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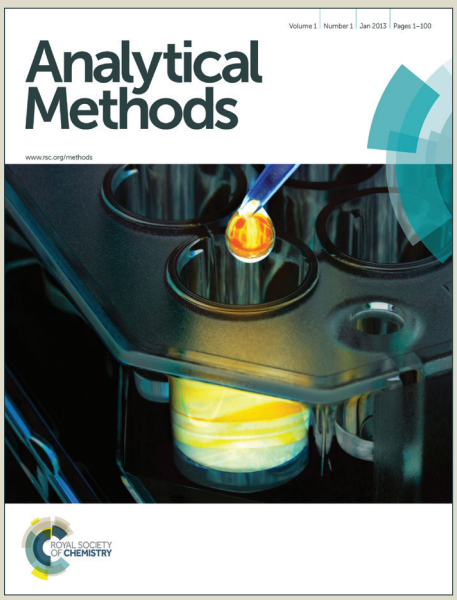

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ARTICLE TYPE

Simultaneous quantification of 17 bioactive constituents in *Sarcandra glabra* by liquid chromatography–electrospray ionisation–mass spectrometry

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A high performance liquid chromatography with electrospray tandem mass spectrometry (HPLC-ESI-MS/MS) method has been developed to quantify 17 bioactive compounds in *Sarcandra glabra* and its preparations simultaneously. These constituents including flavones, coumarins, caffeoyl derivatives and sesquiterpenoids, were detected in the negative ion mode ESI-MS and quantified by multiple reaction monitor (MRM). All 17 constituents were separated and determined within 17 minutes. The linear regressions were acquired with $r^2 > 0.9990$, respectively. The precision was evaluated by intra- and inter-day tests, and relative standard deviation (R.S.D.) values were reported within the range of 0.86–2.29%, 1.16–4.83% and 0.87–4.94%, 0.69–4.92% for mixed standard solutions and extract solutions, respectively. The recovery studies for the assayed constituents were observed over the range of 80.20–119.68%. It demonstrated that the method developed was successfully applied for quantification of 17 main constituents, which provided a new basis for overall assessment on quality of *S. glabra* and its preparations.

Introduction

Sarcandra glabra (Thunb) Nakai (Chloranthaceae family) is a well known traditional Chinese medicine (TCM) which grows in south of China and Japan, and southeastern Asian. The whole plant of *S. glabra* and its water extract have been officially listed in the Chinese Pharmacopoeia, while its medicinal preparations made from *S.glabra* extract, such as Zhongjiefeng tablets and Xiekang capsules, are mainly used to treat inflammation related diseases, such as acute influenza, pharyngolaryngitis, thrombocytopenia, pneumonia, cellulites, appendicitis, shigellosis, leukoderma vitiligo, abscess and cancer, etc [1]. Many bioactive compounds have been isolated from this plant, including caffeoylquinic acids, rosmarinic acid derivatives, flavonoids, coumarins and sesquiterpenoids, etc [2-4]. These types of components should be responsible for its medicinal use, due to their wide range of biological activities, including antioxidant, anti-inflammatory, anti-angiogenic, and antitumor activities [5-8].

At present, the quality control of *S.glabra* is mainly conducted according to the Chinese Pharmacopoeia, in which only isofraxidin and rosmarinic acid are determined by high-performance liquid chromatography–ultraviolet detection [1], and the analytical method is inadequate for the comprehensive quality control. We have developed an ultra-high pressure liquid chromatography (UPLC-UV) technique for simultaneous quantitative analysis of 10 bioactive components [9]. However, time-consuming analytical procedures, relatively low sensitivity and selectivity, and serious matrix interference are the major obstacles for further comprehensive quality control. Since mass spectrometry coupled to liquid chromatography (LC/MS) has proved to be a very powerful analytical technique, and we have developed a fast and reliable method based on an ultra-high pressure liquid chromatography coupled with photodiode-array (PDA) detection and a high-resolution mass spectrometer for comprehensive identification of constituents in *S. glabra* and its related preparations [10]. An HPLC–ESI-TOF-MS analyses method has also been reported for identifying herb-markers in *S. glabra* [11]. However, the specific content of many main constituents in *S. glabra* with different origination and preparations are still unknown. So it is urgent to further establish a rapid and sensitive LC-MS analytical method for simultaneous quantification of the major constituents in *S. glabra*.

In this study, we aimed at developing an effective LC-ESI-MS method for simultaneous quantification of 17 bioactive constituents in *S. glabra* and its related preparations.

Experimental

Chemicals, reagents and materials

Acetonitrile was of HPLC grade from Fisher Chemicals (Fairlawn, NJ, USA). Formic acid and other reagents were of analytical grade from Guangzhou Chemical Reagent Corporation (Guang zhou, China). Distilled water was obtained from Watsons Co.,Ltd. (Guang zhou, China).

The internal standard substance (I.S.) of naringin (> 98%) was purchased from the National Institute for the Control of Pharmaceutical and Biological Products (Beijing, China). Fraxin (**5**) and quercitrin (**16**) were bought from Weikeqi Standards Corporation (Sichuan, China). Protocatechuic acid (**1**), neochlorogenic acid (**2**), eleuthe roside B_1 (**3**), chlorogenic acid (**4**), cryptochlorogenic acid (**6**), caffeic acid (**7**), isofraxidin (**8**), neoastilbin (**9**), astilbin (**10**), rosmarinic acid 4-*O*-*B*-D-glucopyrannoside (**11**), neoisoastilbin (**12**), isoastilbin (**13**), rosmarinic acid (**14**), quercetin-3-*O*-*β*-D-glucuronide (**15**), and

chloranthalactone E (**17**) were isolated from the whole plant of *S. glabra* by the author. Their structures were determined by ^{1}H NMR, ^{13}C NMR, DEPT and MS spectral analysis (supporting information), and they were further purified by semi-preparative HPLC until their purities were no less than 98% by UPLC analysis. The chemical structures of the determined constituents and the internal standard substance were shown in Figure 1.

Twenty-two batches of *S. glabra* originating from eleven habitats including Sichuan, Fujian, Zhejiang, Anhui, Jiangxi, Guizhou, Yunan, Hebei, Hubei, Guangxi, and Guangdong province of China, were purchased from local producing area or local drug stores; Zhongjiefeng tablets (Batch number: 090201) was got from Shanghai Xinyi Jiahua Pharmaceutical Corporation, China; Qingre Xiao yanning capsule (Batch number: N12015) was got from Guangzhou Jingxiutang Pharmaceutical Corporation, China; Xiekang capsule (Batch number: Z36021677) was got from Jiang Xi Herbi-sky Co., Ltd, China. Zhongjiefeng dispersible tablet (Batch number: 20120606) was got from Zhejiang Weikang Pharmaceutical Corporation, China. The voucher specimens authenticated by the author and deposited in the Center for Laboratory, Second Affiliated Hospital, Guang zhou University of Traditional Chinese Medicine.

Chromatographic and mass spectrometry conditions

The LC system consisted of an Agilent Technologies Series 1200 system (Agilent Technologies, Inc., USA) equipped with a G1322A degasser, a G1312B SL binary pump, a G1367C high-performance autosampler (HiP ALS SL), and a G1316B TCC SL thermostatted column compartment (Santa Clara, CA, USA). Chromatographic separations were performed on an Inertsil ODS-3 column (3 μm, 100 × 2.1 mm, GL Sciences Inc., Tokyo, Japan) using methanol (A)–0.1% formic acid (B) (20:80, v/v) as initial proportion of the gradient elution. The gradient profile was set as follows: 0.0 min 80% B eluent, 2.8 min 75% B eluent, 3.0 min 70% B eluent, 6.0 min 68% B eluent, 6.5 min 65% B eluent, 12.0 mim, 60% B eluent, 13.5 mim, 45% B eluent, 15.0 mim, 35% B eluent, 17.0 mim, 20% B eluent. The set column temperature was 40 °C. The HPLC flow rate was 0.4 mL/min. Aliquots of 5 μL were injected into HPLC system for LC-MS analysis.

A 6460 triple-quadrupole mass spectrometer (Agilent Technologies, Inc., USA) was operated with an Agilent G1958-65138 ionization source in the negative electrospray ionization (ESI) mode. An Agilent Mass Hunter workstation (Agilent Technologies, Inc., USA) was used for the control of equipment, data acquisition, and analysis. For the optimization of MS/MS parameters, the standard solutions of 17 constituents and naringin (IS) prepared in 30% methanol were infused in the mobile phase (0.4 mL. min^{-1}) at a flow rate of 20 μL min^{-1} using a syringe pump (Harvard Apparatus, Holliston, MA, USA). The instrument was operated with the capillary voltage at -3.0 kV, and nozzle voltage at -1.5 kV. The drying gas and sheath gas temperature were 350 ºC and 150 ºC, respectively, while the flow rate were 11 L/min and 7 L/min, respectively. Nitrogen was used as nebulizer gas of 30 psi. Multiple reaction monitoring (MRM) was employed for data acquisition (Table 1). Both quadrupoles were set to 1.0 unit mass resolution, respectively, and the dwell times were 50 ms for each *m/z* channel. Data acquisition and quantification were performed by Mass Hunter Workstation software B.02.01 (Agilent Technologies, Torrance, USA).

Preparation of standard solutions

A stock solution containing all 17 reference standards was prepared by dissolving the reference standards in 30% methanol to final concentration of 3.45 μg/mL for protocatechuic acid (**1**), 2.59 μg/mL for neochlorogenic acid (**2**), 1.28 μg/mL for eleuthe roside B_1 (**3**), 3.89 μg/mL for chlorogenic acid (**4**), 0.65 μg/mL for fraxin (**5**), 7.79 μg/mL for cryptochlorogenic acid (**6**), 6.41 μg/mL for caffeic acid (**7**), 2.56 μg/mL for isofraxidin(**8**), 3.83 μg/mL neoastilbin (**9**), 7.69 μg/mL for astilbin (**10**), 1.27 μg/mL for rosmarinic acid 4-O-*β*-D-glupyrannoside (**11**), 3.84 μg/mL for neoisoastilbin (**12**), 5.10 μg/mL for isoastilbin(**13**),7.69 μg/mL for rosmarinic acid (**14**), 8.98 μg/mL for quercetin-3-*O*-*β*-D-glucuronide (**15**), 1.02 μg/mL for quercitrin (**16**) and 1.27 μg/mL for chloranthalactone E (**17**), respectively. The internal standard stock solution of 0.05 mg/ml for naringin was also prepared in 30% methanol. For construction of calibration plots, the standard stock solution was further diluted with 30% methanol to make 7 different concentrations at 1/2, 1/4, 1/8, 1/16, 1/32, 1/64, 1/128 of the original concentration and addition of the IS solution with a concentration of 1000 ng/ml. All solutions were stored in a refrigerator at 4 ºC for analysis.

Preparation of sample solutions

The dried whole plant of *S. glabra* samples were ground into a powder (60 mesh), 0.05 g of the powder was accurately weighed into 25 mL volumetric flask and extracted in an ultrasonic bath with 25 mL 80% MeOH for 30 min at room temperature, then, the extract solutions were prepared by the method of weight relief. 0.48 mL extract solution was diluted with 0.5 mL water after addition of 20 uL IS (0.05 mg/ml). For preparations, 0.01 g of the content was accurately weighed and processed the same as raw materials. All the solution was filtered through a 0.22 um nylon filter and aliquot of 5 uL was injected for LC/MS analysis.

Results and discussion

Optimization of LC–MS/MS conditions

The type of chromatographic columns (Inertsil ODS-3 column, 100 × 2.1 mm i.d., 3 μm; Hypersil GOLD, 100 × 2.1 mm i.d., 5 μm; Hypersil GOLD, 50 × 2.1 mm i.d., 1.9 μm; Eclipse XDB-C18, 150 × 2.1 mm i.d., 3.5 μm), the chromatographic conditions of the mobile phase systems (methanol-aqueous with 0.1% formic acid, methanol-aqueous with 0.1% acetic acid, acetonitrile-aqueous with 0.1% formic acid, and acetonotrile aqueous with 0.1% acetic acid), gradient program and the column temperature (25, 30, and 40 ºC) were optimized in order to obtain overall constituents of *S. glabra* with good resolution within a short analysis. Subsequently, the MS conditions including the drying gas temperature (300, 325 and 350 ºC), the drying gas flow rate (8 and11 L/min), capillary voltage (−3.0 and −3.5 kV), were optimized in order to acquire better separation and detection of multi-compounds in *S. glabra* within shorter analytical time. As a result, the optimum conditions were decided as described in Experimental Section. As shown in Figure 2, the typical extract ions chromatograms (XIC) of MRM chromatograms of standards

and sample obtained under the above-optimised conditions.

Calibration curves and limits of quantification and detection

The calibration curves were prepared by assaying standard solutions as described above. A weighted 1/concentration (1/X) linear regression was used to construct the calibration curve by plotting analyte/IS ratio vs. nominal analyte concentration. (Peak area ratios between the area of each reference compound and those of IS were plotted against the corresponding reference concentration). Detailed information regarding calibration curves and linear ranges is displayed in Table 1.

The limits of detection (LOD) is determined at signal-to-noise ratios (S/N) of about 3, and the lower limit of quantification (LLOQ) is defined as the lowest concentration of standard that can be measured within an acceptable accuracy and precision (≤20% for both parameters). The LOD and LLOQ for each compound analyzed were also shown in Table 1.

Precision, repeatability and stability

The intra-day variation was determined by assaying six replicate injections of a standard solution, and within-batch precision was evaluated by analysis of a sample of *S. glabra* (Sample 12) six times on the same day. The inter-day variation was determined on three consecutive days. The intra- or inter-day precisions calculated as relative standard deviation (RSD) were within the range of 0.86–2.29%, 1.16–4.83% and 0.87–4.94%, 0.69–4.92% for mixed standard solution and sample solution, respectively (Table 2).

Repeatability of this method was obtained by analyzing six different samples from the same batch (Sample 12) using the same preparation procedure as described in Section 2.4. RSD values of component content of seventeen compounds were all less than 9.0% (Table 2).

Stability was tested by analysis of the same sample solution (Sample 12) at room temperature at different times for 2 days. The results are shown in Table 2. RSD values of the peak area of 17 analytes were less than 5.0%. These results suggested that it was feasible to analyze samples within 2 days.

Accuracy

The recovery of the method was determined by the standard addition method applied on a selected *S. glabra* extract sample (Sample 12). A known amount (low, medium and high) of each standard solution were spiked into known amounts of *S. glabra* samples, and then extracted according to the section of sample pretreatment. The recoveries of analytes varied from 80.2.0% to 119.7% (Table 3). The result indicated the reliability and accuracy for the quantitative determination of the constituents.

Sample analysis

The established HPLC-MS method has been successfully applied to the simultaneous determination of 17 constituents in *S. glabra* herbal extract, its crude drug from different factories, and three different parts of the herb. The content of 17 compounds is summarized in Table 4, and the data is further presented in column charts as supporting information. It can be seen that the main constituents detected in *S. glabra* are eleutheroside B_1 (**3**), chlorogenic acid (**4**), cryptochlorogenic acid (**6**), isofraxidin (**8**), rosmarinic acid-4-*O*-*β*-D-glucoside (**11**), rosmarinic acid (**14**) and quercetin-3-*O*-*β*-D-glucuronide (**15**). Among these major constituents, isofraxidin was found to be most abundant in Sichuan, Fujian and Guangdong provinces, and chlorogenic acid was found to be most abundant in Sichuan and Fujian provinces, while rosmarinic acid-4-*O*-*β*-D-glucoside (**11**) and rosmarinic acid (**14**) were found to be most abundant in Yunnan province. The total contents of these constituents in different places of China had a certain degree of difference, with1- to 15-fold variation, and the most abundant amount of the total constituents are samples originating from Sicuan, Fujian and Yunnan province. As shown in Table 4, the difference of the total content of 17 constituents between Sample 6 (Picking in July) and Samples 7-8 (Picking in September and October, *resp*.) originating the same producing area was not obvious, thus the picking season seems not to be the key factor affecting the levels of bioactive constituents in *S. glabra*, and this is also consistent with previous reports [12-13]. Although the content of each analyte varied greatly among different samples, which was probably due to other factors, such as growing condition, climate, and drug processing of the crude herbs, they generally had identical main bioactive constituents in both water extracts and preparations. Since most of these constituents are reported to be anti-inflammatory agents [14-17], the best producing areas for *S. glabra* should be Sicuan, Fujian and Yunnan provinces. For isomers of dihydroflavonols such as **9**, **10**, **12** and **13**, they are abundant in samples collected from Fujian and Sichuan province, but few in other producing areas such as Guangxi, Anhui, Hubei, Guizhou and Guangdong province, and they are almost undetectable in all solid preparations. As for different parts of the herb originating from Sichuan and Fujian provinces, the contents of coumarins (**3** and **8**) in roots were much higher than those in leaves and stems, while the contents of flavonoids such as **9**, **10**, **12**, **13** and **15** were much higher in leaves than those in stems and roots. When compared with the *S. glabra* extract, the major constituents detected in its preparations are protocatechuic acid (**1**), neochlorogenic acid (**2**), eleutheroside B_1 (**3**), chlorogenic acid (**4**), cryptochlorogenic acid (**6**), isofraxidin (**8**), rosmarinic acid-4-*O*-*β*-D-glucoside (**11**) and rosmarinic acid (**14**), and the content of **3**, **8** and **14** are obviously increased in its preparations.

Conclusions

A fast and reliable HPLC-ESI-MS/MS method has been successfully developed to quantify 17 bioactive compounds in *S. glabra* and its preparations simultaneously. These constituents including flavonoids, coumarins, caffeoyl derivatives and sesquiterpenoids, were separated and determined within 17 minutes. Based on the total amount of 17 components, the quality of *S. glabra* originating from Sichuan, Fujian and Yunan province was superior than other originations in China. In short, the determination of a single or only several components could not comprehensively control the quality of *S. glabra* effectively, and simultaneous determination of multiple gradients for natural medicine is essential.

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Notes and references

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Table 1
The MRM transitions and fragmentation parameters for the detection of the analytes and internal standard, calibration curves, linear response range, and Pearson's coefficient of correlation (R^2) for calibration curves, and LLOQ of 17 standards

Analytes	Q_1	Q_3	Fragmentor	Collision Energy	Regression equation	r^2(n = 3)	Linear range (ng/ml)	LOD ng/ml)	LLOQ (ng/ml)
Naringin(**IS**)	579.0	271.1	210	32				0.41	
protocatechuic acid (**1**)	153.3	109.2	80	6	y = 0.0003 x –0.0036	0.9991	26.97-1726.20	10.50	26.25
3-*O*-caffeoylquinic acid (**2**)	353.2	191.1	90	11	y = 0.0006 x –0.0006	0.9991	20.25-1296.00	4.50	15.00
eleutheroside B_1 (**3**)	429.1	221.1	70	2	y = 0.0022x –0.0116	0.9990	10.04-642.40	0.35	0.92
5-*O*-caffeoylquinic acid (**4**)	353.2	191.1	90	11	y = 0.0003 x –0.0067	0.9996	30.42-1947.00	2.06	8.25
Fraxin (**5**)	369.1	207.1	80	16	y = 0.0013x –0.0033	0.9993	5.06-324.00	0.38	1.13
4-*O*-caffeoylquinic acid (**6**)	353.1	178.9	130	12	y = 0.0012 x –0.0020	0.9995	60.84-3894.00	7.38	14.75
caffeic acid (**7**)	179.0	135.0	70	2	y = 0.0006x –0.0046	0.9999	50.05-3203.20	30.00	49.00
Isofraxidin (**8**)	221.0	190.8	80	12	y = 0.0002x –0.0002	0.9994	20.02-1281.10	2.19	5.83
Neoastilbin (**9**)	449.0	303.0	80	16	y = 0.0003 x –0.0008	0.9997	29.93-1915.80	2.33	9.30
Astilbin (**10**)	449.0	303.0	80	16	y = 0.0002 x –0.0015	0.9993	60.04-3842.72	2.43	4.87
Rosmarinic acid-4-*O*-*β*-D-glucoside (**11**)	521.3	359.1	210	32	y = 0.0001 x + 0.0051	0.9992	77.63-4968.00	0.38	1.13
Neoisoastilbin (**12**)	449.0	303.0	80	16	y = 0.0002 x –0.0020	0.9997	39.85-2550.60	2.27	13.63
Isoastilbin (**13**)	449.0	303.0	80	16	y = 0.0002 x –0.0014	0.9994	29.96-1917.60	2.27	13.60
rosmarinic acid (**14**)	358.9	161.1	88	11	y = 0.0001 x –0.0014	0.9997	99.98-6399.00	0.90	2.70
quercetin-3-*O*-*β*-D-glucuronide (**15**)	477.0	301.3	90	16	y = 0.0006x –0.0143	0.9996	70.12-4488.00	1.10	2.75
Quercitrin (**16**)	447.0	300.1	220	23	y = 0.0005 x +0.0002	0.9991	7.96-509.60	0.36	1.08
Chloranthalactone E (**17**)	261.0	217.1	90	10	y = 0.0010 x –0.0019	0.9998	9.94-636.00	1.25	3.75

Table 2
Precision, repeatability and stability of the 17 constituents assayed

Analytes	Precision (RSD%)				Repeatability		Stability(RSD%)
	Standard solution		*S.glabra* extract				
	Intra-day(n=6)	Inter-day(n=3)	Intra-day (n= 6)	Inter-day(n= 3)	Mean (ng/ml)	RSD%	(n = 6)
protocatechuic acid (**1**)	2.29	1.23	4.94	1.69	149.15	7.55	3.41
neochlorogenic acid (**2**)	2.36	3.97	3.20	4.61	251.08	7.30	2.67
eleutheroside B_1 (**3**)	0.94	2.68	0.91	1.05	313.05	7.35	1.05
chlorogenic acid (**4**)	1.36	4.01	0.44	2.15	1334.74	6.67	3.26
Fraxin (**5**)	1.52	2.61	1.62	0.69	77.38	8.30	1.64
cryptochlorogenic acid (**6**)	0.86	3.12	2.25	3.88	640.90	5.87	4.82
caffeic acid (**7**)	0.88	2.03	4.04	3.37	84.07	8.89	3.99
isofraxidin (**8**)	1.54	3.48	3.88	2.41	436.34	6.86	4.53
neoastilbin (**9**)	1.73	2.34	0.97	2.47	933.82	5.80	2.66
astilbin (**10**)	1.25	2.69	0.87	1.45	3245.68	5.47	2.06
Rosmarinic acid-4-*O*-*β*-D-glucoside (**11**)	1.54	4.83	1.34	1.30	763.90	6.10	2.55
neoisoastilbin (**12**)	1.44	3.64	2.26	4.92	180.03	4.81	4.66
isoastilbin (**13**)	1.55	2.41	4.52	1.53	927.88	6.15	1.33
rosmarinic acid (**14**)	1.63	2.48	1.39	4.86	2218.95	5.36	4.29
quercetin-3-*O*-*β*-D-glucuronide(**15**)	1.91	4.25	1.65	2.05	465.08	7.66	2.97
quercitrin (**16**)	1.84	1.16	2.88	3.98	253.54	5.50	2.30
Chloranthalactone E(**17**)	1.31	4.13	2.03	1.81	285.51	7.38	2.64

Table3
Recoveries of the 17 determined constituents

No.	Initial amount (μg)	Added amount (μg)	Detected amount (μg)	Recovery (%)	No.	Initial amount (μg)	Added amount (μg)	Detected amount (μg)	Recovery (%)
1	3.73	1.86	5.79	110.82	**10**	81.14	40.57	116.71	87.67
		3.74	6.98	87.0			81.15	152.86	88.38
		5.59	8.47	84.82			121.71	187.76	87.60
2	6.28	3.14	9.09	89.70	**11**	19.40	9.70	29.24	101.44
		6.28	13.63	117.10			19.40	39.61	104.20
		9.42	17.05	114.41			29.10	50.76	107.78
3	7.83	3.91	10.96	80.20	**12**	4.50	2.25	6.96	109.18
		7.83	15.24	94.65			4.56	9.53	110.45
		11.74	18.05	87.08			6.75	10.87	94.36
4	33.37	16.68	53.20	118.85	**13**	23.20	11.60	36.72	116.62
		33.39	71.78	115.04			23.33	47.76	105.79
		50.05	86.79	106.73			34.80	58.20	100.58
5	1.93	0.97	2.79	88.76	**14**	56.45	27.85	87.98	106.52
		1.94	3.85	98.53			55.69	121.03	115.96
		2.90	4.81	99.04			83.54	143.45	104.14
6	17.60	8.80	27.89	116.92	**15**	11.63	5.81	17.98	109.36
		17.62	35.43	101.21			11.66	22.56	93.76
		26.40	44.57	102.17			17.44	26.75	86.72
7	2.44	1.22	3.86	115.88	**16**	6.34	3.17	9.97	114.64
		2.46	5.15	109.81			6.34	13.39	111.18
		3.67	6.23	103.20			9.51	17.20	114.25
8	10.91	5.45	17.14	114.33	**17**	7.14	3.57	10.98	107.61
		10.92	23.98	119.68			7.14	14.60	104.54
		16.36	27.27	100.02			10.71	17.68	98.48
9	23.35	11.67	33.75	89.10					
		23.44	45.34	93.85					
		35.02	56.52	94.75					

Table 4
Contents of the 17 constituents determined in samples of *S. glabra* and its preparations

Samples[a]	Content (μg/g)																	Total content
	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	
1	45.45	86.22	157.43	465.49	29.88	281.34	67.92	227.50	17.23	42.49	414.04	tr	tr	703.75	519.64	33.38	tr	3091.76
2	29.44	41.73	129.49	333.95	16.12	98.00	58.16	365.99	tr	tr	140.08	tr	tr	414.68	197.09	27.31	409.14	2261.18
3	24.26	37.74	50.37	214.18	11.99	98.21	54.86	159.04	tr	tr	140.64	tr	tr	334.51	185.27	13.47	tr	1324.56
4	55.64	55.71	138.83	302.32	17.56	183.65	66.16	246.44	tr	tr	664.79	tr	tr	640.49	211.90	13.63	tr	2597.14
5	282.65	30.92	211.53	209.77	9.99	69.76	73.12	518.87	tr	tr	92.84	tr	tr	500.79	171.33	52.32	103.67	2327.56
6	85.05	378.24	289.80	2268.08	88.30	1044.92	86.31	406.25	366.10	2605.53	767.02	44.68	236.48	1626.70	364.06	182.94	36.66	10877.11
7	49.64	316.66	244.68	2940.39	47.14	936.06	423.35	657.95	451.85	2520.66	1622.38	97.44	863.44	1472.00	331.98	142.51	tr	13118.12
8	143.04	412.93	171.55	2090.68	87.00	1144.02	83.24	297.03	593.16	4959.74	425.57	54.86	410.05	1372.62	236.91	231.26	67.88	12781.53
9	137.95	160.51	317.52	797.33	79.48	802.80	104.48	482.53	986.44	3362.49	846.02	194.87	990.67	3099.65	467.15	293.51	300.18	13423.58
10	162.60	300.63	287.50	1282.06	75.11	626.87	116.37	446.89	907.91	3324.96	653.85	147.53	883.75	1804.99	342.14	271.25	350.50	11984.90
11	183.64	266.65	245.62	1036.43	89.14	635.22	134.03	481.18	1107.12	2170.63	691.90	87.56	644.25	1325.60	395.53	137.03	283.36	9914.91
12	100.91	159.67	585.98	935.92	62.35	376.24	141.72	505.23	65.53	476.39	883.98	tr	82.21	1616.62	490.12	77.75	248.94	6809.56
13	167.98	91.53	220.11	496.07	48.77	206.59	82.21	326.27	148.01	356.96	573.03	tr	123.39	579.27	281.20	46.70	112.85	3860.93
14	47.28	132.56	83.00	390.35	28.20	290.97	122.56	248.52	tr	tr	250.75	tr	tr	1429.98	380.55	23.79	tr	3428.51
15	137.07	67.01	40.73	72.40	5.23	75.06	50.77	176.35	5.79	21.71	45.11	tr	tr	202.90	46.80	tr	tr	946.94
16	56.03	117.60	162.77	513.73	38.65	356.06	84.14	240.66	26.54	36.93	572.13	tr	tr	761.78	714.29	36.23	tr	3717.55
17	82.03	15.75	235.67	167.15	12.42	39.06	36.61	580.02	tr	tr	183.08	tr	tr	213.45	38.82	9.93	321.10	1935.10
18	88.34	99.39	186.03	459.85	55.37	226.53	43.23	187.50	330.32	443.37	268.16	19.66	141.23	391.53	179.78	68.43	137.39	3326.12
19	67.17	23.33	87.16	138.53	20.72	52.82	40.67	266.16	tr	tr	207.49	tr	tr	323.66	103.97	5.29	157.10	1494.07
20	125.89	151.02	443.68	787.88	121.20	253.41	126.77	303.38	420.49	1072.55	23.35	56.60	340.88	2819.76	65.31	211.90	tr	7324.06
21	29.95	324.60	211.53	419.54	60.31	1261.82	55.93	227.86	tr	29.25	2409.25	tr	tr	3900.54	945.48	43.90	455.17	10375.13
22	278.91	85.70	159.95	433.49	24.04	241.56	395.09	491.53	410.96	930.54	286.42	tr	387.58	463.77	376.50	129.45	176.93	5272.44
23	54.73	428.16	368.70	5170.25	92.97	1253.37	575.84	647.06	200.97	751.41	3599.40	31.52	238.52	1549.84	655.35	107.67	tr	15725.76
24	22.87	133.28	667.04	1266.83	22.39	316.43	124.94	928.11	206.90	534.30	585.97	tr	164.71	799.03	tr	39.14	11.45	5823.40
25	41.05	561.49	103.63	2499.56	65.22	2135.97	281.32	194.76	669.77	8595.18	1168.63	182.42	1904.39	2078.47	1962.86	279.30	tr	22724.03
26	101.17	461.74	1539.62	3575.45	62.91	1068.68	72.00	1009.07	505.35	864.05	1217.62	56.42	319.39	1949.63	tr	96.60	94.17	12993.88
27	99.50	303.61	454.48	1225.35	81.74	637.53	72.39	438.27	213.16	217.63	1297.88	tr	86.43	1674.44	110.53	53.04	81.59	7047.57
28	180.37	307.40	107.35	658.97	54.31	712.52	84.30	135.70	840.89	2768.62	484.84	118.71	757.26	2159.86	1226.61	241.63	138.00	10977.34
29	891.91	237.26	379.64	358.58	33.02	502.63	37.76	1425.72	tr	tr	216.30	tr	tr	1686.94	tr	tr	138.13	5907.87
30	1019.99	821.37	886.97	1103.00	95.61	1626.78	147.90	2167.00	tr	tr	1471.82	tr	tr	3557.66	160.04	tr	346.27	13404.43
31	1215.03	2067.66	591.91	2346.38	86.01	3253.48	105.33	2316.45	176.12	tr	1970.96	tr	tr	14060.17	tr	15.66	tr	28205.16
32	457.09	74.19	41.78	137.86	11.34	114.30	tr	369.89	tr	tr	87.17	tr	tr	505.24	tr	22.97	71.19	1893.01
33	3779.84	651.56	230.70	1065.74	74.42	1587.95	tr	4323.78	tr	tr	832.20	tr	tr	1778.23	tr	tr	86.68	14411.09

Notice: tr:trace; [a]: Samples 1–3 originating from Guangxi Province, Samples 4–5 originating from Anhui Province, Samples 6–8 originating from Fujian Province, Samples 9–11 originating from Sichuan Province, Samples 12–14 originating from Jiangxi Province, Sample 15 originating from Hubei Province, Sample 16 originating from Guizhou Province, Samples 17–19 originating from Jiangxi Province, Sample 20 originating from Hebei Province, Sample 21 originating from Yunan Province, Sample 22 originating from Zhejiang Province, Sample 23 from the stem of *S. glabra* with the origination from Fujian Province, Sample 24 from the root of *S. glabra* with the origination from Fujian Province, Sample 25 from the leaf of *S. glabra* with the origination from Fujian, Sample 26 from the root of *S. glabra* with the origination from Sichuan Province, Sample 27 from the stem of *S. glabra* with the origination from Sichuan Province, Sample 28 from the leaf of *S. glabra* with the origination from Sichuan Province, Province, Sample 29 was Zhongjiefeng dispersible tablet, Samples 30 and 31 were Zhongjiefeng tablets, Sample 32 was Qingrexiaoyanning capsule, Sample 33 was Xiekang capsule.

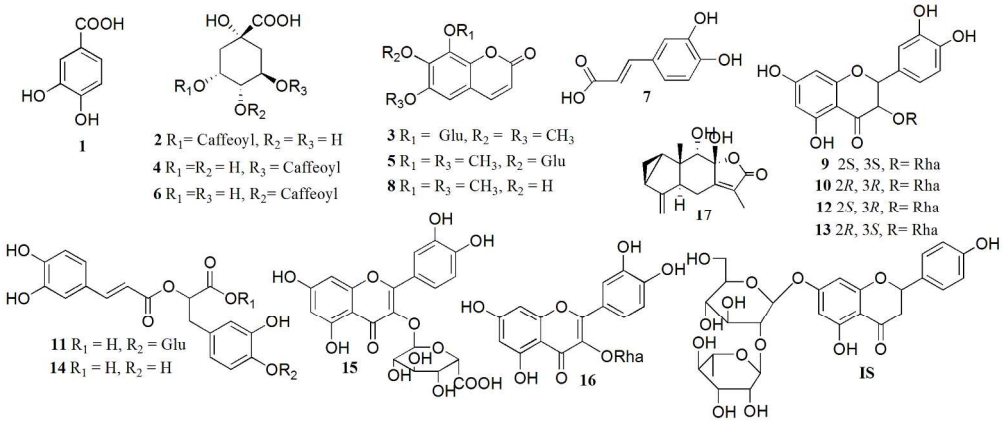

Fig.1 Chemical structures of 17 compounds and naringin (I.S.)
213x89mm (300 x 300 DPI)

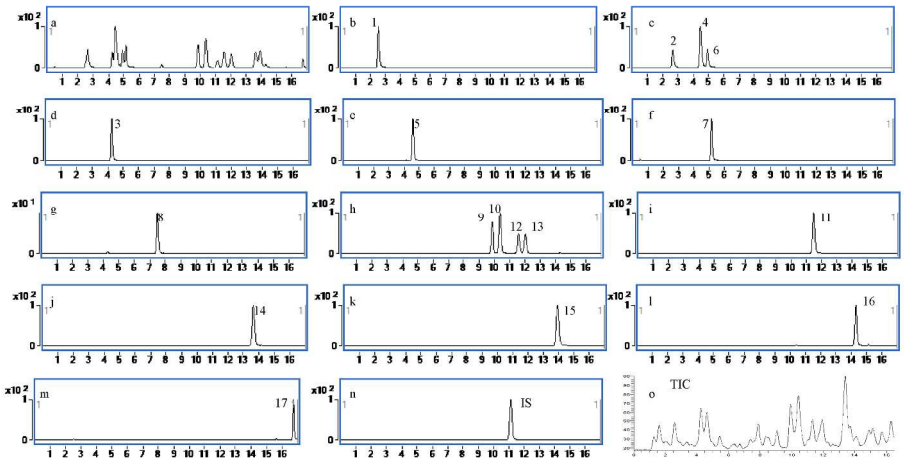

Fig.2. MRM chromatograms of 17 compounds assayed along with I.S and the total ion chromatogram (TIC) of S. glabra. (a): The MRM chromatograms of 17 compounds; (b): Protocatechuic acid (1); (c): 3-O-caffeoylquinic acid (2), 5-O-caffeoyl quinic acid (4) and 4-O-caffeoylquinic acid (6); (d): Eleutheroside B1 (3); (e): Fraxin (5); (f): Caffeic acid (7); (g): Isofraxidin (8); (h): Neoastilbin (9), Astilbin (10), Neoisoastilbin (12) and Isoastilbin (13); (i): Rosmarinic acid-4-O-β-D-glucoside (11); (j): Rosmarinic acid (14); (k): Quercetin-3-O-β-D-glucuronide (15); (l): Quercitrin (16); (m): Chloranthalactone E (17); (n): Naringin(IS) and (o): The total ion chromatogram (TIC) of S. glabra.
297x210mm (300 x 300 DPI)