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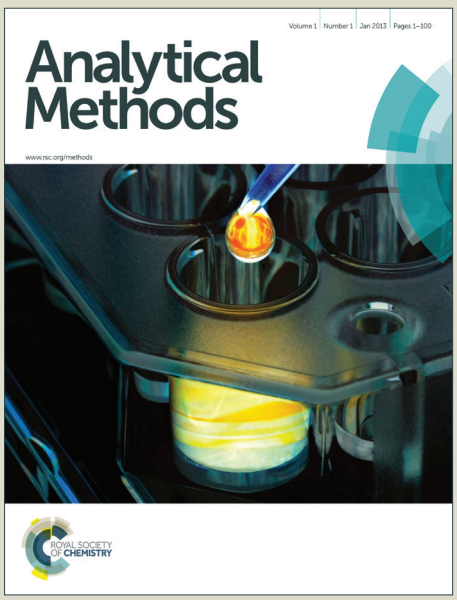

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A practical strategy for chemical profiling of herbal medicines using ultra-high performance liquid chromatography coupled with hybrid triple quadrupole-linear ion trap mass spectrometry, a case study of Mori Cortex

Wang-Hui Jing, Ru Yan*, Yi-Tao Wang*

State Key Laboratory of Quality Research in Chinese Medicine, Institute of Chinese Medical Sciences, University of Macau, Taipa, Macao SAR, China

*To whom correspondence should be addressed

Prof. Yi-Tao Wang

Tel: 853-83974691

Fax: 853-28841358

Email: ytwang@umac.mo

Dr. Ru Yan

Tel: 853-83974876

Fax: 853-28841358

Email: ruyan@umac.mo

Abstract

Herbal medicines (HMs) are believed to produce holistic action through the synergistic effects of both major and minor components. In current study, a practical strategy was proposed for comprehensive characterization of the chemical constituents in HMs. Mori Cortex (MC), which contains diverse chemical constituents and consequently exhibits a broad spectrum of pharmacological activities, was chosen as a model case. The workflow included three steps: First, a thorough literature review was performed to summarize the information of the phytochemistry and the biosynthetic pathways for the genus *Morus*; Second, seven compounds, namely morin (polyhydroxyflavonoid), morusin (prenylflavone), mulberroside A and oxyresveratrol (stilbenes), mulberroside C (2-arylbenzofuran derivative), sanggenon C and kuwanon G (DA-type adducts), were selected to propose mass fragmentation pathways for the primary chemical types in MC; Third, a set of parent to parent ion transitions was constructed using quasi-molecular and sodium adduct ions of the identified compounds and their potential derivatives, and multiple ion monitoring-information dependent acquiring-enhanced product ion (MIM-IDA-EPI) method was thereby used to detect and identify the constituents. As a result, a total of 140 components were detected with 133 identified in the MC extract, including 10 polyhydroxyflavonoids, 4 stilbenes, 16 2-arylbenzofuran derivatives, 60 prenylflavones, and 43 DA type adducts, while the identities of 7 ones could not be elucidated due to insufficient structural information. Collectively, the strategy was demonstrated to be efficient, reliable and sensitive for global chemical profiling of HMs.

Keyword: Comprehensive chemical profiling; Mori Cortex; Multiple ion monitoring-information dependent acquiring-enhanced product ion; Herbal medicines

1. Introduction

It is well-known that herbal medicine (HM) is the mixture of hundreds of components and its activities are based on the synergistic effects of both major and minor components in the material [1]. Therefore, the detection and identification of minor components may be equal to the primary ones contributing to quality control and to understand the health benefits of HMs. In most cases, only the abundant components can be detected using conventional full scan modes during LC-MS measurement [2], while the characterization of those minor constituents usually suffers from co-eluting and unsatisfactory sensitivity of the method.

There are many studies concerning the development of data mining technologies, such as diagnostic fragment ion filtering and mass defect filtering [3–7], to extract compounds from the high-resolution mass spectrometric dataset. In contrast, data acquisition research is relatively rare. On-line data acquisition and off-line data mining are equally important, and sensitivity of data acquisition technique is the foundation of the latter [7]. Multiple reaction monitoring (MRM), which is performed on multi-stage mass spectrometer, has been regarded as the most sensitive and selective data acquisition method, while reference compounds are usually required for the optimization of precursor to product ion transitions and mass spectrometric parameters, especially collision energy (CE). Multiple ion monitoring (MIM) is a special MRM mode, in which quasi-molecular ions or adduct ions are used as both parent and product ions (called parent to parent ion transition) to compose MIM ion transitions and the lowest CE (5 eV) is adopted in the collision cell, indicating that reference compounds are not necessary to optimize this parameter. This technique has been revealed comparable sensitivity with MRM to monitor numerous analytes [8]. Given the chemical complexity of herbal medicine, MIM mode is hence superior to MRM mode in global chemical profiling of HMs. MIM mode has been successfully

applied for universal detection and identification of flavonoids in Astragali Radix [9].

Generally, different plants from the same genus generate similar chemical profiles, at least similar chemical types. Taken ginseng, notoginseng and American ginseng as examples, dammarane-type saponins are demonstrated to be the primary components in these three species in the genus *Panax* [10]. Furthermore, from an evolutionary standpoint, different species of the same genus might exhibit similar biosynthetic pathways because they own similar genomes, and consequently show similar enzyme spectra. For instance, glycosidation and prenylation of flavones were observed as the dominant pathways in the seven species in the genus *Epimedium* [11]. Consequently, it is reasonable to speculate the potential metabolites on the basis of the identified compounds coupled with the biosynthetic pathways. Previously, the information from the literatures is only adopted for the identification of the detected components. In current study, the quasi-molecular and adduct ions of the identified compounds and their potential derivatives were used to construct MIM ion transition list for data acquisition.

As a folk medicine in Eastern Asia, Mori Cortex (MC, "Sang-Bai-Pi" in Chinese) is derived from the dried root barks of *Morus alba* L. MC has been widely applied for the treatment of patients with edema and dysuria for centuries in traditional Chinese medical practices, while its prescriptions have been extensively used as anti-tussive and anti-asthmatic agents. Modern pharmacological evaluations have revealed a variety of pharmacological features for this HM, such as hypoglycemic [12], anti-oxidant [13], anti-inflammatory [14,15], anti-stress and adaptogenic activities [16–18]. The phytochemical investigations demonstrated a wide spectrum of chemical components in the genus *Morus* and various biosynthetic pathways [19,20]. Although a vast number of components have been identified from this genus, the comprehensive chemical characterization hasn't been achieved. Moreover, the quality control of MC only

focused on a couple of chemical components [2,21–22], which cannot meet the demands for the explanation of its holistic actions through the synergistic effects of both major and minor components. Hence, it is crucial to globally characterize the chemical constituents in MC.

With the aim to develop an efficient, sensitive and reliable strategy for comprehensive chemical profiling of HMs, a MIM-based workflow was proposed in current investigation. Despite the highly sensitive, selective and intrinsic multiplexing potential of the MRM methodology, the key bottleneck in MRM-based metabolomics locates at the limited analyte coverage and throughput capacity [9]. In order to address the drawbacks, our strategy was using multiple ion monitoring-information dependent acquiring-enhanced product ion (MIM-IDA-EPI) mode. In view of the wide distribution, abundant chemical types, diverse biosynthetic pathways in this genus and definite activities, MC was chosen as the model case.

2. Experimental

2.1. Chemicals

Morin (MW: 302 Da), mulberroside A (MW: 568 Da), oxyresveratrol (MW: 244 Da), and mulberroside C (MW: 458 Da) were purchased from Shanghai Traditional Chinese Medicine Research Center (Shanghai, China). Morusin (MW: 422 Da), sanggenon C (MW: 708 Da), and kuwanon G (MW: 692 Da) were obtained from BioBioPha Co. Ltd. (Kunming, China). All the chemical structures and purities (greater than 98% for all references) were determined by NMR and LC-MS/MS analysis.

HPLC grade formic acid, acetonitrile (ACN) and methanol were purchased from Merck (Darmstadt. Germany). Deionized water was prepared in-house using Milli-Q plus water purification system (Millipore, Bedford, MA, USA). All other chemicals

were of analytical grade and obtained commercially.

2.2. Preparation of samples

2.2.1. Reference samples

Each authentic compound was dissolved in methanol at 0.3 mg/mL. All the solutions were stored in a refrigerator (4°C) until use.

2.2.2. Mori Cortex extract sample

Crude materials were purchased from Chinese herbal medicine market in Guangzhou (Guangdong, China) and authenticated as the root barks of *Morus alba* L. by Dr. Chunfeng Qiao from our institute. The specimen was deposited at the State Key Laboratory of Quality Research in Chinese Medicine, Institute of Chinese Medical Sciences, University of Macau (Macao, China).

The dried roots (100.0 g) were pulverized into powder and extracted with 80% aqueous ethanol for three times (1.0 L × 3, 1 hour for each time) using heating reflux. Afterwards, the solvent of the combined extract solution was removed under reduced pressure at 55°C to yield a total of 15.3 g residue. An aliquot (100.0 mg) of the extract was dissolved in 10 mL of methanol. The solution was then centrifuged at 10 000×*g* for 10 min and the supernatant was filtered through 0.45 μm membrane to obtain the extract sample.

2.3. LC-MS/MS analysis

An Agilent 1200SL liquid chromatography system (Agilent Technologies, Santa Clara, CA, USA) comprising a binary solvent delivery unit, an autosampler, a column oven, and a diode array detector (DAD), was connected to an API4000 QTrap mass spectrometer (ABSciex, Foster City, CA, USA) equipped with a TurboIonspray interface. All instruments were controlled and synchronized by Analyst software (Version 1.5.1, ABSciex).

The chromatographic separation was achieved on a Zorbax SB-C_{18} column (150

mm × 2.1 mm, 1.8 μm, Agilent Technologies). The column temperature was set at 55°C. The mobile phase consisted of 0.1% aqueous formic acid (A) and ACN containing 0.1% formic acid (B) and was delivered at 0.3 mL/min with a gradient program: 20–55%B (0–25 min) and 55–95%B (25–60 min). Then, 20%B was delivered for another 10 min for system re-equilibration.

The ion spray temperature was maintained at 400°C. Nitrogen was used as nebulizer and auxiliary gas. Both nebulizer gas (GS1) and heater gas (GS2) were set at 50 psi. The curtain gas was fixed at 25 psi and the interface heater was maintained at "On" channel. Both positive and negative modes were utilized to analyze all samples in separate analytical runs. Parent to parent ion transitions (MIM ion transitions) were listed in Table S1 (Supplemental information). For positive mode, the ion spray voltage was set as 5500 V. The declustering potential (DP) for all experiments was set as 100 V, and the dwell time of each ion transition was 10 ms. In the information dependent acquisition (IDA) criteria, dynamic exclusion was set to 15 s to allow the detection of co-eluting substances, and a threshold of 300 counts per second (cps) was set to trigger two separate EPI scans (4000 Da/s). The CE of enhanced product ion (EPI) was set at 25 eV with a collision energy spread (CES) as 15 eV. Under negative ionization mode, ion spray voltage, DP, CE and CES were set as –4500 V, –100 V, –25 eV and –15 eV, respectively. The total duration for a cycle was 1.9 s, which could guarantee enough points for each peak in the mass chromatogram. In addition, Q1 and EMS full scans were adopted as the complementary techniques to avoid detection omission.

All the standard solutions were diluted to appropriate concentration levels and infused directly into the ion source using a syringe pump (Harvard, Quebec, Canada) to obtain MS^1 and MS^2 spectra.

3. Results and discussion

3.1 Strategy for universal chemical characterization

The attempt to propose a practical strategy for the comprehensive chemical characterization of MC was made using ultra-high performance liquid chromatography hyphenated with Qtrap mass spectrometry (UHPLC-Qtrap-MS). The whole workflow (Fig. 1) included three steps: First, a thorough review was carried out on the chemical constituents of the genus *Morus* to reveal the main chemical types and primary biosynthetic pathways; Second, fragmentation patterns of the dominant chemical types, including prenylflavones, stilebenes, 2-arylbenzofuran derivatives, and Diels-Alder (DA) type adducts, were proposed using representative components; Third, chemical detection of MC was performed using a set of parent to parent ion transitions (MIM ion pairs) for both of the identified compounds and the proposed derivatives, and the identification of constituents was achieved by combining the structural information from EPI experiments and the proposed fragmentation rules.

3.2. A summary of the chemical constituents from the genus *Morus*

Based on the data collected through PubMed, ACS, CNKI, Google Scholar, Baidu Scholar, and Web of Science, so far, approximate 300 components have been isolated and identified from the genus *Morus*, and most of them belong to flavonoids [23], stilbenes [21], 2-arylbenzofuranderivatives [24], and DA type adducts [25]. 2-arylbenzofuran derivatives are regarded as the dehydration products of 2-hydroxyl stilbenes. Flavonoids in *Morus* can be divided into polyhydroxyl flavones (for instance morin) and their glycosides [26], as well as prenylflavonoids (for example morusin) [27], the glycoside of which hasn't been isolated from the genus *Morus* yet. Prenylfavonoids coupled with prenylated 2-arylbenzofurans are the biosynthetic precursors of DA-type adducts, which are the diagnostic components for the genus *Morus* [25]. Those adducts can be classified into several different types based on the biosynthetic precursors (Fig.

S1, Supplemental information): 1. adducts of chalcones and prenylflavone/prenylflavanones (for instance kuwanon L); 2. adducts of chalcones and prenylated 2-arylbenzofurans (for instance mulberrofuran C); 3. adducts of chalcones and prenylated stilbenes (for instance kuwanon P); 4. adducts of chalcones and prenylated chalcones (for instance kuwanon Q); 5. adducts of chalcones and prenylated benzaldehydes (for instance guangsangon L); 6. other compounds, such as kuwanon M, dimoracin, and sanggenon B [25].

On the basis of phytochemical review about the genus *Morus* (Table S2, Supplemental information), the sodium adduct ions, the deprotonated and protonated molecular ions, were summarized in Table S1 and used for MIM mode (Supplemental information).

3.3. Mass spectrometric behaviors of authentic compounds

In current study, seven references belonging to five chemical families were analyzed to summarize their fragmentation pathways.

As a polyhydroxylflavone, morin afforded the sodium adduct ion and protonated ion at *m/z* 325 and 303, respectively, under positive ionization, corresponding to a molecular weight of 302 Da. The product ions at *m/z* 285, 257, 239, and 229 in the MS^2 spectrum of the protonated ion were resulted from the successive cleavages of H_2O (18 Da), CO (28 Da), and H_2O (18 Da)/CO (28 Da) groups, respectively (Fig. 2). Under the negative mode, the pseudo-molecular ion was observed at *m/z* 301$[M–H]^-$, while its product ion was observed at *m/z* 257, corresponding to the neutral loss of a CO_2 molecule (44 Da). These findings agreed well with the information archived in the literature [28].

Mulberroside A is the *di*-glycosidation product of oxyresveratrol, and its sodium adduct ion was observed at *m/z* 591, and quasi-molecular ion at *m/z* 569. Under negative ionization mode, the deprotonated molecular ion was yielded at *m/z* 567, and

the predominant product ions were observed at *m/z* 405, 243, 225, and 199 (Fig. 3A), corresponding to the successive neutral losses of a glucosyl group (162 Da), a glucosyl group, and a H_2O moiety (18 Da) or a CO_2 molecule (44 Da) (Fig. 3B). On the other side, the diagnostic product ions of the deprotonated ion (*m/z* 243 $[M–H]^-$) of oxyresveratrol were generated at *m/z* 225 and 199 (Fig. 3C), corresponding to the successive cleavages of a H_2O and a C_2H_2 groups.

Mulberroside C was introduced as a typical 2-arylbenzofuran derivative, and its sodium adduct ion and protonated ion were afforded at *m/z* 481 and 459, respectively. Under the negative ionization mode, deprotonated ion was detected at *m/z* 457 and its characteristic fragment ions were observed at *m/z* 325 and 253 (Fig. 4A), suggesting the successive cleavages of a xylosyl group (132 Da) and a C_4H_8O group (72 Da) through retro-Diels-Alder (RDA) reaction (Fig. 4B).

Morusin consists of the flavone skeleton and two isopentenyl substituents. Its sodium adduct ion and protonated ion were detected at *m/z* 443 and 421, respectively. A C_4H_8 group (56 Da) was expelled initially from the uncyclized isopentenyl substituent, and the subsequent cleavages of CO and H_2O molecules were obviously detected in the MS^2 spectrum of the protonated ion (*m/z* 419 $[M+H]^+$) (Fig. 5), whereas none characteristic cleavage was observed for the cyclized isopentenyl segment (Fig. 5B).

Kuwanon G is the DA-type adduct of a chalcone and a prenylflavone. Under negative mode, the diagnostic product ions of the deprotonated ion (*m/z* 691 $[M–H]^-$) were detected at *m/z* 581 and 471 (Fig. 6A), corresponding to the successive neutral losses of two resorcinol ($C_6H_6O_2$, 110 Da) groups, and then a subsequent neutral loss of H_2 (2 Da) occurred to generate product ion at *m/z* 469 *via* intra-molecular esterification (Fig. 6B). On the other side, successive neutral losses of two resorcinol ($C_6H_6O_2$, 110 Da) groups were also observed for the deprotonated ion (*m/z* 707 [M–

H]$^-$) of sanggenon C under negative ionization (data not shown).

3.4. Mass fragmentation patterns of the chemical families in MC

Based on our findings and previous report [28], the fragmentation pathways of polyhydroxylflavones, for instance morin, mainly include the neutral losses of CO (28 Da) and water (H_2O, 18 Da) groups under positive ion mode. If methyl groups exist in these compounds, the radical cleavage of methyl group (15 Da) could be observed. Meanwhile, the neutral cleavage of CO_2 molecule can be detected for polyhydroxylflavones under negative ion mode.

Prenylflavonoids were regarded as one of the characteristic chemical families in the genus *Morus*. In current study, the characteristic fragmentation pathways were observed as the neutral losses of C_4H_8 (56 Da), CO, and H_2O groups under positive ion mode, which were fully coincided with the fragmentation pattern proposed in the literature [29,30].

Stilbenes and 2-arylbenzofuran derivatives can exist as aglycones or glycosides. For glycosides, the neutral losses of the glycosyl groups can be observed initially, and then the neutral losses of H_2O and CO_2 groups can be detected using negative ionization. If those components were prenylated and/or cyclized, for example mulberroside C, the diagnostic cleavage of C_4H_8O (72 Da) group can be detected under negative ionization, while the characteristic neutral loss of an isobutene group (C_4H_8, 56 Da) can be observed for uncyclized prenylstilbene/2-aryl-prenylbenzofuran under positive ionization, which is similar to the fragmentation pattern of prenylflavonoids.

Due to the presence of resorcinol substituents for DA-type adducts, the characteristic cleavage was detected as the successive losses of $C_6H_6O_2$ (110 Da) groups, which were also revealed for two adducts of chalcone and prenylated 2-arylbenzofuran, mulberrofuran G and isomulberrofuran G [31].

3.5. Development of UHPLC-MS/MS

Our preliminary experiments revealed that a great number of peaks were detected in MC extract using DAD detector. Therefore, an UHPLC system equipped with a rapid resolution high definition column was chosen to provide efficient separation for the chemical constituents in the herbal extract. Given most of the components in MC contain large aromatic conjugated systems, ultraviolet (UV) length of 280 nm was chosen to monitor the column eluent. As judged from the UHPLC-UV chromatogram (Fig. 7), most of the compounds were obtained good separation and more peaks were eluted after 30 min.

To improve the coverage of the mass spectrometric analysis, in particular those minor constituents in the herbal extract, Q1 full scan, EMS full scan and MIM scan were compared. Fig. 8 showed the representative chromatograms obtained using different scan modes under both positive (Fig. 8A-D) and negative (Fig. 8E-H) ion modes.

Because Q1 and EMS scans recorded all ions in the full *m/z* range, they provided very complex profiles with many minor ions obscured by high-abundance species and high level background (Figs. 8A, 8B, 8E and 8F). In MIM mode, ions were isolated twice in Q1 and Q3 with a relatively long dwell time (10 ms), contributing to superior selectivity and sensitivity (e.g. the peak at 27.5 min, marked with an arrow in Fig. 8A-D), and more sensitive triggering of MS/MS spectra for structure elucidation.

The 295 compounds (Table S2, Supplemental information) that were identified from the genus *Morus* were used to construct a list of parent to parent ion transitions for MIM mode (Table S1, Supplemental information). Then the EPI scans were employed to obtain the fragment ion information. Moreover, preliminary experiments were used to assay the sensitivity of the proposed method, and the result suggested that the method featured at sensitive.

3.6. Characterization of the chemical constituents in MC extract

In total, 140 compounds were detected from the MC extract, including 10 polyhydroxyflavonoids, 4 stilbenes, 16 2-arylbenzofuran derivatives, 43 DA-type adducts, and 60 prenylflavones, as well as 7 unknown compounds. Six ingredients were unambiguously identified using authentic compounds (except sanggenon C), while the identities of 127 compounds were tentatively assigned using the proposed fragmentation profiles. The retention times, precursor ions, molecular weights, fragment ions, and plausible identities of those compounds are summarized in Table 1. The general information of each chemical type and the detailed identification process of some representative compounds are described below, whereas the structural characterization process of the other compounds are narrated in Supplemental information.

Under positive ionization, the neutral losses of CO and H_2O molecules act as the major evidences for the discrimination of the polyhydroxyflavonoids, and the additional cleavages of sugar residues are used for structural characterization of polyhydroxyflavonoid glycosides. On the other hand, the diagnostic neutral loss of CO_2 molecule can be utilized as a characteristic of polyhydroxyflavonoids using negative ionization. Compounds **1**, **2**, and **8** were eluted at the retention times (t_R) of 5.0, 5.5, and 11.4 min (Table 1 and Fig. 9), respectively, and shared the identical sodium adduct and protonated ions at *m/z* 649 and 627 under positive mode, suggesting the molecular weight as 626 Da. In the MS^2 spectrum of the protonated ion (*m/z* 627 $[M+H]^+$), diagnostic product ions were afforded at *m/z* 465, 303, 285, and 257 (Fig. S2, Supplemental information), corresponding to the sequential neutral losses of two glucosyl groups (2 × 162 Da), a H_2O moiety (18 Da), and a CO moiety (28 Da) (Fig. S2), respectively. Under negative mode, the ions of *m/z* 625$[M–H]^–$, 463$[M–H–Glc]^–$, 301$[M–H–Glc–Glc]^–$, and 257$[M–H–Glc–Glc–CO_2]^–$ were yielded.

Thus, these three compounds were tentatively characterized as the *di*-glycosidation products of morin, which is the most abundant polyhydroxylflavone in MC. Similarly, compounds **13** (t_R: 12.8) and **17** (t_R: 14.9) were plausibly identified as the *mono-O*-glycosidation products of morin due to the observation the ions of *m/z* 487$[M+Na]^+$, 465$[M+H]^+$, 463$[M–H]^–$, 301$[M–H–Glc]^–$, and 257$[M–H–Glc–CO_2]^–$ (Table 1 and Fig. 9). On the other hand, compounds **21** and **24** (morin, confirmed using authentic compound) were eluted at 21.0 min and 18.8 min, respectively, with identical ions of *m/z* 325$[M+Na]^+$, 303$[M+H]^+$, 301$[M–H]^–$ and 257$[M–H–CO_2]^–$, indicating compound **21** is a regio-isomer of morin (Table 1 and Fig. 9).

Under negative mode, neutral cleavages of H_2O and CO_2 groups are the characteristic behaviors of stilbenes, and another neutral loss of glycosyl group could be detected for stilbene glycoside. As the *di*-glycosidation product of oxyresveratrol, mulberroside A (**3**) was detected at 8.7 min by comparison with the reference compound, while oxyresveratrol (**16**) was observed at 14.7 min (Table 1 and Fig. 9). Compound **9** (t_R: 11.5 min) showed ions of *m/z* 429$[M+Na]^+$, 407$[M+H]^+$, and 405$[M–H]^–$, and prominent fragment ions of *m/z* 243$[M–H–Glc]^–$, 225$[M–H–Glc–H_2O]^–$, and 199$[M–H–Glc–CO_2]^–$ (Table 1 and Fig. S3). Collectively, compound **9** was tentatively identified as oxyresveratrol-*O*-glucoside.

Owing that 2-arylbenzofuran derivatives are the dehydration products of 2-hydroxyl stilbenes, the ions of 2-arylbenzofuran derivatives are usually 2 Da lower than their corresponding stilbenes. And also, neutral loss of CO_2 group is a typical feature for 2-arylbenzofuran derivatives under negative mode. The sodium adduct, protonated and deprotonated ions of compound **22** were exhibited at *m/z* 265, 243 and 241 (Table 1), respectively, suggesting a molecular weight as 242 Da, 2 Da less than oxyresveratrol. The predominant product ion (*m/z* 197) was also 2 Da less than that of oxyreseveratrol (*m/z* 199) (Fig. 4SA), suggesting that compound **22** is the dehydration

product of oxyreseveratrol. Therefore, this compound was tentatively identified as moracin M. Furthermore, moracin M *di*-glycosidation (**7**) and *mono*-glycosidation (**14**) products which were detected at 10.9 min and 13.7 min (Table 1 and Fig. 9) based on the two sets of mass spectral data: *m/z* 589$[M+Na]^+$, 567$[M+H]^+$, 565$[M–H]^-$, 403$[M–H–Glc]^-$, 241$[M–H–Glc–Glc]^-$, 197$[M–H–Glc–Glc–CO_2]^-$ for compound **7**, and *m/z* 427$[M+Na]^+$, 405$[M+H]^+$, 403$[M–H]^-$, 241$[M–H–Glc]^-$ and 197$[M–H–Glc–Glc–CO_2]^-$ for compound **14** (Table 1 and Figs. S4B & S4C), respectively. Prenylated 2-arylbenzofuran derivatives are important subtype of 2-arylbenzofuran derivatives, and diagnostic cleavage of C_4H_8O group can occur for the prenylated 2-arylbenzofuran derivatives under positive ionization mode. Compounds **35** and **36** were eluted at the retention times of 26.5 and 27.1 min, respectively (Fig. 9). The ions of *m/z* 349 $[M+Na]^+$, 327$[M+H]^+$ and 325$[M–H]^-$ (Table 1) indicated a molecular weight of 326 Da. The predominant fragment ions of protonated molecular ion were observed at *m/z* 253 and 211, suggesting the successive neutral losses of a C_4H_8O group and a C_3H_6 group (Figs. S5A and S6A). Thus, these two components were tentatively characterized as moracin O and moracin P. At the meanwhile, their glycosidation products were observed at 21.1 min (**25**) and 21.6 min (**26**) based on the mass spectral signals including: *m/z* 511$[M+Na]^+$, 489$[M+H]^+$, 487$[M–H]^-$, 325$[M–H–Glc]^-$, 253$[M–H–Glc–C_4H_8O]^-$ and 211 $[M–H–Glc–C_4H_8O–C_3H_6]^-$ (Table 1 and Figs. S5B & S6B). Moreover, as the xylosyl substituted moracin P, mulberroside C (identified using reference compound) was detected at 23.1 min (**29**). The isomer of mulberroside C which was observed at 22.1 min (**27**) and exhibited identical mass spectral profile with mulberroside C, was tentatively identified as moracin O-*O*-xyloside (Table 1 and Figs. S5C).

DA-type adducts usually possess higher molecular weights and generate characteristic cleavage of $C_6H_6O_2$ group. Compounds **43**, **54**, **58**, **67**, and **71** were

detected at 30.2 min, 33.1 min, 33.8 min, 35.5 min, and 37.7 min, respectively. The sodium adduct ion and pseudo-molecular ions of them were exhibited at *m/z* 603$[M+Na]^+$, 581$[M+H]^+$, and 579$[M–H]^–$, suggesting that the molecular weight of these components was 580 Da. The characteristic product ions of the deprotonated ion (*m/z* 579 $[M–H]^–$) were exhibited at *m/z* 561, 469, 451, and 359, corresponding to the neutral losses of a H_2O group, a $C_6H_6O_2$ group, a H_2O plus $C_6H_6O_2$ group, and two $C_6H_6O_2$ groups, respectively (Table 1 and Fig. S7). Thus, the identities of these components were tentatively assigned as mulberrofuran C, mulberrofuran J, albafuran C, australisine C, and their isomer.

The neutral loss of C_4H_8 (56 Da), which is generated from the uncyclized prenyl substituent, is regarded as the most important feature for prenylflavonoids. Sixteen components that exhibited quasi-molecular ions at *m/z* 423$[M+H]^+$ and 421$[M–H]^–$ and dominant fragment ions at *m/z* 405$[M+H–H_2O]^+$, 367$[M+H–C_4H_8]^+$, 311$[M+H–C_4H_8–C_4H_8]^+$, 299, and 231 (Table 1 and Fig. S8), were detected at the retention times of 26.3 min (**34**), 32.8 min (**53**), 40.5 min (**84**), 43.9 min (**93**), 44.1 min (**94**), 44.3 min (**95**), 44.9 min (**98**), 45.3 min (**100**), 45.8 min (**101**), 46.3 min (**103**), 47.7 min (**105**), 47.9 min (**106**), 48.9 min (**109**), 49.2 min (**111**), 49.7 min (**113**) and 50.5 min (**116**) (Table 1 and Fig. 9). These components were tentatively identified as kuwanon C and its isomers based on the aforementioned diagnostic fragmentation behaviors. Morusin (420 Da), consisting of a flavones skeleton and two isopentene substituents, was the most abundant constituent in MC. In sight of the wide distribution of prenylflavonoids in this herbal medicine, prenyltransferases should play important roles in the biosynthesis of the secondary metabolites [32]. When morusin was prenylated, metabolites exhibiting molecular weights of 488, 490, 492, 556, 558, 560, 562 and 564 Da might be generated in the plant. As expected, compounds with quasi-molecular ions at *m/z* 491$[M+H]^+$ and 489$[M–H]^–$ were observed at retention

times of 50.0 min (**114**), 52.0 min (**122**), 52.6 min (**124**), 53.4 min (**126**), 57.3 min (**135**), and 58.2 min (**139**) (Table 1 and Fig. 9), and the diagnostic product ions of them were observed at *m/z* 435, 423, 367, and 311, corresponding to the neutral losses of C_4H_8 group (56 Da), C_5H_8 group (68 Da), C_9H_{16} group (124 Da), and $C_{13}H_{24}$ group (180 Da), respectively (Fig. S9), these components were thus identified as sanggenol B and its isomers. Pseudo-molecular ions at *m/z* 489$[M+H]^+$ and 487$[M–H]^–$ were observed at 52.2 min (**123**), 52.8 min (**125**), 57.4 min (**136**), 57.8 min (**138**), and 59.3 min (**140**), and these compounds were tentatively assigned as the dehydrogenation products of and alasin A and its isomers. Similarly, quasi-molecular ions at *m/z* 493$[M+H]^+$ and 491$[M–H]^–$ were detected at 54.6 min (**129**), 55.1 min (**130**), and 56.1 min (**132**) (Table 1 and Fig. 9), suggesting that these components were cathayanon J, sanggenol D or their isomers. The quasi-molecular ions at *m/z* 559$[M+H]^+$ and 557$[M–H]^–$ detected at 57.7 min (**137**) (Table 1 and Fig. 9), corresponds to *di*-prenylated product of morusin based on the mass spectral profiles.

4. Conclusion

In the present study, a practical strategy was proposed and applied for the comprehensive characterization of the chemical profile of MC: First, a compound library was constructed for the phytochemistry of the genus *Morus* to understand the potential secondary metabolites in the extract; Second, the mass fragmentation patterns of seven representative compounds were obtained to propose the fragmentation pathways of respective chemical homologues; Third, a MIM-IDA-EPI method was adopted to analyze the extract to achieve comprehensive detection and identification of the chemical components with the assistance of the proposed fragmentation rules. A total of 140 components were detected with 133 tentatively identified from MC, which demonstrated that the proposed strategy can be adopted as

a useful technique for comprehensive chemical profiling of HMs.

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Appendix Supplementary data

Supplementary data (Supplemental information and Supplemental figures) associated with this article can be found, in the online version, at http://dx.doi.org/.....

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Figure legends

Fig. 1 The strategy for chemical profiling of Mori Cortex using ultra-high performance liquid chromatography coupled with hybrid triple quadrupole-linear ion trap mass spectrometry.

Fig. 2 MS^2 spectra, the chemical structures and the fragment ions assignment of morin.

Fig. 3 MS^2 spectrum of mulberroside A (A), the proposed mass fragmentation scheme of mulberroside A under negative ionization (B) and MS^2 spectra, the chemical structures and the fragment ions assignment of oxyresveratrol (C).

Fig. 4 MS^2 spectrum of mulberroside C (A) and the proposed mass fragmentation scheme under negative ionization (B).

Fig. 5 MS^2 spectrum of morusin (A) and the proposed mass fragmentation scheme under positive ionization (B).

Fig. 6 MS^2 spectrum of kuwanon G (A) and the proposed mass fragmentation scheme under negative ionization (B).

Fig. 7 UHPLC-UV (280 nm) chromatogram of the Mori Cortex extract.

Fig. 8 Representative total ion current chromatograms of Mori Cortex extract acquired using positive ionization with Q1 full scan mode (A), EMS full scan mode (B) and MIM mode (C), and extracted ion current chromatogram of MIM mode (D); using negative ionization with Q1 full scan mode (E), EMS full scan mode (F) and MIM mode (G), and extracted ion current chromatogram of MIM mode (H).

Fig. 9 UHPLC-Total ion current (TIC) chromatograms of the Mori Cortex extract using multiple ion monitoring (MIM) mode under negative (A) and positive (B) ionization.

Table 1 The retention time, UV, MS^1, MS^2 and identities of the components in 80% aqueous ethanol extract of Mori cortex

No.	t_R	Negative mode		Positive mode			MW	Identity
		$[M–H]^–$	MS^2	$[M+H]^+$	$[M+Na]^+$	MS^2		
1	5.0	625	463[M–H–Glc]$^–$;301[M–H–Glc–Glc]$^–$;257	627	649	-	626	Morin *di*-*O*-glucoside
2	5.5	625	463[M–H–Glc]$^–$;301[M–H–Glc–Glc]$^–$;257	627	649	-	626	Morin *di*-*O*-glucoside
3	8.7	567	405 [M–H–Glc]$^–$; 243 [M–H–Glc–Glc]$^–$; 225 [M–H–Glc–Glc–H_2O]$^–$;199 [M–H–Glc–Glc–CO_2]$^–$	569	591	-	568	Mulberroside A
4	9.8	-	-	331	353	313[M+H–H_2O]$^+$;289[M+H–C_3H_6]$^+$;153	330	Unknown
5	10.6	-	-	331	353	313[M+H–H_2O]$^+$;289[M+H–C_3H_6]$^+$;153	330	Unknown
6	10.6	477	293;233	479	501	-	478	Moracin W
7	10.9	565	403[M–H–Glc]$^–$;241[M–H–Glc–Glc]$^–$;197	567	589	-	566	Moracin M *di*-*O*-glucoside
8	11.4	625	463[M–H–Glc]$^–$;301[M–H–Glc–Glc]$^–$;257	627	649	-	626	Morin *di*-*O*-glucoside

9	11.5	405	243[M–H–Glc]$^-$;225 [M–H–Glc–H_2O]$^-$;199[M–H–Glc–CO_2]$^-$	407	429	-	406	Oxyresveratrol-*O*-glucoside
10	11.6	449	431[M–H–H_2O]$^-$; 329[M–H–$C_4O_4H_8$]$^-$;203;175	-	-	-	450	Dihydrokaempferol *C*-glucoside/ Norartocarpanone *C*-glucoside
11	12.1	449	431[M–H–H_2O]$^-$; 329[M–H–$C_4O_4H_8$]$^-$;203;175	-	-	-	450	Dihydrokaempferol *C*-glucoside/ Norartocarpanone *C*-glucoside
12	12.3	625	463[M–H–Glc]$^-$;301[M–H–Glc–Glc]$^-$	627	649	-	626	Morin *di*-*O*-glucoside
13	12.8	463	301[M–H–Glc]$^-$;257	465	487	-	464	Morin-*O*-glucoside
14	13.7	403	241[M–H–Glc]$^-$;199	405	427	-	404	Moracin M-*O*-glucoside
15	13.9	563	535[M–H–H_2O]$^-$;517[M–H–H_2O–H_2O]$^-$;487[M–H–H_2O–C_4H_{10}]$^-$;459;429;403;	565	587	-	564	Kuwanol A/ Isomer
16	14.7	243	225;199	245	267	-	244	Oxyresveratrol
17	14.9	463	301[M–H–Glc]$^-$;257	465	487	-	464	Morin-*O*-glucoside
18	16.2	-	-	595	617	567[M+H-CO]$^+$;536;531;472;444;398	594	Kuwanon Z/ Isomer
19	16.4	-	-	595	617	567[M+H-CO]$^+$;536;531;472;444;398	594	Kuwanon Z/ Isomer
20	17.4	243	225;199	245	267	-	244	Oxyresveratrol isomer

21	18.8	301	257[M–H–CO_2]$^-$	303	325	-	302	Morin isomer
22	19.4	241	197	243	265	-	242	Moracin M
23	20.9	-	-	595	617	567[M+H-CO]$^+$;536;531;472;444;398	594	Kuwanon Z/ Isomer
24	21.0	301	257[M–H–CO_2]$^-$	303	325	285[M+H-H_2O]$^+$;257[M+H-H_2O-CO]$^+$;239[M+H-H_2O-CO-H_2O]$^+$; 229	302	Morin
25	21.1	487	325[M–H–Glc]$^-$;253;211	489	511	-	488	Moracin O-*O*-glucoside/ Moracin P-*O*-glucoside
26	21.6	487	325[M–H–Glc]$^-$;253;211	489	511	-	488	Moracin O-*O*-glucoside/ Moracin P-*O*-glucoside
27	22.1	457	325[M–H–Xyl]$^-$;253;211	459	481	-	458	Mulberroside C isomer
28	22.2	-	-	629	651	601;570;423;395;367	628	Mulberrofuran K/ Yunanensin E
29	23.1	457	325[M–H–Xyl]$^-$;253;211	459	481	-	458	Mulberroside C
30	23.7	-	-	437	459	419[M+H–H_2O]$^+$; 365[M+H–C_4H_8O]$^+$; 309[M+H–C_4H_8O– C_4H_8]$^+$	436	Benzokuwanon E/ Sanggenon A/ Isomer
31	24.9	-	-	437	459	419[M+H–H_2O]$^+$; 365[M+H–C_4H_8O]$^+$; 309[M+H–C_4H_8O–C_4H_8]$^+$	436	Benzokuwanon E/ Sanggenon A/ Isomer
32	25.6	-	-	437	459	419[M+H–H_2O]$^+$;365[M+H–C_4H_8O]$^+$; 309[M+H–$C_4H_{10}O$–C_4H_8]$^+$	436	Benzokuwanon E/ Sanggenon A/ Isomer
33	26.1	691	581[M–H–$C_6H_6O_2$]$^-$; 459; 419	693	715	637;421;365;355;299	692	Kuwanon G Isomer

34	26.3	-	-	423	445	405[M+H–H_2O]$^+$;367[M+H–C_4H_8]$^+$; 311[M+H–C_4H_8–C_4H_8]$^+$;299;231	422	Kuwanon C/ Jisang/ Cathayanon G/ Broussoflavonol F/ Isomer
35	26.5	325	253[M–H–C_4H_8O]$^-$;211	327	349	-	326	Moracin O/ Moracin P
36	27.1	325	253[M–H–C_4H_8O]$^-$;211	327	349	-	326	Moracin P/ Moracin O
37	27.6	409	391[M–H–H_2O]$^-$;327;309;209	-	-	-	410	Wittifuran A/ Wittifuran U
38	28.1	-	-	421	443	365[M+H–C_4H_8]$^+$;337[M+H–C_4H_8–H_2O]$^+$;299	420	Morusin isomer
39	28.4	409	391[M–H–H_2O]$^-$;327;309;209	-	-	-	410	Wittifuran A/ Wittifuran U
40	29.5	329	311;293;229;211;171	331	353	-	330	Unknown
41	30	711	601[M–H–$C_6H_6O_2$]$^-$;549;491[M–H–$C_6H_6O_2$–$C_6H_6O_2$]$^-$	713	735	-	712	Sanggenon T/ Isomer
42	30.1	-	-	421	443	365[M+H–C_4H_8]$^+$;337;299	420	Morusin isomer
43	30.2	579	561;469[M–H–$C_6H_6O_2$]$^-$;451;359[M–H–$C_6H_6O_2$–$C_6H_6O_2$]$^-$;305;241;227	581	603	563;471;443;361;309;293;243	580	Mulberrofuran C/ Mulberrofuran J/ Albafuran C/ Australisine C/ Isomer
44	30.2	711	601[M–H–$C_6H_6O_2$]$^-$;549;491[M–H–$C_6H_6O_2$–$C_6H_6O_2$]$^-$	713	735	-	712	Sanggenon T/ Isomer
45	30.6	759	-	761	783	705[M+H–C_4H_8]$^+$;687[M+H–C_4H_8–	760	Kuwanon N/ Kuwanon H/ Isomer

						$H_2O]^+$;649$[M+H–C_4H_8]^+$;421;365;355		
46	31.1	591	481$[M–H–C_6H_6O_2]^-$;463	593	615	575;483;465;457;399;	592	Mulberrofuran Q
47	31.4	709	599$[M–H–C_6H_6O_2]^-$;489;437;371;309	711	733	693;655;637;601;557;517;421;365	710	Moracenin D/ Isomer
48	31.5	711	601$[M–H–C_6H_6O_2]^-$;549;491[M–H–$C_6H_6O_2–C_6H_6O_2]^-$	713	735	-	712	Sanggenon T/ Isomer
49	32.3	709	599$[M–H–C_6H_6O_2]^-$;436;371;309	711	733	693;655;637;601;557;517;421;365	710	Moracenin D/ Isomer
50	32.5	563	545;453$[M–H–C_6H_6O_2]^-$	565	587	-	564	Kuwanol A
51	32.6	711	601$[M–H–C_6H_6O_2]^-$;549;491[M–H–$C_6H_6O_2–C_6H_6O_2]^-$	713	735	-	712	Sanggenon T/ Isomer
52	32.8	-	-	423	445	405;367$[M+H–C_4H_8]^+$; 311$[M+H–C_4H_8–C_4H_8]^+$; 299;231	422	Kuwanon C/ Jisang/ Cathayanon G/ Broussoflavonol F/ Isomer
53	32.8	711	601$[M–H–C_6H_6O_2]^-$;549;491[M–H–$C_6H_6O_2–C_6H_6O_2]^-$	-	-	-	712	Sanggenon T/ Isomer
54	33.1	579	561;469$[M–H–C_6H_6O_2]^-$;451;359[M–H–$C_6H_6O_2–C_6H_6O_2]^-$;305;241;227	581	603	563;471;443;361;309;293;243	580	Mulberrofuran C/ Mulberrofuran J/ Albafuran C/ Australisine C/ Isomer
55	33.4	353	335;227	355	377	337$[M+H–H_2O]^+$; 299$[M+H–C_4H_8]^+$;281	354	Glyasperin F/ Licoisoflavanone/ Morachalcone C/ Isomer

56	33.6	607	597[M–H–$C_6H_6O_2$]$^-$;487[M–H–$C_6H_6O_2$–$C_6H_6O_2$]$^-$;455;335	609	631	499;433;415;363;337;323	608	Guangsangon G/ Guangsangon I/ Isomer
57	33.7	-	-	421	443	365[M+H–C_4H_8]$^+$;337;299	420	Morusin Isomer
58	33.8	579	561;469[M–H–$C_6H_6O_2$]$^-$;451;359[M–H–$C_6H_6O_2$–$C_6H_6O_2$]$^-$;305;241;227	581	603	563;471;443;361;309;293;243	580	Mulberrofuran C/ Mulberrofuran J/ Albafuran C/ Australisine C/ Isomer
59	33.9	625	499[M–H–$C_6H_6O_3$]$^-$; 389[M–H–$C_6H_6O_3$–$C_6H_6O_2$]$^-$; 279	627	649	609;517;499;475;433;365;297	626	Kuwanon L/ Guangsangon K
60	34.8	353	335[M–H–H_2O]$^-$;227	355	377	337; 299[M+H–C_4H_8]$^+$;281	354	Glyasperin F/ Licoisoflavanone/ Morachalcone C/ Isomer
61	34.9	561	451[M–H–$C_6H_6O_2$]$^-$;	563	585	453;441;425;387	562	Mulberrofuran G/ Isomulberrofuran G/ Kuwanol A
62	35.2	-	-	695	717	677;639[M+H–C_4H_8]$^+$;567[M+H–C_4H_8–C_4OH_8]$^+$;519;499;341;323	694	Kuwanon O/ Isomer
63	35.2	711	693[M–H–H_2O]$^-$;585[M–H–$C_6H_6O_3$]$^-$;389	-	-	-	712	Sanggenon T/ Isomer
64	35.3	607	597[M–H–$C_6H_6O_2$]$^-$;487[M–H–$C_6H_6O_2$–$C_6H_6O_2$]$^-$;455;335	609	631	499;433;415;363;337;323	608	Guangsangon G/ Guangsangon I/ Isomer
65	35.4	353	335;227	355	377	337; 299[M+H–C_4H_8]$^+$;281	354	Glyasperin F/ Licoisoflavanone/ Morachalcone C/

								Isomer
66	35.5	561	451[M–H–$C_6H_6O_2$]$^-$;439	563	585	453;441;425;387	562	Mulberrofuran G/ Isomulberrofuran G/ Kuwanol A
67	35.5	579	561;469[M–H–$C_6H_6O_2$]$^-$;451;359[M–H–$C_6H_6O_2$–$C_6H_6O_2$]$^-$;305;241;227	581	603	563;471;443;361;309;293;243	580	Mulberrofuran C/ Mulberrofuran J/ Albafuran C/ Australisine C/ Isomer
68	36.7	-	-	761	783	705[M+H–C_4H_8]$^+$;687;649[M+H–C_4H_8–C_4H_8]$^+$;421;365;355	760	Kuwanon N/ Kuwanon H/ Isomer
69	37.2	353	335;227	355	377	337; 299[M+H–C_4H_8]$^+$;281	354	Glyasperin F/ Licoisoflavanone/ Morachalcone C/ Isomer
70	37.5	-	-	353	375	323; 295	352	Cyclocommunol
71	37.7	579	561;469[M–H–$C_6H_6O_2$]$^-$;451;359[M–H–$C_6H_6O_2$–$C_6H_6O_2$]$^-$;305;241;227	581	603	563;471;443;361;309;293;243	580	Mulberrofuran C/ Mulberrofuran J/ Albafuran C/ Australisine C/ Isomer
72	37.9	691	581[M–H–$C_6H_6O_2$]$^-$; 459; 419	693	715	637;421;365;355;299	692	Kuwanon G isomer
73	38.1	691	581[M–H–$C_6H_6O_2$]$^-$; 459; 419	693	715	637;421;365;355;299	692	Kuwanon G
74	38.4	437	419;379;315	439	461	383[M+H–C_4H_8]$^+$; 365;	438	Morunigrol E/ Morunigrol G/ Mornigrol F/ Mornigrol G/ Hydroxymorusin
75	38.5	693	583[M–H–$C_6H_6O_2$]$^-$;531;473[M–H–$C_6H_6O_2$–$C_6H_6O_2$]$^-$;421;405;295;259	695	717	677; 621; 567;499;457;341;323	694	Kuwanon O/ Isomer

76	38.9	423	282	425	447	-	424	Kuwanon E/ Cathayanon H/ Lespedezaflavanone C
77	38.9	-	-	761	783	705[M+H–C_4H_8]$^+$;687;649[M+H–C_4H_8–C_4H_8]$^+$;421;365;355	760	Kuwanon N/ Kuwanon H/ Moracenin C/ Isomer
78	39.1	693	583[M–H–$C_6H_6O_2$]$^-$;531;473[M–H–$C_6H_6O_2$–$C_6H_6O_2$]$^-$;421;405;295;259	695	717	677; 621; 567;499;457;341;323	694	Kuwanon O/ Isomer
79	39.4	691	581[M–H–$C_6H_6O_2$]$^-$; 459; 419	693	715	637;421;365;355;299	692	Kuwanon G isomer
80	39.5	-	-	421	443	365[M+H–C_4H_8]$^+$;337;299	420	Morusin isomer
81	39.5	427	297;257;243;191	-	-	-	428	Unknown
82	39.6	437	419;379	439	461	421[M+H–H_2O]$^+$;365[M+H–C_4H_8–H_2O]$^+$;347;311	438	Morunigrol E/ Morunigrol G/ Mornigrol F/ Mornigrol G/ Hydroxymorusin
83	39.9	693	567;389;347;	695	717	677[M+H–H_2O]$^+$; 621[M+H–H_2O–C_4H_8]$^+$; 567;499;457;341;323	694	Kuwanon O/ Isomer
84	40.5	421	352; 309; 231	423	445	405;367[M+H–C_4H_8]$^+$; 311[M+H–C_4H_8–C_4H_8]$^+$; 299;231	422	Kuwanon C/ Jisang/ Cathayanon G/ Broussoflavonol F/ Isomer
85	40.5	-	-	693	715	637[M+H–C_4H_8]$^+$;421;365;355;299	692	Kuwanon G isomer
86	41.4	693	583[M–H–$C_6H_6O_2$]$^-$;531;473[M–H–	695	717	677; 621; 567;499;457;341;323	694	Kuwanon O/ Isomer

			$C_6H_6O_2$–$C_6H_6O_2]^-$;421;405;295;259					
87	42.4	693	675[M–$H_2O]^-$; 583[M–H–$C_6H_6O_2]^-$; 567; 457[M–H–$C_6H_6O_3]^-$; 359; 277	695	717	677; 621; 567;499;457;341;323	694	Kuwanon O/ Isomer
88	42.6	-	-	761	783	705[M+H–$C_4H_8]^+$;687;649[M+H–C_4H_8–$C_4H_8]^+$;421;365;355	760	Kuwanon N/ Kuwanon H/ Isomer
89	42.8	693	675[M–$H_2O]^-$; 583[M–H–$C_6H_6O_2]^-$; 567; 457[M–H–$C_6H_6O_3]^-$; 359; 277	695	717	677; 621; 567;499;457;341;323	694	Kuwanon O/ Isomer
90	43	759	581;539;471;419;379	761	783	705[M+H–$C_4H_8]^+$;687;649[M+H–C_4H_8–$C_4H_8]^+$;421;365;355	760	Kuwanon N/ Kuwanon H/ Isomer
91	43.2	-	-	631	653	575[M+H–$C_4H_8]^+$;521;509;453;323	630	Mulberrofuran F/ Mongolicin A
92	43.3	627	609[M–$H_2O]^-$; 517[M–H–$C_6H_6O_2]^-$; 407[M–H–$C_6H_6O_2$–$C_6H_6O_2]^-$	629	651	519; 507; 491;354;387;321	628	Mulberrofuran K/ Yunanensin E
93	43.9	421	352; 309; 231	423	445	405;367[M+H–$C_4H_8]^+$; 311[M+H–C_4H_8–$C_4H_8]^+$; 299;231	422	Kuwanon C/ Jisang/ Cathayanon G/ Broussoflavonol F/ Isomer
94	44.1	421	352; 309; 231	423	445	405;367[M+H–$C_4H_8]^+$; 311[M+H–C_4H_8–$C_4H_8]^+$; 299;231	422	Kuwanon C/ Jisang/ Cathayanon G/ Broussoflavonol F/ Isomer
95	44.3	421	352; 309; 231	423	445	405;367[M+H–$C_4H_8]^+$; 311[M+H–C_4H_8–	422	Kuwanon C/ Jisang/ Cathayanon G/

						$C_4H_8]^+$; 299;231		Broussoflavonol F/ Isomer
96	44.4	759	741[M–$H_2O]^-$;649[M–H–$C_6H_6O_2]^-$;509;417	761	783	705;687;649;421;365;355	760	Kuwanon N/ Kuwanon H/ Moracenin C/ Isomer
97	44.5	419	375; 350; 309; 297	421	443	365[M+H–$C_4H_8]^+$;337;299	420	Morusin Isomer
98	44.9	421	352; 309; 231	423	445	405;367[M+H–$C_4H_8]^+$; 311; 299;231	422	Kuwanon C/ Jisang/ Cathayanon G/ Broussoflavonol F/ Isomer
99	45.1	423	405[M–H+$H_2O]^-$;297	425	447	301	424	Kuwanon E/ Cathayanon H/ Lespedezaflavanone C
100	45.3	421	352; 309; 231	423	445	405;367[M+H–$C_4H_8]^+$; 311; 299;231	422	Kuwanon C/ Jisang/ Cathayanon G/ Broussoflavonol F/ Isomer
101	45.8	421	352; 309; 231	423	445	405;367[M+H–$C_4H_8]^+$; 311; 299;231	422	Kuwanon C/ Jisang/ Cathayanon G/ Broussoflavonol F/ Isomer
102	46.1	419	375; 350; 309; 297	421	443	365[M+H–$C_4H_8]^+$;337;299	420	Morusin Isomer
103	46.3	421	352; 309; 231	423	445	405;367[M+H–$C_4H_8]^+$; 311; 299;231	422	Kuwanon C/ Jisang/ Cathayanon G/ Broussoflavonol F/ Isomer
104	47.2	419	375; 350; 309; 297	421	443	365[M+H–$C_4H_8]^+$;337;299	420	Morusin Isomer
105	47.7	421	352; 309; 231	423	445	405;367[M+H–$C_4H_8]^+$; 311; 299;231	422	Kuwanon C/ Jisang/ Cathayanon G/

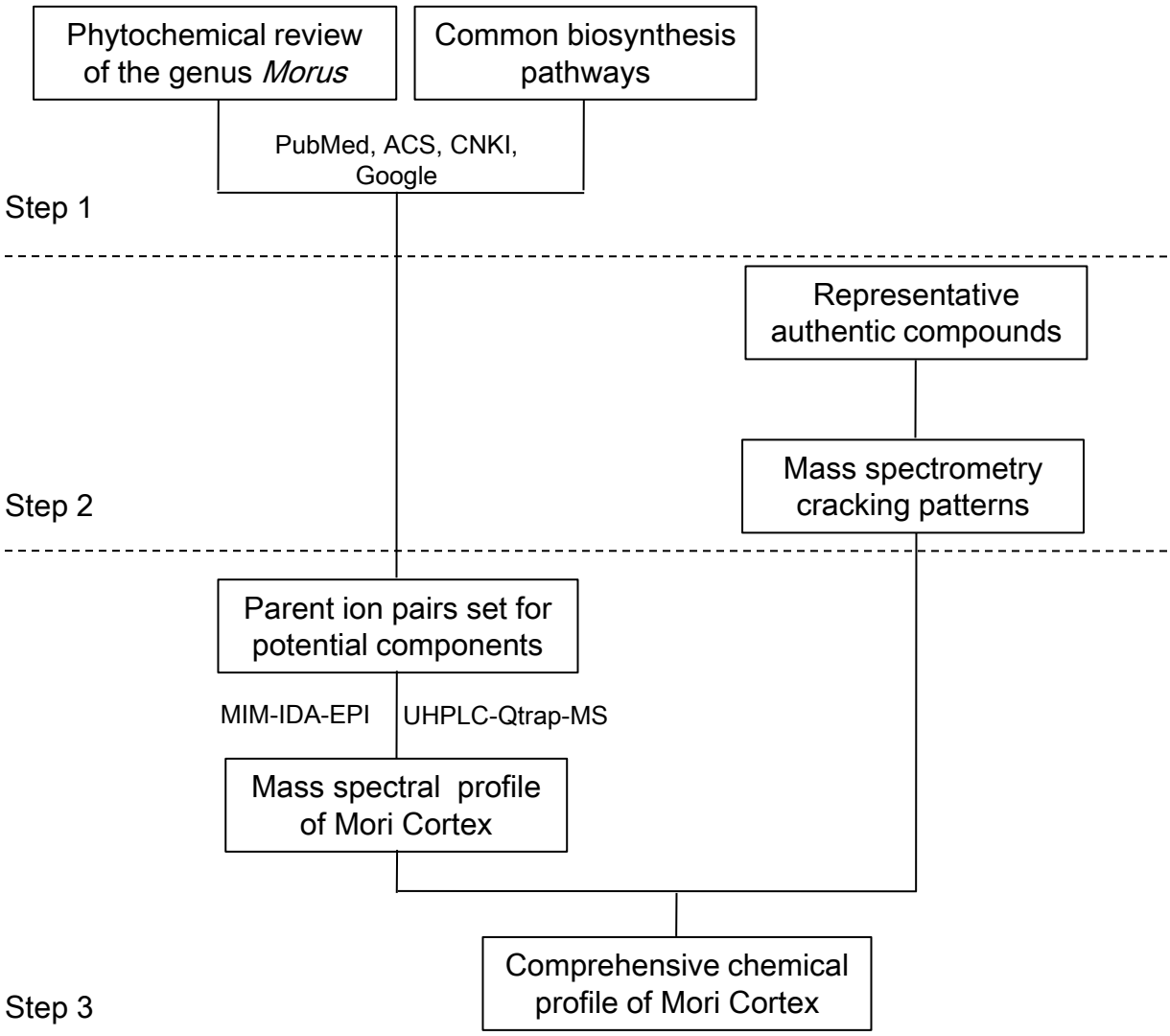
								Broussoflavonol F/ Isomer
106	47.9	421	352; 309; 231	423	445	405;367[M+H–C_4H_8]$^+$; 311; 299;231	422	Kuwanon C/ Jisang/ Cathayanon G/ Broussoflavonol F/ Isomer
107	48	419	375; 350; 309; 297	421	443	365;337;299	420	Morusin
108	48.4	405	335;321;282	407	429	351[M+H–C_4H_8]$^+$;339;295[M+H–C_4H_8–C_4H_8]$^+$;283	406	6-Geranylapigenin/ Kuwanon S/ Cathayanon F
109	48.9	421	352; 309; 231	423	445	405;367[M+H–C_4H_8]$^+$; 311; 299;231	422	Kuwanon C/ Jisang/ Cathayanon G/ Broussoflavonol F/ Isomer
110	49	419	375; 350; 309; 297	421	443	365;337[M+H–C_4H_8]$^+$;299	420	Morusin isomer
111	49.2	421	352; 309; 231	423	445	405;367[M+H–C_4H_8]$^+$; 311; 299;231	422	Kuwanon C/ Jisang/ Cathayanon G/ Broussoflavonol F/ Isomer
112	49.5	419	375; 350; 309; 297	421	443	365[M+H–C_4H_8]$^+$;337;299	420	Morusin Isomer
113	49.7	421	352; 309; 231	423	445	405;367[M+H–C_4H_8]$^+$; 311; 299;231	422	Kuwanon C/ Jisang/ Cathayanon G/ Broussoflavonol F/ Isomer
114	50	-	-	491	513	435[M+H–C_4H_8]$^+$;417[M+H–C_4H_8–H_2O]$^+$;361[M+H–C_4H_8–H_2O–C_4H_8]$^+$;319;311;283	490	Sanggenol B/Isomer

115	50.3	419	375; 350; 309; 297	421	443	365[M+H–C_4H_8]$^+$;337;299	420	Morusin Isomer
116	50.5	421	352; 309; 231	423	445	405;367[M+H–C_4H_8]$^+$; 311; 299;231	422	Kuwanon C/ Jisang/ Cathayanon G/ Broussoflavonol F/ Isomer
117	50.8	437	311;285;259	439	461	-	438	Morunigrol E/ Morunigrol G/ Mornigrol F/ Mornigrol G/ Hydroxymorusin
118	51	-	-	405	427	349[M+H–C_4H_8]$^+$; 321; 283	404	Unknown
119	51	437	311;285;259	439	461	-	438	Morunigrol E/ Morunigrol G/ Mornigrol F/ Mornigrol G/ Hydroxymorusin
120	51.2	437	311;285;259	439	461	-	438	Morunigrol E/ Morunigrol G/ Mornigrol F/ Mornigrol G/ Hydroxymorusin
121	51.6	419	375; 350; 309; 297	421	443	365[M+H–C_4H_8]$^+$;337;299	420	Morusin isomer
122	52	489	445;377;365;309;299;244	491	513	435[M+H–C_4H_8]$^+$;417;319;361;311;283	490	Sanggenol B/ Isomer
123	52.2	487	349;309;231;	489	511	433[M+H–C_4H_8]$^+$;377;365;311;255;	488	Andalasin A/ Isomer
124	52.6	489	445;364;351;309;257;243;231	491	513	435[M+H–C_4H_8]$^+$;379;367;311;283	490	Sanggenol B/ Isomer
125	52.8	487	349;309;231;	489	511	433[M+H–C_4H_8]$^+$;377;365;311;255;	488	Andalasin A/ Isomer
126	53.4	489	377;367	491	513	435[M+H–C_4H_8]$^+$;417;319;361;311;283	490	Sanggenol B/ Isomer
127	53.8	-	-	405	427	349[M+H–C_4H_8]$^+$; 321; 283	404	Unknown

128	54.2	417	335;265	419	441	363[M+H–C_4H_8]$^+$;348	418	Morunigrol A/ Cyclomorusin
129	54.6	491	473;365;339;313	493	515	475;437[M+H–C_4H_8]$^+$;419;369;341	492	Cathayanon J/ Sanggenol D
130	55.1	491	473;365;339;313	493	515	475;437[M+H–C_4H_8]$^+$;369;351;313;295	492	Cathayanon J/ Sanggenol D
131	55.7	403	333;319;293	405	427	349[M+H–C_4H_8]$^+$; 321; 283	404	Unknown
132	56.1	491	287;203	493	515	475;437[M+H–C_4H_8]$^+$;419;369;341	492	Cathayanon J/ Sanggenol D
133	56.4	455	-	-	-	-	456	Mulberrofuran R/ Isomer
134	57.1	455	-	-	-	-	456	Mulberrofuran R/ Isomer
135	57.3	489	471;261;227	491	513	435[M+H–C_4H_8]$^+$;379[M+H–C_4H_8–C_4H_8]$^+$;367;311;283	490	Sanggenol B/ Isomer
136	57.4	487	443;417;243;231	489	511	433[M+H–C_4H_8]$^+$;377[M+H–C_4H_8–C_4H_8]$^+$;365;311;255;	488	Andalasin A/ Isomer
137	57.7	557	445;419;311;259	559	581	503[M+H–C_4H_8]$^+$;447[M+H–C_4H_8–C_4H_8]$^+$;435;379;323	558	Albanol B/ Isomer
138	57.8	487	443;417;243;231	489	511	433[M+H–C_4H_8]$^+$;377[M+H–C_4H_8–C_4H_8]$^+$;365;311;255;	488	Andalasin A/ Isomer
139	58.2	-	-	491	513	435[M+H–C_4H_8]$^+$;417;319;361;311;283	490	Sanggenol B/ Isomer
140	59.3	487	349;243	489	511	433[M+H–C_4H_8]$^+$;377[M+H–C_4H_8–	488	Andalasin A/ Isomer

$C_4H_8]^+$;365;311;255;

Graphic abstract



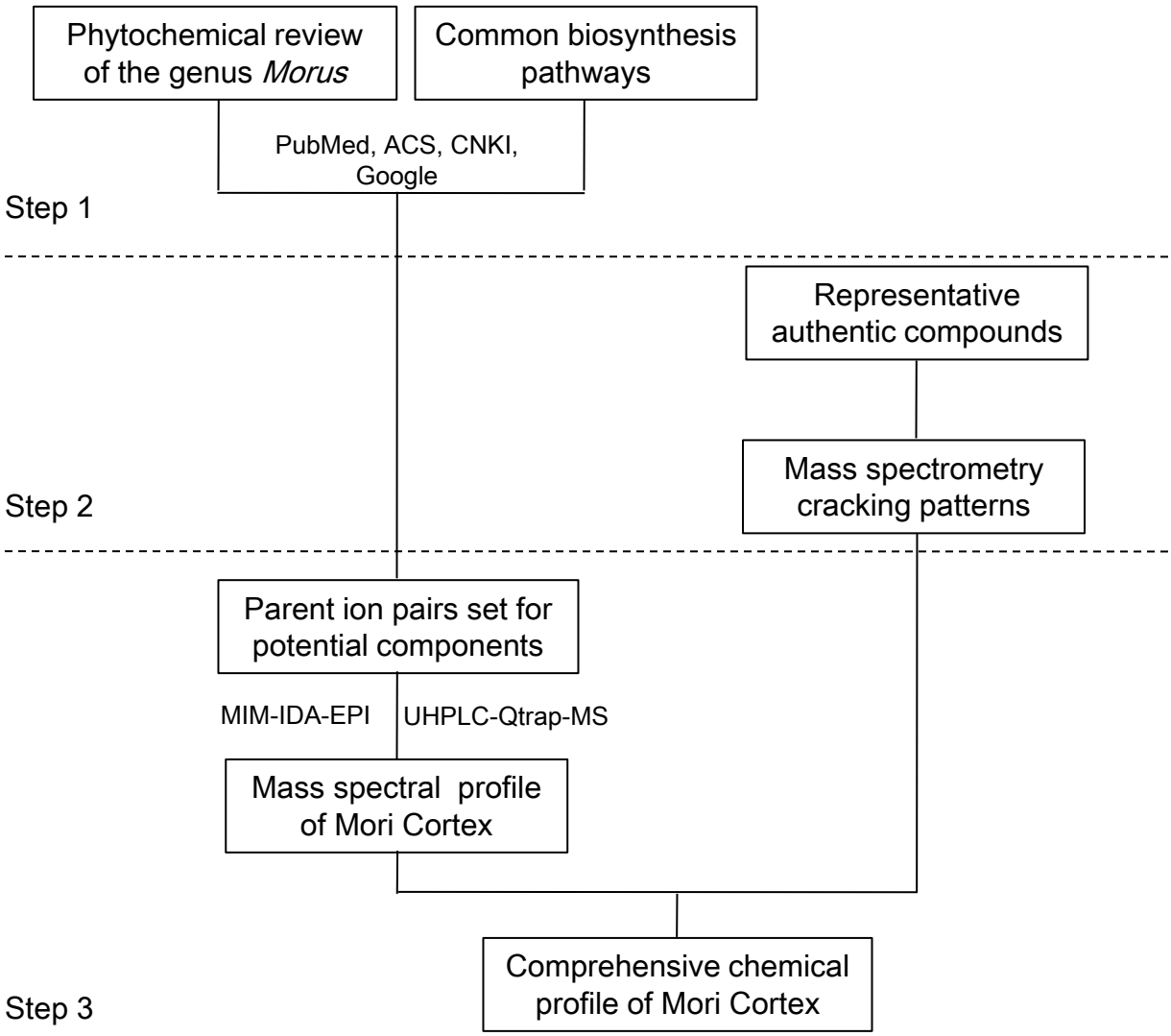


Fig. 1

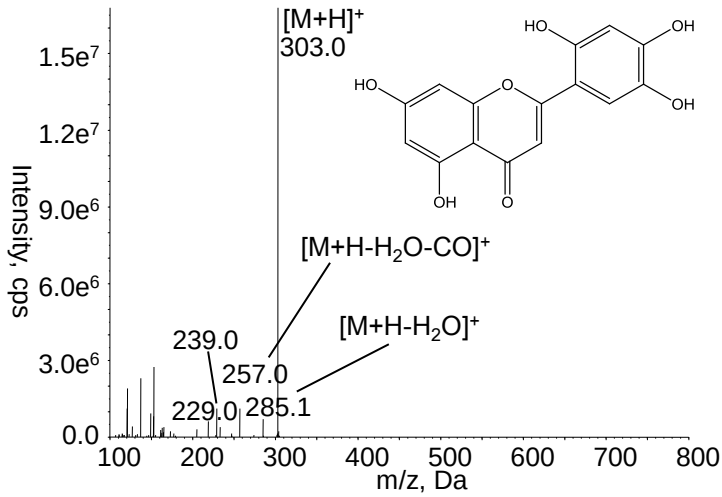


Fig. 2

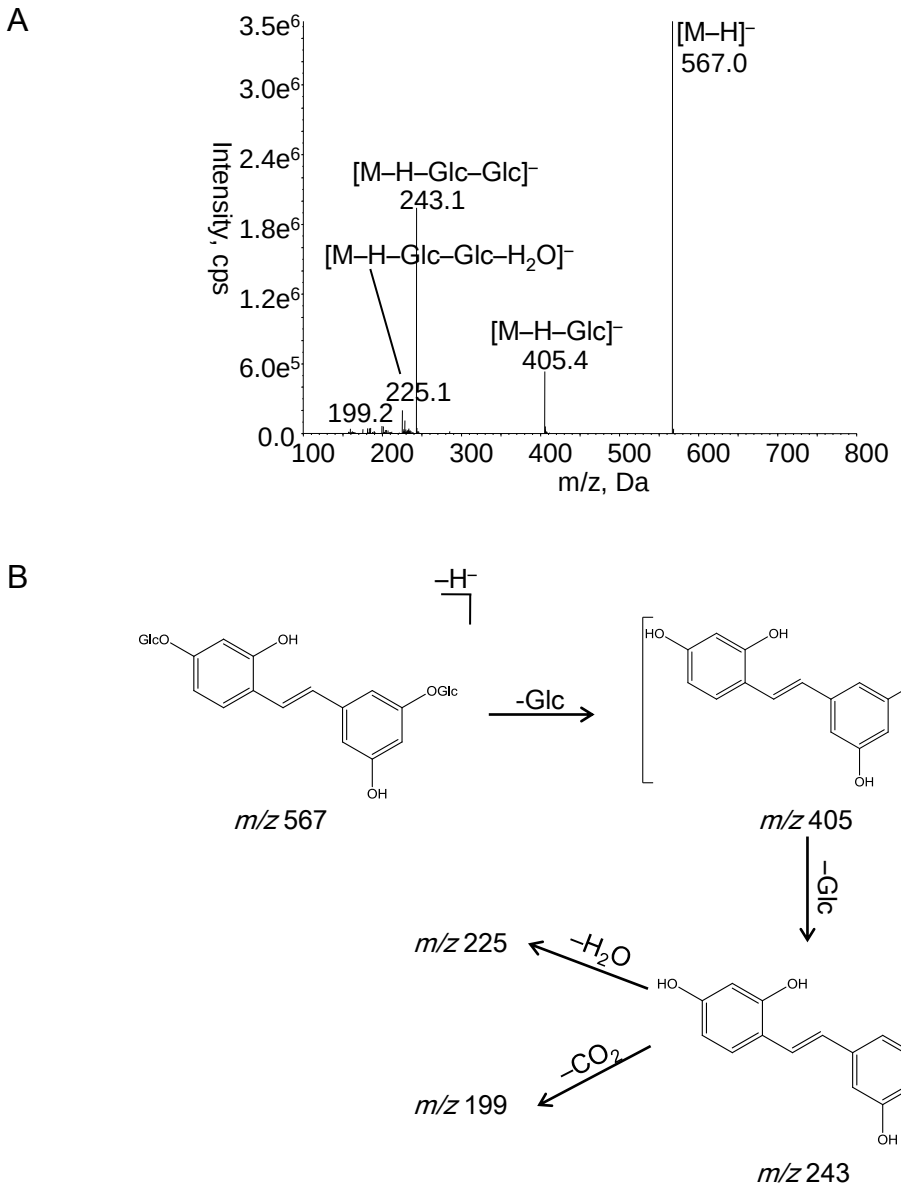
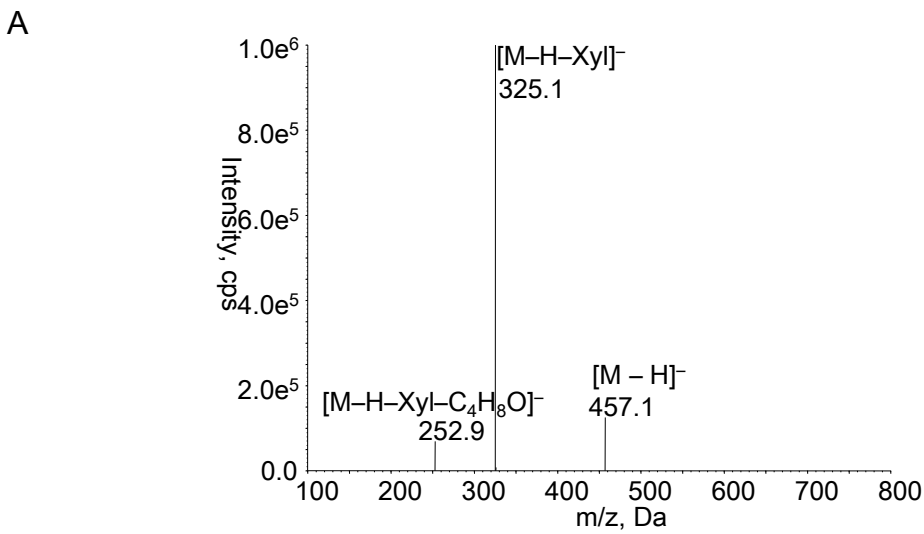


Fig. 3



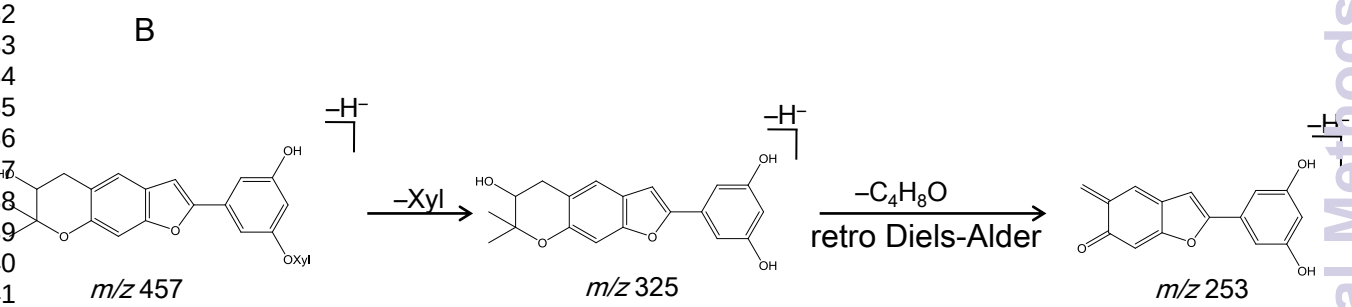
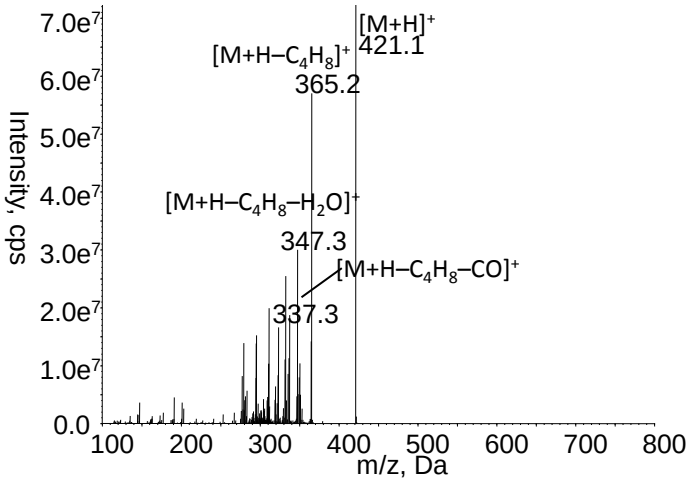


Fig. 4

A



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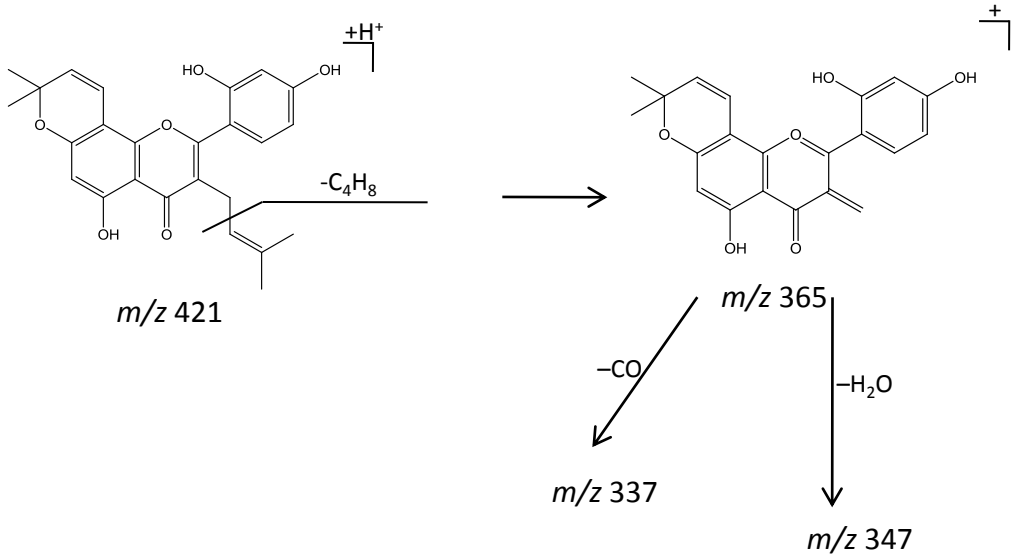
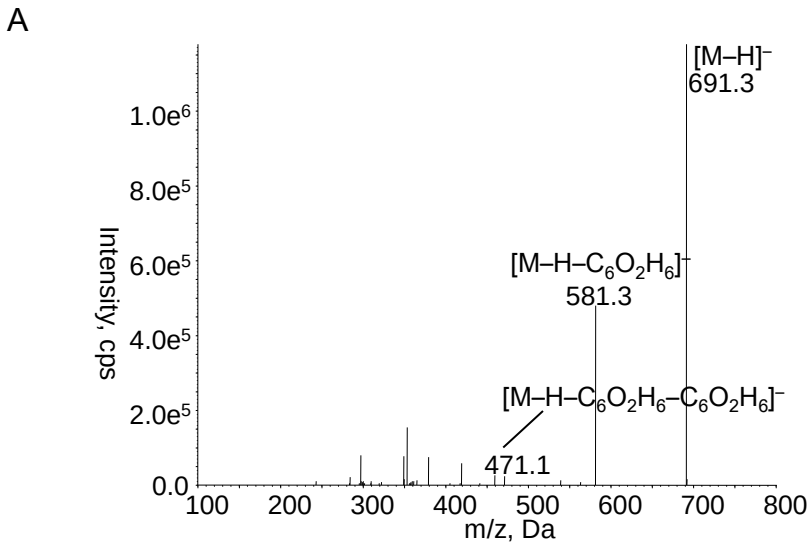


Fig. 5



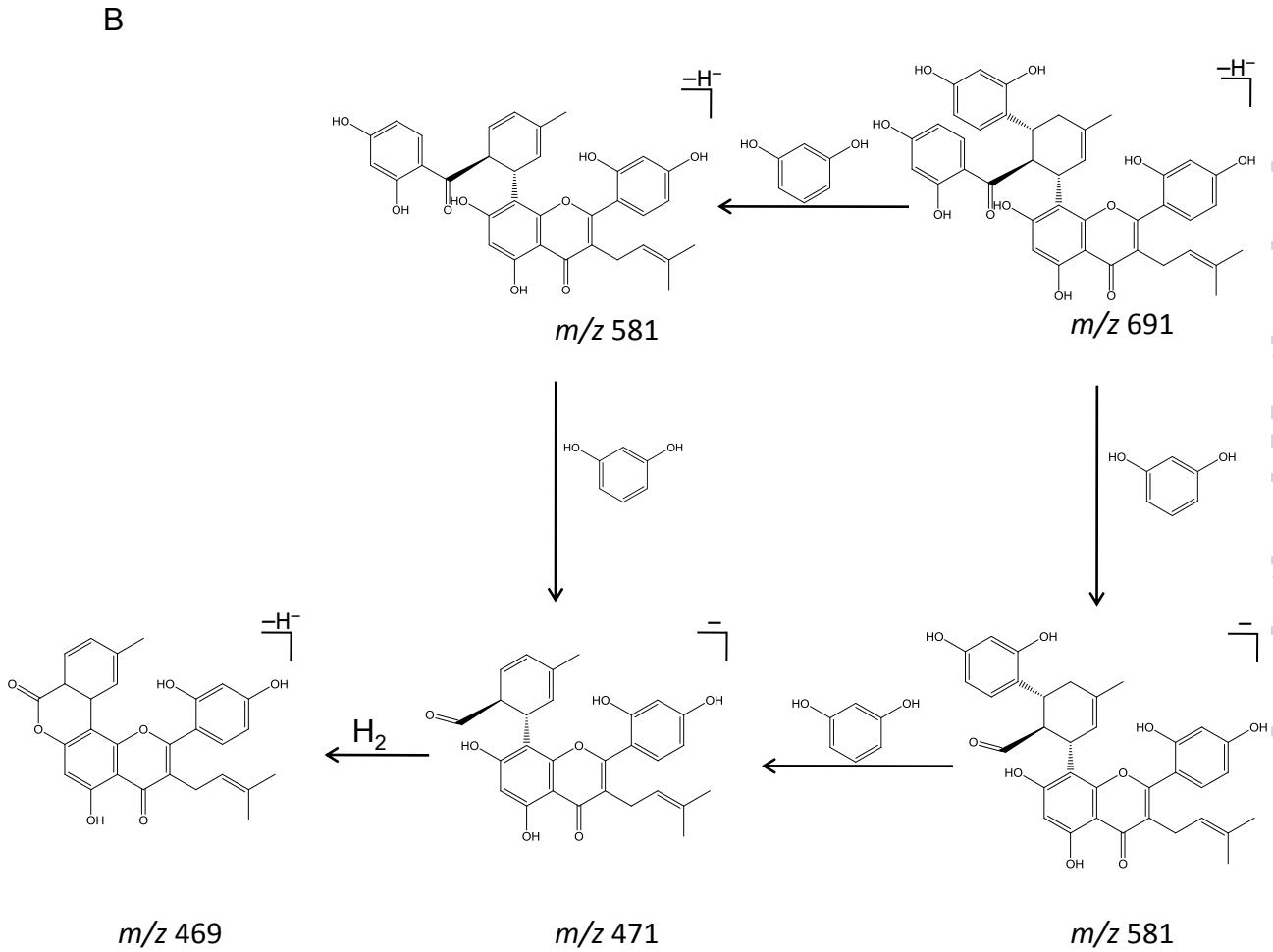


Fig. 6

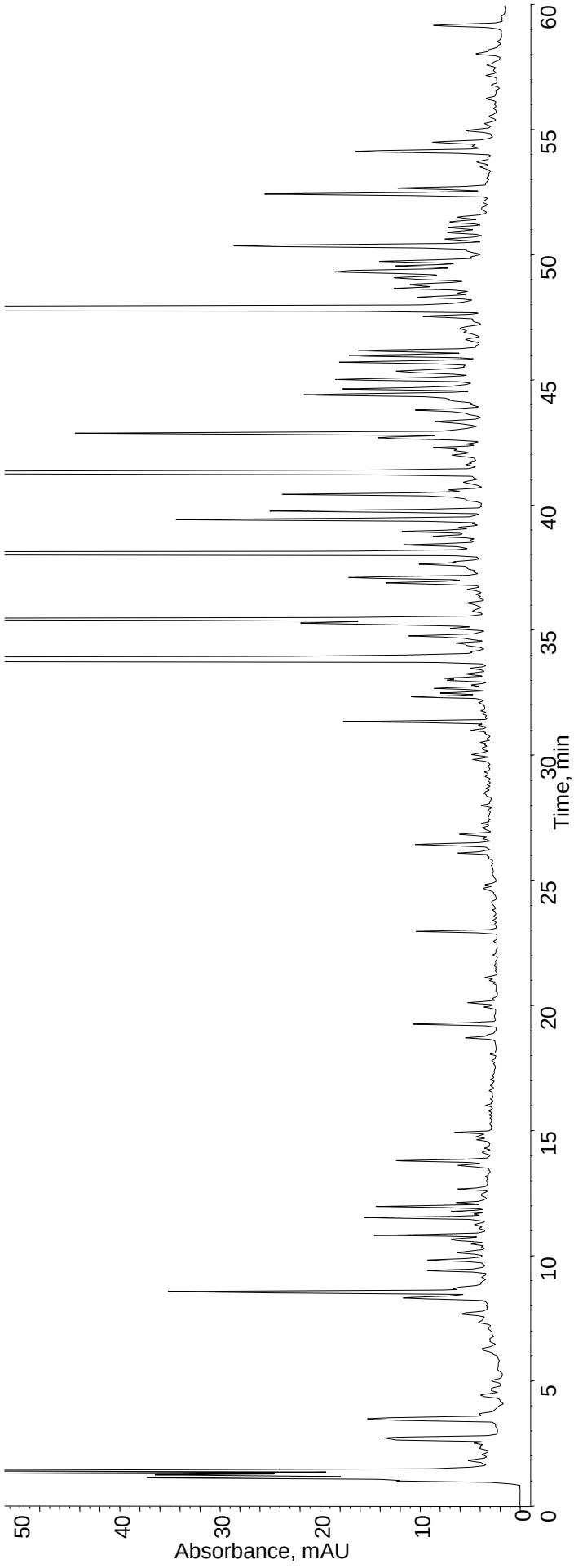


Fig. 7

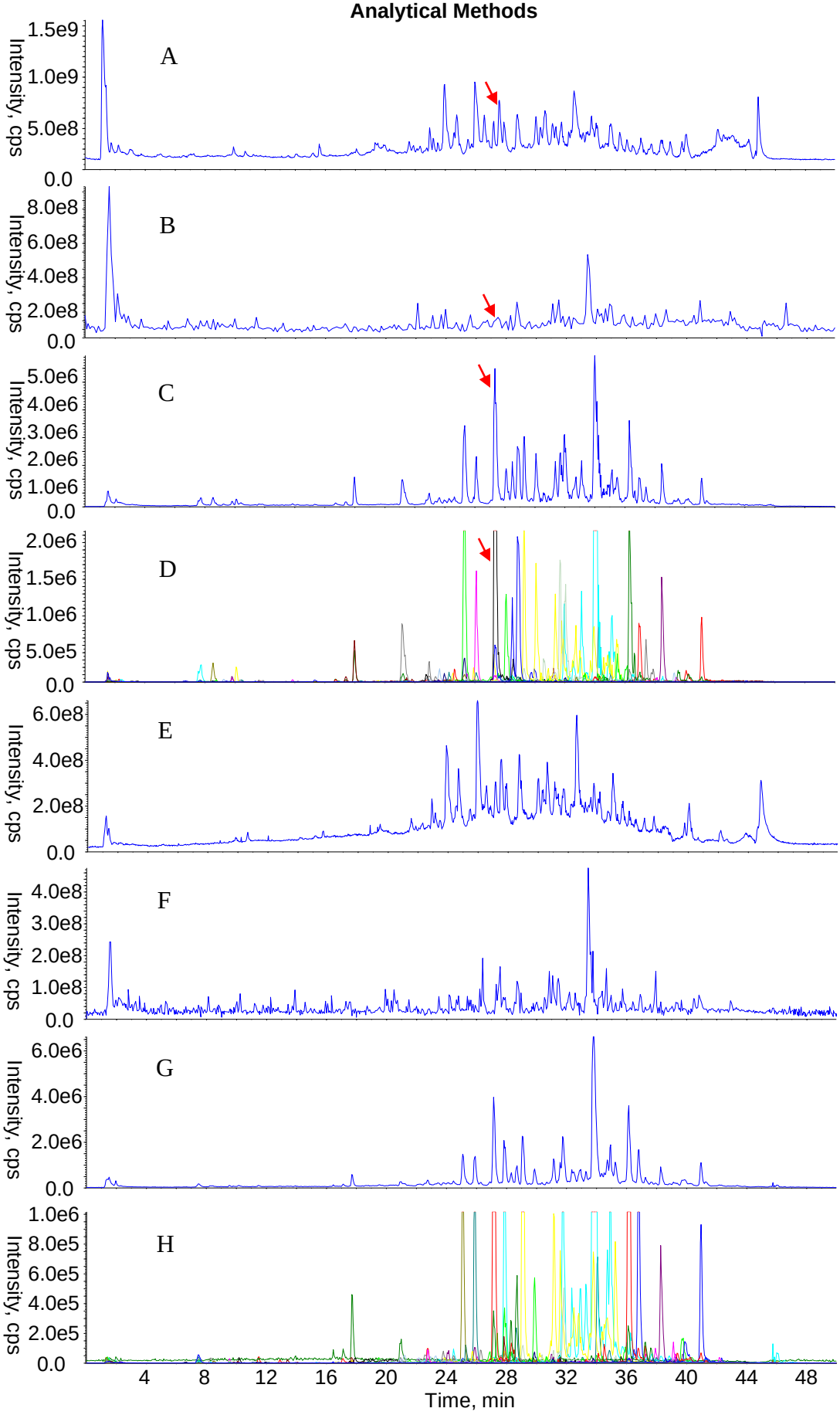


Fig. 8

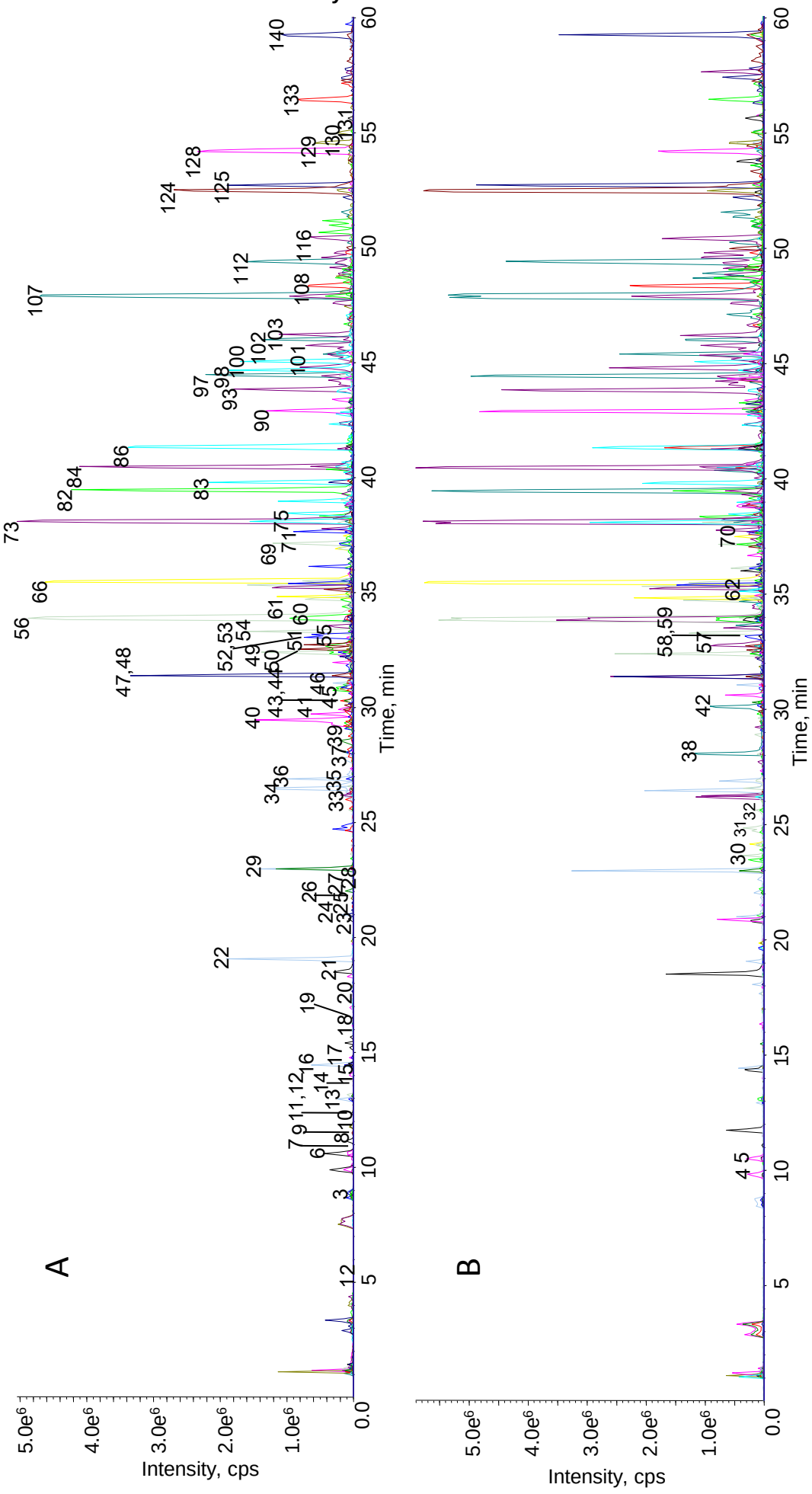


Fig. 9