

Characterization of thirty-nine polymethoxylated flavonoids (PMFs) in the branches of *Murraya paniculata* by HPLC-DAD-ESI-MS/MS

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[ABSTRACT] AIM: To investigate the polymethoxylated flavonoids (PMFs) in the branches of *Murraya paniculata* (L.) Jack. **METHODS:** A sensitive HPLC-DAD-ESI-MS/MS method was established to screen PMFs in the branches of *M. paniculata* based on the analysis of six PMF standards in the positive mode by CID-MS/MS. **RESULTS:** The diagnostic fragmentation pathways for polymethoxylated flavones, polymethoxylated flavanones, polymethoxylated chalcones and PMF glycosides were summarized, respectively. According to the MS fragmentation pathways, 39 PMFs, including 24 flavones, 10 flavanones or chalcones and 5 PMFs glycosides were screened. **CONCLUSION:** The results indicated that the developed analytical method could be employed as a rapid, effective technique for the chemical screening of PMFs in TCMS extracts.

[KEY WORDS] HPLC-DAD-ESI-MS/MS; characterization; polymethoxylated flavonoids (PMFs); *Murraya paniculata*

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1 Introduction

Murraya paniculata (L.) Jack, named Qianlixiang in Chinese, is a traditional Chinese medicine (TCM) officially listed in the Chinese Pharmacopoeia^[1], whose dried leaves or tender branches have been used worldwide as a folk medicine with many strong medical effects, such as febrifuge, astringent, antidiarrheal, toothache remedy, antidiarrheal and stimulant, among others^[2-3].

Previous studies indicated that polymethoxylated flavonoids (PMFs) were considered to be the representative

constituents of the plant^[4], which possess a number of biological properties, such as anti-allergic, anti-oxidant, anti-bacterial, anti-proliferative and anti-inflammatory activities^[5-10]. A sensitive method to screen and identify 70 PMFs in the leaves of *M. paniculata* was recently established in our laboratory^[11]. However, the constituents in its branches are apparently still unknown. It was therefore important to screen the PMFs of the branches of *M. paniculata*, which may provide a wider outlook on the applications of this Chinese herb.

It is well-known that some individual constituents could not be detected owing to low abundance, co-elution and high background of HPLC. Therefore, high-resolution chromatographic methods coupled to highly sensitive and selective detectors are needed. Mass spectrometry, especially coupled to soft ionization-source, such as electrospray ionization (ESI), has turned the possibility of coupling with HPLC instrument into reality and provides rich information, including molecular weight and structural information, on-line. Recently, HPLC-ESI-MS and HPLC-ESI-MS/MS have become very powerful approaches for the rapid identification of constituents in botanical extracts and the crude plant materials of

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TCMs^[12-16].

There has been no study dealing with the systematic report of PMFs in the branches of *M. paniculata* until now. Therefore, an HPLC-DAD-ESI-MS/MS method is described in this paper to evaluate the constituents in the branches of *M. paniculata*, which could provide evidence for its medicinal application.

2 Experimental

2.1 Chemicals and materials

Six PMFs reference compounds, 5, 6, 7, 3', 4'-pentamethoxyflavanone (10), 6'-hydroxy-3, 4, 5, 2', 4',

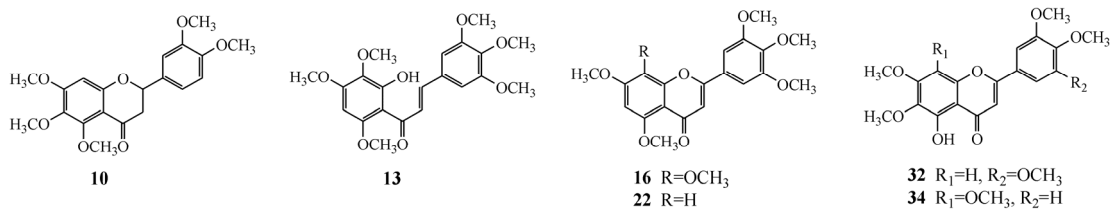


Fig. 1 Structures of six PMFs reference standards isolated from *Murraya paniculata*

HPLC-grade acetonitrile and methanol were purchased from Fisher Scientific (Fair Lawn, NJ, USA). Formic acid was purchased from Sigma Aldrich (St. Louis, MO, USA). Ultrapure water (Wahaha, Hangzhou, China) was used throughout the experiments. The 0.22 μm membranes used were purchased from Xinjinghua Co. (Shanghai, China).

The branches of *M. paniculata* were collected from Longzhou in Guangxi Province, China. The sample was authenticated by Prof. TU Peng-fei, and a voucher specimen deposited at the Department of Natural Medicines, Peking University, China.

2.2 Sample preparation for analysis

Powdered dried branches of *M. paniculata* were weighed accurately (0.5 g) and placed into a 50 mL flask containing 5 mL of methanol/water (70 : 30, V/V), then the mixture was extracted in an ultrasonic bath (Eima Ultrasonics Corp., Germany) at room temperature for 0.5 h. The methanol solution was filtered through a 0.22 μm membrane, and then an aliquot of 10 μL of the filtrate was injected into the HPLC-MS system for analysis.

2.3 HPLC-DAD-ESI-MS/MS analysis

The HPLC-DAD analysis was carried out on an Agilent 1100 series liquid chromatograph system (Agilent Technologies, USA), equipped with a binary pump, an auto-sampler, a photodiode array detector and a column temperature controller. The analytical column was an Agilent Zorbax Extend C₁₈ (250 mm \times 4.6 mm, i.d., 5 μm) with the oven temperature maintained at 25 $^{\circ}\text{C}$. 0.1% formic acid aqueous solution (V/V, solvent A) and acetonitrile (solvent B) were used as mobile phase for the LC separation. The elution conditions were applied with a linear gradient as follows: 0–5 min, 20%–28% B; 5–70 min, 28%–42% B; 70–90 min, 42%–64% B; 90–95

5'-hexamethoxychalcone (13), 5, 7, 8, 3', 4', 5'-hexamethoxyflavone (16), 5, 7, 3', 4', 5'-pentamethoxyflavone (22), 5-hydroxy-6, 7, 3', 4', 5'-pentamethoxyflavone (32), 5-hydroxy-6, 7, 8, 3', 4'-pentamethoxyflavone (34), were previously extracted, isolated and identified from *M. paniculata* in our laboratory. Their structures (shown in Fig. 1) were fully elucidated by comparison of their spectra data (ESI-MS and ¹H, ¹³C NMR) with those published literature values^[17-19]. The purities of the six PMFs standards were determined to be no less than 95% by HPLC-UV.

min, 64%–100% B. The flow rate was at 1.0 mL \cdot min⁻¹ and peaks were detected at 330 nm.

For ESI-MS/MS analysis, a 6320 ion trap mass spectrometer (Santa Clara, CA, USA) was connected to the same Agilent 1100 HPLC instrument via an electrospray ionization (ESI) interface. The HPLC effluent was introduced into the ESI source in a post-column splitting ratio of 1:3. The ESI-MS was performed in a positive ionization mode with source settings as follows: nebulizer gas pressure of 30.0 psi; dry gas flow rate of 12.0 L \cdot min⁻¹; electrospray voltage of the ion source of 3 000 V; capillary temperature of 350 $^{\circ}\text{C}$; capillary exit of 121.0 V; skimmer of 40.0 V; compound stability of 50%; trap drive level of 100%; target mass of m/z 400; scan range of m/z 100–700; AutoMS(n) operation mode; collision energy of 1 V; SmartFrag Start Ampl of 30%, SmartFrag End Ampl of 200%. A data-dependent program was used in the HPLC-ESI-MSⁿ analysis so that the protonated ions could be selected for further MSⁿ analysis. Nitrogen (> 99.99%) and He (> 99.99%) were used as sheath and damping gas, respectively. The Agilent 6300 Series Trap Control workstation (Version 6.1) was used for the data processing.

3 Results and Discussion

3.1 Optimization of HPLC conditions

In order to obtain satisfactory extraction efficiency for all the constituents, extraction conditions, including extraction methods, extraction solvents and extraction time were assessed based on single factor experiments. The best extraction efficiency was obtained by ultrasonication extraction with 70% ethanol for 30 min. Meanwhile, it was found that the choice of detection at 330 nm could provide an optimum S/N for most of PMFs compounds. Because the ingredients in the sample could not be separated with isocratic elution, gra-

dient elution was carried out. The different HPLC parameters, including mobile phases (methanol/water and acetonitrile/water), the concentration of formic acid in water (0.05%, 0.1% and 0.3%, V/V), category of RP-ODS columns (Agilent Zorbax Eclipse SB C₁₈ column, 250 mm × 4.6 mm i.d., 5 μm; Agilent Zorbax Extend C₁₈, 250 mm × 4.6 mm i.d., 5 μm and Waters Symmetry Shield C₁₈ column, 250 mm × 4.6 mm i.d., 5 μm), column temperature (20, 25 and 30 °C) and flow rate (0.8, 1.0 and 1.2 mL·min⁻¹), were examined. The addition of formic acid was advantageous to obtain the best resolution of adjacent peaks during chromatographic separation.

3.2 Optimization of ESI-MS/MS conditions

In order to achieve optimum conditions, all factors related to MS performance, including ionization mode, nebulizer gas pressure, electrospray voltage of the ion source and collision energy, were assessed. The results showed that ESI in positive ion mode was more sensitive for PMFs than in the negative ion mode. The major constituents were well detected, and most of the investigated compounds exhibited quasi-molecular ions [M + H]⁺ and product-ions with rich structural information in the positive mode of Collision-Induced Dissociation (CID)-MS/MS.

Table 1 Characterization of PMFs in the branches of *Murraya paniculata* by HPLC-DAD-ESI-MS/MS

No.	<i>t_R</i> ^a /min	[M + H] ⁺ (<i>m/z</i>)	MS ² / <i>m/z</i>	MS ³ / <i>m/z</i>	MS ⁴ / <i>m/z</i>
			P-ion (% loss) ^b	P-ion (% loss) ^b	P-ion (% loss) ^b
1	6.21	509	347*(100, 162)	332*(100, 15)	289(100, 43), 317(28.4, 15)
2	7.04	461	299*(100, 162)	268*(100, 33), 238(78.0, 61), 269(29.7, 30)	-
3	7.31	491	329*(100, 162)	314*(100, 15), 299(15.6, 30)	-
4	9.26	493	331*(100, 162)	316*(100, 15)	301(100, 15), 168(73.0, RDA), 298(7.9, 18)
5	17.10	521	359*(100, 162)	344(100, 15), 313(16.0, 46), 329(9.9, 30)	-
6	17.39	329	314*(100, 15)	286(100, 28), 285(54.4, 29)	-
7	18.42	389	328*(100, 61), 345(29.0, 44), 374(22.5, 15), 373(21.0, 16), 359(20.1, 30), 356(12.5, 33) 181*(100, RDA), 177(43.9, RDA), 313(7.3, 18)	312(100, 16), 313(61.5, 15)	-
8	19.93	331	344*(100, 15), 343(55.9, 16), 298(11.1, 61), 326(3.1, 33)	298(100, 46), 328(54.0, 16), 299(49.9, 45), 326(41.0, 18), 315(32.5, 29)	-
9	20.33	359	211*(100, RDA), 191(37.9, RDA), 357(16.1, 18)	196*(100, 15), 178(23.0, 33), 183(15.5, 28)	150(100, 46), 178(88.5, 18)
10	21.35	375	374*(100, 15), 359(87.5, 30), 341(25.2, 48), 356(10.7, 33)	359(100, 15), 341(44.7, 33), 356(17.0, 18)	-
11	21.81	389	343*(100, 30), 358(69.0, 15)	315(100, 28), 325(13.5, 18)	-
12	22.90	373	221*(100, RDA), 387(31.5, 18), 211(28.2, RDA)	193*(100, 28), 190(51.7, 31), 191(42.2, 30), 206(31.7, 15)	163(100, 30)
13	24.14	405	374*(100, 15), 359(98.6, 30), 328(20.2, 61)	359*(100, 15)	288(100, 61), 341(91.0, 18)
14	25.85	389	328*(100, 15), 327(59.5, 16), 299(15.1, 44)	299*(100, 29), 312(33.3, 16), 298(5.8, 30), 300(5.4, 28)	284(100, 15)
15	27.62	343	373*(100, 30), 343(33.9, 60), 388(28.4, 15)	345*(100, 28), 340(34.5, 33), 312(20.1, 61), 343(10.2, 30), 358(12.0, 15)	317(100, 28)
16	28.72	403	373*(100, 30), 342(43.3, 61), 388(27.6, 15)	345(100, 28), 327(76.2, 46)	-
17	29.05	403	312*(100, 61), 358(71.6, 15), 329(28.1, 44), 343(22.1, 30), 340(20.3, 33)	297*(100, 15), 269(35.1, 43), 281(23.1, 31)	175(100, RDA)
18	30.56	373	297*(100, 18), 285(70.2, 30), 257(52.3, 58), 255(12.9, 60)	255(100, 42), 241(65.4, 56), 187(58.4), 177(54.8), 227(48.9), 145(35.2), 203(31.6), 201(26.9), 133(26.5)	-
19	31.38	315			

Continued

No.	t_R^a /min	$[M + H]^+$ (m/z)	$MS^2/(m/z)$		$MS^3/(m/z)$		$MS^4/(m/z)$	
			P-ion (% loss) ^b		P-ion (% loss) ^b		P-ion (% loss) ^b	
20	31.77	345	181*(100, RDA), 191(77.5, RDA), 327(14.3, 18)		125(100, 56), 166(67.9, 15), 107(45.1, 74)		-	
21	32.29	343	313*(100, 30), 328(92.4, 15)		285(100, 28), 183(47.5, RDA)		-	
22	33.54	373	313*(100, 60), 358(23.2, 15), 343(15.9, 30), 357(11.7, 16)		284*(100, 29), 283(62.4, 30), 297(3.9, 16)		268(100, 16)	
23	35.07	375	221*(100, RDA), 181(18.8, RDA)		193*(100, 28), 190(72.3, 31), 206(37.0, 15), 191(11.8, 30)		163(100, 30)	
24	35.68	375	211*(100, RDA)		168(100, 33), 196(72.9, 15), 327*(100, 15), 151(51.4, RDA), 281(48.0, 61), 312*(20.3, 30), 309(17.9, 33), 298(14.0, 44)		-	
25	37.13	403	342(100, 61), 373(28.2, 30), 359(24.8, 44), 388(18.6, 15), 370*(16.9, 33), 387(16.1, 16)		327*(100, 46), 358(52.8, 15), 355(36.5, 18), 345(26.8, 28), 330(26.0, 43)		151(100, RDA), 284(38.4, 43), 281(31.1, 46), 299(29.9, 28), 312(25.5, 15), 296(21.7, 31)	
26	41.01	403	373*(100, 30), 388(69.4, 15), 342(11.0, 31), 355 (7.2, 18)		327*(100, 46), 358(52.8, 15), 355(36.5, 18), 345(26.8, 28), 330(26.0, 43)		312(100, 15), 297(29.2, 30), 299(22.3, 28), 284(18.3, 43), 298(16.9, 29)	
27	42.27	343	282*(100, 61), 328(65.2, 15), 310(18.2, 33), 299*(17.9, 44), 313(12.8, 30)		254 (100, 28)		-	
28	48.09	433	403*(100, 30), 418(55.7, 15), 417(19.8, 16), 385(12.9, 48)		388*(100, 15), 373(58.0, 30), 375(41.7, 28), 387(39.7, 16), 385(23.7, 18), 360(21.2, 43), 371 (20.6, 32), 388*(100, 15), 357(98.8, 46), 360(70.6, 43)		360(100, 28), 345(83.6, 43), 357(72.7, 31), 206(65.3, RDA), 327(62.5, 61)	
29	49.27	433	403*(100, 30), 418(62.1, 15), 372(14.0, 61), 385(11.4, 48)		342(62.6, 61), 375(57.4, 28), 373(32.6, 30), 385(31.9, 18), 370(27.3, 33)		-	
30	54.11	373	358*(100, 15), 343(61.9, 30), 312(10.9, 61), 325(6.5, 46)		343*(100, 15), 312(13.6, 46), 325(5.5, 33)		297(100, 46), 328(52.8, 15), 325(44.4, 18), 300(30.8, 43), 315(21.1, 28)	
31	54.55	373	358*(100, 15), 343(57.8, 30), 312(13.5, 61)		343*(100, 15), 325(11.8, 33), 312(11.7, 46), 329 (10.8, 29)		297(100, 61)	
32	61.07	389	356*(100, 33), 328(68.4, 61), 374(35.8, 15), 359(10.5, 30)		328*(100, 28), 295(8.5, 61), 341(8.0, 15)		-	
33	64.40	389	359*(100, 30), 341(45.2, 48), 374(42.6, 15), 356(31.4, 33), 328(16.1, 61)		344*(100, 15), 341(79.8, 18), 343(56.7, 16), 331(49.5, 28), 316(44.5, 43)		-	
34	64.53	389	359*(100, 30), 341(43.3, 48), 374(39.5, 15), 356(23.9, 33), 328(18.6, 61)		341*(100, 18), 328(62.6, 31), 329(26.5, 30), 344(20.6, 15)		326(100, 15)	
35	65.91	389	359*(100, 30), 341(48.9, 48), 374(39.9, 15), 356(33.3, 33), 328(22.2, 61)		197 (100, RDA), 344 (72.5, 15)		-	
36	68.33	375	211*(100, RDA), 191(41.2, RDA), 357(17.1, 18)		196(100, 15)		-	
37	74.95	405	221*(100, RDA), 211(29.2, RDA)		193* (100, 28), 190 (55.0, 31), 206 (37.0, 15), 191(28.9, 30), 178(12.7, 43)		107(100, 86)	
38	84.86	345	181*(100), 191(70.3), 327 (10.1), 199(4.9)		122(100, 59), 124.8(39.2, 56)		-	
39	87.19	375	221*(100), 181(24.6), 357(9.3), 193(6.9)		193*(100, 28), 190 (58.6, 31), 206 (32.6, 15), 191 (31.4, 30)		135(100), 163(66.1), 119(55.1), 107(49.6)	

^a t_R , retention time;^bP-ion (% loss), the product ions, the relative intensity and the loss (Da);

*Precursor-ion for next stage MS;

Compounds identified by comparison with reference standards.

3.3 HPLC-DAD-MS/MS analysis of authentic compounds

In order to identify the structures of the constituents in *M.**paniculata*, six reference compounds were first analyzed by HPLC-DAD-ESI-MS/MS techniques. According to their

chemical structures, UV absorption maxima and dominant fragmentation pathways, the authentic compounds could be classified into three groups, including polymethoxylated flavones, flavanones and chalcones. In the full scan mass spectra, most of PMF standards exhibited $[M + H]^+$ ions of sufficient abundance that could be subsequently isolated automatically and subjected to CID-MS/MS analysis (shown in Table 1). The proposed fragmentation patterns were helpful to clarify the structural identification of the constituents in *M. paniculata*. The nomenclature commonly used for the mass fragments of flavonoids was adopted in this work^[20].

In the CID-MS/MS experiment, four polymethoxylated flavone standards were analyzed first. Comparing the product-ion spectra of the standards, some characteristic dissociation pathways could be summarized for further characterization of the other polymethoxylated flavones. First, most of the $[M + H]^+$ ions of the standards, except compound 32, could lose one to four methyl radicals ($\text{CH}_3\cdot$) in their MS/MS spectra, and formed the base peaks of $[M + H - n \times 15]^+$. However, compound 32 also eliminated both one methyl radical and one H_2O to yield the $[M + H - 33]^+$ ion as the base peak of MS spectrum. This fragmentation pathway could be taken as the major diagnostic characteristic for polymethoxylated flavones. Meanwhile, the other dissociation pathways by loss of 16 (CH_4), 18 (H_2O), 28 (CO), 29 ($\text{HCO}\cdot$), 31 ($\text{CH}_4 + \text{CH}_3\cdot$), 33 ($\text{H}_2\text{O} + \text{CH}_3\cdot$), 43 ($\text{CO} + \text{CH}_3\cdot$), 44 (CO_2), 46 ($\text{H}_2\text{O} + \text{CO}$), and 61 ($\text{CO} + \text{H}_2\text{O} + \text{CH}_3\cdot$) were also frequently detected as diagnostic fragments in their MS/MS and MS/MS/MS spectra. These main product-ions mentioned above could form the characteristic ESI-MSⁿ “fingerprint” of PMFs, which could be used to separate out the polymethoxylated flavones from the complex extract of TCMs rapidly. The “fingerprint” set up in the study was highly similar to the one that was achieved by APCI-MS/MS^[21]. Some diagnostic fragment losses, such as 18, 28 and 44 amu, detected in the product-ion spectra were also frequently reported in the characterization of ordinary flavonoids^[22].

As for polymethoxylated flavanone derivatives, compound 10 gave the $[M + H]^+$ ion at m/z 375 in the CID-MS/MS experiment, which further generated the prominent ion at m/z 211 as base peak in its MS/MS spectrum. It could be deduced that the dominating fragmentation pathway was retro-Diels-Alder (RDA) cleavage from the 1,3-position of the C-ring. Meanwhile, the minor ion at m/z 191 was also detected, owing to the RDA fragmentation from the 1, 4-position of the C-ring. The loss of 15 ($\text{CH}_3\cdot$), 18 (H_2O), 28 (CO), 33 ($\text{H}_2\text{O} + \text{CH}_3\cdot$) and 61 ($\text{CO} + \text{H}_2\text{O} + \text{CH}_3\cdot$) amu from the base peak at m/z 211, could be also detected as minor fragmentation ions in the CID-MS/MS spectrum. This kind of fragmentation pathway, namely that the $[M + H]^+$ ion underwent RDA reaction prior to the neutral losses of $\text{CH}_3\cdot$, H_2O , CO , etc., was different noticeably from ordinary flavanones. Therefore, it could be adopted as a shortcut to distinguish polymethoxylated flavanones from ordinary flavones

rapidly.

Compound 13 was taken as an example to summarize the fragmentation pathways of polymethoxylated chalcones by the CID-MS/MS method. The RDA cleavage at bond X to yield the base peak ion $^X\text{B}^+$ at m/z 221 and at bond Y to yield the minor ion $^Y\text{A}^+$ at m/z 211 could also be simultaneously detected in the MS/MS spectrum first. The fragmentation pathway was highly similar with what happened to flavanones. This is reasonable because cyclization of 6'-hydroxychalcones to flavanones has been reported in a number of studies demonstrating the presence of an intramolecular equilibrium between a flavanone-type and a chalcone-type molecular ion^[23-24]. At the same time, the loss of 15 ($\text{CH}_3\cdot$), 16 (CH_4), 18 (H_2O), 28 (CO), 30 ($2 \text{CH}_3\cdot$) and 31 ($\text{CH}_4 + \text{CH}_3\cdot$) amu from the base peak at m/z 221 could also be detected. Thus, according to their fragmentation pathways, it was easy to tell the difference between polymethoxylated chalcones and flavones, but difficult to distinguish them from polymethoxylated flavanones. However, the differences of the UV spectra between polymethoxylated chalcones and polymethoxylated flavanones provided a method to classify them, because the maximum UV absorption of chalcones usually ranged from 330 to 370 nm, whereas the flavanones maintained their UV maximum at about 320 nm.

3.4 HPLC-DAD-MS/MS analysis of the PMFs in *M. paniculata*

The purpose of this study was to separate and evaluate the PMFs in the branches of *M. paniculata*. PMFs have regularity in elemental composition as they have the basic aglycone structure with a maximum of seven substituents, such as methoxyl group (OCH_3) and/or hydroxyl group (OH) on their A, B and C rings. The MWs of the basic structures of aglycone are 222, 224 and 224 Da for flavones, flavanones and chalcones, respectively, which are increased by 30 or 16 Da, respectively when a methoxyl or hydroxyl was attached. Based on the numbers and the types of the substituent groups, the chemical formula and mass of every possible PMFs isomer can be designated in advance.

Because of the complexity and the similarity of the ingredients in *M. paniculata*, EIC-MS (extracted ion chromatogram) method was employed to analyze the PMFs in the plant (shown in Fig. 2 and Table 1).

In the study, the abundances of most of the unknown peaks especially the chalcones and flavanones were too low to afford the online UV absorption spectra, so it was difficult to distinguish them from flavanones and chalcones. Therefore, they were evaluated together.

Meanwhile, from the results of the full-scan of LC-MS, some compounds with the MWs (molecular weights) between 450 and 550 had the distinct possibility to be PMF glycosides, owing to the fragmentation pathways of their $[\text{aglycone} + \text{H}]^+$ ions which were similar to the diagnostic characteristics of PMFs. In their MS/MS spectra, all of the

$[M + H]^+$ ions readily eliminated the sugar moiety to produce the corresponding $[\text{aglycone} + H]^+$ ions as the base peak. Neutral loss scan of 162 Da revealed the presence of hexose moieties, such as glucose, in their molecules. Then the $[\text{aglycone} + H]^+$ ions were selected to trace the structural information of the PMF aglycones. The neutral losses detected in their MS/MS spectra were in accord with the “fingerprint” of the corresponding polymethoxylated flavones, demonstrating they were probable PMF glycosides present in *M. paniculata*. The study provided significant information for the phytochemical research on the branches of *M. paniculata* and the plants of the genus *Murraya* in general.

After screening the molecular masses with the EIC-MS method, 39 PMFs, including 24 flavones (4 known), 10 flavanones or chalcones (2 known) and 5 PMF glycosides were tentatively identified (as shown in Table 2). Some EIC-MS peaks were too weak to be seen clearly in the total ion chromatogram (TIC) spectra. Meanwhile, the retention times of some EIC-MS peaks were so similar that they could not be identified simultaneously in the TIC spectra. Thus, the EIC-MS method adopted in this study was confirmed to be a powerful method to evaluate the ingredients preliminarily in highly complex extracts of TCMs, and other medicinal plants.

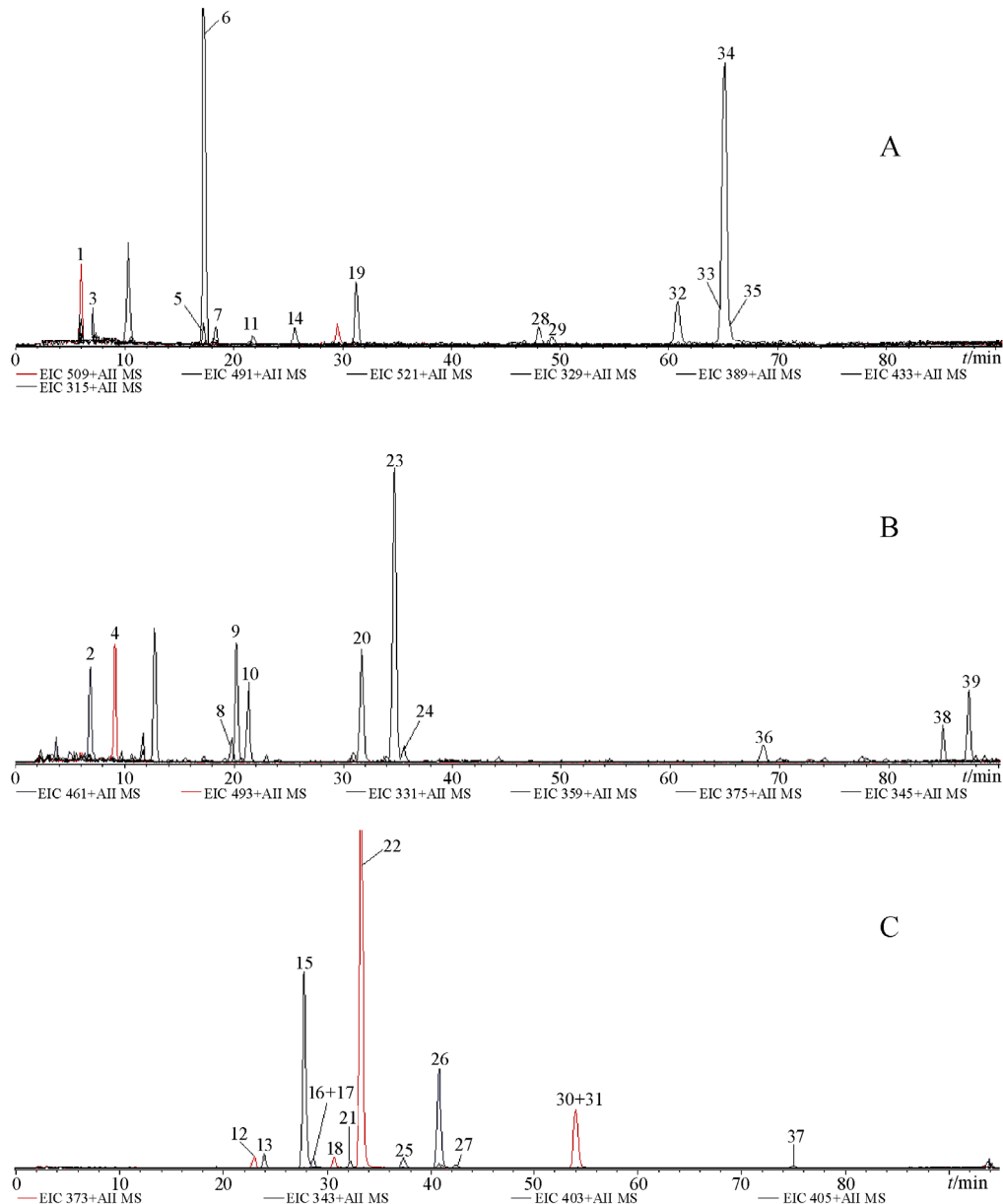


Fig. 2 The EIC-MS peaks of all possible PMFs in the branches of *Murraya paniculata*. (A) m/z 521, 509, 491, 433, 389, 329, 315; (B) m/z 493, 461, 375, 359, 345, 331; (C) m/z 405, 403, 373, 343.

Table 2 The MWs and structural identification of all possible PMFs detected in the branches of *Murraya paniculata*

Peaks	Number	PMFs	No. of -OCH ₃	No. of -OH	MW
1	1	Tetrahydroxy-dihydroxyflavone glycoside	2	4	508
2	1	Monohydroxy-dimethoxyflavone glycoside	2	1	460
3	1	Monohydroxy-trimethoxyflavone glycoside	3	1	490
4	1	Trihydroxy-dimethoxyflavone glycoside	2	3	492
5	1	Monohydroxy-tetramethoxyflavone glycoside	4	1	520
6	1	Monohydroxy-trimethoxyflavone	3	1	328
7, 11, 14, 32-35	7	Monohydroxy-pentamethoxyflavone	5	1	388
8	1	Monohydroxy-trimethoxyflavanone or Monohydroxy-trimethoxychalcone	3	1	330
9	1	Monohydroxy-trimethoxyflavone	3	1	358
10, 23, 24, 36, 39	5	Pentamethoxyflavanone or Pentamethoxychalcone	5	0	374
12, 18, 22, 30, 31	5	Pentamethoxyflavone	5	0	372
13, 37	2	Hexamethoxyflavanone or Hexamethoxychalcone	6	0	404
15, 21, 27	3	Tetramethoxyflavone	4	0	342
16, 17, 25, 26	4	Hexamethoxyflavone	6	0	402
19	1	Dihydroxy-dimethoxyflavone	2	2	314
20, 38	2	Tetramethoxyflavanone or Tetramethoxychalcone	4	0	344
28, 29	2	Heptamethoxyflavone	7	0	432

4 Conclusions

A sensitive HPLC-DAD-ESI-MS/MS method was established to screen the PMFs present in the branches of *M. paniculata*. Six PMF standards, including four flavones, one flavanone and one chalcone, were analyzed by CID-MS/MS first to obtain the respective characterizations of the fragment pathways, which could be adopted as the basis for further analysis the PMFs in the extract. Meanwhile, owing to regularities of PMFs in elemental composition, the EIC-MS method by MWs was employed to screen the homoeomorphic PMFs from the extract. In the end, 34 PMFs and five PMF glycosides were screened preliminarily. Among them, six PMFs could be unambiguously identified by comparison with reference substances. The results indicated that the developed analytical method could be employed as a rapid, effective technique for the structural characterization of PMFs in complex mixtures.

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千里香枝中 39 个多甲氧基黄酮的 HPLC-DAD-ESI-MS/MS 分析

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【摘要】 目的: 定性分析千里香枝中的多甲氧基黄酮类成分。方法: 建立一种灵敏的 HPLC-DAD-ESI-MS/MS 方法, 筛选千里香枝中的多甲氧基黄酮。结果: 分别研究总结了多甲氧基黄酮、二氢黄酮、查耳酮以及多甲氧基黄酮苷的诊断性断裂途径。结合这些特征和 EIC-MS/MS 实验, 筛选了 39 个多甲氧基黄酮类成分, 包括 24 个多甲氧基黄酮、10 个多甲氧基二氢黄酮或查耳酮、5 个多甲氧基黄酮苷。结论: 本研究为从复杂物质体系中筛选多甲氧基黄酮提供了一种快速有效的方法。

【关键词】 HPLC-DAD-ESI-MS/MS; 定性鉴定; 多甲氧基黄酮; 千里香枝

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