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Article

Simultaneous Screening and Identifying Four Categories of Particular Flavonoids in the Leaves of *Murraya exotica* L. by HPLC–DAD–ESI-MS-MS

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Polymethoxylated flavonoids (PMFs), the particular flavonoid subclass in which all or almost all hydroxyls are capped by methylation, have high oral bioavailability and various activities. A sensitive high-performance liquid chromatography–diode array detection–electrospray ionization tandem mass spectrometry (HPLC–DAD–ESI-MS-MS) method was established to screen and identify the PMFs in the leaves of *Murraya exotica*. Eight PMF standards, including two polymethoxylated flavones, two polymethoxylated flavanones, two polymethoxylated chalcones and two PMF glycosides, were first to be analyzed in positive mode by collision-induced dissociation MS-MS. On the basis of the ESI-MS, characterizations were deduced, and in the results of the extracted ion chromatogram MS-MS experiment, 26 PMFs, including 18 flavones, five flavanones or chalcones and three PMF glycosides, were screened out from the complex extract of the leaves of *M. exotica*. Among them, 24 PMFs were hydroxylated polymethoxyflavonoids, whereas the rest were all permethoxylated PMFs. This was the first systematic report on the presence of PMFs in the leaves of *M. exotica*. The results indicated that the established analytical method could be adopted as a rapid, effective technique for the structural characterization of PMFs from the complex extracts of traditional Chinese medicines.

Introduction

Dietary flavonoids and other polyphenols show great potential as cancer chemopreventive agents in cell culture studies (1, 2). However, their low bioavailability as a result of conjugative metabolism does not translate well into *in vivo* activity (3). Polymethoxylated flavonoids (PMFs), the flavonoid subclass in which all or almost all hydroxyls are capped by methylation, have high oral bioavailability, displaying antiallergic, antioxidant, antibacterial, antiproliferative, anti-inflammatory and anticancer activities (4–8), Meanwhile, hydroxylated polymethoxyflavonoids (OH-PMFs), which are less abundant than permethoxylated PMFs (9), have recently drawn more attention, because accumulating evidence has suggested that OH-PMFs have much stronger health-promoting biological activities than their permethoxylated counterparts. For example, 5-hydroxy polymethoxyflavones exhibited greater potency with anticarcinogenic and anti-inflammatory effects (10).

Previous studies indicated that PMFs were regarded to be one type of the representative constituents in *Murraya exotica* L. (Jiulixiang in Chinese) (11–13). A sensitive method has previously been established to screen and identify 70 PMFs in the leaves of *M. paniculata* (14), which is another source for *Folium et cacumen murrayae*, officially listed in the Chinese Pharmacopoeia. However, the constituents in *M. exotica* are still unknown. Therefore, there is a great need to screen out PMFs from *M. exotica*, which can provide a wider outlook on the applications of the Chinese herb.

Early reported methods for analysis of PMFs were based on high-performance liquid chromatography (HPLC) separation coupled with ultraviolet (UV) detection (15, 16). However, some constituents could not be detected, owing to low abundance, coelution and high background of HPLC. Therefore, high-resolution chromatographic methods coupled to highly sensitive and selective detectors are needed. Mass spectrometry (MS), especially coupled to a soft ionization source such as electrospray ionization (ESI), has turned the possibility of coupling with an HPLC instrument into reality and has provided rich information, including online molecular mass and structural information. Recently, HPLC–ESI-MS and HPLC–ESI-tandem mass spectrometry (MS-MS) have become very powerful approaches for the rapid identification of the constituents in traditional Chinese medicine (TCM) extracts (17–20).

Therefore, for the purpose of selective phytochemical screening and structural characterization of PMFs in the leaves of *M. exotica*, a developed HPLC–diode array detection (DAD)–ESI-MS-MS method was adopted to investigate the fragment patterns of eight PMF standards and was applied in the rapid identification of PMF compounds from TCM extracts.

Experimental

Materials, chemicals and reagents

HPLC-grade acetonitrile and methanol were purchased from Fisher Scientific (Fair Lawn, NJ). Formic acid was purchased from Sigma-Aldrich (St. Louis, MO). Deionized water (18.2 MΩ) used throughout the experiment was purified by a Milli-Q water system (Millipore, Bedford, MA). The leaves of *M. exotica* were collected from Fusui County, Guangxi Province, China, and authenticated by Professor Pengfei Tu in the authors' laboratory. The voucher specimen was deposited at the Center of Scientific Experiment (Beijing University of Chinese Medicine, Beijing, China). Eight PMF standards, including 5, 7, 8, 3', 4',

5'-hexamethoxyflavone (P-1), 5-hydroxy-6, 7, 8, 3', 4'-pentamethoxyflavone (P-2), 5, 6, 7, 3', 4'-pentamethoxyflavanone (P-3), 5, 7, 3', 4', 5'-pentamethoxyflavanone (P-4), 6'-hydroxy-3, 4, 5, 2', 4', 5'-hexamethoxychalcone (P-5), 6'-hydroxy-3, 4, 5, 2', 5'-pentamethoxychalcone (P-6), 5, 3'-dihydroxy-6, 4'-dimethoxyflavone-7-O-β-D- glucopyranoside (P-7), 5, 3'-dihydroxy-6, 7 and 4'-trimethoxyflavone-8-O-β-D-glucopyranoside (P-8), were previously extracted, isolated and identified from *M. paniculata* in the authors' laboratory for qualitative analysis (Figure 1) (21, 22). Their purities were determined to be no less than 95% by HPLC–UV.

Sample preparation

Powdered dried leaves of *M. exotica* were weighed accurately (0.3 g), and then the mixture was extracted in an ultrasonic bath (Eima Ultrasonics, Germany) with 25 mL of methanol–water (70:30, v/v) at room temperature for 0.5 h. The extraction solvent was filtered through a 0.45 μm membrane before injection into the HPLC–MS system for analysis.

HPLC system

The Agilent 1100 series HPLC-MS system (Agilent Technologies, Palo Alto, CA) used in the experiment was equipped with a binary pump, an autosampler, a photodiode array detector and a column temperature controller. The separations were conducted on an Agilent Zorbax Extended C18 column (250 × 4.6 mm i.d., 5 μm) at 25°C. Formic acid aqueous solution (0.1% v/v, solvent A) and acetonitrile (solvent B) were used as mobile phases for the HPLC separation. The elution conditions were applied with a linear gradient as follows: 20% B (0 min), 28% B (5 min), 42% B (70 min), 64% B (90 min) and 100% B (95 min). The DAD acquisition wavelength was recorded in the range of 200–400 nm. The flow rate was 1.0 mL/min and the detection wavelength was 330 nm.

Mass spectrometry

After passing through the flow cell of the DAD, the column eluate was split to 0.25 mL/min, which was directed to a trap mass spectrometer for analysis. The ESI-MS was performed in positive ionization mode with source settings as follows: nebulizer gas (nitrogen) pressure of 35.00 psi; dry gas (nitrogen) flow rate of 11.00 L/min; electrospray voltage of the ion source of 3,500 V; capillary temperature of 350°C; compound stability of 50%; trap drive level of 100%; target mass of m/z 400; scan range of m/z 100–700. A data-dependent program was adopted in the ESI-MS^n analysis so the protonated or deprotonated ions could be selected for further MS^n analysis. Nitrogen (>99.99%) and He (>99.99%) were used as sheath and damping gas, respectively.

Results and Discussion

Optimization of HPLC conditions

To obtain the best extraction efficiency for all PMFs, extraction conditions, such as extraction methods (standing overnight, ultrasonication and refluxing), extraction solvents (50, 70 and 100% methanol) and extraction time (20, 30 and 40 min), were assessed based on single factor experiments. The results demonstrated that the best extraction efficiency could be achieved by ultrasonication extraction with 70% ethanol for 30 min. Meanwhile, to achieve proportionate peak-to-peak resolutions, the different HPLC parameters were tested, including mobile phase (acetonitrile–water and methanol–water), the concentration of formic acid in water (0.05, 0.1 and 0.3%), category of RP-ODS columns (Agilent Zorbax Extended C18 column, 250 × 4.6 mm i.d., 5 μm; Agilent Zorbax Eclipse Plus C18, 250 × 4.6 mm i.d., 5 μm; and Waters Symmetry Shield C18 column, 250 × 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). The addition of formic acid provided the best resolutions of chromatographic peaks in HPLC (Figure 2).

Optimization of ESI-MS-MS conditions

To achieve optimum conditions to screen PMFs in the leaves of *M. exotica*, all relevant parameters were studied, including ionization mode, nebulizer gas pressure, electrospray voltage of

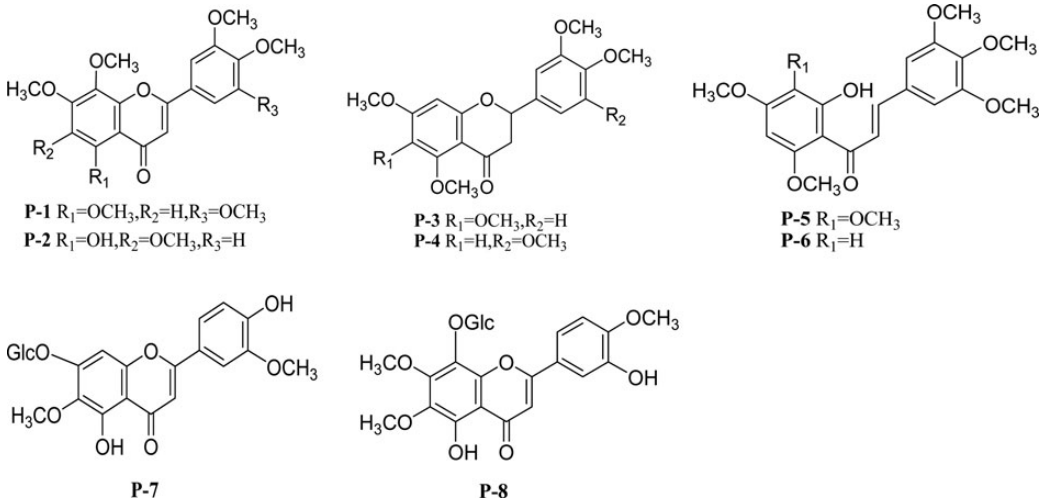

Figure 1. Chemical structures of PMF standards P1–P8 (glc: glucose).

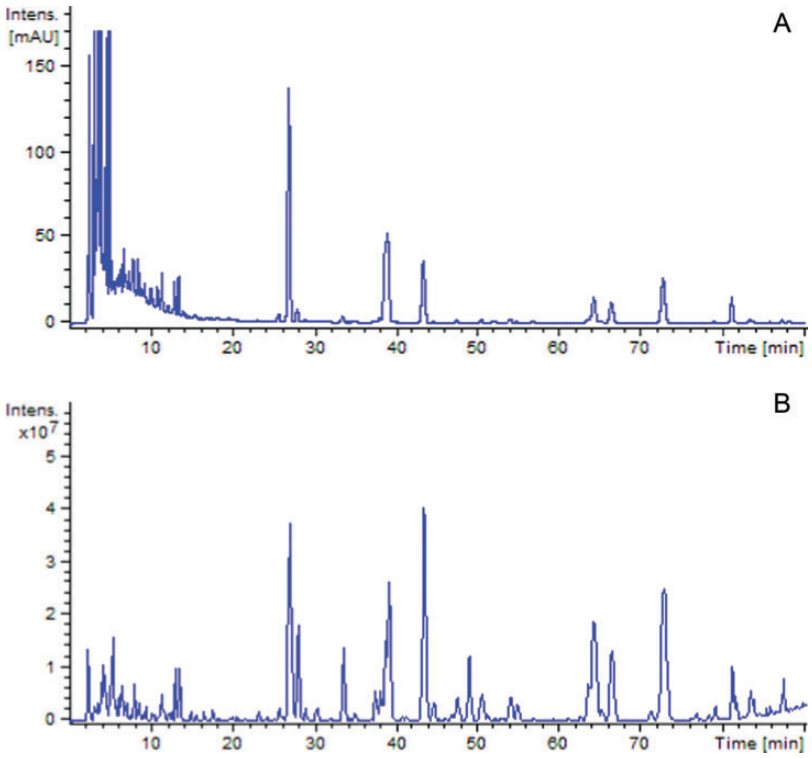

Figure 2. HPLC–DAD–MS-MS analysis of PMFs in the leaves of *Murraya exotica*: HPLC–DAD chromatogram of the extract at 330 nm (A); the ESI-MS TIC of the extract in positive mode (B).

Table I
Characterizations of Eight PMF Standards by CID-MS-MS*

Peak	$[M+H]^+$ (m/z)	MS^2 (m/z)			MS^3 (m/z)		
		P-ion (%)†	Loss‡	Radical loss	P-ion (%)†	Loss‡	Radical loss
P-1	403	**373** (100)	30	$2CH_3^{\bullet}$	345 (100)	28	CO
		343 (33.9)	60	$4CH_3^{\bullet}$	340 (34.8)	33	$H_2O + CH_3^{\bullet}$
		388 (28.8)	15	$CH_3^{\bullet}$	343 (10.1)	30	$2CH_3^{\bullet}$
P-2	389	**359** (100)	30	$2CH_3^{\bullet}$	341 (100)	18	H_2O
		341 (43.6)	43	$CH_3^{\bullet} + CO$	328 (62.8)	31	$CH_4 + CH_3^{\bullet}$
		374 (38.6)	15	$CH_3^{\bullet}$	329 (26.4)	30	$2CH_3^{\bullet}$
		356 (23.3)	33	$H_2O + CH_3^{\bullet}$	344 (19.3)	15	$CH_3^{\bullet}$
P-3	375	**211** (100)	RDA	$^{1,3}A^{+\ddagger}$	196 (100)	15	$CH_3^{\bullet}$
		191 (37.1)	RDA	$^{1,4}B^{+\ddagger}$	178 (34.7)	33	$H_2O + CH_3^{\bullet}$
		357 (16.6)	18	H_2O	150 (19.2)	61	$CO + H_2O + CH_3^{\bullet}$
					183 (15.5)	28	CO
P-4	375	**221** (100)	RDA	$^{1,4}B^{+\ddagger}$	193 (100)	28	CO
		181 (24.1)	RDA	$^{1,3}A^{+\ddagger}$	190 (61.9)	31	$CH_4 + CH_3^{\bullet}$
					191 (40.0)	30	$2CH_3^{\bullet}$
					206 (30.5)	15	$CH_3^{\bullet}$
P-5	405	**221** (100)	RDA	$^{x}B^{+\S}$	193 (100)	28	CO
		387 (31.6)	18	H_2O	190 (51.5)	31	$CH_4 + CH_3^{\bullet}$
		211 (28.3)	RDA	$^{y}A^{+\S}$	191 (43.4)	30	$2CH_3^{\bullet}$
					206 (31.6)	15	$CH_3^{\bullet}$
P-6	375	**221** (100)		$^{x}B^{+\S}$	193 (100)	28	CO
		181 (30.0)		$^{y}A^{+\S}$	190 (61.9)	31	$CH_4 + CH_3^{\bullet}$
					191 (40.0)	30	$2CH_3^{\bullet}$
					206 (22.3)	15	$CH_3^{\bullet}$
P-7	493	**331** (100)	162	Glucose	316 (100)	15	$CH_3^{\bullet}$
					301 (74.9)	30	$2CH_3^{\bullet}$
					273 (19.4)	58	$2CH_3^{\bullet} + CO$
P-8	523	**361** (100)	162	Glucose	346 (100)	15	$CH_3^{\bullet}$
					328 (46.6)	33	$CH_3^{\bullet} + H_2O$
					331 (9.6)	30	$2CH_3^{\bullet}$

*Note: P-ion (%): the product ions and the relative intensity. Precursor ions for next stage MS are in bold.
†Loss (Da).
‡$^{1,3}A^+$ and $^{1,3}B^+$ stand for the fragment ions from the RDA cleavage from 1,3-position on the C-ring of flavanones.
§$^{y}A^+$ and $^{x}B^+$ stand for the fragment ions from the RDA cleavage from the C-ring of chalcones.

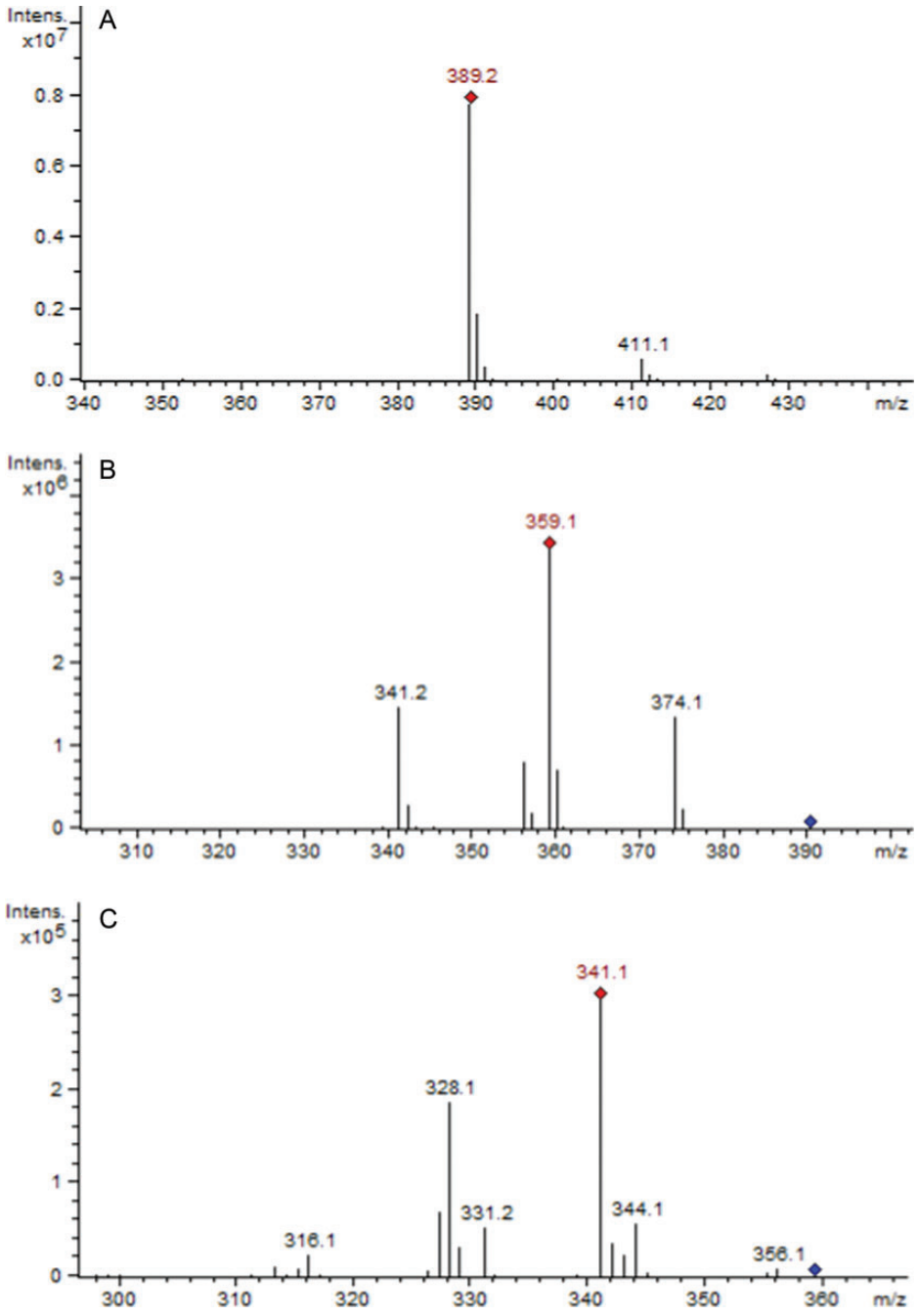

Figure 3. MS^n spectra of P-2: MS spectrum (A); MS^2 spectrum (precursor ion was *m/z* 389) (B); MS^3 spectrum (precursor ion was *m/z* 359) (C).

the ion source and collision energy. The results demonstrated that ESI in positive ion mode was more sensitive to PMFs than in negative ion mode. The PMFs were well detected (Figure 2), and most of them exhibited $[M + H]^+$ ions and product ions with abundant structural information in the positive mode of collision-induced dissociation (CID)-MS/MS.

HPLC–DAD-MS-MS analysis of authentic compounds

To identify structures of the constituents in the leaves of *M. exotica*, eight PMF standards were analyzed by HPLC–DAD–ESI-MS-MS techniques. According to their chemical structures, UV absorption maxima and dominant fragmentation pathways, the standards could be classified into four types, including polymethoxylated flavones (Type A), flavanones (Type B), chalcones (Type C), and PMF glycosides (Type D). In the full scan mass spectra, all of them exhibited $[M + H]^+$ ions of sufficient abundance to be subsequently isolated and subjected to CID-MS-MS analysis (Table I). The proposed fragmentation patterns were helpful to study the structures of PMFs. The nomenclature commonly used for mass fragments of flavonoids was adopted in this work (23).

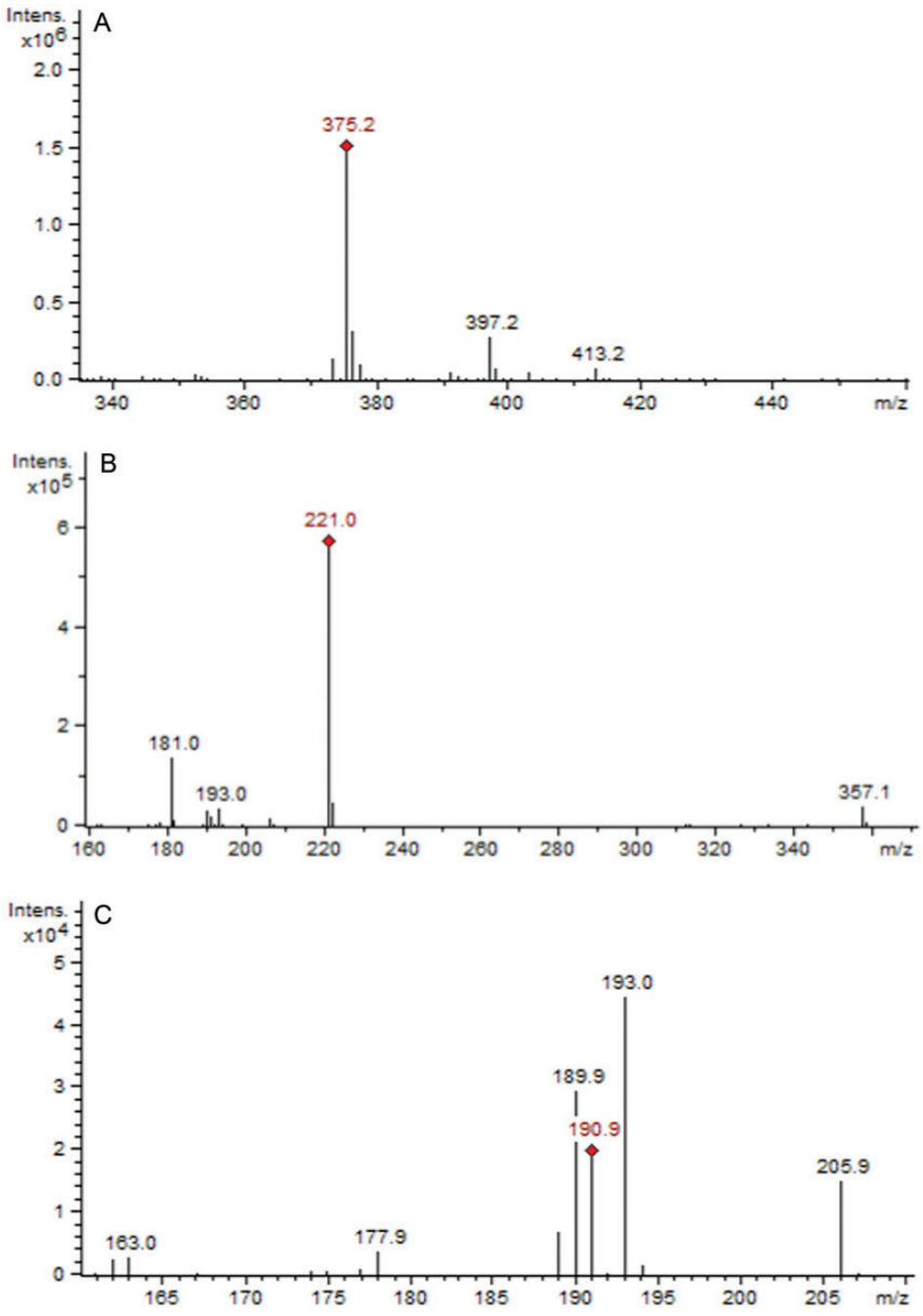

Figure 4. MS^n spectra of P-4: MS spectrum (A); MS^2 spectrum (precursor ion was m/z 375) (B); MS^3 spectrum (precursor ion was m/z 211) (C).

Type A

Two polymethoxylated flavone standards (Compounds P-1 and P-2) were analyzed first in the CID-MS-MS experiment. Comparing the product ion spectra of the standards (Figure 3), some characterized dissociation pathways could be summarized for further characterization of the other polymethoxylated flavones. First, all of their $[M + H]^+$ ions of standards could lose one to four methyl radicals ($CH_3^\bullet$) in their MS/MS spectra, and formed the base peaks of $[M + H - n \times 15]^+$. This fragmentation pathway can be assumed as the major diagnostic characteristic for polymethoxylated flavones. Second, the other dissociation pathways by the loss of 16 (CH_4), 18 (H_2O), 28 (CO), 31 ($CH_4 + CH_3^\bullet$), 33 ($H_2O + CH_3^\bullet$), 43 ($CO + CH_3^\bullet$) and 61 ($CO + H_2O + CH_3^\bullet$) were also frequently detected in the ESI-MS^n spectra. These product ions could form their characteristic ESI-MS^n fingerprint, which could be used to rapidly screen out the polymethoxylated flavones from the complex TCM system.

Type B

The fragmentation pathways of two polymethoxylated flavanone derivatives (Compounds P-3 and P-4) were similar to each other in the CID-MS-MS experiment. P-4, for example, gave the $[M + H]^+$ ion at m/z 375, which further generated

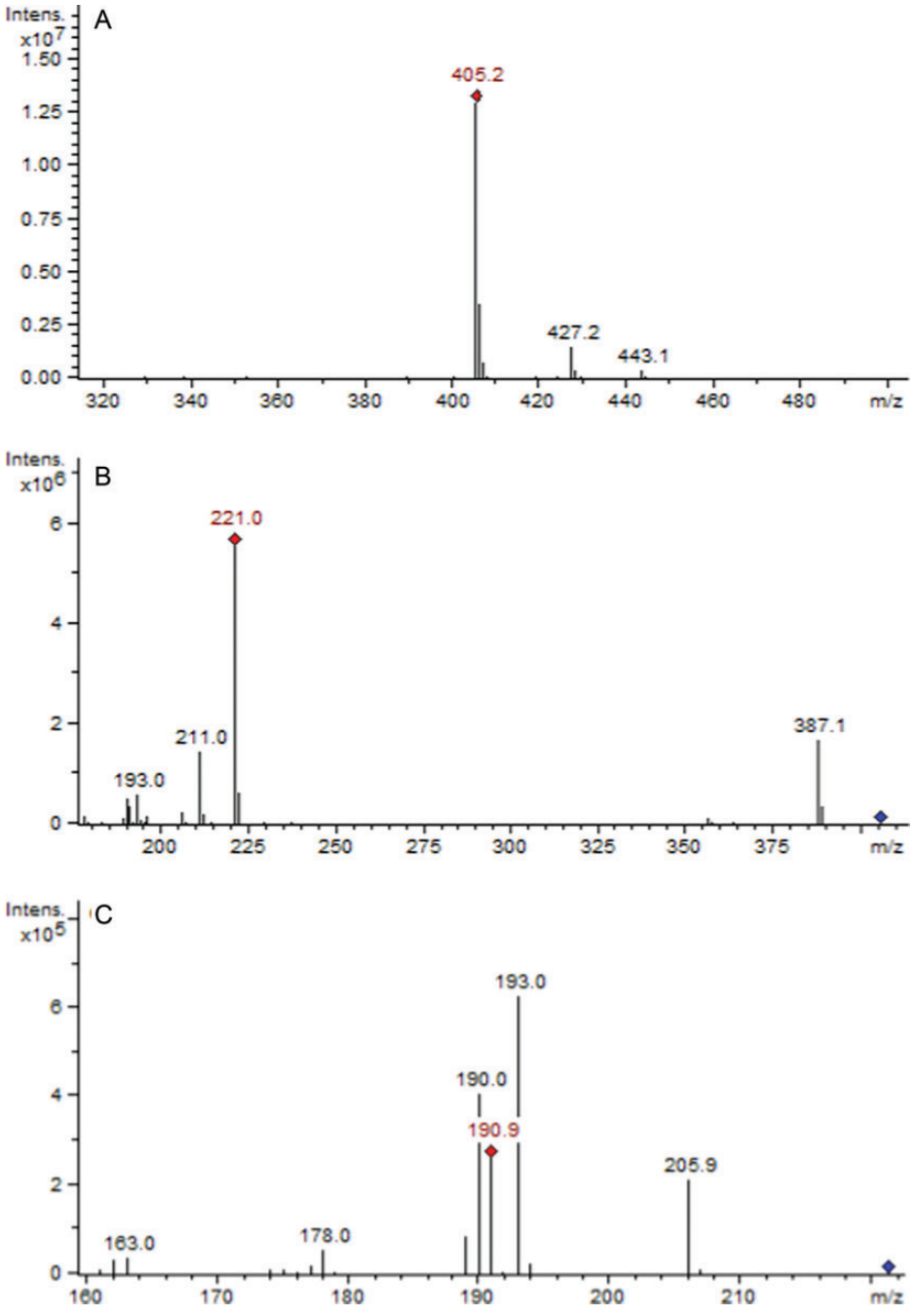

Figure 5. MS^n spectra of P-5: MS spectrum (A); MS^2 spectrum (precursor ion was m/z 405) (B); MS^3 spectrum (precursor ion was m/z 221) (C).

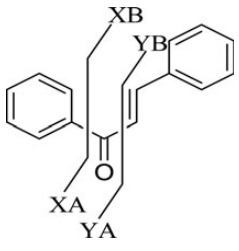

Figure 6. Proposed MS fragmentation pathway for chalcone derivatives.

the base peak at m/z 221 in the MS^2 spectrum (Figure 4). It could be deduced that its dominating fragmentation pathway was Retro-Diels-Alder (RDA) cleavage from the 1, 4-position of the C-ring. Meanwhile, the minor ion at m/z 181 was also detected in the result of the RDA fragmentation from the 1, 3-position of the C-ring. The loss of 15 ($CH_3^{\bullet}$), 28 (CO), 30 ($2CH_3^{\bullet}$) and 31 ($CH_3^{\bullet} + CH_4$) from the base peak at m/z 221 could also be detected as minor fragmentation ions in the CID-MS-MS spectra. The fragmentation pathway that the $[M + H]^+$ ion underwent in RDA reaction prior to the neutral

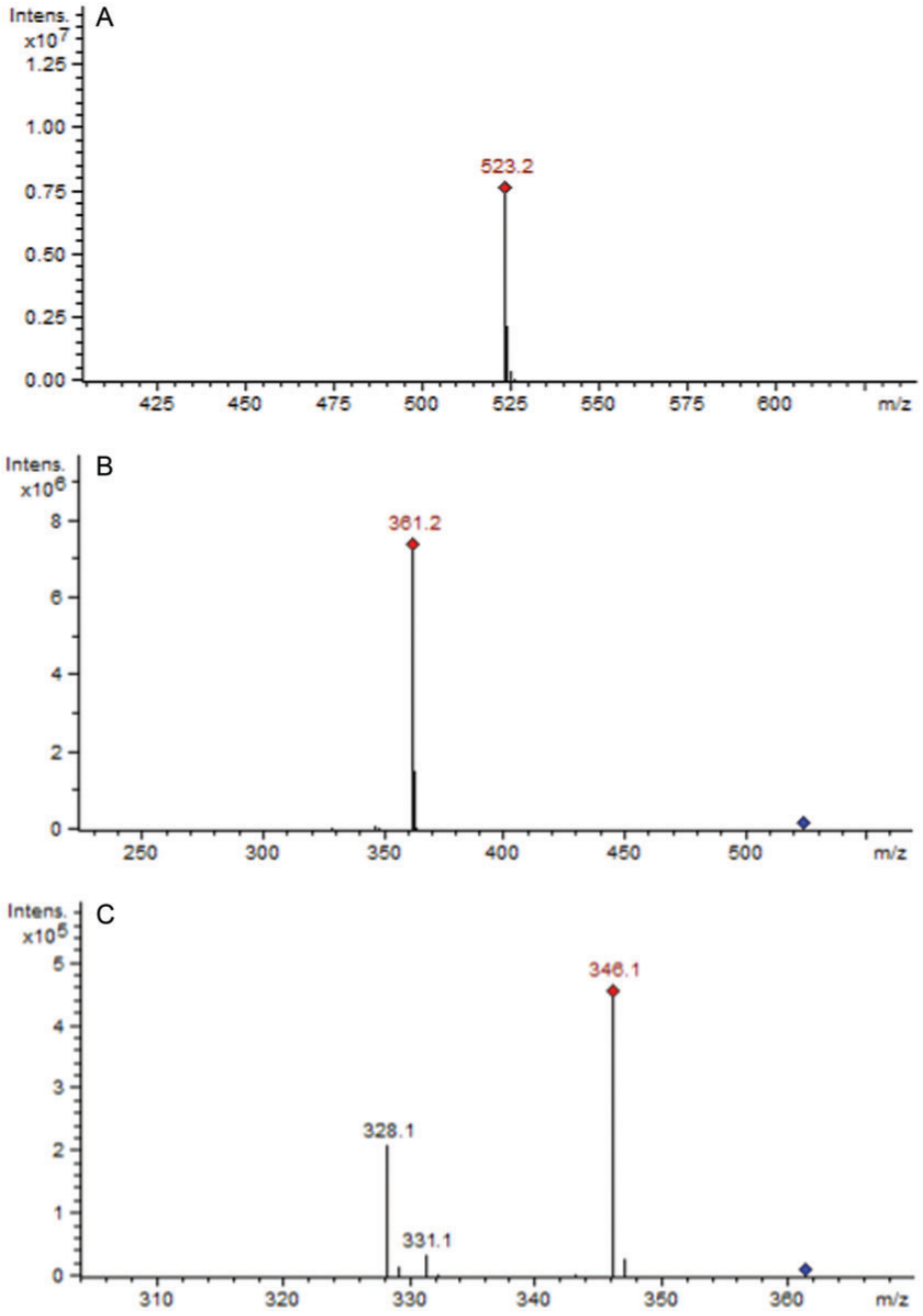

Figure 7. MS^n spectra of P-8: MS spectrum (A); MS^2 spectrum (precursor ion was m/z 523) (B); MS^3 spectrum (precursor ion was m/z 361) (C).

loss of $CH_3^{\bullet}$, CH_4, H_2O and CO was noticeably different from ordinary flavanones Therefore, it could be adopted as a shortcut to rapidly distinguish them from ordinary flavones.

Type C

Two polymethoxylated chalcone standards (Compounds P-5 and P-6) were also analyzed by the CID-MS-MS method. Their fragmentation pathways were similar to each other. Using P-5 as an example (Figure 5), the RDA cleavage at bond X to yield the base peak ion $^{X}B^{+}$ at m/z 221 and at bond Y to yield the minor ion $^{Y}A^{+}$ at m/z 211 (Figure 6) could also be simultaneously detected in the MS-MS spectrum. The fragmentation pathway was highly similar with flavanones. This is reasonable because the cyclization of 6'-hydroxychalcones to flavanones has been reported in many studies, which demonstrated that an intramolecular equilibrium is present between a flavanone-type and a chalcone-type of molecular ion (24, 25). Therefore, according to their fragmentation pathways, it was easy to distinguish polymethoxylated chalcones from polymethoxylated flavones, but difficult to distinguish them from polymethoxylated

Table II
Chemical Formulas and Masses of All Possible Polymethoxylated Flavones

Substituents	Substituents	OH	2OH	3OH	4OH	5OH
$2OCH_3$	$C_{17}H_{14}O_4$ 282	$C_{17}H_{14}O_5$ 298	$C_{17}H_{14}O_6$ 314	$C_{17}H_{14}O_7$ 330	$C_{17}H_{14}O_8$ 346	$C_{17}H_{14}O_9$ 362
$3OCH_3$	$C_{18}H_{16}O_5$ 312	$C_{18}H_{16}O_6$ 328	$C_{18}H_{16}O_7$ 344	$C_{18}H_{16}O_8$ 360	$C_{18}H_{16}O_9$ 376	
$4OCH_3$	$C_{19}H_{18}O_6$ 342	$C_{19}H_{18}O_7$ 358	$C_{19}H_{18}O_8$ 374	$C_{19}H_{18}O_9$ 390		
$5OCH_3$	$C_{20}H_{20}O_7$ 372	$C_{20}H_{20}O_8$ 388	$C_{20}H_{20}O_9$ 404			
$6OCH_3$	$C_{21}H_{22}O_8$ 402	$C_{21}H_{22}O_9$ 418				
$7OCH_3$	$C_{22}H_{24}O_9$ 432					

Table III
Chemical Formulas and Masses of All Possible Polymethoxylated Flavanones or Chalcones

Substituents	Substituents	OH	2OH	3OH	4OH	5OH
$2OCH_3$	$C_{17}H_{16}O_4$ 284	$C_{17}H_{16}O_5$ 300	$C_{17}H_{16}O_6$ 316	$C_{17}H_{16}O_7$ 332	$C_{17}H_{16}O_8$ 348	$C_{17}H_{16}O_9$ 364
$3OCH_3$	$C_{18}H_{18}O_5$ 314	$C_{18}H_{18}O_6$ 330	$C_{18}H_{18}O_7$ 346	$C_{18}H_{18}O_8$ 362	$C_{18}H_{18}O_9$ 378	
$4OCH_3$	$C_{19}H_{20}O_6$ 344	$C_{19}H_{20}O_7$ 360	$C_{19}H_{20}O_8$ 376	$C_{19}H_{20}O_9$ 392		
$5OCH_3$	$C_{20}H_{22}O_7$ 374	$C_{20}H_{22}O_8$ 390	$C_{20}H_{22}O_9$ 406			
$6OCH_3$	$C_{21}H_{24}O_8$ 404	$C_{21}H_{24}O_9$ 420				
$7OCH_3$	$C_{22}H_{26}O_9$ 434					

flavanones. However, the differences of UV absorption spectra between polymethoxylated chalcones and polymethoxylated flavanones provided an easy way to distinguish them, because the maximum absorption of chalcones usually ranged from 330 to 370 nm, whereas flavanones maintained at approximately 320 nm.

Type D

Compounds P-7 and P-8, all attributed to PMF glycoside standards, were analyzed last by the CID-MS-MS method. First, their $[M + H]^+$ ions readily eliminated the sugar moiety to produce the corresponding $[Aglycone + H]^+$ ions as the base peaks in MS spectra. Next, the other dissociation pathways of $[Aglycone + H]^+$ by the loss of 15 ($CH_3^\bullet$), 18 (H_2O), 30 ($2CH_3^\bullet$), 31 ($CH_3^\bullet + CH_4$), 33 ($H_2O + CH_3^\bullet$), 43 ($CH_3^\bullet + CO$) and 46 ($H_2O + CO$) were detected as diagnostic fragments in their MS^n spectra, which were in accordance with the fragmentation pathways of polymethoxylated flavones (Figure 7). These primary product ions could also form the characteristic ESI-MS^n fingerprint of PMF glycosides, which could be used to rapidly screen them out from the complex system.

HPLC–DAD–MS-MS analysis of the PMFs in the leaves of M. exotica

PMFs in the leaves of *M. exotica* have the basic aglycone structure with a maximum of seven substituents, such as a methoxyl group (OCH_3) and/or hydroxyl group (OH) on their A, B and C rings. The molecular weights of basic structures of aglycone are 222, 224 and 224 Da for flavones, flavanones and chalcones, respectively, which are increased by 30 or 16 Da when a methoxyl or hydroxyl is attached. On the basis of the numbers and types of substituent groups, the chemical formula and mass of every possible PMF isomer can be designated in advance (Tables II and III).

Because of the complexity and similarity of PMFs in the leaves of *M. exotica*, the extracted ion chromatogram (EIC)-MS method was adopted to analyze the PMFs (Figure 8 and Table IV). The abundances of most of the unknown compounds, especially the chalcones and flavanones, were too low to use the online UV absorption spectra, so it was difficult to distinguish them from each other. Therefore, they were screened and identified together at last.

After screening the molecular weights with the EIC-MS method, 36 candidates for PMFs were preliminarily screened from the leaves of *M. exotica*. However, the $[M + H]^+$ of 10 candidates (Peaks 1–2, 21, 23, 25, 27, 30, 31, 34 and 36) have undergone completely different fragmentation pathways than those of PMF standards, so they were not characterized as PMFs. The other 26 candidates were tentatively identified as 18 polymethoxylated flavones, five flavanones or chalcones and three PMF glycosides (Table V) according to the diagnostic fragmentation pathways of the PMF standards. Among them, 24 PMFs were identified as OH-PMFs, whereas the rest were all permethoxylated PMFs.

Meanwhile, the intensities of some EIC-MS peaks were too weak to be observed in the total ion chromatogram (TIC) spectra. Moreover, the retention times of some EIC-MS peaks were so similar that they could not be simultaneously identified in TIC spectra. However, the EIC method has been helpful to display every peak, especially the weak peaks in the highly complex mixtures. Thus, the EIC-MS method was confirmed to be powerful enough to preliminarily screen the constituents in highly complex TCM extracts.

Conclusion

In the paper, a sensitive HPLC–DAD–ESI-MS-MS method was established that could be used to simultaneously identify and screen the PMFs present in the leaves of *M. exotica*. Eight PMF standards, including two flavones, two flavanones, two chalcones and two glycosides, were analyzed by CID-MS-MS to obtain the respective characteristics of fragment pathways, which could be used as the basis for further analysis of the PMFs in the extract. Meanwhile, owing to regularities of PMFs in elemental composition, the EIC-MS method by molecular weights was used to screen the homoeomorphic PMFs in its extract. In the end, 26 PMFs were identified preliminarily, including 23 PMFs and three PMF glycosides. This was the first systematic report on the presence of PMFs in the leaves of *M. exotica*, especially for polymethoxylated flavanones, polymethoxylated chalcones and PMF glycosides. The results indicated that the developed HPLC–DAD–ESI-MS-MS method could be employed as a rapid, effective technique to screen and identify PMFs from TCM extracts.

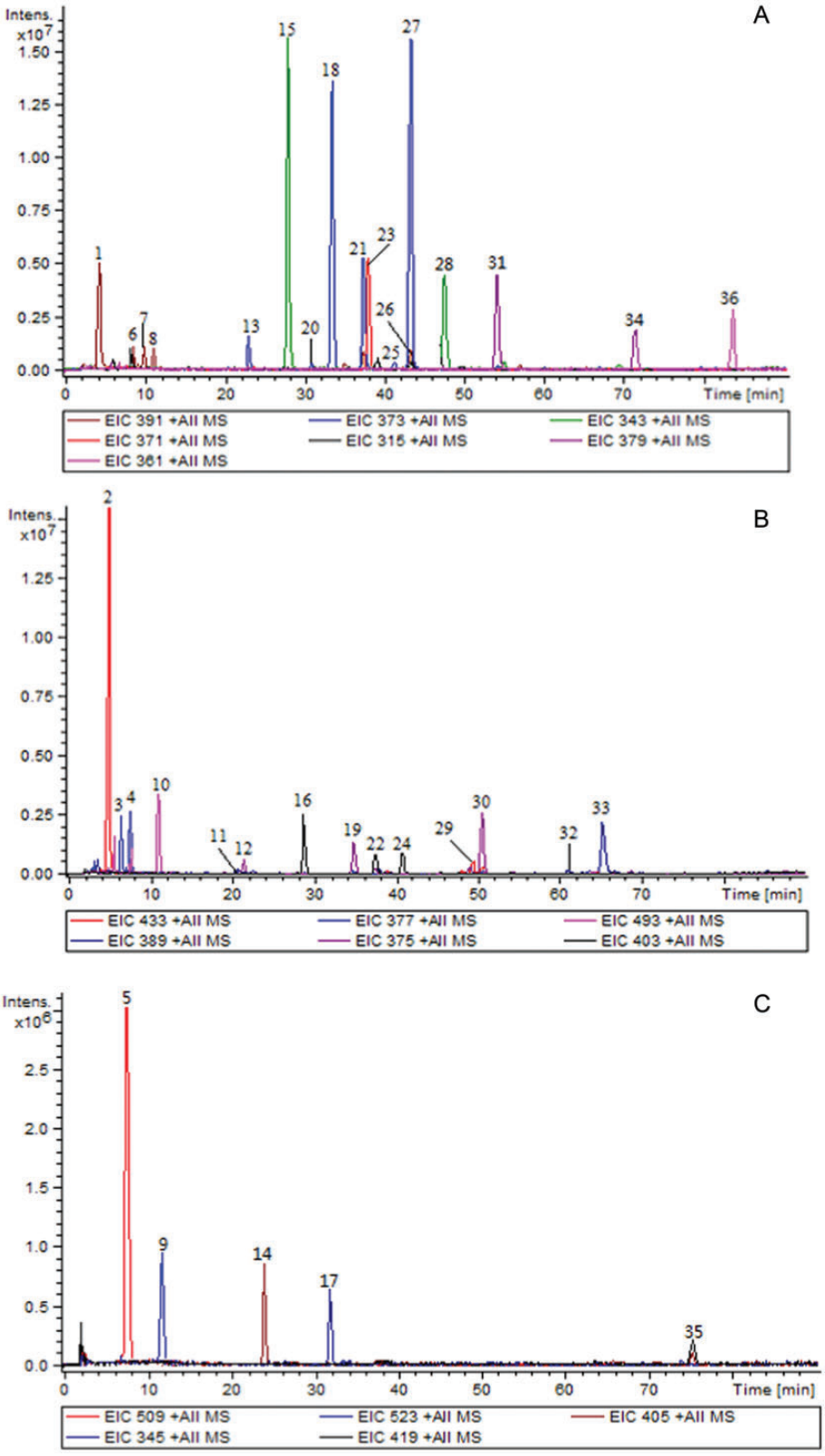

Figure 8. EIC-MS peaks of all possible PMF candidates in the leaves of *Murraya exotica* (peaks are exhibited in three figures for clarity).

Table IV

Characterization of PMF Candidates in the Leaves of *Murraya exotica* by Molecular Weights and the EIC-MS-MS Method*

Number	$t_R^{\dagger}$ (min)	$[M + H]^+$ (m/z)	MS^2 (m/z) P-ion (%, loss)‡	MS^3 (m/z) P-ion (%, loss)‡
1	4.6	391	**215** (100, 176), 373 (75.6, 18), 199 (68.7, 192)	197 (100, 18)
2	5.2	433	**367** (100, 66), 419 (94.4, 14), 415 (69.3, 18), 337 (50.6, 96), 397 (38.6, 36)	283 (100, 84), 271 (4.2, 96)
3	6.7	377	**362** (100, 15), 344 (31.2, 33), 347 (11.6, 30), 316 (2.4, 61)	344 (100, 18), 347 (41.6, 14), 316 (19.7, 46)
4	7.7	377	**362** (100, 15), 344 (34.7, 33), 347 (12.1, 30), 316 (3.5, 61)	344 (100, 18), 347 (33.2, 14), 316 (19.8, 46), 319 (5.8, 43)
5	8.0	509	**347** (100, 162)	332 (100, 15), 317 (80.6, 30), 287 (34.6, 60), 314 (17.2, 33)
6	8.8	391	**376** (100, 15), 358 (61.9, 33), 361 (25.5, 30), 330 (6.0, 61)	358 (100, 18), 361 (31.1, 16), 330 (16.5, 46), 333 (5.8, 43)
7	10.1	391	**376** (100, 15), 358 (61.0, 33), 361 (23.7, 30)	358 (100, 18), 361 (25.4, 16), 330 (8.0, 46), 333 (4.9, 43)
8	11.1	391	**376** (100, 15), 358 (59.1, 33), 361 (21.6, 30), 330 (7.2, 61)	358 (100, 18), 361 (27.6, 16), 330 (7.1, 46)
9	11.4	523	**461** (100, 162)	430 (100, 31), 431 (92.2, 30), 217 (80.4, RDA)
10	12.2	493	**431** (100, 162)	400 (100, 31), 416 (15.9, 15), 413 (14.3, 18)
11	20.8	389	**361** (100, 28), 346 (47.6, 43), 328 (42.5, 61), 374 (17.1, 15), 343 (14.6, 46)	346 (100, 15), 328 (45.0, 33), 343 (23.7, 18), 300 (9.7, 61)
12	21.4	375	**211** (100, RDA), 191 (37.7, RDA), 357 (18.9, 18)	196 (100, 15), 178 (37.0, 33), 150 (17.8, 61), 183 (17.6, 28)
13	23.0	373	**343** (100, 30), 358 (64.7, 15), 357 (8.1, 16), 329 (4.1, 44)	315 (100, 28), 328 (11.5, 15), 273 (9.9, 70), 153 (7.9, 190)
14	24.1	405	**221** (100, RDA), 211 (32.7, RDA), 387 (32.3, 18),	193 (100, 28), 190 (53.3, 31), 206 (33.9, 15), 191 (31.8, 30)
15	27.9	343	**328** (100, 15), 327 (62.7, 16), 299 (13.6, 44), 329 (2.7, 14)	299 (100, 29), 312 (21.9, 16), 313 (8.9, 15), 283 (6.9, 45)
16	28.7	403	**373** (100, 30), 342 (36.9, 61), 388 (29.3, 15), 374 (6.6, 29)	345 (100, 28), 340 (32.4, 33), 358 (15.7, 15), 343 (12.9, 30), 312 (12.1, 61)
17	31.8	345	**181** (100, RDA), 191 (66.0, RDA), 327 (10.1, 18)	166 (100, 15), 125 (63.5, 56), 122 (50.6, 59)
18	33.2	373	**312** (100, 61), 358 (24.0, 15), 343 (20.6, 30), 340 (15.2, 33), 357 (13.2, 16)	284 (100, 28), 283 (67.6, 29), 297 (6.5, 15)
19	34.7	375	**221** (100, RDA), 181 (24.3, RDA), 357 (8.6, 18)	193 (100, 28), 190 (58.2, 31), 191 (38.7, 30), 206 (30.6, 15)
20	35.2	391	**376** (100, 15), 358 (62.5, 33), 361 (19.0, 30), 330 (9.7, 61)	358 (100, 18), 361 (33.9, 15), 330 (20.8, 46)
21	37.1	373	**355** (100, 18), 337 (18.0, 36), 319 (8.8, 54)	337 (100, 18), 319 (55.8, 36), 159 (36.7, 196), 295 (16.8, 60)
22	37.5	403	**342** (100, 61), 373 (30.2, 30), 359 (27.1, 44), 388 (18.9, 15), 387 (16.3, 16)	327 (100, 15), 151 (54.0, RDA), 281 (49.7, 61), 309 (28.6, 33), 314 (15.8, 28)
23	37.9	371	**261** (100, 110), 219 (49.8, 152)	219 (100, 42), 233 (9.1, 28), 181 (1.0, 80)
24	40.8	403	**373** (100, 30), 388 (67.7, 15), 342 (14.5, 61), 355 (8.1, 48)	327 (100, 46), 358 (59.9, 15), 355 (37.2, 18), 345 (31.0, 28), 330 (25.6, 43)
25	41.5	373	**219** (100, 154), 273 (53.6, 100), 355 (8.7, 18)	161 (100, 58), 189 (26.4, 30), 191 (8.9, 28)
26	42.4	391	**361** (100, 30), 376 (57.5, 15), 343 (30.9, 48), 358 (24.8, 33)	333 (100, 28), 318 (7.6, 43), 343 (2.5, 18)
27	43.2	373	**355** (100, 18), 319 (14.3, 54), 337 (13.4, 36)	337 (100, 18), 319 (97.3, 36), 209 (92.4, 146), 227 (87.4, 128)
28	47.0	315	**181** (100, RDA), 161 (13.1, RDA)	153 (100, 28), 125 (41.1, 56), 121 (28.1, 60)
29	49.5	433	**403** (100, 30), 418 (52.9, 15), 372 (14.3, 61), 385 (9.1, 48)	357 (100, 76), 388 (92.8, 45), 342 (67.4, 61), 360 (55.9, 43), 331 (43.9, 72), 375 (39.1, 28), 370 (28.1, 33), 385 (25.9, 18)
30	50.6	375	**219** (100, 156), 273 (86.8, 102), 291 (38.9, 84)	161 (100, 58), 189 (13.2, 30), 191 (5.4, 84)
31	53.7	379	**297** (100, 82)	199 (100, 98), 266 (30.7, 31), 265 (22.1, 32)
32	60.6	389	**356** (100, 33), 328 (76.8, 61), 374 (37.5, 15), 359 (15.8, 30)	328 (100, 28), 295 (2.9, 61), 225 (2.9, RDA)
33	65.6	389	**359** (100, 30), 341 (49.4, 48), 374 (44.0, 15), 356 (28.9, 33), 328 (19.9, 61)	344 (100, 15), 341 (79.1, 18), 343 (64.2, 16), 331 (59.2, 28), 197 (35.9, RDA)
34	70.9	379	**335** (100, 44), 279 (83.6, 100), 297 (62.4, 82), 249 (6.2, 130)	291 (100, 44)
35	75.0	419	**389** (100, 30), 358 (45.4, 61), 371 (42.5, 48), 404 (29.5, 15), 386 (27.5, 33)	328 (100, 61), 356 (66.0, 33), 361 (55.5, 28), 371 (46.4, 18), 359 (39.3, 30), 346 (35.1, 43), 374 (30.1, 15)
36	83.1	361	**259** (100, 102), 189 (14.9, 172), 203 (14.6, 158), 231 (11.4, 130)	189 (100, 70), 231 (19.4, 28), 203 (4.3, 56), 190 (3.1, 69)

*Note: Precursor ions for next stage MS are in bold.

†t_R: retention time.

‡P-ion (%): the product ions and the relative intensity.

Table V
Structural Identifications of All Possible PMFs Detected in the Leaves of *Murraya exotica*

Peaks	Amounts	PMFs	Number of $-OCH_3$	Number of $-OH$	MW
3, 4	2	Tetrahydroxy-trimethoxyflavone	3	4	376
5	1	Tetrahydroxy-dimethoxyflavone glucose	2	4	508
6–8, 20, 26	5	Trihydroxy-tetramethoxyflavone	4	3	390
9	1	Trihydroxy-trimethoxyflavone glucose	3	3	522
10	1	Trihydroxy-dimethoxyflavone glucose	2	3	492
11, 32, 33	3	Monohydroxy-pentamethoxyflavone	5	1	389
12, 19	2	Pentamethoxyflavanone or Pentamethoxychalcone	5	0	374
13, 18	2	Pentamethoxyflavone	5	0	372
14	1	Hexamethoxyflavanone or Hexamethoxychalcone	6	0	404
15	1	Pentamethoxyflavone	5	0	342
16, 22, 24	3	Hexamethoxyflavone	6	0	402
17	1	Tetrathoxyflavanone or Tetramethoxychalcone	4	0	344
28	1	Trihoxyflavanone or Trimethoxychalcone	3	0	314
29	1	Heptamethoxyflavone	7	0	432
35	1	Monohydroxy-hexamethoxyflavone	6	1	418

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