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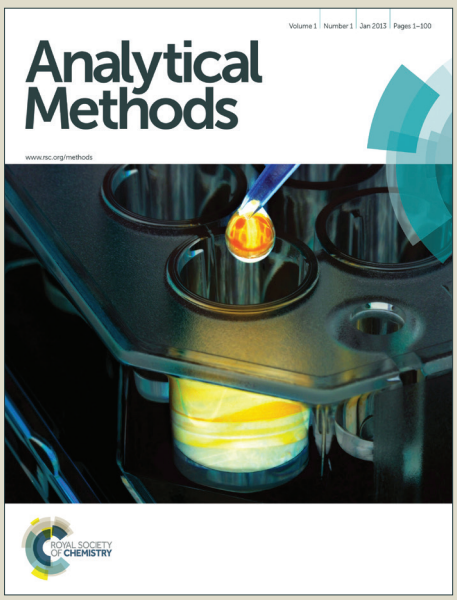

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Screening and Analyzing the Potential Bioactive Components from Rhubarb, Using Multivariate Data Processing Approach and Ultra-High Performance Liquid Chromatography Coupled with Time-of-flight Mass Spectrometry

De-zhu Yang[1], Ge Sun[1], Aihua Zhang[2], Shuang Fu[1], Jian-hua Liu[1]*

1. Institute of Medicine and Drug Research, Qiqihar Medical University, Qiqihar 161006, Heilongjiang, China.

2. College of Pharmacy, Heilongjiang University of Chinese Medicine, Heping Road 24, Harbin 150040, China

Address correspondence to:

Prof. Jian-hua Liu

Institute of Medicine and Drug Research

Qiqihar Medical University

Qiqihar 161006,

Heilongjiang, China.

Tel. & Fax +86-452-2663702

Email: oplsda@126.com

Abstract

Root of *Rheum palmatum L.*, known as rhubarb, is widely used in the treatment of obstipation, gastrointestinal indigestion and other diseases in China and other Asian countries for thousands of years. However, the constituents absorbed into blood after oral administration of rhubarb remain well unknown. Here, a sensitive and rapid method by UPLC/Q-TOF-MS/MS technology combined with multivariate data processing approach (Mdpa) was established to investigate the absorbed constituents in rats after oral administration of rhubarb. Chromatographic fingerprints of the rhubarb samples were firstly established *in vitro* and *in vivo*, with 80 compounds in rhubarb and 41 compounds in rat plasma after oral administration of rhubarb were detected in negative mode. Of the 80 detected compounds *in vitro*, 78 were tentatively characterized. Of the identified 41 compounds in rat plasma, 19 were the original form of compounds absorbed from the 30 detected compounds in vitro, 11 were the metabolites of the compounds existed in rhubarb. The integrative UPLC/Q-TOF-MS/MS and Mdpa method were successfully applied for rapid discovery of multiple components from rhubarb. Based on the significance of these results, this method demonstrated that this method was a useful technique for rapid screening and identifying bioactive components from complex herbal medicines.

Keywords

UPLC/Q-TOF-MS/MS; herbal medicine; rhubarb; identification; multivariate data processing approach

1. Introduction

Herbal medicines are globally accepted to possess antibacterial, antifungal, anticancer, antiviral, antiparasitic, anti-inflammatory activity and other pharmacological activities of benefit to humankind and express their effects through multi-components and multi-targets [1,2]. They have always been the mainstay of disease therapy, and still considered to be a rich resource for new drug discovery [3]. The screening bioactive components from herbal medicines are a serious challenge for researchers, since the compounds of herbal medicines are complicated and the biologically active compounds are only partially known [4]. For the screening analysis of bioactive components in herbal medicine, conventional phytochemical approach which isolate and identify individual components one by one could be used to search the lead compounds in herbal medicine, but this strategy is a time-consuming, and labor intensive. In views of this, development of sensitive and effective methods that meet the demand of high-throughput, high-fidelity screening of bioactive components from natural products are important to drug discovery. Recently, a new method based on UPLC/Q-TOF-MS/MS combined with multivariate data processing approach (Mdpa) has been widely introduced for the screening and analysis of natural compounds, making this method the most efficient analytical technology in quantitative and qualitative analysis of herbal medicine [5-8]. It would provide an efficient strategy for rapid screening, and identification of bioactive components of lead drug candidates from complex biological or chemical mixtures [9]. With the advantages of short analysis time, high chromatographic resolution and improved sensitivity, as well as high accuracy of mass values, UPLC/Q-TOF-MS/MS with Mdpa is very straightforward and efficient and has been accepted to screen the active components in traditional Chinese medicine (TCM) [10].

Use of plants as a source of medicine has been inherited and is an important component of the health care system [11]. China is the largest producer of medicinal herbs and plants used for traditional medicine contain a wide range of substances that can be used to treat diseases. The root of Rheum palmatum L. (Fig.1A), rhubarb (Fig.1B) is one of the most used TCM, is officially listed in Chinese, European and Japanese Pharmacopoeia [12]. Rhubarb is a widely used traditional medicine and can exert a number of biological effects including anti-inflammatory and antiplatelet effects [13-15]. Although it has been applied in clinical, the bioactive compounds in rhubarb are not fully known. In recent years, a few phytochemical and pharmacological studies of rhubarb have isolated and characterized a few of anthracene derivatives [16], which are generally agreed upon to be the major bioactive components of rhubarb. Although it is used largely in Chinese hospitals, there is no substantial evidence to screen the main effective ingredients of rhubarb *in vivo*. Therefore, the fully active components of rhubarb still largely remain unknown. In the present study, a new method employing UPLC/Q-TOF-MS/MS and Mdpa for the screening of bioactive compounds in rhubarb has been proposed. The results of this work demonstrated that the developed method could be employed for the rapid screening, and identification of the absorbed bioactive components and metabolites from rhubarb. To our best knowledge, this is the first systematical study on screening the bioactive components in rhubarb.

2. Material and methods

2.1 Chemicals and materials

HPLC grade methanol was purchased from Merck (Germany)； HPLC grade acetonitrile was purchased from Fisher (USA). Distilled water was purchased from Watson's Food & Beverage Co., Ltd. (Guangzhou, China). Leucine enkephalin was purchased from Sigma–Aldrich (MO, USA). HPLC grade formic acid was purchased from Kermel Chemical Reagent Co., Ltd (Tianjin, China)； formic acid was purchased from (DIKMA, USA). Rhubarb was purchased from Harbin Tongrentang Drug Store (Harbin, China), and authenticated by Prof. Jian-hua Liu, College of Pharmacy, Qiqihar Medical University. Voucher specimens were deposited at the authors' laboratory.

2.2 Preparation of rhubarb samples for analysis in vitro

The sliced rhubarb (50.0 g) was extracted by 1.0 L volumes of 70% ethanol three times, each for 3 h. T The extracted solution was filtered through 5 layer gauzes and made to a concentration of 1 g crude drug per milliliter, and finally the solution was freezedried. The freeze-dried powder (1g) was extracted with 50mL methanol for 10 min under ultrasonics, centrifuged at 13000 rpm (5 ◦C) for 10 minutes. The solution was filtered through 0.22 um membranes prior to use, and 5 ul aliquot was injected for analysis.

2.3 Animals handling

Male Wistar rats (240±20 g) were purchased from the Vital River Laboratory Animal Technology Co., Ltd. (Beijing, China). The rats were housed in an animal room (24 ± 2◦C, 40% relative humidity). A 12-h dark/light cycle was set, and given water and fed normal food for 1week before the experiment. All rats were randomly divided into 2 groups of 3 rats each: control group and dosed group. Rats were orally administered rhubarb extract at a dose of 0.504g /100g body weight. The control group was orally administrated with an equivalent volume of distilled water. After 30 min, the rats were anaesthetized by intraperitoneal injection of 5% pentobarbital sodium (0.10 mL/100g body weight). The experimental procedures were approved by the Animal Care and Ethics Committee at Qiqihar Medical University (approval number: QMU-E-2013-06085).

2.4. Preparation of serum samples in vivo

The blood samples were collected from hepatic portal vein at 0.5h after administration (after optimization screening of the time point) and the rats were sacrificed. Then, the serum was separated immediately by centrifuging at 13 000rpm for 10min at 4 ◦C. All samples were stored at −80 ◦C until analysis. The phosphoric acid (40 μL) was added to 2.0 mL blood samples and then vortexed for 60 s. The mixed solution was applied to pre-actrbated OASIS HLB

solid phase extraction columns (Waters, USA). Before that, the column was washed with 3mL of methanol and 3mL of water. Then, 2.0 mL 100% methanol elutes was collected and dried under a stream of nitrogen gas at 45 ◦C. Each dried sample was reconstituted in 100 µl methanol prior to analyses, centrifuged at 13000 rpm for 10min at 4 ◦C. The sample was filtered through a 0.22 um membrane, and 5ul aliquot was injected for UPLC/MS analysis.

2.5 Ultra-High Performance Liquid Chromatography Platform

Chromatographic separation for samples was performed using a Waters Acquity™ ultra performance LC system controlled with Masslynx (V4.1). Separation was performed on an Acquiry UPLC HSS T_3 Column (2.1×100 mm, 1.7 µm, Waters Corporation, Milford, USA) held at 30 ◦C. A gradient with eluent A (HCOOH: CH_3CN= 0.1:100, v/v) and eluent B (HCOOH: H_2O=0.1: 100, v/v) was used at a flow rate of 0.4 ml/min. The linear elution gradient program was used as follows: 0-3.0 min, 99-88 % A; 3.0-4.5 min, 88-87% A; 4.5-5.5 min, 87-86 % A; 5.5-8.0 min, 86-80% A; 8.0-15.0 min, 80-66 % A; 15.0-16.0 min, 66-53% A; 16-18 min, 53-0 % A; 18-19.5 min, 0% A; 19.5-20min, 0-99 % A; 20-22 min, 99% A.

2.6 Mass Spectrometry Condition

MS instrument consisted of a Waters QTOF/MS (Waters Corp., Milford, MA, USA). Ionization was performed in the negative electrospray (ESI) mode. The optimal conditions of analysis in ESI^- mode were as follows: the capillary voltage of 2400 V, the sampling cone voltage was 35 V, the extraction cone voltage was 3.5 V, the ion source temperature was set at 110 ◦C, the desolvation gas temperature was 300 ◦C, the desolvation gas flow was 600L/h. Ar gas was used as collision gas at a pressure of 0.2Mpa. Collision energy was recorded in the range of 30-50 V. The full-scan MS data were produced across the mass range of 50-1000 Da. A 200 pg/mL solution of leucine-enkephalin via a lockspray interface generates a reference ion at m/z 554.2615 Da ($[M-H]^-$) for negative ion mode.

2.7 Multivariate data processing approach for preliminary phytochemical screening

The raw data of all tested samples were analyzed by the MarkerLynx and EZINFO software (Waters Corp., USA) for preliminary phytochemical screening. For data analysis, a list of the intensities of the peaks detected was generated using RT and mass data (m/z) pairs as the identifier of each peak. The resulting three-dimensional data comprising peak number, sample name and ion intensity were analyzed by principal component analysis (PCA) and orthogonal partial least-squared discriminant analysis (OPLS-DA). For further confirmed the structure and the source of the metabolites, all data were introduced to MassFragment tool. The ions which were present in the dosed group and absent in the control group were extracted with the help of the VIP plot of OPLS-DA, and further these ions were identified with a combination of elemental composition tool and MS/MS fragment mass spectra.

3. Results and discussion

3.1 Optimization of chromatographic and mass spectrometry conditions

Natural products are often highly complex system with different types and concentrations of components, proved to be a crucial and challenging task to thoroughly separate and analyze them. The mobile phase played an important role in achieving good chromatographic behavior and appropriate ionization. A mixture of 0.1% v/v aqueous acetic acid and acetonitrile was finally chosen as the preferred mobile phase because it produced the desired separation and acceptable tailing factors within the 20 min run time. The acid is known to achieve better separation for compounds by reducing the peak tailing; moreover, the acid as additive to mobile phase could provide higher MS signal intensity, so acid is added into the mobile phase. In the course of optimizing separation conditions, mobile phase, gradient program, column temperature and detection wavelength were investigated. The final results showed that best resolution, shortest analysis time and lowest pressure variations were achieved when a gradient elution mode composed of acetonitrile (containing 0.1% formic acid) and water (containing 0.1% formic acid) was programmed as follows: 0-3.0 min, 99-88 % A; 3.0-4.5 min, 88-87% A; 4.5-5.5 min, 87-86 % A; 5.5-8.0 min, 86-80% A; 8.0-15.0 min, 80-66 % A; 15.0-16.0 min, 66-53% A; 16-18 min, 53-0 % A; 18-19.5 min, 0% A; 19.5-20min, 0-99 % A; 20-22 min, 99% A. When formic acid was added to the mobile phase, however, higher and narrower peaks were achieved. The flow rate was 0.4 mL min^{-1} while the column temperature was set at 30 ◦C. The negative ion mode provided higher signal intensity and had ability to detect more peak signal perhaps because of the existence of some compounds easy to ionize in the negative mode. The negative ion mode was employed because of its increased sensitivity to the signals of the common constituents compared with the positive ion mode. Furthermore, some ions were only observed in the negative ion mode, which is helpful for the structural determination. As a result, the negative ion mode was used.

3.2 Identification of bioactive compounds by UPLC/Q-TOF-MS/MS

All information of MS data obtained using the aforementioned protocol indicated the retention time and precise molecular mass was necessary for the identification of bioactive compounds. The precise molecular mass was determined within a reasonable degree of measurement error using Q-TOF/MS, and degree of unsaturation and the potential element composition were also obtained. Global profiling in negative ion mode was analyzed by UPLC/Q-TOF-MS/MS. The mass spectrum with MS and MS/MS of sennoside A was shown in Fig 2A. The precise molecular weight is 861.1853, and the main fragment ions that were analyzed via the MS/MS screening were observed at m/z 699$[M\text{-}H\text{-}C_6H_{10}O_5]^-$, 655$[M\text{-}H\text{-}C_7H_{10}O_7]^-$, 386$[M\text{-}H\text{-}C_{22}H_{19}O_{12}]^-$ in the negative ion spectrum. Its calculated molecular formula was speculated to be $C_{42}H_{38}O_{20}$ based on the analysis of its elemental composition and fractional isotope abundance, this ion was tentatively identified as sennoside A. Their mass spectrum and proposed fragmentation pathway are illustrated in Fig.2B. As demonstrated above, 80 interested ions were extracted, among

them, 78 components including anthracene derivatives, glycosides, tannins, and organic acids were identified or tentatively characterized based on their retention times, exact mass measurement for each molecular ion and subsequent fragment ions or via the matching of empirical information with those of published components in the in-house library, and their information was shown in Table 1. The mass error for molecular ions of all identified compounds was within ± 7 ppm.

3.3 Multivariate statistical analysis

Mdpa converts the multidimensional data space into two matrices known as scores and loadings. In the PCA score plot (Fig. 3A and B), each coordinate represents a sample, and it could be observed that the determined samples are clearly divided into two clusters. For the analysis of the differences in chemical compositions between dosed rat serum and blank serum samples, OPLS-DA, a supervised multivariate analysis method was performed. The interest ions which were present only in the dosed group and absent in the control group were extracted easily by VIP plot of OPLS-DA (Fig. 3C). In the VIP-plot, each point represents an ion RT–m/z pair; the X-axis represents variable contribution, and the further the ion RT–m/z pair point departs from zero, the more the ion contributes to the difference between the dosed rat serum and blank serum samples. As demonstrated above, 41 interested ions in blood samples were extracted, among them, 30 prototype components absorbed in rat serum and 11 metabolites were identified *in vivo*, and their information was shown in Table 2. As a result, global chromatogram of constituents after oral administration rhubarb *in vivo* was shown in Fig. 4.

Previous studies examined the phenolic compounds in rhubarbs using UPLC/Q-TOF-MS/MS [17]. The results suggested that the phenolic compounds including sennosides, anthraquinones, stilbenes, glucose gallates, naphthalenes, and catechins were tentatively characterized based on their mass spectra. In a study, an effective LC/MS method for rapid screening of antioxidative phenolic compounds in rhubarb is presented [18]. Fifty compounds, including phenolic acids, phenolic glucosides and hydroxyanthraquinones, were detected by LC/MS/MS analysis. Wang Z and co-authors used UPLC-PDA detector developed for the simultaneous determination of six anthraquinone glycosides in rhubarb [19]. HPLC methods for the systematic determination of 30 compounds in rhubarb were developed by Komatsu K and co-workers [20]. A study showed that potential differences in the chemical markers between raw and processed R. palmatum samples [21]. It was analyzed by UPLC/Q-TOF-MS coupled with multivariate statistical analysis using PCA and OPLS-DA. Emodin-8-O-glucoside and gallic acid-3-O-glucoside were determined to be the best markers for the raw and processed R. palmatum. UPLC/Q-TOF-MS with multivariate statistical analysis represents an efficient method for exploring the chemical markers in the raw and processed R. palmatum material.

In this study, integrative UPLC/Q-TOF-MS/MS and Mdpa method were performed to screen the bioactive compounds in *rhubarb*. Rhubarb is one of the most well-known herbal medicines, which is clinically effective for

various diseases, was chosen as a model. Although rhubarb has been used in clinic widely, the bioactive ingredients and metabolites of rhubarb are not fully understood. By comparative analysis of the chemical profiles of rhubarb extracts, control serum and dosed serum, bioactive compounds in rhubarb may be discovered. The potential bioactive compounds were then identified based on their MS and MS/MS spectra. The data sets of retention time (RT)-m/z pairs, ion intensities and sample codes were further processed with Mdpa to generate a VIP-plot. The identification of compounds in biosamples was achieved by accurate mass measurement and detailed fragmentation pathway analysis [22,23]. Furthermore, the method allowed the detection of low-abundance metabolites along with their structural elucidation. The results obtained from a comprehensive comparative analysis of the fingerprints of the rhubarb and its metabolic fingerprints in rat biological samples indicated that 41 components in the rhubarb were absorbed into the rat's body. In addition, 11 components might be metabolites of some components in the rhubarb. From these results, it could be concluded that the proposed method could be used to rapidly and simultaneously analyze and screen the multiple absorbed bioactive constituents and metabolites in rhubarb.

5. Conclusion

High-throughput screening and identification of bioactive compounds from complex mixtures is a challenging work. In this study, a reliable and sensitive method has been established for identification of the major bioactive compounds of rhubarb extract and their metabolites in rat plasma by UPLC/Q-TOF-MS/MS. Using negative ion mode and applying the MS fragmentation rules, 80 compounds were detected and 78 compounds were tentatively characterized in rhubarb extract. The proposed method was appropriate for rapid screening and identification of absorbed and metabolic components of rhubarb. In this study, using integrative UPLC/Q-TOF-MS/MS technique and Mdpa method, 41 components including 30 components from rhubarb and 11 metabolites were simultaneously observed, and provided essential data for further pharmacological studies of rhubarb. This approach yielded a series of potential bioactive components, which is both useful for the drug discovery of compounds from rhubarb and provides a strategy for characterizing and identifying bioactive compounds in a high-throughput manner. In summary, an efficient strategy based on Mdpa-guided fractionation, UPLC/Q-TOF-MS/MS was established and successfully applied for discovery and preparation of active constituents from Chinese herbal medicine and to better clarify its pharmacological action mechanism.

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Competing financial interests

The authors declare no competing financial interests.

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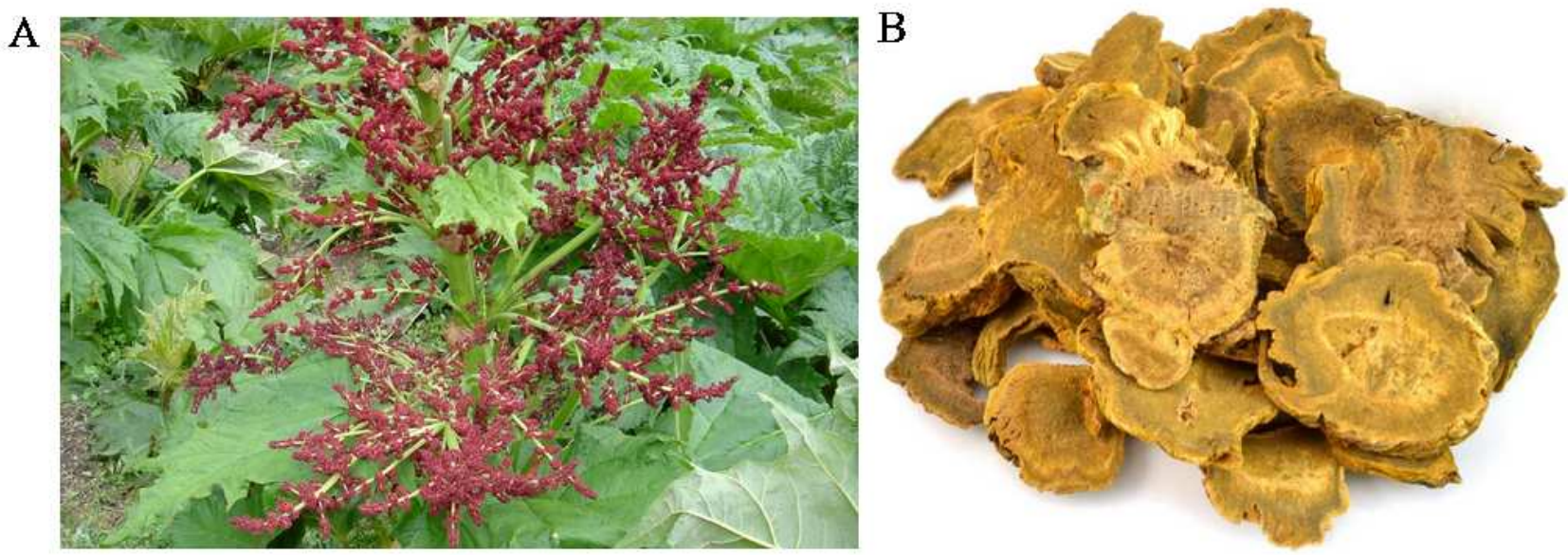

Figure 1. *Rheum palmatum L* (A), and its root (B), also known as rhubarb.

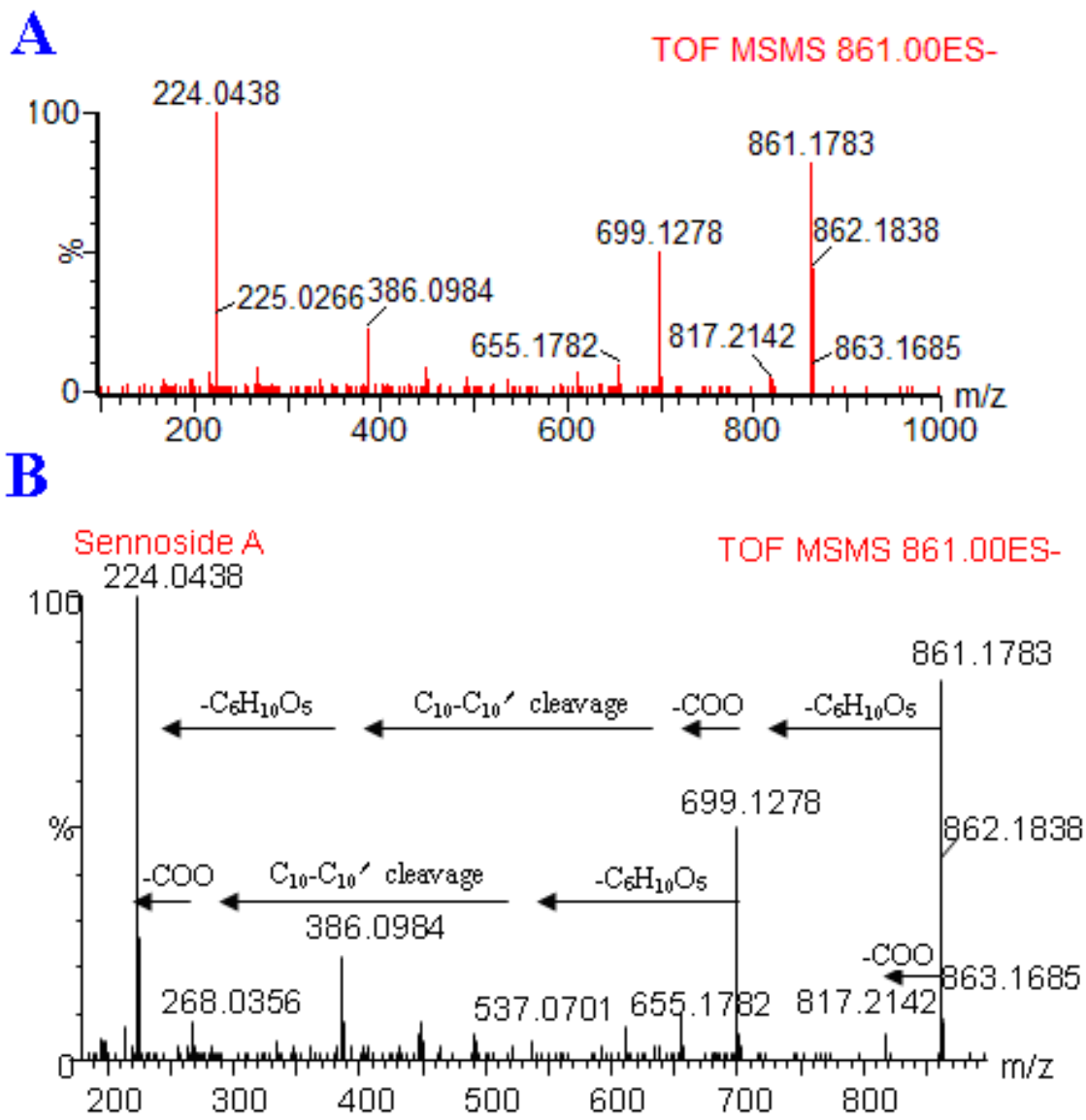

Figure 2. The mass spectrum with MS (A) and MS/MS (B) of ingredient sennoside A measured on UPLC/Q-TOF-MS/MS operating in negative ion mode.

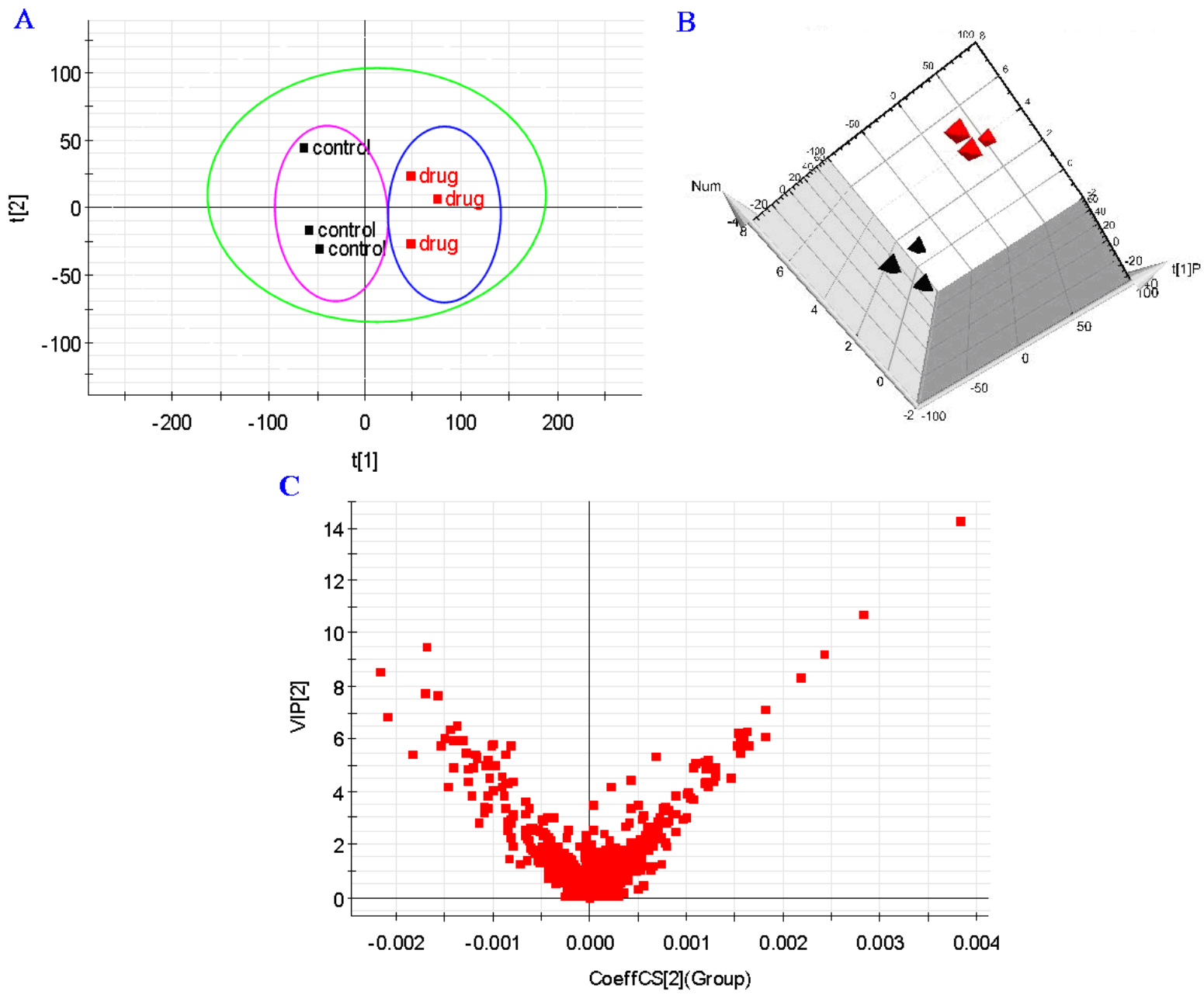

Figure 3. Multivariate statistical analysis of constituents in plasma dosed with rhubarb in the negtive ESI mode. PCA scores plot (A) and 3D scores plot (B) of dosed rat serum and control rat serum; the VIP plot of OPLS-DA of dosed rat serum and control rat serum (C).

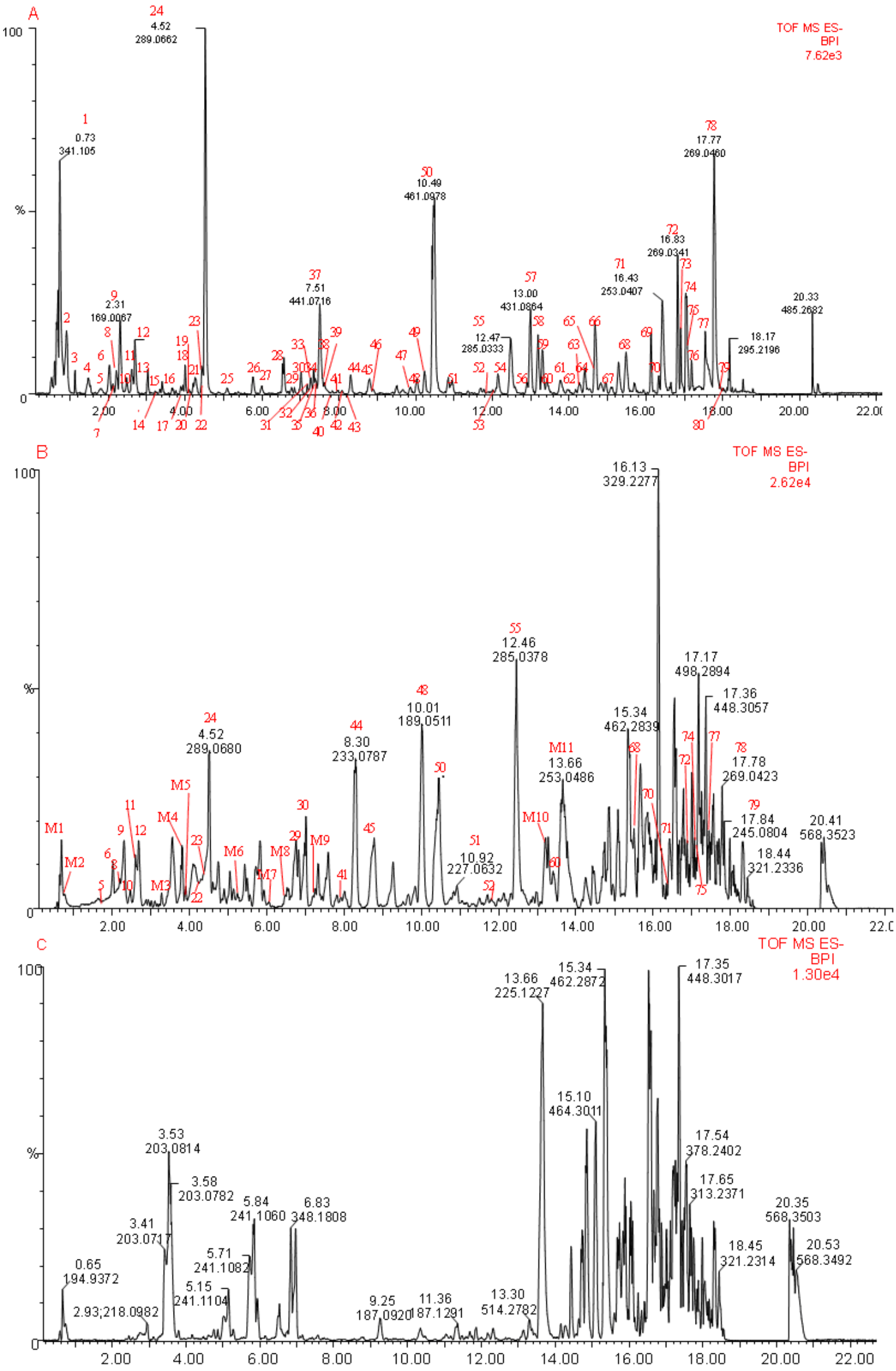

Figure 4. The UPLC/Q-TOF-MS/MS chromatograms of rhubarb extract (A), rat dosed serum (B) and control rat serum (C) in the negative ESI mode. Peaks marked with numbers were assigned to the absorbed components of rhubarb and peaks marked with M were metabolites. Structural characterization of these peaks was shown in Table 1 and each peak number was consistent with Table 1.

Table 1 Identification and structural characterisation of compounds detected in Rhubarb by UPLC/Q-TOF-MS/MS in the negtive ESI mode.

No.	tR	[M-H]$^-$ (m/z)	Molecular formula	PPM	Fragment ions (m/z)	Compounds identified
1	0.73	341.1098	$C_{12}H_{22}O_{11}$	-4.1	179[M-H-$C_6H_{10}O_5$]$^-$161[M-H-$C_6H_{12}O_6$]$^-$113[M-H-$C_7H_{16}O_8$]$^-$	Sucrose
2	0.91	341.1078	$C_{12}H_{22}O_{11}$	-4.1	179[M-H-$C_6H_{10}O_5$]$^-$161[M-H-$C_6H_{12}O_6$]$^-$113[M-H-$C_7H_{16}O_8$]$^-$	β-Maltose
3	1.13	503.1600	$C_{18}H_{32}O_{16}$	-2.4	179[M-H-$C_{12}H_{20}O_{10}$]$^-$161[M-H-$C_{12}H_{22}O_{11}$]$^-$131[M-H-$C_{13}H_{24}O_{12}$]$^-$ 113[M-H-$C_{13}H_{26}O_{13}$]$^-$	Melezitose
4	1.48	383.1195	$C_{14}H_{24}O_{12}$	+1.3	341[M-H-C_2H_2O]$^-$323[M-H-$C_2H_4O_2$]$^-$179[M-H-$C_8H_{12}O_6$]$^-$	acetylmaltose
5	1.80	331.0677	$C_{13}H_{16}O_{10}$	+3.6	271[M-H-$C_2H_4O_2$]$^-$211[M-H-$C_4H_8O_4$]$^-$169[M-H-$C_6H_{10}O_5$]$^-$125[M-H-$C_7H_{10}O_7$]$^-$	Glucopyranosyloxyl gallic acid
6	2.03	331.0673	$C_{13}H_{16}O_{10}$	+2.4	271[M-H-$C_2H_4O_2$]$^-$211[M-H-$C_4H_8O_4$]$^-$169[M-H-$C_6H_{10}O_5$]$^-$125[M-H-$C_7H_{10}O_7$]$^-$	Glucopyranosyloxyl gallic acid
7	2.12	193.0706	$C_7H_{14}O_6$	-3.1	271[M-H-$C_2H_4O_2$]$^-$211[M-H-$C_4H_8O_4$]$^-$169[M-H-$C_6H_{10}O_5$]$^-$125[M-H-$C_7H_{10}O_7$]$^-$	Methyl α-D-Glucopyranoside
8	2.20	331.0660	$C_{13}H_{16}O_{10}$	-1.5	271[M-H-$C_2H_4O_2$]$^-$211[M-H-$C_4H_8O_4$]$^-$169[M-H-$C_6H_{10}O_5$]$^-$125[M-H-$C_7H_{10}O_7$]$^-$	Glucopyranosyloxyl gallic acid
9	2.31	169.0094	$C_7H_6O_5$	-1.8	125[M-H-CO_2]$^-$107[M-H-CH_2O_3]$^-$	Gallic acid
10	2.48	331.0676	$C_{13}H_{16}O_{10}$	+3.3	271[M-H-$C_2H_4O_2$]$^-$211[M-H-$C_4H_8O_4$]$^-$169[M-H-$C_6H_{10}O_5$]$^-$125[M-H-$C_7H_{10}O_7$]$^-$	Glucopyranosyloxyl gallic acid
11	2.60	493.1171	$C_{19}H_{26}O_{15}$	-4.5	313[M-H-$C_6H_{12}O_6$]$^-$ 283[M-H- $C_7H_{14}O_7$]$^-$ 169[M-H- $C_{12}H_{20}O_{10}$]$^-$ 125[M-H- $C_{13}H_{20}O_{12}$]$^-$	Glucopyranosyl-galloyl-glucose
12	2.69	493.1191	$C_{19}H_{26}O_{15}$	-0.4	313[M-H-$C_6H_{12}O_6$]$^-$ 169[M-H- $C_{12}H_{20}O_{10}$]$^-$ 125[M-H- $C_{13}H_{20}O_{12}$]$^-$	Glucopyranosyl-galloyl-glucose
13	3.03	243.0493	$C_{10}H_{12}O_7$	-4.9	169[M-H-$C_3H_6O_2$]$^-$125[M-H-$C_4H_6O_4$]$^-$124[M-H-$C_4H_7O_4$]$^-$ 107[M-H-$C_4H_8O_5$]$^-$	Glycerin-monogallete 20481201
14	3.21	483.0818	$C_{20}H_{20}O_{14}$	-1.7	315[M-H-$C_7H_4O_5$]$^-$299[M-H-$C_7H_4O_6$]$^-$169[M-H-$C_{13}H_{14}O_9$]$^-$ 125[M-H-$C_{14}H_{14}O_{11}$]$^-$	Digalloyl-glucose
15	3.41	451.1231	$C_{21}H_{24}O_{11}$	-2.0	289[M-H- $C_6H_{10}O_5$]$^-$ 245[M-H- $C_7H_{11}O_7$]$^-$	catechin-glucopyranoside
16	3.67	451.1266	$C_{21}H_{24}O_{11}$	+5.8	289[M-H- $C_6H_{10}O_5$]$^-$ 245[M-H- $C_7H_{11}O_7$]$^-$	Catechin-glucopyranoside
17	3.88	451.1260	$C_{21}H_{24}O_{11}$	4.4	289[M-H- $C_6H_{10}O_5$]$^-$ 245[M-H- $C_7H_{11}O_7$]$^-$	Catechin-glucopyranoside
18	3.99	577.1345	$C_{30}H_{26}O_{12}$	-0.2	407[M-$C_8H_{10}O_4$]$^-$289[M-H-$C_{15}H_{12}O_6$]$^-$125[M-H-$C_{24}H_{20}O_9$]$^-$ 109[M-H- $C_{24}H_{20}O_{10}$]$^-$	Catechin dimers
19	4.11	451.1223	$C_{21}H_{24}O_{11}$	-3.8	289[M-H- $C_6H_{10}O_5$]$^-$ 245[M-H- $C_7H_{11}O_7$]$^-$	Catechin-glucopyranoside
20	4.14	483.0782	$C_{20}H_{20}O_{14}$	1.4	315[M-H-$C_7H_4O_5$]$^-$299[M-H-$C_7H_4O_6$]$^-$169[M-H-$C_{13}H_{14}O_9$]$^-$ 125[M-H-$C_{14}H_{14}O_{11}$]$^-$	Digalloyl-glucose

21	4.26	577.1345	$C_{30}H_{26}O_{12}$	-0.2	407[M-H-$C_8H_{10}O_4$]$^-$289[M-H-$C_{15}H_{12}O_6$]$^-$245[M-H-$C_{16}H_{12}O_8$]$^-$ 125[M-H- $C_{24}H_{20}O_9$]$^-$109[M-H- $C_{24}H_{20}O_{10}$]$^-$	Catechin dimers
22	4.34	137.0236	$C_7H_6O_3$	-2.2	121[M-H- O]$^-$93[M-H-CO_2]$^-$	hydroxy-benzoic acid
23	4.43	183.0284	$C_8H_8O_5$	-4.9	168[M-H- CH_3]$^-$124[M-H- $C_2H_3O_2$]$^-$	Methyl gallate
24	4.52	289.0712	$C_{15}H_{14}O_6$	-3.1	271[M-H-H_2O]$^-$245[M-H-CHO_2]$^-$125[M-H-$C_9H_8O_3$]$^-$ 109[M-H-$C_9H_8O_4$]$^-$	Catechin
25	5.08	577.1342	$C_{30}H_{26}O_{12}$	-0.7	407[M-H-$C_8H_{10}O_4$]$^-$289[M-H-$C_{15}H_{12}O_6$]$^-$245[M-H-$C_{16}H_{12}O_8$]$^-$ 125[M-H- $C_{24}H_{20}O_9$]$^-$109[M-H- $C_{24}H_{20}O_{10}$]$^-$	Catechin dimers
26	5.76	289.0706	$C_{15}H_{14}O_6$	-2.1	245[M-H-CHO_2]$^-$125[M-H-$C_9H_8O_3$]$^-$ 109[M-H-$C_9H_8O_4$]$^-$	epicatechin
27	5.99	729.1473	$C_{37}H_{30}O_{16}$	+2.3	577[M-$C_7H_4O_4$]$^-$559[M-H-$C_7H_6O_5$]$^-$407[M-H-$C_{15}H_{14}O_8$]$^-$289[M-$C_{22}H_{16}O_{10}$]$^-$ 269[M-H-$C_{22}H_{20}O_{11}$]$^-$169[M-H-$C_{30}H_{24}O_{11}$]$^-$125[M-H-$C_{31}H_{24}O_{13}$]$^-$	Procyanidin B-13'-O-gallate
28	6.53	389.1226	$C_{20}H_{22}O_8$	-2.6	227[M-H-$C_6H_{10}O_5$]$^-$	3,5,4'-Trihydroxystilene-4'-glucoside
29	6.77	163.0391	$C_9H_8O_3$	-2.5	147[M-H-O]$^-$119[M-H-CO_2]$^-$	p-coumaric acid
30	7.01	233.0446	$C_{12}H_{10}O_5$	-1.7	189[M-H-CO_2]$^-$175[M-H-$C_2H_2O_2$]$^-$ 147[M-H-$C_3H_2O_3$]$^-$	(5Z)-6-Hydroxy-3,4-dioxo-6-phenyl-5-hexenoic acid
31	7.18	431.0984	$C_{21}H_{20}O_{10}$	0.7	269[M-H-$C_6H_{10}O_5$]$^-$240[M-H-$C_7H_{11}O_6$]$^-$	Aloe-emodin 8-glucoside\7
32	7.23	861.1873	$C_{42}H_{38}O_{20}$	-0.6	699[M-H- $C_6H_{10}O_5$]$^-$655[M-H-$C_7H_{10}O_7$]$^-$ 386[M-H-$C_{22}H_{19}O_{12}$]$^-$	Sennoside A isomer
33	7.27	309.0929	$C_{15}H_{18}O_7$	+4.2	147[M-$C_6H_{10}O_5$]$^-$ 131[M- $C_6H_{10}O_6$]$^-$103[M-H- $C_7H_{10}O_7$]$^-$	Cinnamoyl-glucose
34	7.35	477.1404	$C_{23}H_{26}O_{11}$	+1.5	313[M-H-$C_{10}H_{12}O_2$]$^-$211[M-H-$C_{14}H_{18}O_5$]$^-$169[M-H-$C_{16}H_{20}O_6$] 125[M-H-$C_{17}H_{20}O_8$]$^-$	Isolindleyin 39189
35	7.38	417.1191	$C_{21}H_{22}O_9$	+1.2	255[M-$C_6H_{10}O_5$]$^-$	Aloe emodin glucoside
36	7.45	445.0770	$C_{21}H_{18}O_{11}$	0.0	283[M-H-$C_6H_{10}O_5$]$^-$239[M-H-$C_7H_{10}O_7$]$^-$	Rhein-8-O-glucopyranoside
37	7.51	441.0822	$C_{22}H_{18}O_{10}$	-3.6	289[M-H-$C_7H_4O_4$]$^-$271[M-H-$C_7H_6O_5$]$^-$ 169[M-H-$C_{15}H_{12}O_5$]$^-$ 125[M-H-$C_{16}H_{12}O_7$]$^-$	(-)-Epicatechin-3-O-gallate
38	7.59	191.0709	$C_{11}H_{12}O_3$	+0.5	175[M-H-CH_4]$^-$147[M-H-CO_2]$^-$ 131[M-H-$C_2H_4O_2$]$^-$	p-Ethoxy cinnamic acid
39	7.62	477.1393	$C_{23}H_{26}O_{11}$	-0.8	313[M-H-$C_6H_{12}O_5$]$^-$211[M-H-$C_{14}H_{18}O_5$]$^-$169[M-H-$C_{16}H_{20}O_6$]$^-$147[M-H-$C_{14}H_{18}O_9$]$^-$	Lindleyin 8565764

40	7.83	441.0812	$C_{15}H_{12}O_4$	-2.3	289[M-H-$C_7H_4O_4$]$^-$271[M-H-$C_7H_6O_5$]$^-$169[M-H-$C_{15}H_{12}O_5$]$^-$ 125[M-H- $C_{16}H_{12}O_7$]$^-$	(-)-Epicatechin-3-O-gallate 97034
41	7.93	167.0334	$C_8H_8O_4$	-6.0	123[M-H-CO_2]$^-$	Dihydroxy methylbenzoic acid18677
42	8.03	861.1878	$C_{42}H_{38}O_{20}$	0.0	699[M-H-$C_6H_{10}O_5$]$^-$655[M-H-$C_7H_{10}O_7$]$^-$557[M-H-$C_{10}H_{26}O_{10}$]$^-$386[M-H- $C_{20}H_{14}O_8$]$^-$	Sennoside A isomer
43	8.30	847.2087	$C_{42}H_{40}O_{19}$	+0.1	685[M-H-$C_6H_{10}O_5$]$^-$ 386[M-H-$C_{22}H_{21}O_{11}$]$^-$ 253[M-H-$C_{27}H_{30}O_{15}$]$^-$	Sennoside C
44	8.31	233.0801	$C_{13}H_{14}O_4$	-5.6	189[M-H-C_2H_4O]$^-$159[M-H-$C_3H_4O_2$]$^-$123[M-H-$C_6H_6O_2$]$^-$	Untitled
45	8.80	231.0620	$C_{13}H_{12}O_4$	-3.7	189[M-H- C_2H_2O]$^-$ 174[M-H-CH_3]$^-$159[M-H-CH_2O]$^-$147[M-H-C_2H_2O]$^-$ 133[M-H-C_2O_2]$^-$	Acetyl Methylformylcinnamate
46	8.88	861.1853	$C_{42}H_{38}O_{20}$	-2.9	699[M-H-$C_6H_{10}O_5$]$^-$655[M-H-$C_7H_{10}O_7$]$^-$ 386[M-H-$C_{22}H_{19}O_{12}$]$^-$	Sennoside A
47	9.84	393.1190	$C_{19}H_{22}O_9$	1.0	231[M-H-$C_6H_{10}O_5$]$^-$	Aloesin
48	10.01	189.0539	$C_{11}H_{10}O_3$	-6.2	174[M-H-CH_3]$^-$159[M-H-CH_2O]$^-$147[M-H-C_2H_2O]$^-$ 133[M-H-C_2O_2]$^-$	Methylformylcinnamate
49	10.28	487.0880	$C_{23}H_{20}O_{12}$	+0.6	283[M-$C_8H_{12}O_7$]$^-$267[M-$C_8H_{12}O_7$]$^-$239[M-$C_9H_{12}O_9$]$^-$	Rhein 1-O-(O-acetyl)-glucoside
50	10.49	461.1090	$C_{22}H_{22}O_{11}$	+1.3	313[M-H-$C_9H_8O_2$]$^-$169[M-H-$C_{15}H_{16}O_6$]$^-$161[M-H-$C_{16}H_{12}O_6$]$^-$ 151[M-H-$C_{15}H_{18}O_7$]$^-$ 147[M-H-$C_{13}H_{14}O_9$]$^-$	Cinnamoyl-glucogallin
51	10.94	227.0707	$C_{14}H_{12}O_3$	-0.4	185[M-H-C_2H_2O]$^-$ 143[M-H-C_2H_2O]$^-$	3,5,4'-Trihydroxy-trans-stilbene
52	11.82	181.0493	$C_9H_{10}O_4$	-4.4	163M-H-H_2O]$^-$151[M-H-C_2H_6]$^-$ 137[M-H-CO_2]$^-$	4-Methoxy-6-methylsalicylic Acid
53	12.01	417.1178	$C_{21}H_{22}O_9$	-1.9	255[M-H-$C_6H_{710}O_5$]$^-$225[M-H-$C_7H_{12}O_6$]$^-$	Aloin
54	12.15	461.1084	$C_{22}H_{22}O_{11}$	+0.4	313[M-H-$C_9H_8O_2$]$^-$169[M-H-$C_{15}H_{16}O_6$]$^-$161[M-H-$C_{16}H_{12}O_6$]$^-$ 151[M-H-$C_{15}H_{18}O_7$]$^-$ 147[M-H-$C_{13}H_{14}O_9$]$^-$	Cinnamoyl-glucogallin
55	12.52	285.0396	$C_{15}H_{10}O_6$	-1.1	255[M-CH_2O]$^-$239[M-CH_2O_2]$^-$	ω-Hydroxyemodin
56	12.90	407.1350	$C_{20}H_{24}O_9$	+2.0	245[M-$C_6H_{10}O_5$]$^-$230[M-$C_7H_{13}O_5$]$^-$ 215[M-H-$C_8H_{16}O_5$]$^-$187[M-H- $C_9H_{16}O_6$]$^-$	Torachrysone-8-o-β-D-glucoside
57	13.00	431.0980	$C_{21}H_{20}O_{10}$	+0.5	269[M-H-$C_6H_{10}O_5$]$^-$ 268[M-H-$C_6H_{11}O_5$]$^-$240[M-H-$C_7H_{11}O_6$]$^-$	Aloe-emodin-o-glucoside
58	13.19	431.0980	$C_{21}H_{20}O_{10}$	+0.5	269[M-$C_6H_{10}O_5$]$^-$241[M-H-$C_7H_{10}O_6$]$^-$225 [M-H-$C_8H_{10}O_8$]$^-$	Emodin-o-glucoside
59	13.30	415.1035	$C_{21}H_{20}O_9$	+1.4	253[M-H-$C_6H_{10}O_5$]$^-$ 225[M-H-$C_6H_6O_7$]$^-$	Chrysophanol-glucoside
60	13.45	299.0194	$C_{15}H_8O_7$	+0.7	255[M-H-CO_2]$^-$ 239[M-H-CHO_3]$^-$	Emodic acid
61	13.78	313.0345	$C_{16}H_{10}O_7$	-1.0	269[M-CO_2]$^-$241[M-H-C_2O_3]$^-$225 [M-H-C_3O_5]$^-$	Endocrocin
62	14.00	613.1182	$C_{29}H_{26}O_{15}$	-1.8	444[M-$C_7H_6O_5$]$^-$169[M-$C_{22}H_{20}O_{10}$]$^-$147[M-H-$C_{20}H_{19}O_{13}$]$^-$	Cinnamoyl digallyl-glucose

63	14.29	233.0818	$C_{13}H_{14}O_4$	+1.7	191[M-H-C_2H_2O]$^-$175[M-H-C_3H_6O]$^-$147[M-H- $C_3H_2O_3$]$^-$	Unidentified
64	14.41	473.1082	$C_{23}H_{22}O_{11}$	-0.4	311[M-H-$C_6H_{10}O_5$]$^-$269[M-H-$C_8H_{12}O_7$]$^-$241[M-H-$C_9H_{12}O_8$]$^-$	emodin 8-*O*-(6'-*O*-acetyl)-glucoside
65	14.65	449.1445	$C_{22}H_{26}O_{10}$	-0.7	245[M-$C_8H_{12}O_6$]$^-$230[M-$C_9H_{15}O_6$]$^-$ 215[M-H-$C_{10}H_{18}O_6$]$^-$187[M-H-$C_{11}H_{18}O_7$]$^-$	torachrysone 8-*O*-(6′-*O*acetyl)-glucoside
66	14.67	473.1077	$C_{23}H_{22}O_{11}$	-1.5	269[M-H-$C_8H_{12}O_7$]$^-$268[M-H-$C_8H_{13}O_7$]$^-$ 240[M-H-$C_9H_{13}O_8$]$^-$224[M-H-$C_9H_{13}O_8$]$^-$	Aloe-emodin 8-*O*-(6'-*O*-acetyl)-glucoside
67	14.95	431.0984	$C_{21}H_{20}O_{10}$	+1.4	269[M-$C_6H_{10}O_5$]$^-$241[M-H-$C_7H_{10}O_6$]$^-$225 [M-H-$C_8H_{10}O_8$]$^-$	Emodin-8-glucoside
68	15.48	285.0396	$C_{15}H_{10}O_6$	-1.1	257[M-H-CO]$^-$241[M-H-CO_2]$^-$	lunatin
69	16.13	329.2330	$C_{18}H_{34}O_5$	+0.6	311[M-OH]$^-$	Unidentified
70	16.33	299.0188	$C_{15}H_8O_7$	-1.3	268[M- CH_3O]$^-$255[M-CO_2]$^-$240M-H-CO_3]$^-$	Hydroxy physcion
71	16.43	297.0401	$C_{16}H_{10}O_6$	+0.7	253[M-H-CO_2]$^-$225[M-H-C_2O_3]$^-$	4,5-Dihydroxy-7-methyl-9,10-dioxo-9,10-dihydro-2-anthracenecarboxylic acid
72	16.83	269.0452	$C_{15}H_{10}O_5$	0.7	255[M-H-O]$^-$240 [M-H-CHO]$^-$	Aloe emodin
73	16.90	607.1874	$C_{32}H_{32}O_{12}$	+2.0	477[M-H-C_9H_6O]$^-$459[M-H-$C_9H_8O_2$]$^-$443[M-$C_{10}H_{12}O_2$]$^-$295[M-H-$C_{19}H_{20}O_4$]$^-$ 169 [M-H-$C_{25}H_{26}O_7$]$^-$ 147[M-H-$C_{23}H_{24}O_{10}$]$^-$	4-(3-Oxobutyl)phenyl6-O-[(2E)-3-phenyl-2-propenoyl]-2-O-(3,4,5-trihydroxybenzoyl)-β-D-glucopyranoside
74	17.05	283.0247	$C_{15}H_8O_6$	1.4	239[M-H-CO_2]$^-$211[M-H-C_2O_3]$^-$183 [M-H-C_3O_4]$^-$	Rhein
75	17.08	311.0553	$C_{17}H_{12}O_6$	-1.0	269[M-H-C_2H_2O]$^-$ 240[M-H-$C_3H_3O_2$]$^-$	(4,5-Dihydroxy-9,10-dioxo-9,10-dihydro-2-anthracenyl)methyl acetate
76	17.19	253.0494	$C_{15}H_{10}O_4$	-2.8	225[M-H-CO_2]$^-$	Chrysophanol
77	17.57	311.0546	$C_{17}H_{12}O_6$	-3.2	269[M-H-C_2H_2O]$^-$ 241[M-H-$C_3H_2O_2$]$^-$225[M-H-$C_4H_2O_3$]$^-$	(1,3-Dihydroxy-9,10-dioxo-9,10-dihydro-2-anthracenyl)methyl acetate
78	17.78	269.0443	$C_{15}H_{10}O_5$	-2.6	241[M-H-CO]$^-$225 [M-H-CO_2]$^-$	Emodin
79	17.86	245.0811	$C_{14}H_{14}O_4$	-1.2	230[M-CH_3]$^-$ 215[M-H-C_2H_6]$^-$ 187[M-H-C_3H_6O]$^-$	Torachrysone
80	18.05	283.0627	$C_{16}H_{12}O_5$	+7.4	269[M-H-CH_2]$^-$	physcion

Table 2 UPLC/Q-TOF-MS/MS identification of compounds and metabolites in rat plasma after the absorption of rhubarb

No.	tR	Metabolite name	Molecular formula	[M-H]⁻(m/z)	mDa	Fragmentions (m/z)	Parent drug
M1	0.69	Glucuronide conjugation	$C_{19}H_{24}O_{16}$	507. 1051	+0.8	507.1051 [M-H]⁻, 331.0655[M-H-$C_6H_8O_6$]⁻	Glucopyranosyloxyl gallic acid
M2	0.71	Glucuronide conjugation	$C_{21}H_{22}O_{12}$	465.1034	+0.2	465.1034 [M-H]⁻, 289.0740[M-H-$C_6H_8O_6$]⁻	Catechin
5	1.80	Parent	$C_{13}H_{16}O_{10}$	331.0665	0.0	331.0665 [M-H]⁻ 169.0095[M-H-$C_6H_{10}O_5$]⁻	Glucopyranosyloxyl gallic acid
6	2.03	Parent	$C_{13}H_{16}O_{10}$	331.0655	-3.0	331.0655 [M-H]⁻ 169.0094[M-H-$C_6H_{10}O_5$]⁻	Glucopyranosyloxyl gallic acid
8	2.20	Parent	$C_{13}H_{16}O_{10}$	331.0657	-2.5	331.0657 [M-H]⁻ 169.0068[M-H-$C_6H_{10}O_5$]⁻	Glucopyranosyloxyl gallic acid
9	2.31	Parent	$C_7H_6O_5$	169.0131	-1.6	169.0131 [M-H]⁻125.0165[M-H-CO_2]⁻	Gallic acid
10	2.47	Parent	$C_{13}H_{16}O_{10}$	331.0656	-2.7	331.0657 [M-H]⁻ 169.0113[M-H-$C_6H_{10}O_5$]⁻	Glucopyranosyloxyl gallic acid
11	2.60	Parent	$C_{19}H_{26}O_{15}$	493.1179	-1.4	493.1179 [M-H]⁻ 331.0656[M-H-$C_6H_{10}O_5$]⁻	Glucopyranosyl-galloyl-glucose
12	2.69	Parent	$C_{19}H_{26}O_{15}$	493.1155	-3.8	493.1155 [M-H]⁻ 331.0679[M-H-$C_6H_{10}O_5$]⁻	Glucopyranosyl-galloyl-glucose
M3	3.28	Glucuronide conjugation	$C_{21}H_{22}O_{12}$	465.1026	-1.5	465.1026 [M-H]⁻, 289.0710[M-H-$C_6H_8O_6$]⁻	Catechin
M4	3.80	Glucuronide conjugation	$C_{21}H_{22}O_{12}$	465.1033	0.0	465.1033 [M-H]⁻, 289.0729[M-H-$C_6H_8O_6$]⁻	Catechin
M5	3.88	Glucuronide conjugation	$C_{15}H_{20}O_{10}$	359.0969	-2.5	359.0969 [M-H]⁻, 183.0267[M-H-$C_6H_8O_6$]⁻	Methyl gallate
22	4.34	Parent	$C_7H_6O_3$	137.0243	+3.9	137.0243 [M-H]⁻93.0328[M-H-CO_2]⁻	hydroxy-benzoic acid
23	4.43	Parent	$C_8H_8O_5$	183.0289	-2.2	183.0289 [M-H]⁻, 124.0138[M-H-$C_2H_3O_2$]⁻	Methyl gallate
24	4.52	Parent	$C_{15}H_{14}O_6$	289.0708	-1.4	289.0708 [M-H]⁻, 245.0769[M-H-CHO_2]⁻	Catechin
M6	5.21	Glucuronide conjugation	$C_{15}H_{18}O_{11}$	373.0719	+1.9	373.0719 [M-H]⁻, 197.0468 [M-H-$C_6H_8O_6$]⁻	Ethyl gallate
M7	5.99	Glucuronide conjugation	$C_{14}H_{16}O_{10}$	343.0663	-0.6	343.0663 [M-H]⁻, 167.0334[M-H-$C_6H_8O_6$]⁻	Dihydroxy methylbenzoic acid
M8	6,67	Esterification	$C_9H_{10}O_5$	197.0442	-4.2	197.0442 [M-H]⁻, 169.0145[M-H-$C_2H_3O_2$]⁻	Gallic acid
29	6.76	Parent	$C_9H_8O_3$	163.0388.	-4.3	163.0388[M-H]⁻, 119.0452[M-H-CO_2]⁻	p-coumaric acid
30	7.01	Parent	$C_{11}H_{10}O_3$	233.0443	-3.0	233.0443 [M-H]⁻, 189.0537[M-H-CO_2]⁻	(5Z)-6-Hydroxy-3,4-dioxo-6-phenyl-5-hexenoic acid
M9	7.24	Glucuronide conjugation	$C_{21}H_{18}O_{11}$	445.0770	-0.2	445.0770 [M-H]⁻, 269.0417[M-H-$C_6H_8O_6$]⁻	Aloe emodin

41	7.93	Parent	$C_8H_8O_4$	167.0332	-7.2	167.0334 $[M-H]^-$,123.0431 $[M-H-CO_2]$	Dihydroxy methylbenzoic acid
44	8.31	Parent	$C_{13}H_{14}O_4$	233.0809	-2.1	233.0809 $[M-H]^-$, 189.0707$[M-H-C_2H_4O]^-$	Untitled
45	8.78	Parent	$C_{13}H_{12}O_4$	231.0658.	+0.4	231.0658. $[M-H]^-$ 189.0522$[M-H-C_2H_2O]^-$	Acetyl Methylformylcinnamate
48	10.03	Parent	$C_{11}H_{10}O_3$	189.0552	0.0	189.0552. $[M-H]^-$ 174$[M-H-CH_3]^-$	Methylformylcinnamate
50	10.46	Parent	$C_{22}H_{22}O_{11}$	461.1086	+0.4	461.1086. $[M-H]^-$ 313.0501$[M-H-C_9H_8O_2]^-$	Cinnamoyl-glucogallin
51	10.94	Parent	$C_{14}H_{12}O_3$	227.0698	-4.4	227.0698 $[M-H]^-$ 185.0586$[M-H-C_2H_2O]^-$	3,5,4'-Trihydroxy-trans-stilbene
52	11.82	Parent	$C_9H_{10}O_4$	181.0509	+4.4	181.0509 $[M-H]^-$,137.0583 $[M-H-CO_2]^-$	4-Methoxy-6-methylsalicylic Acid
55	12.47	Parent	$C_{15}H_{10}O_6$	285.0404	+1.8	285.0404$[M-H]^-$239.0888$[M-CH_2O_2]^-$	ω-Hydroxyemodin
M10	13.24	Glucuronide conjugation	$C_{20}H_{22}O_{10}$	421.1145	+2.4	421.1145 $[M-H]^-$, 245.0776 $[M-H-C_6H_8O_6]^-$	Torachrysone
60	13.41	Parent	$C_{15}H_8O_7$	299.0189	-1.0	299.0189 $[M-H]^-$, 255.0308 $[M-H-H_2O]^-$	Emodic acid
M11	13.66	Glucuronide conjugation	$C_{21}H_{18}O_{10}$	429.0822	0.0	429.0822 $[M-H]^-$, 253.0449 $[M-H-C_6H_8O_6]^-$	Chrysophanol
68	15.49	Parent	$C_{15}H_{10}O_6$	285.0398	-0.4	257$[M-H]^-$241.0489$[M-H-CO_2]^-$	lunatin
70	16.37	Parent	$C_{15}H_8O_7$	299.0188	-1.3	299.0188 $[M-H]^-$, 255.00276 $[M-H-H_2O]^-$	Hydroxy physcion
71	16.43	Parent	$C_{16}H_{10}O_6$	297.0402	+1.0	297.0402 $[M-H]^-$,253.0477 $[M-H-CO]^-$	4,5-Dihydroxy-7-methyl-9,10-dioxo-9,10-dihydro-2-anthracenecarboxylic acid 2278948
72	16.83	Parent	$C_{15}H_{10}O_5$	269.0455	+1.9	269.0455 $[M-H]^-$, 240.0724 $[M-H-CHO]^-$	Aloe emodin
74	17.05	Parent	$C_{15}H_8O_6$	283.0250	+2.5	283.0250 $[M-H]^-$,239.0313$[M-H-CO_2]^-$	Rhein
75	17.08	Parent	$C_{17}H_{12}O_6$	311.0543	-4.2	311.0543 $[M-H]^-$, 283.0158 $[M-H-CO]^-$	(4,5-Dihydroxy-9,10-dioxo-9,10-dihydro-2-anthracenyl)methyl acetate
77	17.57	Parent	$C_{17}H_{12}O_6$	311.0547	-2.9	311.0547 $[M-H]^-$, 269.0517 $[M-H-C_2H_3O]^-$	acetat(1,3-Dihydroxy-9,10-dioxo-9,10-dihydro-2-anthracenyl)methyl acetate
78	17.78	Parent	$C_{15}H_{10}O_5$	269.0443	-2.6	269.0443 $[M-H]^-$, 241.0432 $[M-H-CO]^-$	Emodin
79	17.84	Parent	$C_{14}H_{14}O_4$	245.0804	-4.1	245.0804 $[M-H]^-$, 230.0546 $[M-H-CH_3]^-$	Torachrysone