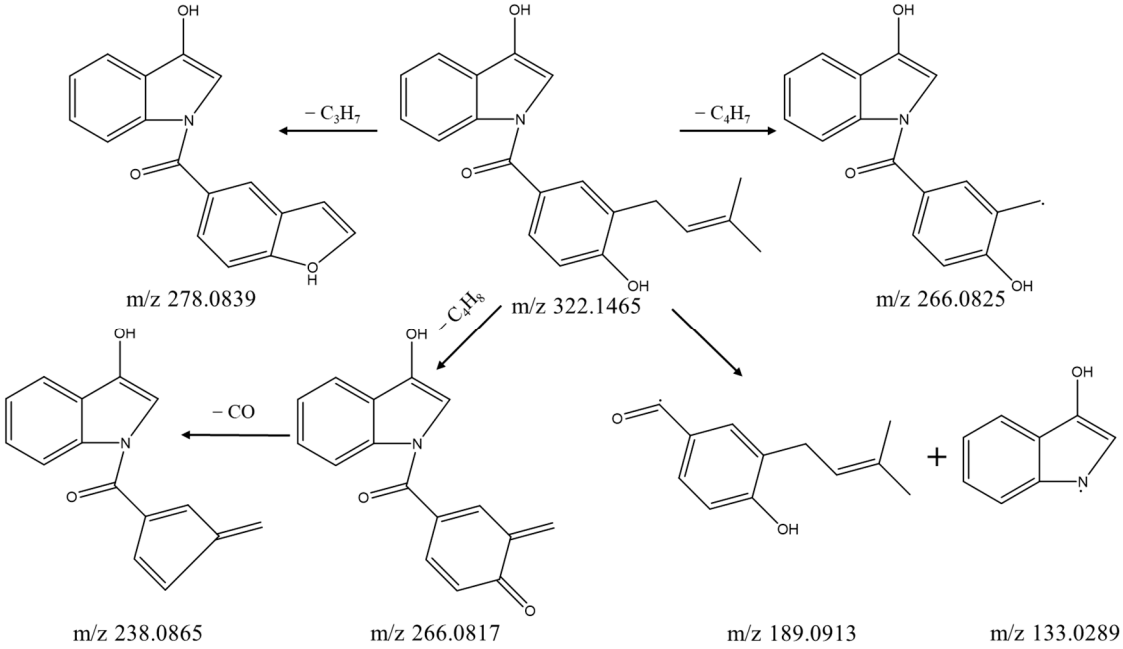


Figure 10. Proposed fragmentation pathways of 3-hydroxy-N-(3-isopentenyl-4-hydroxybenzoyl) indoles.

Table 2. Chromatographic and mass spectrometric data (positive ion) of compounds identified from OMDC using UHPLC-QTOF-MS/MS.

No.	Formula	$[M+H]^-$	Error (ppm)	tR (min)	MS/MS (Characteristic Product Ions)	Identity	Identity
93	$C_{15}H_{11}NO_3$	254.0812	0.5	21.29	236.0703, 208.0760, 165.0695, 121.0288, 107.0498, 93.0677, 65.0383	3-Hydroxy-N-(3-hydroxybenzoyl) indole	6243
94	$C_{15}H_{26}O_2$	239.2006	−0.8	22.19	221.1896, 203.1798, 147.1171, 135.1166, 133.1010, 107.0862, 95.0863, 81.0710	3-Methyl-5-(1,3,3-trimethyl-7-oxabicyclo [2.2.1] hept-2-yl)-pent-1-en-3-ol	18,924
95	$C_{15}H_{11}NO_3$	254.0812	0.4	22.85	236.0713, 208.0754, 165.0694, 121.0289, 107.0493, 93.0342, 65.0396	3-Hydroxy-N-(3-hydroxybenzoyl) indole	33,513
96	$C_{16}H_{13}NO_4$	284.0917	0.7	22.91	269.0690, 226.0628, 150.0299, 121.0280,	3-Methoxy-4-hydroxy-n-(p-hydroxybenzoyl) indole	9202
97	$C_{16}H_{13}NO_4$	284.0917	0.8	23.79	269.0685, 150.0309, 120.0442	3-Methoxy-4-hydroxy-n-(p-hydroxybenzoyl) indole	165,711
98	$C_{15}H_{11}NO_3$	254.0812	0.9	24.9	236.0716, 208.0768, 181.0657, 165.0705, 135.0449, 121.0291, 107.0499, 93.0341, 65.0399	3-Hydroxy-N-(p-hydroxybenzoyl) indole	251,175
99	$C_{15}H_{15}NO$	226.1226	0.7	25.7	122.0602, 105.0342, 103.0553, 77.0399	N-Benzoyl-phenylethylamine	1,428,756

Table 2. *Cont.*

No.	Formula	[M+H]$^-$	Error (ppm)	tR (min)	MS/MS (Characteristic Product Ions)	Identity	Identity
100	$C_{15}H_{11}NO_3$	254.0812	0.9	26.75	236.0714, 209.0585, 181.0654, 165.0708, 153.0689, 135.0453, 121.0286, 107.0482, 93.0338, 65.0401	3-Hydroxy-N-(p-hydroxybenzoyl) indole	50,895
101	$C_{32}H_{32}O_9$	561.2119	−1.3	27.96	509.1637, 424.1442, 385.1068, 373.1072, 259.0970, 167.0699, 123.0433, 107.0498	(3R)-Propterol-B-(α,6)-(−)-isomucronulatol	4620
102	$C_{15}H_{26}O_2$	239.2006	0.6	28.48	221.1925, 203.1806, 161.1315, 147.1172, 119.0849, 95.0860, 81.0722	3-Methyl-5-(2,2,4-trimethylcyclohexanol-3-yl)-pent-l-ene-3-ol	18,924
103	$C_{16}H_{13}NO_4$	284.0917	1	28.57	269.0685, 137.0245, 91.0530	3-Methoxy-4-hydroxy-n-(p-hydroxybenzoyl) indole	7924
104	$C_{17}H_{19}NO$	254.1539	0.8	29.84	131.0490, 122.0962, 105.0699, 103.0545, 79.0548	N-Hydrocinnamoyl-2-phenylethylamine	113,125
105	$C_{17}H_{17}NO$	252.1383	1.1	30.75	148.0760, 131.0492, 105.0699, 103.0543, 79.0550, 77.0395	N-Cinnamoyl-2-phenylethylamine	5,724,222
106	$C_{15}H_{11}NO_2$	238.0863	0.8	30.84	220.0760, 165.0700, 121.0291, 91.0548	N-p-Hydroxybenzoyl indole	369,512
107	$C_{15}H_{11}NO_2$	238.0863	0	31.83	220.0758, 165.0698, 121.0289, 93.0336, 65.0405,	N-p-Hydroxybenzoyl indole	52,164
108	$C_{16}H_{13}NO_3$	268.0968	0.7	32.1	253.0742, 225.0792, 210.0675, 149.0602, 134.0366, 121.0291, 107.0496, 93.0347, 65.0397	3-Methoxy-N-(p-hydroxybenzoyl) indole	520,562
109	$C_{16}H_{13}NO_3$	268.0968	1.1	32.63	253.0745, 150.0318, 121.0298, 105.0342, 77.0398	3-Methoxy-hydroxy-n-benzoyl indole	503,247
110	$C_{30}H_{48}O_3$	457.3676	−1	33.09	439.3568, 421.3458, 376.1913, 245.1907, 233.1914, 185.1312, 163.1505, 147.1145, 109.0989, 81.0724	Soyasapogenol E	13,542
111	$C_{16}H_{13}NO_3$	268.0968	0.5	33.3	253.0737, 255.0783, 150.0320, 134.0367, 121.0285, 105.0349, 93.0333, 77.0394, 65.0390	3-Methoxy-hydroxy-n-benzoyl indole	29,002
112	$C_{15}H_{11}NO_2$	238.0863	1	33.57	221.0604, 220.0762, 193.0655, 165.0705, 135.0442, 105.0342, 77.0397	3-Hydroxy-N-benzoyl indole	1,270,624
113	$C_{16}H_{14}O_3$	255.1016	1.6	34.48	240.0793, 209.0972, 194.0735, 177.0560, 165.0707, 151.0395, 131.0500, 103.0553, 95.0504, 77.0400	2′-Hydroxy-4′-methoxychalcone	2,708,762
114	$C_{30}H_{46}O_4$	471.3469	−1	35.03	453.3366, 435.3233, 395.2965, 199.1518, 173.1328, 159.1160, 145.0998, 97.0654	3,22,24-Trihydroxy-γ-lactone-olean-12-en-29-oic acid	15,709
115	$C_{30}H_{48}O_3$	457.3676	−0.7	35.18	439.3575, 421.3461, 409.3465, 381.3164, 309.2588, 259.2056, 245.1891, 233.1904, 205.1586, 145.1007, 135.1165, 119.0863, 81.0704	Melilotigenin C	64,956
116	$C_{16}H_{13}NO_3$	268.0968	0.8	35.67	253.0736, 165.0544, 150.0313, 137.121.0291, 105.0341, 77.0393	3-Methoxy-hydroxy-n-benzoyl indole	59,907
117	$C_{20}H_{19}NO_3$	322.1438	1	35.79	266.0819, 238.0874, 211.0753, 133.0322, 121.00286	3-Hydroxy-N-(3-isopentenyl-4- hydroxybenzoyl) indole	11,031

Table 2. *Cont.*

No.	Formula	[M+H]$^-$	Error (ppm)	tR (min)	MS/MS (Characteristic Product Ions)	Identity	Identity
118	$C_{20}H_{19}NO_3$	322.1438	0.7	37.49	266.0825, 254.0814, 211.0757, 189.0913, 165.0703, 133.0289, 105.0336, 77.0389	3-Hydroxy-N-(3-isopentenyl-4-hydroxybenzoyl) indole	261,939
119	$C_{16}H_{14}O_3$	255.1016	0.9	41.5	240.0783, 209.0972, 194.0725, 177.0551, 165.0699, 151.0392, 131.0492, 103.0545, 95.0498, 77.0385	2′-Hydroxy-4′-methoxychalcone	129,251
120	$C_{30}H_{46}O_4$	471.3469	−0.6	42.6	453.3360, 435.3254, 423.3261, 395.2930, 287.2016, 259.1681, 247.1705, 201.1631, 189.1624, 147.1173, 109.1043, 95.0854	Unknown	17,953
121	$C_{30}H_{48}O_3$	457.3676	−0.9	45.42	421.3458, 399.2707, 297.2570, 215.1794, 173.1332, 135.1171, 109.1006	Unknown	54,664
122	$C_{30}H_{46}O_4$	471.3469	−5.7	45.93	387.2866, 325.1404, 233.1514, 148.0852	24-Hydroxy-3-oxoolean-12-en-29-oic acid	52,681
123	$C_{30}H_{50}O_3$	459.3833	−0.5	46.13	441.3717, 423.3615, 355.1895, 335.2005, 247.2064, 203.1779, 163.0758, 131.0486, 81.0691	Soyasapogenol B	8837
124	$N_{19}H_{21}NO_4$	366.2127	−1.7	46.43	244.1758, 145.0256, 121.0996, 85.0651	3-Methoxyaegeline	64,028
125	$C_{30}H_{50}O_4$	475.3782	−0.5	46.52	457.2366, 321.1122, 267.0679, 219.1767, 179.0354	Wistariasapogenol B	12,829
126	$C_{30}H_{50}O_3$	459.3833	−0.2	47.04	441.3741, 423.3649, 323.1291, 311.1293, 219.0657, 203.1810, 161.1330, 135, 1163, 123.1181, 95.0873	Unknown	25,684
127	$C_{30}H_{50}O_3$	459.3833	−0.4	48.77	441.3746, 323.1950, 311.1284, 293.1184, 201.1654, 179.0698, 109.1012, 107.0856	Unknown	18,190

3.6. Identification of 22 Metabolites (M1-M22) in Mice

Since drug metabolism largely determines the pharmacokinetic characteristics and bioavailability of most drugs, in order to better understand the metabolic pathway of OMDC, in vivo metabolite analysis was carried out. To analyze the in vivo metabolites, a megadose of OMDC solution (150 mg/kg body weight) was administered orally to rats. Plasma samples from three animals at different sampling sites (1, 2, 4, 6, and 12 h) were mixed and gathered for LC/MS. In total, 22 metabolites from plasma were tentatively identified (Table 3) based on fragment ions. These compounds were metabolized by glucuronidation and sulfation (Figures 11 and 12).

Eight glucuronidated, 9 sulfated, 3 both glucuronidated and glucuronidated, and 2 both glucuronidated and sulfated metabolites were identified. M10 showed [M-H]$^-$ at m/z 431.0984 and its molecular formula was predicted as $C_{21}H_{20}O_{10}$. The fragment ion was formed by the neutral loss of 176.0326 Da ($C_6H_8O_6$) in the MS^2 spectra; thus, it was determined to be pinocembrin glucuronide. M20 showed [M-H]$^-$ at m/z 335.0231 and its molecular formula was predicted to be $C_{15}H_{12}O_7S$. Based on the fragment ion at m/z 255.0650, 171.0463, and 151.0000, the molecule was determined to be pinocembrin sulfate. M1 showed [M-H]$^-$ at m/z 607.1304 and its chemical formula was $C_{27}H_{28}O_{16}$. The fragment ion at m/z 255.0650, forecasted as $C_{15}H_{12}O_4$, was generated by the loss of two 176.0326 Da ($C_6H_8O_6$). Subsequently, the molecule was provisionally determined to be pinocembrin diaglucuronide. M9 showed [M-H]$^-$ at m/z 511.0552 and its chemical formula was $C_{21}H_{20}O_{13}S$. It was generated by a loss of 79.95 Da (SO_3) and 176.0326 Da ($C_6H_8O_6$). Hence, the metabolite was provisionally confirmed as pinocembrin glucuronide sulfate.

Four corresponding metabolites (M4, M7, M8, and M18) of 2′,4′-dihydroxychalcone were detected (Figure 11). Other metabolites are also derived from flavonoids. As the major chemical components and major in vivo metabolites of OMDC, flavonoids may be crucial for the pharmacological effects of OMDC.

Table 3. In vivo metabolites after the administration of OMDC to rats.

No.	Formula	[M-H]$^-$	Error (ppm)	tR (min)	MS/MS (Characteristic Product Ions)	Identity
M1	$C_{27}H_{28}O_{16}$	607.13046	3.3	15.94	431.0981, 255.0655	Dia-glucuronidation of 71
M2	$C_{22}H_{20}O_{11}$	459.09329	0.4	16.3	283.0594, 268.0382	Glucuronidation of 24
M3	$C_{21}H_{20}O_{11}$	447.09329	−48	16.42	271.0603, 151.0036, 119.0506	Glucuronidation of 43
M4	$C_{27}H_{28}O_{15}$	591.13554	2.7	16.67	415.1037, 239.0711	Dia-glucuronidation of 82
M5	$C_{27}H_{30}O_{15}$	593.15119	4.8	17.05	417.1143, 241.0861	Dia-glucuronidation of 80
M6	$C_{21}H_{18}O_{10}$	429.08272	3.5	17.36	253.0505, 195.0454	Glucuronidation of 69
M7	$C_{21}H_{20}O_9$	415.10346	1.4	19.78	239.0709, 197.0611, 135.0085	Glucuronidation of 82
M8	$C_{21}H_{20}O_{12}S$	495.06027	5.2	21.37	319.0283, 239.0682	Sulfation and glucuronidation of 82
M9	$C_{21}H_{20}O_{13}S$	511.05519	1.1	22.12	431.0999, 255.0656	Sulfation and glucuronidation of 71
M10	$C_{21}H_{20}O_{10}$	431.09837	1.9	23.03	255.0667, 213.0552, 211.0769, 171.0448	Glucuronidation of 71
M11	$C_{22}H_{20}O_{12}$	475.0882	−61.9	23.27	299.0558, 284.0336, 255.0281, 227.0366	Glucuronidation of 51
M12	$C_{15}H_{12}O_8S$	351.01801	0.4	23.58	271.0618, 177.0213, 151.0031, 119.0517, 107.0156	Sulfation of 43
M13	$C_{22}H_{22}O_{10}$	445.11402	0.9	25.99	269.0817, 254.0572, 226.0632, 165.0196	Glucuronidation of 84
M14	$C_{21}H_{22}O_9$	417.11911	0.2	27.33	241.0865, 197.0986, 150.0330	Glucuronidation of 80
M15	$C_{16}H_{12}O_8S$	363.01801	1	27.59	283.0616, 268.0378	Sulfation of 24
M16	$C_{16}H_{10}O_8S$	361.00236	−1	28.34	281.0453, 253.0508,	Sulfation of 57
M17	$C_{16}H_{12}O_9S$	379.01293	0	29.92	299.0560, 284.0319, 165.0181	Sulfation of 51
M18	$C_{15}H_{12}O_6S$	319.02818	0	30.36	239.0707, 197.0598, 148.0168, 135.0084	Sulfation of 82
M19	$C_{16}H_{14}O_7S$	349.03875	−0.7	32.49	269.0813, 165.0194, 149.9945	Sulfation of 84
M20	$C_{15}H_{12}O_7S$	335.0231	−0.5	35.08	255.0653, 213.0538, 171.0463, 151.0000, 145.0642, 107.0139	Sulfation of 71
M21	$C_{15}H_{14}O_7S$	337.03875	0.3	40.05	257.0818, 239.0714, 151.0031	Sulfation of 52
M22	$C_{15}H_{14}O_6S$	321.04383	−0.8	41.09	241.0864, 197.0967, 150.0318, 135.0087	Sulfation of 80

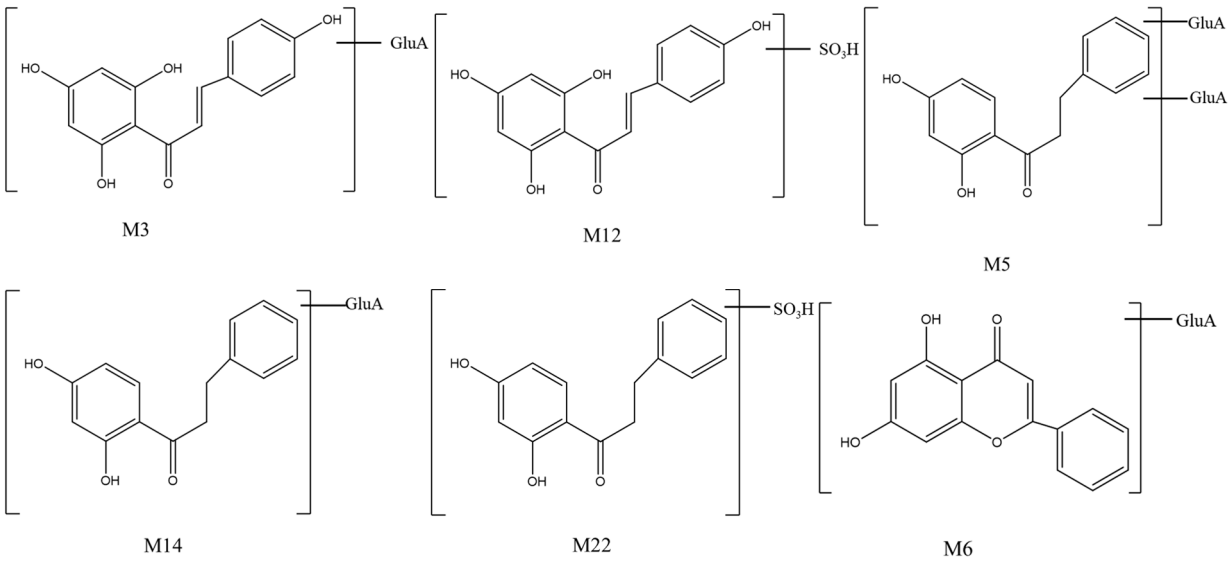


Figure 11. Structures of identified metabolites of drugs.

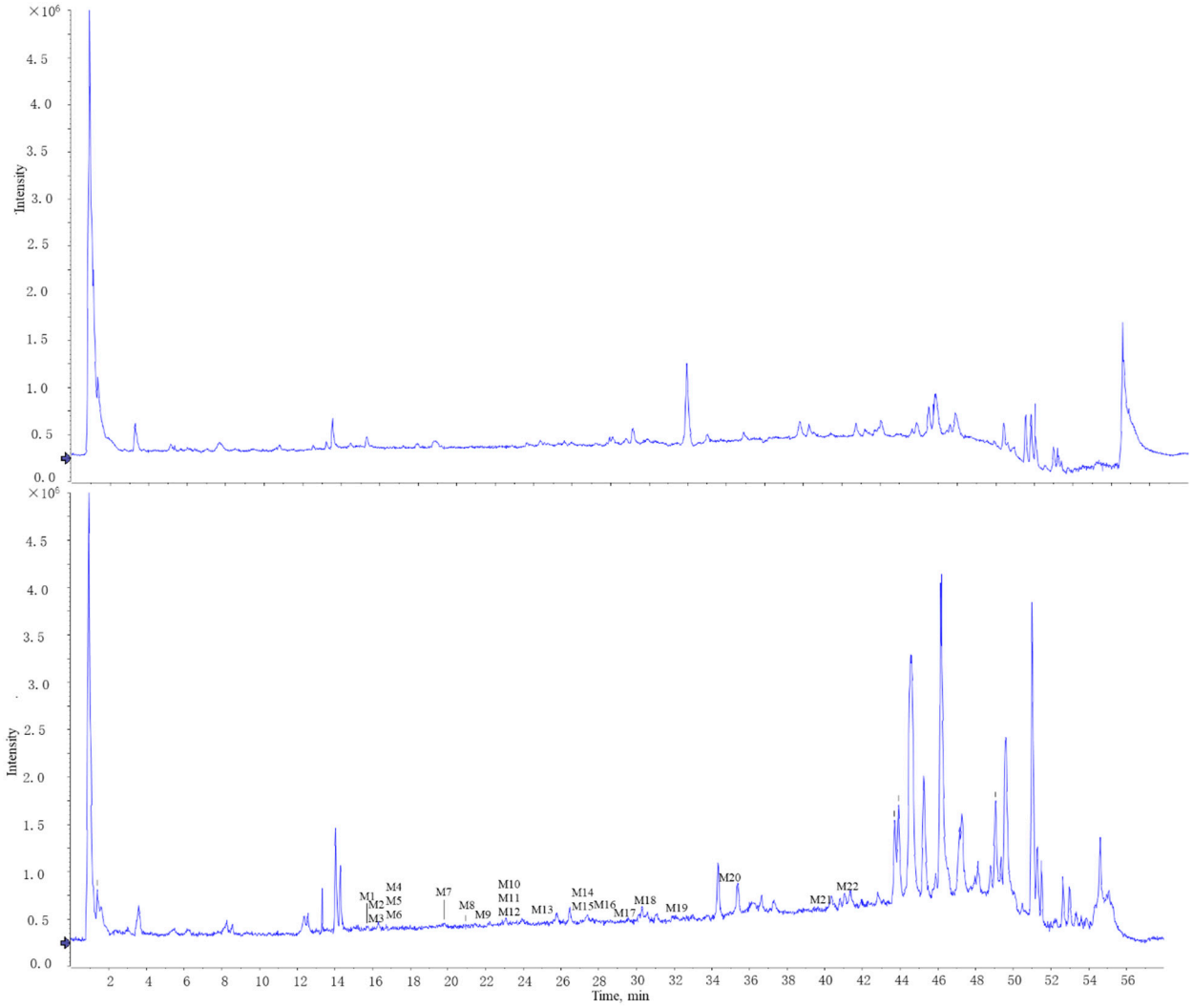


Figure 12. The EICs of 22 metabolites in rat plasma after administration of OMDC.

4. Conclusions

A rapid method using UHPLC-QTOF-MS was established for the isolation and authentication of the chemical constituents of OMDC. The compounds in OMDC extract were identified by NL and DPI sifting schema. Our results indicated that a total of 127 compounds, including 92 flavonoids, 15 indole alkaloids, and 20 others, could be identified in the OMDC extract. After the oral administration of OMDC extract to rats, 22 different compounds were found in the plasma, which appeared to be flavonoid metabolites. The primary in vivo metabolic reactions undergone by OMDC were glucuronidation and sulfation. This UHPLC-MS method is the first of its kind to determine the chemical constituents of OMDC in the positive and negative ion modes. Our findings revealed that the combination of UHPLC-MS and effective data mining is a logical, practical, and systematic method for the characterization of the chemical constituents and metabolites of OMDC.

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