Profiling of components of rhizoma et radix polygoni cuspidati by high-performance liquid chromatography with ultraviolet diode-array detector and ion trap/time-of-flight mass spectrometric detection

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ABSTRACT

Introduction: Rhizoma et Radix Polygoni Cuspidati (Huzhang in Chinese, HZ) is a traditional medicinal plant in China. Many of the components of HZ have been proved to be bioactive while it is difficult to conduct a comprehensive chemical profiling of HZ as a consequence of the absence of efficient separation system and sensitive detective means. We developed a simple and effective method for comprehensive characterization of constituents in HZ. **Objective:** To develop a simple and effective method to characterize the components in HZ and provide useful information for subsequent metabolic studies of HZ. **Materials and Methods:** The components in HZ aqueous extract were characterized by using high performance liquid chromatography with UV diode-array detector (HPLC-DAD) and ion trap/time-of-flight mass spectrometric detection (HPLC-IT/TOF). Stilbenes, anthraquinones, gallates and tannins, naphthalenes and some other compounds were identified and confirmed by diagnostic fragment ions with accurate mass measurements, characteristic fragmentation pathways and relevant published literatures. **Results:** Among the 238 constituents detected in HZ, a total number of 74 constituents were identified unambiguously or tentatively, including 29 compounds reported for the first time in HZ. **Conclusion:** The identification and structure elucidation of these chemicals provided essential data for quality control and further in vivo metabolic studies of HZ.Key words: *Polygonum cuspidatum*, HPLC-DAD, HPLC-IT/TOF, qualitative analysis.

Key words: *Polygonum cuspidatum*, high-performance liquid chromatography-diode array detector, high-performance liquid chromatography-ion trap/time-of-flight, qualitative analysis

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INTRODUCTION

Rhizoma et Radix Polygoni Cuspidati, well-known as Huzhang (HZ) in China and Japanese knotweed in Japan, comes from the stem and root of *Polygonum cuspidatum* Sieb. Et Zucc. This herb has been widely used in Chinese and Japanese folk medicine for the treatment of atherosclerosis, hypertension, cough, suppurative dermatitis, and gonorrhea. HZ contains a variety of chemical groups such as stilbenes, anthraquinones, flavones, and tannins. Previous pharmacological and clinical studies have indicated that several chemical components in HZ are bioactive.[1-6]

Various methods have been used for qualitative analysis of major chemical constituents in HZ. Yi *et al.* developed a high-performance liquid chromatography-electrospray ionization/mass spectrometry (HPLC-ESI/MS method for simultaneously qualitative and quantitative analysis of 9 compounds.[7] Xia *et al.* separated HZ methanol extract by HPLC-diode array detector (HPLC-DAD) and characterized 22 major constituents using HPLC-time-of-flight (TOF/MS).[8] Sun *et al.* detected 20 volatile oil in HZ by gas chromatography-MS.[9] Electrophoresis and NMR have also been adopted for qualitative assessment of HZ.[10,11]

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Despite much effort has been dedicated to chemical profiling of HZ, limited and repeated information about HZ constituents was acquired as a consequence of the absence of efficient separation system and sensitive detector. To comprehensively identify the complex chemicals of HZ, a fast and high sensitive method need to be developed. Recently, HPLC coupled with ion trap and TOF-MS (HPLC-IT/TOF-MS) has been widely used in characterization and chemical profiling of plants.[12] The hybrid ion trap and TOF MS can integrate the advantages of ion trap in producing multistage tandem fragmentations and that of TOF in high resolution and accurate mass measurement, thus providing higher sensitivity and accuracy (error within 5 ppm) than those of TOF and IT-MS. This tandem mass technique has raised the qualitative analysis of herb medicines (HMs) to a new height both in

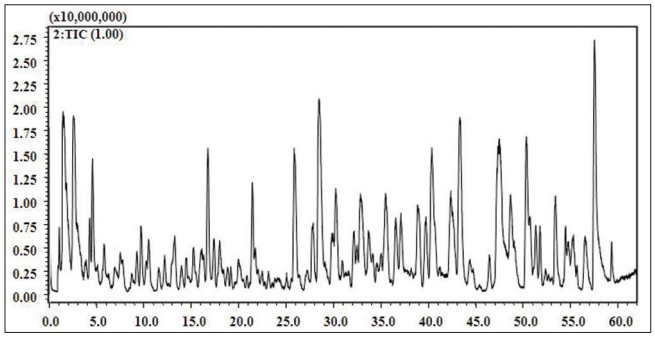


Figure 1: Total ion chromatogram of Huzhang aqueous extraction

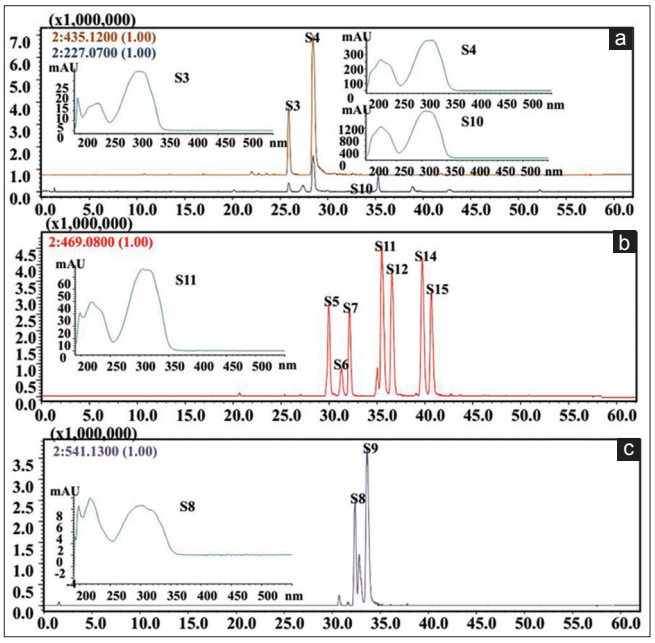


Figure 2: Extracted ion chromatograms of ions at m/z 435.12 (a), 469.08 (b) and 541.13 (c) and ultraviolet spectra of some stilbenes

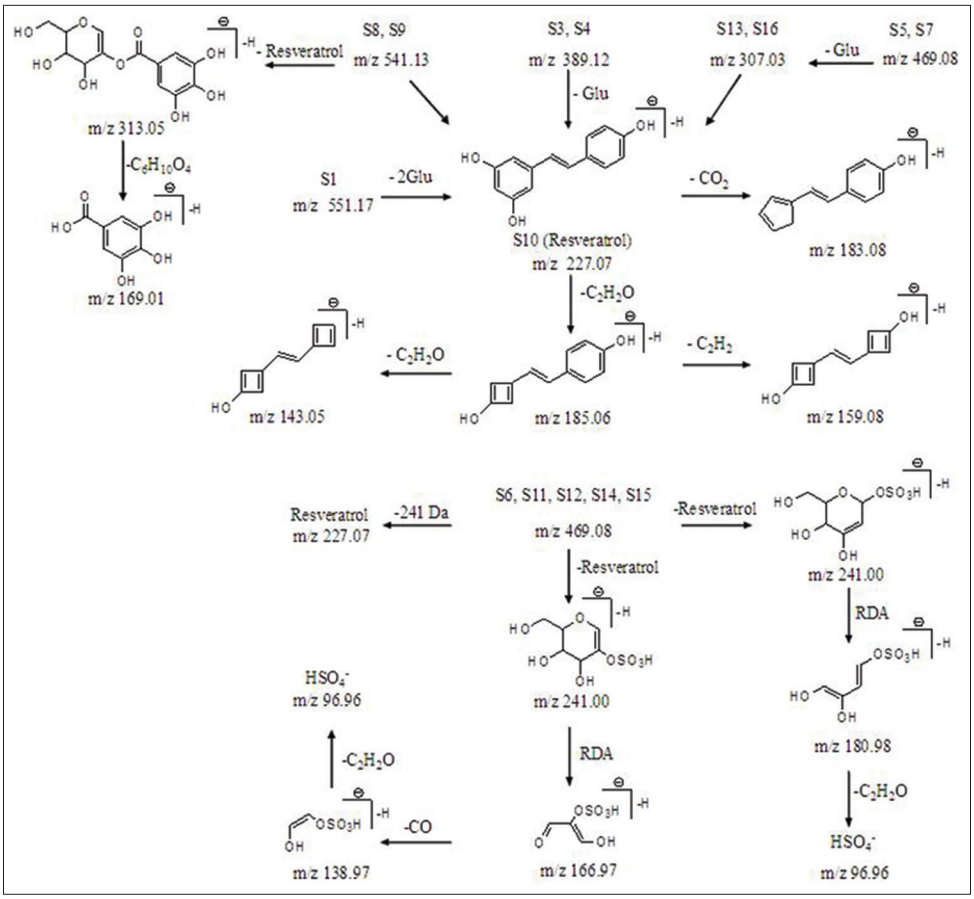


Figure 3: The proposed fragmentation pathways of stilbenes

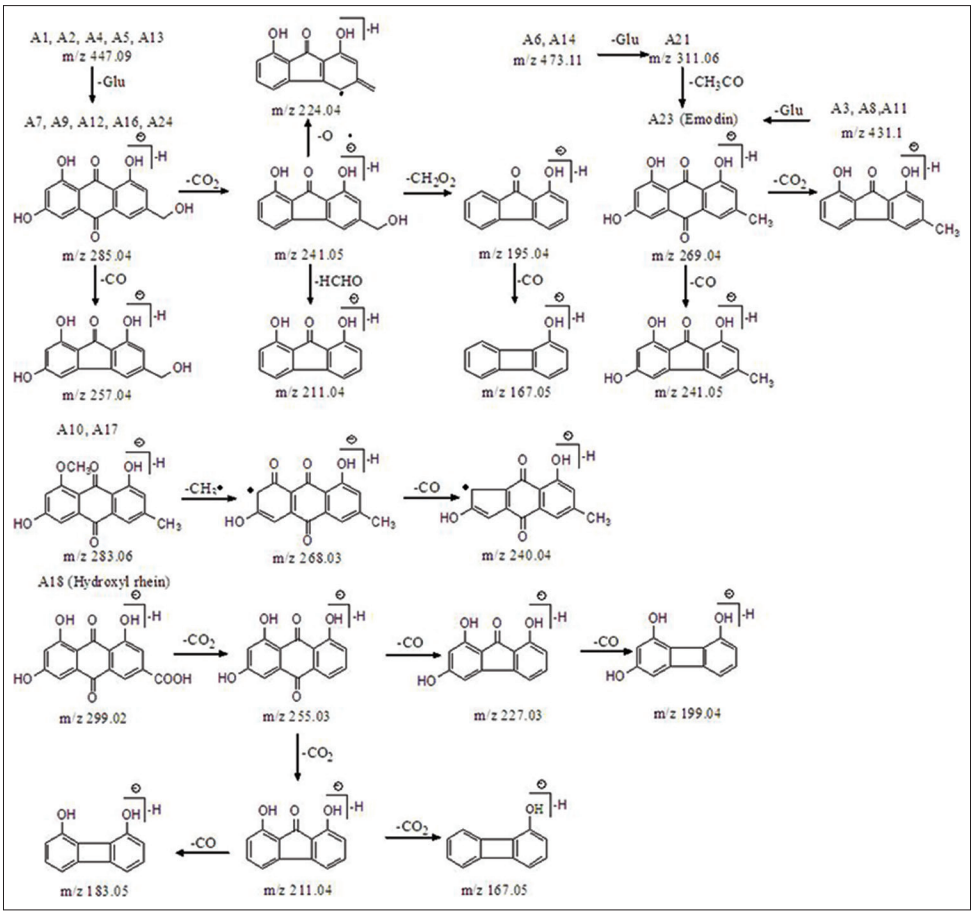


Figure 4: The proposed fragmentation pathways of anthraquinone derivatives

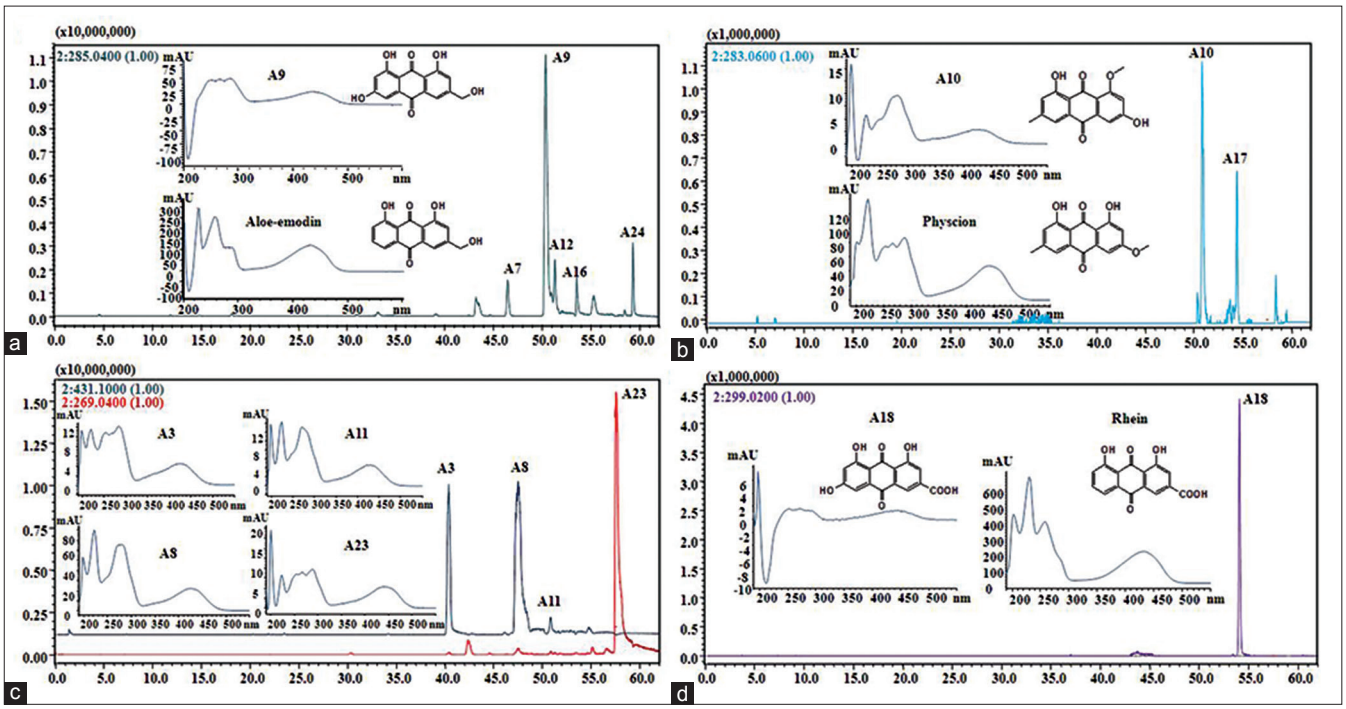


Figure 5: Extracted ion chromatograms of ions at m/z 285.04 (a), 283.06 (b), 431.10 (c) and 299.02 (d); and ultraviolet spectra of some anthraquinone derivatives

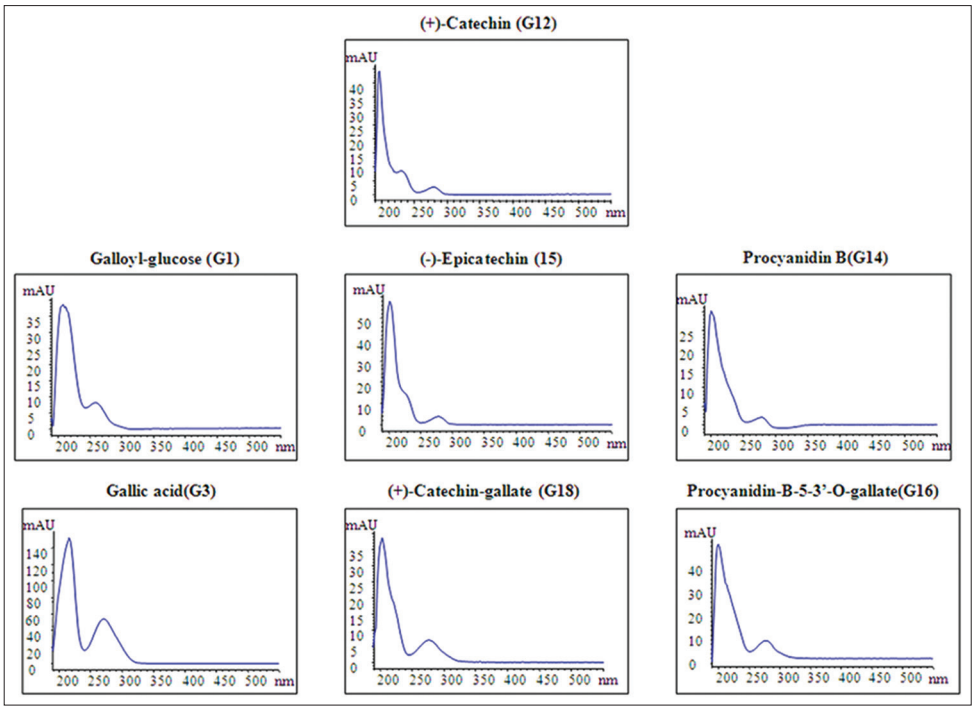


Figure S1: Ultraviolet spectra of some gallates and tannins

analytical speed and accuracy and has been confirmed to be a very powerful tool on the comprehensive identification of both target and nontarget components. Moreover, our previous investigation had clearly indicated the relevance and usefulness of the combination of chromatographic, spectrophotometric, and mass-spectrometric analysis to detect and identify components in complicated samples.[13] Therefore, in the present study, HPLC with diode-array and IT/TOF MS detection was used for separation and identification of the components in HZ extract. Compared with previous methods, more components (74) were characterized or tentatively identified, and 29 of these compounds were reported in HZ for the first time.

MATERIALS AND METHODS

Reagents and chemicals

Reference standards of gallic acid (HPLC >98%), (+)-catechin (HPLC >98%), (-)-epicatechin (HPLC >98%), emodin (HPLC >98%), rhein (HPLC >98%), aloe-emodin (HPLC >98%), physcion (HPLC >98%) were purchased from the National Institutes for Food and Drug Control (Beijing, China). Emodin-1-O-β-D-glucopyranoside (HPLC >98%), emodin-8-O-β-D-glucopyranoside (HPLC >98%), resveratrol (HPLC >98%), and polydatin (HPLC >98%) were purchased from Shanghai Yilin Biotech Co. Ltd (Shanghai, China). Methanol of HPLC grade was purchased from Merck (Darmstadt, Germany). Formic acid (analytical reagent) was purchased from the First Chemical Company of Nanjing (Jiangsu, China). Deionized water was prepared with a Milli-Q system (Millipore, MA, USA).

Plant materials

The rhizome et radix of *P. cuspidatum* was collected in Hubei Province, China and authenticated by Associate Professor Rui Song (State Key Laboratory of Natural Medicine, China Pharmaceutical University, Nanjing, China). The voucher specimens (No. zzj-P-20130813) were deposited at the Herbarium of China Pharmaceutical University, Nanjing, China.

Sample preparation

The preparation of HZ aqueous extraction took published literature as reference with some modifications.[14] 25 g of HZ was immersed in 500 mL distilled water for 1 h and heated with a heating mantle. After boiling, gentle heating was continued until the volume reduced below 250 mL. The decoction was filtered while hot and condensed to 50 mL and stored at −80°C for later use.

High-performance liquid chromatography-diode array detector analysis

The analysis was performed using an Agilent Series 1100 liquid chromatograph (Agilent Technologies, Palo Alto, CA, USA). Chromatography was carried out on a Shimadzu Inertsil C8-3 reversed phase column (150 mm × 2.1 mm, 3 μm). The mobile phase was composed of A (methanol) and B (0.1% formic acid aqueous solution) under gradient elution conditions: 5% A at 0–5 min, 5–30% A at 5–25 min, 30–50% A at 25–45 min, 50–90% A at 45–60 min, and

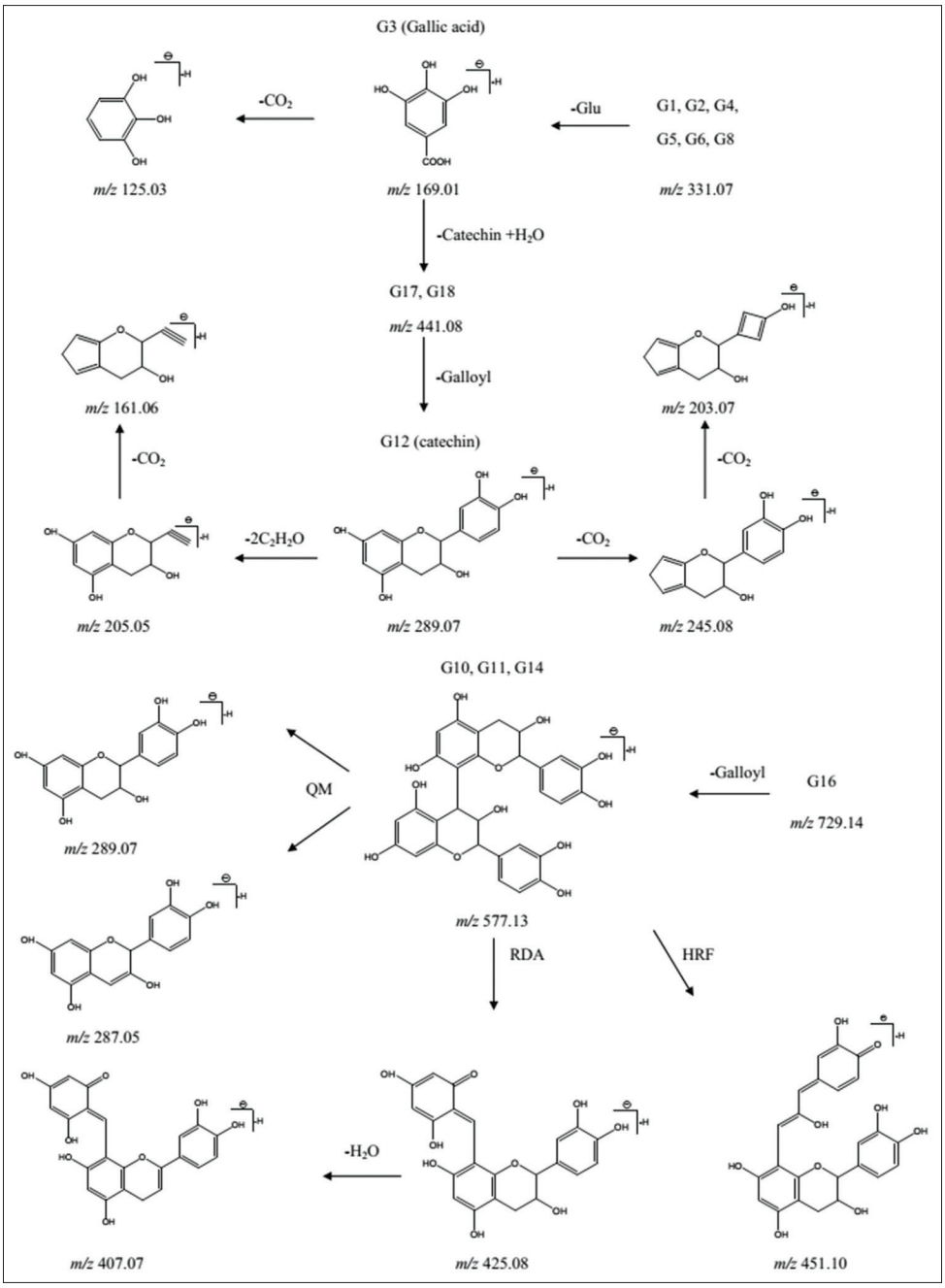


Figure S2: The proposal fragmentation pathways of gallates and tannins

then maintained at 90% A for 2 min. The flow rate was 0.3 mL/min. The column oven temperature was set at 35°C. The DAD detector scanned from 190 nm to 600 nm.

High performance liquid chromatography-ion trap/time-of-flight analysis

The experiments were performed using a Shimadzu (Kyoto, Japan) LC system and a hybrid IT/TOF MS. The LC condition was the same as described above. IT/TOF-MS was operated with ESI source in negative mode. The optimized analytical conditions were as follows: Interface voltage, −3.5 kV; detector voltage, 1.7 kV; nebulizing gas (N_2) flow, 1.5 L/min; drying gas (N_2) flow, 100 kPa; curved desorption line temperature and heat block temperature, 200°C; ion accumulated time, 30 ms; precursor ion selected width, 3.0 amu; collision energy, 30–80%. Mass spectra and chromatograms were acquired and processed with LC-MS solution version 3.6 (Shimadzu, Japan). Shimadzu's Composition Formula Predictor software was adopted to predict chemical formulas for both precursor and product ions.

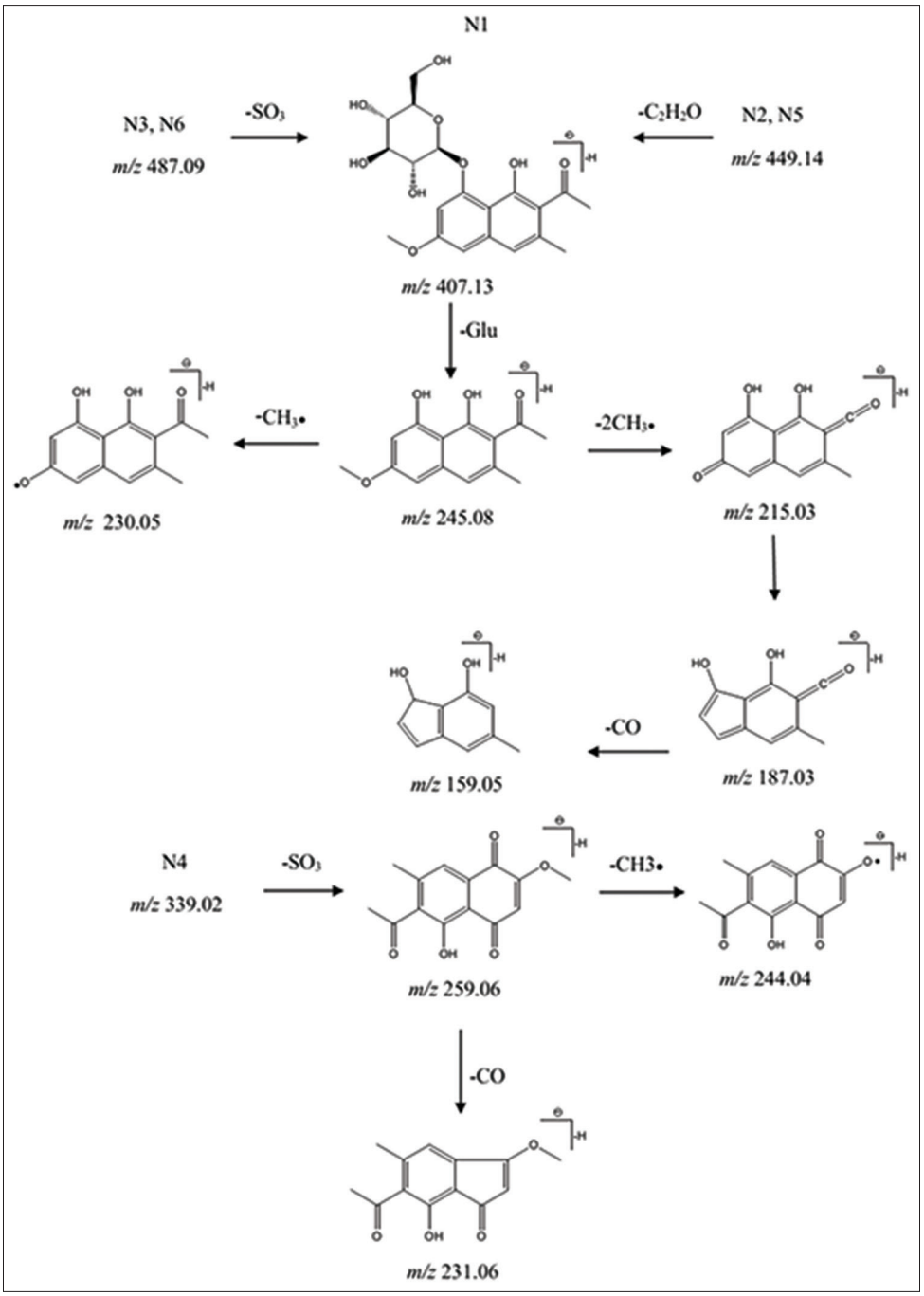


Figure S3: The proposed fragmentation pathways of naphthalenes

RESULTS AND DISCUSSION

Identification of major constituents in Huzhang

Figure 1 shows the typical total ion chromatogram (TIC) of HZ extract acquired in negative mode. In the present study, 238 components were detected in the HZ extract and 74 of them had been confirmed or tentatively identified. Compounds were first classified into several categories based on the characteristic fragment ions obtained from the HPLC-IT/TOF MS analysis. The formulae of detected components were then predicted by the formula predictor software according to the accurate mass provided by their deprotonated ions $[M - H]^-$ or $[M + HCOO]^-$. Following that, the fragmentation pathways of these compounds were proposed on account of their MS^n behaviors. By comparison with the available standard samples, previously published data and the path of biosynthesis, the components were ultimately identified or putatively characterized. The obtained ultraviolet (UV) spectra of some compounds were hired for auxiliary confirmation.

Identification of stilbenes derivatives

A total of 16 compounds were assigned to stilbenes derivatives [Tables S1 and S2], among which three components (S1, S13, S16) were first reported in HZ. By comparison with the reference substance, S4 and S10 were identified as polydatin and resveratrol, respectively. A resveratrol-O-diglucoside (S1), which showed $[M + HCOO]^-$ ion at m/z 597.18 and $[M - H]^-$ ion at m/z 551.17, was detected in HZ extract. It formed fragment ions at m/z 389.12 ($[M\text{-}H\text{-}Glu]^-$) and 227.07 ($[M\text{-}H\text{-}2Glu]^-$). S3 showed the same $[M + HCOO]^-$ and $[M - H]^-$ ions with those of polydatin. Meanwhile, the MS^2 spectra of S3 displayed characteristic fragment ions at m/z 227.07 and 185.06, indicating that it was resveratrol-O-glucoside as well. By comparing the UV spectra of S3 with resveratrol [Figure 2], it was tentatively identified as resveratroloside.[1,15,16] Both S8 and S9 displayed deprotonated ion at m/z 541.13 and shared fragment ions at m/z 227.07 and 185.06. The neutral loss of 314 Da and the dominant fragment ions at m/z 313.05 and 169.01 were the symbols of the galloyl glucose. Hence, they were tentatively characterized as resveratrol 3-O-D-(6' or 2'-galloyl)-glucopyranoside.[17]

Besides the glucosides discussed above, several sulfates were detected in HZ extract. S13 and S16 all gave $[M - H]^-$ ion at m/z 307.03. The following neutral loss of 80 Da forming ions at m/z 227.07 ($[M\text{-}H\text{-}SO_3]^-$) and other MS^2 fragmentation ions consistent with those of resveratrol led to the tentative characterization of S13 and S16 as resveratrol-O-sulfates. S5 and S7 both showed deprotonated ion at m/z 469.08 and characteristic MS^2 fragmentation ions at m/z 307.02 ($[M\text{-}H\text{-}Glu]^-$), 227.07($[M\text{-}H\text{-}Glu\text{-}SO_3]^-$) and 185.06 ($[M\text{-}H\text{-}Glu\text{-}SO_3\text{-}C_2H_2O]^-$), revealing that they might be sulfonylresveratrol-3-O-glucosides.[17] S11, S12, S14, and S15 shared the same precursor ion but almost distinct

Table S1: Identification of stilbenes derivatives

Number	t_R (min)	Precursor ion	MS^n (negative mode)	Identification
S1	20.173	597.18 $[M+HCOO]^-$	MS^2 [597.18]: 227.0680 (44.01); 321.0541 (2.52); 389.1206 (100); 551.1708 (48.79)	**Resveratrol-O-diglucoside**
S2	25.733	405.12 $[M-H]^-$	MS^2 [405.12]: 243.06 (100)	Piceatannol-O-glucoside
S3	25.907	435.12 $[M+HCOO]^-$	MS^2 [435.12]: 227.07 (100); 389.12 (46.12)	Resveratrol-4'-O-β-D-glucopyran oside
		389.12 $[M-H]^-$	MS^2 [389.12]: 143.05 (1.49); 159.08 (1.20); 183.08 (1.05); 185.06 (1.63); 225.0526 (1.05); 227.07 (100)	
S4	28.467	435.12 $[M+HCOO]^-$	MS^2 [435.12]: 227.0679 (100); 389.12 (61.91)	Polydatin*
		389.12 $[M-H]^-$	MS^2 [389.12]: 185.06 (1.17); 227.07 (100)	
S5	29.960	469.08 $[M-H]^-$	MS^2 [469.08]: 185.06 (3.64); 227.07 (100); 307.02 (6.01)	Sulfonylresveratrol-3-O-β-D-gluco pyranoside
S6	31.307	469.08 $[M-H]^-$	MS^2 [469.08]: 227.07 (3.78); 241.00 (100); 306.02 (3.51)	Resveratrol-3-O-β-D-(sulfonyl)-glu copyranoside
			MS^3 [241.00]: 96.96 (100)	
S7	32.120	469.08 $[M-H]^-$	MS^2 [469.08]: 183.08 (1.44); 185.06 (5.57); 227.07 (100); 269.08 (1.71); 307.02 (8.36)	Sulfonylresveratrol-3-O-β-D-gluco pyranoside
S8	32.467	541.13 $[M-H]^-$	MS^2 [541.13]: 169.01 (17.80); 185.06 (1.94); 227.07 (73.55); 313.05 (100); 379.08 (1.06)	Resveratrol 3-O-D-(6' or 2'-galloyl)-glucopyranoside
S9	33.72	541.13 $[M-H]^-$	MS^2 [541.13]: 169.01 (4.01); 227.07 (1.26); 313.05 (100); 495.24 (2.88)	Resveratrol 3-O-D-(6' or 2'-galloyl)-glucopyranoside
S10	35.293	227.07 $[M-H]^-$	MS^2 [227.07]: 143.05 (48.19); 159.08 (21.72); 183.08 (18.20); 185.06 (100)	Resveratrol*
S11	35.507	469.08 $[M-H]^-$	MS^2 [469.08]: 180.98 (2.53); 222.99 (13.55); 227.07 (23.21); 241.00 (100); 281.08 (2.48); 299.09 (2.87); 307.02 (8.36)	Resveratrol-3-O-β-D-(sulfonyl)-glucopyranoside
			MS^3 [241.00]: 96.96 (100); 138.97 (14.51)	
S12	36.573	469.08 $[M-H]^-$	MS^2 [469.08]: 166.96 (0.27); 227.07 (0.47); 241.00 (100)	Resveratrol-3-O-β-D-(sulfonyl) glucopyranoside
			MS^3 [241.00]: 96.96 (100); 138.97 (9.25); 166.97 (3.89)	
S13	38.880	307.03 $[M-H]^-$	MS^2 [307.03]: 143.05 (1.23); 157.06 (0.79); 159.08 (1.28); 183.08 (1.03); 185.06 (5.63); 227.07 (100)	**Resveratrol-O-sulfate**
S14	39.747	469.08 $[M-H]^-$	MS^2 [469.08]: 152.99 (1.21); 180.98 (2.83); 227.07 (1.75); 241.00 (100)	Resveratrol-3-O-β-D-(sulfonyl) glucopyranoside
			MS^3 [241.00]: 96.96 (100); 180.98 (6.55)	
S15	40.693	469.08 $[M-H]^-$	MS^2 [469.08]: 227.07 (1.93); 241.00 (100); 269.08 (3.96)	Resveratrol-3-O-β-D-(sulfonyl) glucopyranoside
			MS^3 [241.00]: 96.96 (100)	
S16	42.733	307.03 $[M-H]^-$	MS^2 [307.03]: 143.05 (2.93); 157.06 (2.16); 159.08 (2.16); 183.08 (1.78); 185.06 (7.02); 227.07 (100); 243.06 (1.19)	**Resveratrol-O-sulfate**

MS: Mass spectrometry. Those components shown in **bold** were reported in Huzhang for the first time. Those components marked with an asterisk (*) were confirmed by comparison with authentic reference.

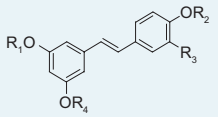
fragment ions with those of S5 and S7. In their MS^2 spectra, the dominant ions were all at m/z 241.00, which was 228 Da less than the precursor ion. With the extra MS^3 spectra of the ion at m/z 241.00, we predicted it as sulfonyl glucose [Figure 3]. Therefore, these four constituents were believed to be resveratrol-3-O-(sulfonyl)-glucosides.[18,19]

IDENTIFICATION OF ANTHRAQUINONES DERIVATIVES

A total of 24 anthraquinone derivatives were characterized or plausibly identified [Tables S3 and S4]. A3, A8, and A23 were separately characterized as emodin-1-O-β-D-glucopyranoside, emodin-8-O-β-D-glucopyranoside and emodin by direct injection of authentic standards.

In the present study, 11 hydroxyl aloe-emodin derivatives were first reported in HZ. A7, A9, A11, A16, A24 shared the $[M - H]^-$ ion at m/z 285.04, 16 Da more than that of aloe-emodin. The sequential loss of CO and CHO radical further indicated that they were aloe-emodin derivatives [Figure 4]. Therefore, they were tentatively identified as hydroxyl aloe-emodin. Compared with aloe-emodin, the UV spectrum of A9 displayed some changes in the shape and bathochromic shift in the band III [Figure 5], revealing that there was a β-OH on the other side of methylol group.[12] Consequently, A9 was tentatively identified as 6-hydroxylaloe-emodin. Five isomers (A1, A2, A4, A5, A13), which could form the aglycone ion at m/z 285.04 after having lost 162 Da and displayed characteristic fragment ions of hydroxyl aloe-emodin, were consequently identified as hydroxyl aloe-emodin-O-glucosides. The precursor ion of A20 produced fragment ion at m/z 285.04 after losing 80 Da, which yield a series of characteristic ions of hydroxyl aloe-emodin at m/z 257.04, 241.05, and 211.04 in MS^3 spectrum, providing evidence for the assumption that A20 was hydroxyl aloe-emodin-O-sulfate.

Table S2: Structures of identified stilbenes derivatives



Compound	R_1	R_2	R_3	R_4
S1	Glc	Glc	H	H
	H	Glc	OGlc	H
S2	Glc	OH	OH	H
	H	Glc	OH	H
	H	H	Glc	H
S3	H	Glc	H	H
S4*	Glc	H	H	H
S5/S7	Glc	SO_3H	H	H
	Glc	H	H	SO_3H
S6/S11/S12/S14/S15	Glc	Sulfonyl-Glc	H	H
S8/S9	Glc	(2"/6"-Galloyl) Glc	H	H
S10*	H	H	H	H
S13/S16	SO_3H	H	H	H
	H	SO_3H	H	H
	H	H	H	SO_3H

Those components shown in **bold** were reported in Huzhang for the first time. Those components marked with an asterisk (*) were confirmed by comparison with authentic reference.

Both A6 and A14 showed the $[M - H]^-$ ion at m/z 473.11, which was identical with that of emodin-O-(acteyl)-glucoside. Nevertheless, the MS^2 fragment ion at m/z 311.05 formed by direct loss of 162 Da from the precursor ion denied the deduction. Based on this evidence, those two compounds were plausibly identified as acetylemodin-O-glucosides. In addition, an acetylemodin (A21), which showed a deprotonated ion at m/z 311.06 and shared most of the fragment ions with emodin, was detected at 55.707 min.

The $[M - H]^-$ ion and a series of characteristic fragment ions of compound A10 and A17 led to the initial assumption that they were physcion and its isomer. The injection of standard sample of physcion overthrow the inference then as both of them had weaker retention than physcion. Besides, the UV spectrum of A10 was quite different from that of physcion [Figure 5]. Those comprehensive hints indicated that they were possibly two more polar isomers of physcion. Taking the retention times of emodin-1-O-β-D-glucopyranoside and emodin-8-O-β-D-glucopyranoside as reference, A10 was tentatively identified as emodin-1-questin which was first reported in HZ, while A17 was characterized as emodin-8-questin reported in literatures.[7]

A11 showed the $[M - H]^-$ ion at m/z 431.10, and shared most of the fragment ions with those of emodin-1 or 8-O-β-D-glucopyranoside. In the UV spectra of A11, the characteristic band III and band V of anthraquinone displayed hypochromatic shift compared with emodin [Figure 5], indicating that the β-OH of emodin was substituted. Consequently, A11 was tentatively identified as emodin-6-O-glucoside.

The formula of A18, which showed $[M - H]^-$ ion at m/z 299.02, was predicted as $C_{15}H_7O_7$. In the MS^2 spectrum, the ion at m/z 255.03 indicated that a carboxyl was attached to the anthraquinone moiety. Thus, A18 was tentatively characterized as hydroxyl rhein. By comparing the UV spectra of A18 with that of rhein [Figure 5], the substitution position was found to be more likely on the

Table S3: Identification of anthraquinones

Number	t_R (min)	Precursor ion	MS^n (negative mode)	Identification
A1	30.907	447.09 $[M-H]^-$	MS^2 [447.09]: 195.05 (0.32); 205.04 (0.27); 211.04 (1.72); 239.04 (0.72); 241.05 (1.24); 256.04 (4.07); 257.04 (0.66); 281.04 (1.96); 285.04 (100); 286.04 (11.60); 298.05 (0.60); 309.04 (5.14); 327.05 (5.14)	**Hydroxyl aloe-emodin-O-glucoside**
A2	39.093	447.09 $[M-H]^-$	MS^2 [447.09]: 169.07 (0.38); 197.06 (9.37); 241.05 (100); 242.05 (7.11); 255.06 (0.38); 265.04 (0.66); 283.06 (1.83); 285.04 (2.04); 403.10 (7.83); 404.11 (1.32); 429.08 (0.49); 430.10 (0.33); 448.09 (1.95); 449.11 (0.38) MS^3 [241.05]: 115.05 (5.05); 131.05 (3.01); 141.07 (4.55); 169.07 (28.32); 182.04 (3.01); 197.06 (100); 199.04 (3.01)	**Hydroxyl aloe-emodin-O-glucoside**
A3	40.373	431.10 $[M-H]^-$	MS^2 [431.10]: 210.03 (1.49); 225.05 (10.14); 240.04 (1.93); 241.05 (2.27); 269.04 (100); 270.05 (5.10) MS^3 [269.04]: 180.05 (1.22); 181.06 (19.52); 182.04 (4.05); 197.06 (12.08); 210.03 (16.56); 224.04 (1.86); 225.05 (100); 241.0458 (23.18)	Emodin-1-O-β-D-glucopyranoside*
A4	42.467	447.09 $[M-H]^-$	MS^2 [447.09]: 185.06 (0.29); 195.05 (0.24); 198.03 (0.45); 212.04 (0.29); 213.05 (0.81); 225.05 (0.61); 226.03 (1.09); 240.04 (0.33); 241.05 (3.32); 253.05 (0.61); 271.07 (0.52); 281.04 (3.24); 284.03 (1.86); 285.04 (100); 286.04 (6.46); 309.04 (3.67); 327.05 (4.14)	**Hydroxyl aloe-emodin-O-glucoside**
A5	44.400	447.09 $[M-H]^-$	MS^2 [447.09]: 211.04 (0.73); 224.05 (0.62); 225.05 (0.95); 227.03 (0.62); 239.04 (1.44); 240.04 (2.30); 241.05 (1.76); 255.03 (0.95); 256.04 (0.73); 268.04 (2.67); 269.04 (7.68); 283.02 (8.90); 284.03 (100); 285.04 (19.95); 286.04 (0.77)	**Hydroxyl aloe-emodin-O-glucoside**
A6	44.773	473.11 $[M-H]^-$	MS^2 [473.11]: 225.05 (0.81); 269.04 (1.37); 307.0534 (1.37); 311.05 (100); 312.05 (8.08); 353.05 (5.56); 353.06 (4.38) MS^3 [311.05]: 224.05 (18.11); 225.05 (19.69); 239.07 (15.69); 240.04 (13.31); 267.06 (35.34); 268.03 (17.32); 269.04 (100); 283.06 (21.28)	**Acetylemodin-O-glucoside**
A7	46.467	285.04 $[M-H]^-$	MS^2 [285.04]: 197.06 (2.24); 241.05 (100); 242.05 (10.50)	Hydroxyl aloe-emodin
A8	47.493	431.10 $[M-H]^-$	MS^2 [431.10]: 210.03 (1.33); 225.05 (9.30); 241.05 (1.56); 265.05 (2.35); 269.04 (100); 270.05 (10.21); 293.04 (6.21); 311.05 (4.10) MS^3 [269.04]: 180.06 (2.84); 181.06 (22.36); 182.04 (4.17); 196.05 (1.39); 197.06 (16.33); 207.04 (2.36); 210.03 (23.41); 224.04 (4.73); 225.05 (100); 241.05 (20.81); 270.0435 (37.09); 271.05 (1.08)	Anthraglycoside B (emodin-8-O-β-D-glucopyranoside)*
A9	50.360	285.04 $[M-H]^-$	MS^2 [285.04]: 167.05 (3.56); 182.04 (1.30); 183.05 (2.44); 195.04 (16.88); 198.03 (2.06); 211.04 (100); 212.04 (9.28); 213.05 (3.65); 223.04 (4.40); 224.04 (18.79); 227.03 (1.87); 228.04 (2.81); 239.03 (11.16); 240.04 (1.87); 241.05 (71.47); 242.05 (3.46); 255.03 (13.40); 256.03 (13.12); 257.04 (54.75); 258.05 (1.12); 268.03 (11.47); 287.04 (14.54)	Hydroxyl aloe-emodin
A10	50.707	283.06 $[M-H]^-$	MS^2 [283.06]: 240.04 (100); 241.04 (8.03); 268.04 (2.03); 269.03 (0.84); 282.05 (1.18); 284.06 (1.02)	**Emodin-1-questin**
A11	50.840	431.10 $[M-H]^-$	MS^2 [431.10]: 225.05 (2.49); 265.04 (1.18); 269.04 (100); 270.05 (4.73); 282.05 (8.51); 283.06 (1.66); 293.04 (3.67); 311.05 (12.75); 335.06 (1.18)	**Emodin-6-O-glucoside**
A12	51.307	285.04 $[M-H]^-$	MS^2 [285.04]: 167.05 (9.20); 195.04 (14.63); 211.04 (91.23); 212.04 (17.30); 224.06 (15.30); 239.03 (13.29); 241.05 (100); 242.06 (7.86); 255.02 (9.20); 256.04 (10.62); 257.04 (28.78); 268.03 (7.86)	Hydroyl aloe-emodin
A13	51.413	447.09 $[M-H]^-$	MS^2 [447.09]: 197.06 (0.53); 198.03 (0.64); 213.06 (2.14); 217.05 (0.96); 240.04 (0.65); 241.05 (1.23); 253.05 (0.44); 257.04 (4.50); 270.06 (0.36); 271.05 (0.36); 281.04 (2.87); 285.04 (100); 286.04 (5.05); 298.04 (0.48); 309.04 (4.33); 327.05 (4.82)	**Hydroxyl aloe-emodin glucoside**
A14	51.880	473.11 $[M-H]^-$	MS^2 [473.11]: 225.05 (6.89); 253.05 (1.60); 265.04 (2.83); 269.04 (100); 270.06 (5.26); 280.03 (1.89); 293.04 (10.95); 311.05 (4.46); 335.05 (1.60); 395.08 (1.60); 413.08 (6.49) MS^3 [269.04]: 225.05 (100); 241.05 (15.23)	**Acetylemodin-O-glucoside**

Contd..

Table S3: Contd...

Number	t_R (min)	Precursor ion	MS^n (negative mode)	Identification
A15	53.413	517.09 $[M-H]^-$	MS^2 [517.09]: 210.03 (0.04); 225.05 (0.47); 241.05 (0.09); 269.04 (30.96); 270.05 (2.41); 293.04 (1.04); 311.06 (1.47); 431.10 (1.86); 473.11 (100); 474.30 (7.02); 475.11 (4.19)	Emodin-8-O-(6'-O-malonyl)-glucoside
A16	53.547	285.04 $[M-H]^-$	MS^2 [285.04]: 167.05 (9.20); 195.04 (14.63); 211.04 (91.23); 212.04 (17.30); 224.04 (15.30); 239.03 (13.29); 241.05 (100); 242.06 (7.86); 255.02 (9.20); 256.04 (10.62); 257.04 (28.78); 268.03 (7.86)	Hydroxyl aloe-emodin
A17	54.280	283.06 $[M-H]^-$	MS^2 [283.06]: 240.04 (100); 241.05 (5.23); 268.03 (4.48)	Emodin-8-questin
A18	54.480	299.02 $[M-H]^-$	MS^2 [299.02]: 167.05 (24.44); 183.05 (18.06); 193.03 (1.85); 199.04 (1.85); 210.03 (8.03); 211.04 (100); 212.04 (2.26); 227.03 (32.43); 237.01 (1.22); 255.03 (67.42); 256.03 (6.16); 271.02 (5.55); 300.02 (1.85)	**6-hydroxylrhein**
A19	54.813	511.05 $[M-H]^-$	MS^2 [511.05]: 210.03 (1.00); 225.06 (6.46); 241.00 (4.86); 241.05 (1.31); 269.04 (100); 270.05 (20.78); 431.09 (32.02)	**Emodin-O-(sulfonyl)-glucoside**
A20	55.280	365.00 $[M-H]^-$	MS^2 [365.00]: 285.04 (100) MS^3 [285.04]: 195.05 (11.89); 198.02 (3.10); 211.04 (100); 212.04 (4.38); 213.05 (7.18); 223.04 (3.10); 224.05 (9.68); 228.04 (5.00); 239.03 (9.99); 240.05 (3.10); 241.05 (79.78); 255.03 (7.18); 256.03 (9.70); 257.04 (69.91); 268.04 (5.92)	**Hydroxy aloe-emodin-O-sulfate**
A21	55.707	311.06 $[M-H]^-$	MS^2 [311.06]: 195.05 (11.25); 196.05 (13.45); 224.05 (100); 225.05 (53.52); 239.07 (20.10); 240.04 (45.57); 265.13 (11.25); 267.07 (25.60); 268.03 (15.64); 269.04 (12.35)	**Acetylemodin**
A22	56.533	511.05 $[M-H]^-$	MS^2 [511.05]: 222.99 (2.24); 225.06 (6.04); 241.00 (73.67); 241.05 (2.29); 269.04 (100); 270.05 (14.29); 311.05 (6.54); 341.06 (1.53); 431.09 (37.77)	**Emodin-O-(sulfonyl)-glucoside**
A23	57.547	269.04 $[M-H]^-$	MS^2 [269.04]: 179.05 (1.04); 180.06 (3.42); 181.06 (18.97); 182.04 (3.68); 197.06 (14.37); 207.04 (2.39); 210.03 (23.51); 224.04 (3.67); 225.05 (100); 226.06 (1.22); 227.03 (1.04); 241.05 (23.78); 270.05 (58.93); 271.05 (4.15)	Emodin*
A24	59.347	285.04 $[M-H]^-$	MS^2 [285.04]: 189.05 (14.03); 213.05 (29.27); 217.05 (16.77); 241.05 (36.21); 257.04 (100)	Hydroxyl aloe-emodin

MS: Mass spectrometry. Those components shown in **bold** were reported in Huzhang for the first time. Those components marked with an asterisk (*) were confirmed by comparison with authentic reference.

other side of the carboxyl. Since the C-6 position was easier to be substituted, A18 was consequently qualified as 6-hydroxylrhein.

A19 and A22 exhibited the same deprotonated ion at m/z 511.05 and roughly identical fragment ions despite little difference in intensity. A consecutive loss of SO_3 and glucoside led to the formation of aglycone ion at m/z 269.04, which was believed to be emodin proved by the diagnostic ions of emodin at m/z 241.05 and 225.05. In the meantime, the fragment ion at m/z 241.00 (sulfonyl glucose) provided further clue that these two compounds were likely to be emodin-O-(sulfonyl)-glucosides.

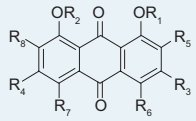
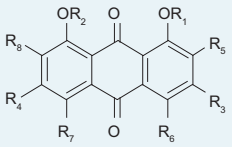
Identification of gallates and tannins

Gallates and tannins can be found in most of the Polygonaceae plants. In the present study, 19 gallates and tannins were detected, and 18 of them were characterized [Tables S5 and S6]. By comparing the retention times, characteristic fragment ions and UV spectra [Figure S1] with references, G3, G12, G15 were identified as gallic acid, (+)-catechin, and (-)-epicatechin, respectively. Five isomers (G1, G2, G4, G5, G6, and G8), which displayed $[M-H]^-$ ion at m/z 331.07 and shared the same characteristic ions at m/z 169.01 and 125.02 [Figure S2], were characterized as galloyl glucose.

Protocatechuic acid was once detected in HZ.[20] In our present study, a protocatechuic acid-O-glucoside (G7) rather than the aglycone itself was found in the herb. The $[M-H]^-$ ion at m/z 315.07 first lost 162 Da to form the deprotonated ion of protocatechuic acid, which produced the ion at m/z 108.02 by a carboxyl crashed down.

G9 and G13 yielded the $[M-H]^-$ ion at m/z 451.12, which could give rise to a further fragment ion at m/z 289.07 after a loss of 162 Da. G13 was eluted between (+)-catechin and (-)-epicatechin, provided evidence that it was more likely to be (-)-epicatechin glucoside under the consideration that (+)-catechin glucoside should have weaker retention than (+)-catechin on reverse phase chromatography. Therefore, G13 was believed to be (-)-epicatechin glucoside while G9 was tentatively

Table S4: Structures of identified anthraquinones derivatives

Compound	R_1	R_2	R_3	R_4	R_5	R_6	R_7	R_8
								
A1/A2/A4/A5/A13	H	Glc	CH_2OH	OH	H	H	H	H
	H	Glc	CH_2OH	H	OH	H	H	H
	H	Glc	CH_2OH	H	H	OH	H	H
	H	Glc	CH_2OH	H	H	H	OH	H
	H	Glc	CH_2OH	H	H	H	H	OH
A3*	H	Glc	OH	CH_3	H	H	H	H
A6/A14	$COCH_3$	Glc	OH	CH_3	H	H	H	H
	Glc	OCH_3	OH	CH_3	H	H	H	H
A7/A9/A12/A16/A24	H	H	CH_2OH	OH	H	H	H	H
	H	H	CH_2OH	H	OH	H	H	H
	H	H	CH_2OH	H	H	OH	H	H
	H	H	CH_2OH	H	H	H	OH	H
	H	H	CH_2OH	H	H	H	H	OH
A8*	Glc	H	OH	CH_3	H	H	H	H
A10	**H**	**CH_3**	OH	CH_3	H	H	H	H
A11	H	H	OGlc	CH_3	H	H	H	H
A15	**(6'-malonyl) Glc**	**H**	OH	CH_3	H	H	H	H
A17	**CH_3**	**H**	OH	CH_3	H	H	H	H
								
A18	H	H	COOH	OH	H	H	H	H
A19/A22	Sulfonyl-Glc	H	OH	CH_3	H	H	H	H
	H	Sulfonyl-Glc	OH	CH_3	H	H	H	H
A20	Glc	H	CH_2OH	OH	H	H	H	H
	H	Glc	CH_2OH	OH	H	H	H	H
	H	H	CH_2OH	OGlc	H	H	H	H
A21	$COCH_3$	H	OH	CH_3	H	H	H	H
	H	$COCH_3$	OH	CH_3	H	H	H	H
	H	H	$OCOCH_3$	CH_3	H	H	H	H
A23*	H	H	OH	CH_3	H	H	H	H

Those components shown in **bold** were reported in Huzhang for the first time. Those components marked with an asterisk (*) were confirmed by comparison with authentic reference.

Table S5: Identification of gallates and tannins

Number	t_R (min)	Precursor ion	MS^n (negative mode)	Identification
G1	3.893	331.07 $[M-H]^-$	MS^2 [331.07]: 168.01 (4.73); 169.01 (11.86); 211.02 (6.61); 271.04 (100); 272.04 (3.67)	Galloyl glucose
G2	4.267	331.07 $[M-H]^-$	MS^2 [331.07]: 125.02 (33.86); 169.01 (100); 170.02 (4.91)	Galloyl glucose
G3	4.560	169.01 $[M-H]^-$	MS^2 [169.01]: 125.02 (100); 126.03 (7.26)	Gallic acid*
		339.03 $[2M-H]^-$	MS^2 [339.03]: 125.02 (7.70); 169.01 (100)	
G4	5.080	331.07 $[M-H]^-$	MS^2 [331.07]: 125.03 (2.61); 169.01 (10.01); 211.02 (8.68); 271.04 (100); 271.19 (1.66); 272.04 (2.92)	Galloyl glucose
G5	7.707	331.07 $[M-H]^-$	MS^2 [331.07]: 151.00 (92.92); 169.01 (100); 193.02 (71.67)	Galloyl glucose
G6	8.933	331.07 $[M-H]^-$	MS^2 [331.07]: 169.01 (100)	Galloyl glucose
G7	9.240	315.07 $[M-H]^-$	MS^2 [315.07]: 108.02 (22.38); 152.01 (49.26); 153.02 (100); 163.04 (16.02)	**Protocatwchuic acid-O-glucoside**
G8	12.947	331.07 $[M-H]^-$	MS^2 [331.07]: 125.02 (19.31); 150.00 (37.65); 151.00 (6.44); 167.04 (7.42); 168.01 (100); 169.01 (25.42); 193.02 (4.12); 313.05 (33.03); 314.05 (3.47)	Galloyl glucose

Contd..

Table S5: Contd...

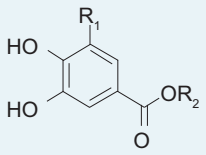
Number	t_R (min)	Precursor ion	MS^n (negative mode)	Identification
G9	14.453	451.12 $[M-H]^-$	MS^2 [451.12]: 203.07 (1.05); 205.05 (1.42); 245.08 (10.39); 289.06 (100); 290.07 (2.10)	(+)-Catechin-5-O-glucoside
G10	14.760	577.13 $[M-H]^-$	MS^2 [577.13]: 245.04 (3.28); 245.08 (3.28); 273.04 (2.89); 287.05 (5.25); 289.07 (40.01); 299.05 (5.64); 407.07 (100); 408.08 (6.36); 425.08 (86.07); 426.08 (2.69); 451.10 (21.27)	Procyanidin B
G11	16.080	577.13 $[M-H]^-$	MS^2 [577.13]: 203.07 (1.20); 205.05 (1.44); 245.04 (4.69); 245.08 (10.30); 269.04 (1.09); 273.04 (7.08); 281.04 (3.35); 285.04 (1.17); 287.05 (12.28); 288.06 (1.64); 289.07 (49.53); 290.07 (7.19); 299.05 (12.53); 300.06 (1.17); 339.08 (1.05); 407.07 (100); 408.08 (24.51); 425.08 (57.96); 426.08 (23.52); 449.08 (1.01); 451.10 (11.69); 452.10 (5.21); 578.13 (8.99); 579.13 (9.96)	Procyanidin B
G12	16.733	289.07 $[M-H]^-$	MS^2 [289.07]: 109.03 (16.59); 125.02 (10.59); 137.02 (16.75); 146.04 (2.17); 151.0402 (13.57); 159.0411 (11.50); 161.0600 (55.15); 164.0096 (12.76); 165.0185 (12.13); 175.0435 (12.76); 179.0335 (23.72); 187.0381 (30.07); 188.0458 (17.38); 203.0687 (100); 205.0475 (95.94); 245.0771 (93.02); 290.0705 (16.26)	(+)-Catechin*
G13	19.160	451.12 $[M-H]^-$	MS^2 [451.12]: 179.04 (3.30); 203.07 (6.26); 205.05 (3.93); 245.08 (20.67); 289.07 (100); 290.07 (5.65)	**(-)-Epicatechin-O-glucoside**
G14	19.947	577.13 $[M-H]^-$	MS^2 [577.13]: 203.07 (1.48); 245.04 (3.08); 245.08 (6.97); 269.04 (1.53); 273.04 (6.76); 281.04 (3.33); 285.04 (1.39); 287.05 (12.02); 289.07 (36.71); 290.07 (3.41); 299.05 (10.40); 300.06 (1.14); 339.08 (1.35); 407.07 (100); 408.08 (25.22); 425.08 (64.44); 425.26 (1.36); 426.08 (31.94); 451.10 (8.16); 452.10 (3.16)	Procyanidin B
G15	21.427	289.07 $[M-H]^-$	MS^2 [289.07]: 109.03 (6.06); 123.05 (2.92); 125.02 (16.38); 135.04 (2.63); 137.02 (13.96); 146.04 (4.07); 149.03 (1.78); 151.04 (10.70); 159.04 (6.63); 161.06 (50.68); 162.03 (3.21); 164.01 (12.40); 165.02 (9.41); 167.03 (5.13); 175.04 (11.11); 175.07 (5.42); 177.06 (1.78); 179.03 (21.37); 180.05 (1.50); 185.06 (1.93); 186.06 (1.50); 187.04 (24.36); 188.05 (17.81); 199.07 (2.36); 203.07 (100); 204.07 (2.08); 205.05 (82.12); 206.05 (3.21); 212.04 (4.07); 217.05 (1.78); 227.07 (8.05); 230.06 (2.78); 231.03 (7.19); 245.08 (77.68); 246.08 (11.68); 247.06 (8.69); 271.06 (3.07); 290.07 (21.08)	(-)-Epicatechin*
G16	23.840	729.14 $[M-H]^-$	MS^2 [729.14]: 243.03 (1.92); 245.04 (1.51); 245.08 (3.82); 255.03 (1.46); 269.04 (1.59); 271.06 (2.81); 285.03 (2.14); 287.05 (5.82); 289.07 (40.73); 290.07 (4.73); 299.05 (2.58); 303.05 (2.03); 331.05 (1.43); 389.06 (4.14); 407.07 (100); 407.25 (2.61); 407.46 (1.46); 408.08 (19.58); 409.08 (1.57); 425.08 (2.48); 433.09 (2.20); 441.08 (34.46); 442.08 (12.75); 443.08 (1.65); 451.10 (27.97); 452.10 (7.81); 541.11 (1.35); 559.09 (23.75); 560.09 (12.78); 561.10 (2.38); 577.11 (9.08); 578.11 (6.48); 603.11 (8.19); 604.11 (5.52); 730.14 (13.17); 731.14 (12.09)	Procyanidin-B-1-3'-O-gallate/ Procyanidin-B-5-3'-O-gallate
G17	27.787	441.08 $[M-H]^-$	MS^2 [441.08]: 137.02 (0.46); 145.02 (0.72); 149.03 (0.55); 161.06 (0.81); 165.02 (1.08); 167.00 (0.55); 169.01 (39.87); 170.02 (2.61); 179.03 (2.92); 187.04 (0.50); 193.01 (15.28); 194.01 (0.64); 203.07 (4.88); 205.01 (1.23); 205.04 (3.64); 211.04 (0.55); 213.02 (0.55); 227.07 (0.77); 245.08 (13.22); 253.04 (3.19); 259.06 (2.38); 271.06 (6.58); 272.06 (1.58); 289.07 (100); 290.07 (17.46); 303.05 (4.12); 331.04 (7.34); 442.08 (9.46); 443.08 (2.38)	(+)-Catechin or (-)-epicatechin gallate
G18	29.787	441.08 $[M-H]^-$	MS^2 [441.08]: 169.01 (29.90); 193.01 (20.83); 203.07 (1.62); 205.01 (1.63); 205.04 (1.63); 245.08 (7.43); 253.05 (1.63); 259.06 (2.16); 271.06 (7.03); 287.05 (1.90); 289.07 (100); 289.22 (1.34); 303.05 (2.84); 331.04 (4.73)	(+)-Catechin or (-)-epicatechin gallate
G19	34.147	505.12 $[M-H]^-$	MS^2 [505.12]: 161.05 (1.63); 189.05 (6.73); 203.07 (3.55); 205.05 (3.07); 215.03 (59.74); 227.07 (1.80); 233.04 (9.32); 245.08 (13.63); 253.05 (2.13); 271.06 (2.92); 289.07 (100); 290.06 (1.63); 367.08 (4.56)	Not identified

MS: Mass spectrometry. Those components shown in **bold** were reported in Huzhang for the first time. Those components marked with an asterisk (*) were confirmed by comparison with authentic reference.

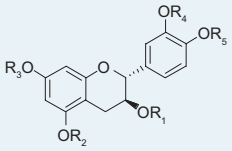
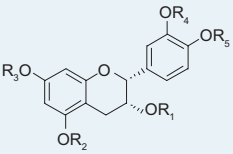
identified as (+)-catechin-5-O-glucoside.[21] Another pair of positional isomers, G17 and G18, gave the deprotonated ion at m/z 441.08. In the MS^2 spectra, the dominant ion at m/z 289.07 and other characteristic fragment ions implied that they were (+)-catechin or (-)-epicatechin derivatives. Since the precursor ion was 152 Da more than the dominant fragment ion at m/z 289.07, thus guiding us to tentatively identify G17 and G18 as (+)-catechin or (-)-epicatechin gallates. The fragment ion at m/z 169.01 also provided evidence for this assumption.

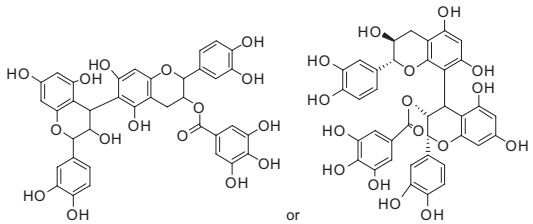
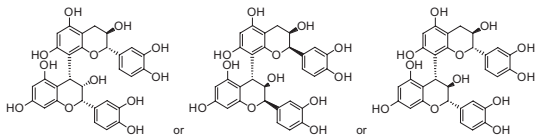
Three enantiomers of procyanidin B (G10, G11, and G14) were found in the EICs at m/z 577.13. The deprotonated molecule of these compounds could further yield the most

Table S6: Structures of identified gallates and tannins



	R_1	R_2
G1/G2/G4/G5/G6/G8	OH	Glc
G3*	OH	H
G7	H	Glc

	R_1	R_2	R_3	R_4	R_5
					
G9	H	Glc	H	H	H
G12*	H	H	H	H	H
G17/G18	Galloyl	H	H	H	H
	H	Galloyl	H	H	H
	H	H	Galloyl	H	H
	H	H	H	Galloyl	H
	H	H	H	H	Galloyl
					
G13	Glc	H	H	H	H
	H	Glc	H	H	H
	H	H	Glc	H	H
	H	H	H	Glc	H
	H	H	H	H	Glc
G15*	H	H	H	H	H
G17/G18	Galloyl	H	H	H	H
	H	Galloyl	H	H	H
	H	H	Galloyl	H	H
	H	H	H	Galloyl	H
	H	H	H	H	Galloyl

Possible structures	
G16	
G10/ G11/ G14	

Those components shown in **bold** were reported in Huzhang for the first time.Those components marked with an asterisk (*) were confirmed by comparison with authentic reference.

abundant ion at m/z 407.07, which might result from Retro Diels-Alder reaction (RDA) and the following loss of H_2O. The fragment ion at m/z 289.07 can be attributed to the quinone methide (QM) fission cleavage of the type-B interflavan bond resulting from the loss of an (+)-catechin or (-)-epicatechin residue.[22] Besides, a type-B galloylated procyanidin (G16) with the precursor ion at m/z 729.14 was detected at 23.840 min. In the MS^n spectrum, RDA reaction, heterocyclic ring fission, and QM fission cleavage had led to the formation of characteristic ions at m/z 407.07, 451.10, and 289.07 [Figure S2].

Identification of naphthalene derivatives

Six naphthalene derivatives were detected and characterized in HZ [Tables S7 and S8]. Five of them (N1, N2, N3, N5, N6) formed the same fragment ion at m/z 245.08, which was predicted as torachrysone supported by the characteristic fragment ions at m/z 230.05, 215.03, 187.03, and 159.05 [Figure S3]. N1 was identified as torachrysone-8-O-β-D-glucoside.[23] N2 and N5 were characterized as two positional isomers of torachrysone-8-O-(acetyl)-glucosides since they directly lost 204 Da (acetyl glucose) and formed the base peak at m/z 245.08. N3 and N6 shared the same $[M-H]^-$ ion at m/z 487.09, which was 80 Da more than that of N1, indicating that the two compounds were highly likely the sulfate ester of N1, and that the sulfate moiety was connected to glucose since there was no fragment ion which formed by directly loss of hexoside.

The MS^2 spectrum of N4 was entirely different from those of the five components discussed above. The $[M-H]^-$ ion at m/z 339.02 lost 80 Da and yielded the dominant ion at m/z 259.06 [Figure S3], which was tentatively identified as 2-methoxy-6-acethyl-7-methyljuglone.[20] Therefore, N4 was tentatively identified as 2-methoxy-6-acethyl-7-methyljuglone-5-O-sulfate. Other fragment ions at m/z 244.04 ($[M\text{-}H\text{-}CH_3\bullet]^{-\bullet}$) and 231.06 ($[M\text{-}H\text{-}CO]^-$) provided further evidence for the conclusion.

Identification of other compounds

Totally, 20 components which could hardly be assigned to categories above were detected, and 10 of them were tentatively identified [Tables S9 and S10]. O1 and O5 were characterized as critic acid[24] and5, 7-dihydroxy-1 (3H)-isobenzofuran-one,[25] respectively. O4 and O6 were believed to be glucosides of O5 since the precursor ion of them was 162 Da more than that of O5, and they share the other fragment ions with O5.

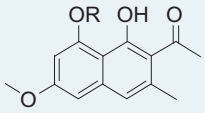
O6 displayed the deprotonated ion at m/z 329.09, which produced the ion at m/z 167.03 after losing 162Da, indicating that it was a glucoside. The aglycone was predicted as vanillic acid proved by the ion at m/z

Table S7: Identification of naphthalene derivatives

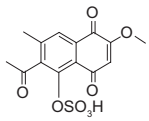
Number	t_R (min)	Precursor ion	MS^n (negative mode)	Identification
N1	43.373	407.13 $[M-H]^-$	MS^2 [407.13]: 215.03 (1.41); 230.06 (13.92); 231.06 (0.47); 245.08 (100); 246.08 (15.42); 247.08 (0.11); 408.13 (3.82); 409.14 (0.88) MS^3 [245.08]: 202.06 (0.37); 215.03 (4.79); 230.06 (100); 231.06 (5.30); 246.08 (6.69)	Torachrysone-8-O-β-D-glucoside
N2	48.587	449.14 $[M-H]^-$	MS^2 [449.14]: 215.03 (2.00); 230.05 (11.87); 245.08 (100) MS^3 [245.08]: 215.0336 (2.78); 230.0557 (100)	Torachrysone-8-O-(acetyl)-glucoside
N3	48.707	487.09 $[M-H]^-$	MS^2 [487.09]: 159.05 (0.19); 187.03 (0.19); 202.06 (0.19); 215.03 (7.10); 216.04 (0.41); 230.05 (27.01); 231.06 (1.32); 241.00 (1.19); 245.08 (100); 246.08 (10.17); 287.08 (0.32); 407.13 (6.75); 408.13 (1.45); 488.08 (5.34); 489.08 (2.26) MS^3 [245.08]: 215.03 (4.38); 230.05 (100); 231.06 (0.57)	**Torachrysone-8-O-(sulfonyl)-glucoside**
N4	49.160	339.02 $[M-H]^-$	MS^2 [339.02]: 231.06 (2.74); 244.04 (7.23); 259.06 (100); 260.06 (2.23)	**2-Methoxy-6-acethyl-7-methyljuglone-5-O-sulfate**
N5	51.133	449.14 $[M-H]^-$	MS^2 [449.14]: 215.03 (1.62); 230.06 (6.92); 245.08 (100); 246.08 (3.02) MS^3 [245.08]: 215.03 (3.11); 230.06 (100)	Torachrysone-8-O-(acetyl)-glucoside
N6	52.387	487.09 $[M-H]^-$	MS^2 [487.09]: 215.03 (2.55); 230.05 (10.96); 245.08 (100) MS^3 [245.08]: 215.03 (4.15); 230.06 (100)	**Torachrysone-8-O-(sulfonyl)-glucoside**

MS: Mass spectrometry. Those components shown in **bold** were reported in Huzhang for the first time. Those components marked with an asterisk (*) were confirmed by comparison with authentic reference.

Table S8: Structures of naphthalene derivatives



	R
N1	Glc
N2/N5	Acetyl-Glc
N3/N6	Sulfonyl-Glc

	Possible structures
N4	

Those components shown in **bold** were reported in Huzhang for the first time. Those components marked with an asterisk (*) were confirmed by comparison with authentic reference.

123.05 ([M-H-Glu-CO_2]).[26] Meanwhile, the occurrence of the two characteristic ions at m/z 239.05 ([M-H-90 Da]$^-$) and 209.04 ([M-H-120 Da]$^-$) proved that O6 was likely to be vanillic acid-C-glucoside though the exact substitution position remained to be further confirmed.

O7 was characterized as a sulfate since the $[M-H]^-$ ion lost 80 Da directly and formed ion at m/z 233.04, which was identical with the deprotonated ion of 5-carboxymethyl-7-hydroxy-2-methyl chromone.[27] Another sulfate, O8, shared the same fragment ion at m/z 189.05 with O7, indicating they might have the same 2, 5-dimethyl-7-hydroxylchromone nucleus. The most abundant ion at m/z 231.06 prompted that there might be an acetyl on the heterocyclic nucleus or connected to the hydroxyl group. Since sulfation usually acted on the hydroxyl group, the acetyl was more possibly substituted on the heterocyclic nucleus. Considering the electronic effect and steric hindrance, O8was tentatively characterized as 3-acetyl-2, 5-dimethylchromone-7-O-sulfate. O9 displayed the $[M-H]^-$ ion at m/z 269.02 and the fragment ion at m/z 189.05, thus indicating that the compound might be 2,5-dimethylchromone-7-O-sulfate.

The $[M-H]^-$ at m/z 285.01 of O10 directly lost 80 Da, forming an ion at m/z 205.05, which was predicted as $C_{11}H_9O_4^-$. The aglycone was tentatively characterized as 7-hydroxy-4-methoxy-5-methylcoumarin.[7] Consequently, O10 was tentatively identified as 4-methoxy-5-methylcoumarin-7-O-sulfate.

SUMMARY

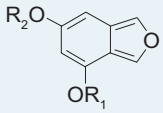
A relatively comprehensive chemical profiling of HZ was achieved by combination of HPLC-DAD and HPLC-IT/TOF analysis. 74 compounds including stilbenes, anthraquinones, gallates and tannins, naphthalenes along with some other minor components were identified or presumed based on their accurate mass, fragment patterns, and characteristic UV spectra. Besides, 29 of these components were reported in HZ for the first time. Although the substitution positions of some components remained to be further confirmed by other ancillary qualitative methods,

Table S9: Identification of other compounds

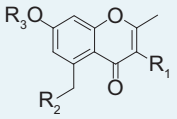
Number	t_R (min)	Precursor ion	MS^n (negative mode)	Identification
O1	2.573	191.02 $[M^-H]^-$	MS^2 [191.02]: 87.01 (1.41); 110.97 (1.21); 111.01 (100); 111.09 (1.61); 173.01 (2.12)	Citric acid
O2	5.787	327.07 $[M-H]^-$ 373.08 $[M+HCOO]^-$	MS^2 [327.07]: 165.02 (100) MS^2 [373.08]: 165.02 (100); 327.07 (25.37) MS^3 [165.02]: 121.03 (100)	**5,7-dyhydroxy-1 (3H)-isobenzofuran-one-O-glucoside**
O3	10.20	301.09 $[M-H]^-$ 347.10 $[M+HCOO]^-$	MS^2 [301.09]: 139.04 (100) MS^2 [347.10]: 124.02 (6.83); 139.04 (100); 161.04 (26.99); 301.09 (84.94)	Tachioside/isotachioside
O4	10.480	327.07 $[M-H]^-$	MS^2 [327.07]: 121.03 (6.33); 165.02 (100); 189.02 (1.01); 207.03 (1.32)	**5,7-dyhydroxy-1 (3H)-isobenzofuran-one-O-glucoside**
O5	13.267	165.02 $[M-H]^-$	MS^2 [165.02]: 77.04 (17.38); 93.04 (49.74); 121.03 (100); 122.04 (28.27); 166.02 (95.92)	5,7-dyhydroxy-1 (3H)-isobenzofuran-one
O6	15.213	329.09 $[M-H]^-$	MS^2 [329.09]: 123.05 (1.99); 167.03 (23.66); 209.04 (100); 210.04 (2.69); 239.05 (7.89)	**Vanillic acid-C-glucoside**
O7	30.240	313.00 $[M-H]^-$	MS^2 [313.00]: 147.04 (0.38); 189.05 (100); 190.06 (2.14); 233.04 (55.31); 234.05 (4.97); 314.00 (1.67)	**5-carboxymethyl-2-methylchromone-7-O-sulfate**
O8	35.320	311.02 $[M-H]^-$	MS^2 [311.02]: 189.05 (2.95); 231.06 (100); 232.07 (12.02)	**3-acetyl-2,5-dimethylchromone-7-O-sulfate**
O9	42.373	269.02 $[M-H]^-$	MS^2 [269.02]: 189.05 (100); 189.18 (1.99); 189.26 (0.65); 189.32 (1.50); 189.47 (0.53); 190.06 (3.08) MS^3 [189.05]: 146.04 (100); 174.03 (71.81)	**2,5-dimethylchromone-7-O-sulfate**
O10	43.240	285.01 $[M-H]^-$	MS^2 [285.01]: 161.03 (0.20); 162.03 (0.20); 190.03 (9.42); 205.05 (100); 206.05 (2.93); 286.01 (5.95)	**4-methoxy-5-methylcoumarin-7-O-sulfate**

MS: Mass spectrometry. Those components shown in **bold** were reported in Huzhang for the first time. Those components marked with an asterisk (*) were confirmed by comparison with authentic reference.

Table S10: Structures of other compounds

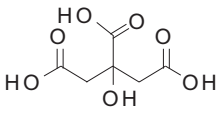


	R_1	R_2
O2/O4	Glc	H
	H	Glc
O5	H	H

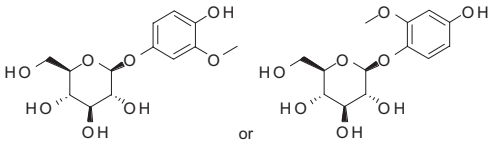


	R_1	R_2	R_3
O7	H	COOH	SO_3H
O8	$COCH_3$	H	SO_3H
O9	H	H	SO_3H

O1

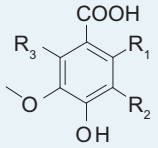


O3



Contd..

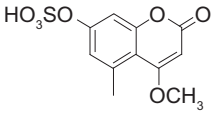
Table S10: Contd...



	R_1	R_2	R_3
O6	Glc	H	H
	H	Glc	H
	H	H	Glc

Possible structures

O10



Those components shown in **bold** were reported in Huzhang for the first time. Those components marked with an asterisk (*) were confirmed by comparison with authentic reference.

the subtle combination of HPLC-DAD and HPLC-IT/TOF method established in the present study could provide reference method for quality control of HZ and provide a basis for further metabolic studies *in vivo* of HZ. In addition, the high sensitive method could yet be regarded as a new perspective of componential analysis of other HMs, especially those containing components with chromophores.

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