Identification of Multiple Constituents in Shuganjieyu Capsule and Rat Plasma after Oral Administration by Ultra-Performance Liquid Chromatography Coupled with Electrospray Ionization and Ion Trap Mass Spectrometry

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Shuganjieyu (SGJY) capsule is a classical formula widely used in Chinese clinical application. In this paper, an ultra-performance liquid chromatography coupled with electrospray ionization and ion trap mass spectrometry has been established to separate and identify the chemical constituents of SGJY and the multiple constituents of SGJY in rats. The chromatographic separation was performed on a C_{18} RRHD column (150 × 2.1 mm, 1.8 μm), while 0.1% formic acid–water and 0.1% formic acid–acetonitrile was used as mobile phase. Mass spectral data were acquired in both positive and negative modes. On the basis of the characteristic retention time (R_t) and mass spectral data with those of reference standards and relevant references, 73 constituents from the SGJY and 15 ingredients including 10 original constituents and 5 metabolites from the rat plasma after oral administration of SGJY were identified or tentatively characterized. This study provided helpful chemical information for further pharmacology and active mechanism research on SGJY.

Keywords: Constituents, rat plasma, UHPLC–ESI–MS/MS, Shuganjieyu capsule

1. Introduction

Shuganjieyu (SGJY) capsule, which contains two medicinal materials, including the dried herbs of *Hypericum perforatum* L. and the dried roots and rhizomes or stems of *Acanthopanax senticosus* (Rupr. et Maxim) Harms, is the first approved Chinese herbal medicine for mild to moderate monopolar depression in Chinese. Currently, the research of the chemical components in SGJY has been mainly based on identification of chemical constituents respectively and systematically from individual herb extracts. The constituents of SGJY are numerous and diverse. However, until now, like most traditional Chinese medicine (TCM), there have been few reports on the absorption and efficacy after oral administration of SGJY, which is valuable for further studies on the pharmaceutical effect and mechanism of the SGJY formula. As tandem mass spectrometry (MS/MS) has been proven to be efficient tool for the rapid on-line analysis for the known compounds and elucidation of unknown compounds in complex matrices, in this study, a high-speed and sensitive technique ultrahigh-performance liquid chromatography (UHPLC)–electrospray ionization (ESI)–MS/MS system was adopted to characterize the constituents of SGJY capsule and the metabolic profile in rat plasma after oral administration of SGJY. Moreover, the result of this study was expected to provide helpful chemical information for further pharmacology and active mechanism research on SGJY formula.

2. Experimental

2.1. Materials and Reagents. Shuganjieyu capsule (batch number 150314), the extract of *H. perforatum* L. (batch number S150402), and the extract of *A. senticosus* Harms (batch number S150102) were offered by Chengdu Kanghong Pharmaceutical Co. Ltd. (Chengdu, China). The reference standards of rutin, hyperoside, quercetin, isofraxidin, epicatechin, chlorogenic acid, and eleutheroside E were purchased from National Institutes for Food and Drug Control (Beijing, P.R. China), hypericin was purchased from Chengdu Munster biotechnology company (Chengdu, China), and hyperforin was purchased from ChromaDex Corporate (California, USA). (6*S*,7*E*,9*R*)-Roseoside was isolated in our laboratory (purity, >98%), and its chemical structure was identified by spectral analysis. HPLC-grade acetonitrile and methanol were purchased from Honeywell Burdick & Jackson (Ulsan, Korea). Ultrapure water for the preparation of samples and mobile phase was prepared with Milli-Q Biocel water system (Millipore, Massachusetts, USA). Other reagents were of analytical grade.

2.2. Instrumentation and Analytical Conditions. The Agilent 1290 Infinity UHPLC system (Agilent Technologies Inc., California, USA) was equipped with quaternary pump, vacuum degasser, a cooling autosampler, and a diode-array detector. An Agilent Eclipse Plus C_{18} RRHD column (150 × 2.1 mm, 1.8 μm) was utilized for separation with the column temperature at 30 °C. A binary gradient elution was adopted with mobile phase consisting of (A) 0.1% formic acid in water and (B) 0.1% formic acid in acetonitrile: 0–3 min, B 5%; 3–15 min, B 5–10%; 15–25 min, B 10–20%; 25–40 min, B 20–40%; 40–45 min, B 40–100%; 45–50 min, B 100%; 50–51 min, B 100–5%; and 51–60 min, B 5%. The flow rate was set at 0.20 mL/min. The autosampler was conditioned at 4 °C, and the injection volume was 10 μL.

ThermoQuest Finnigan LTQ system equipped with an electrospray ionization source (ThermoQuest LC/MS Division, San Jose, CA, USA) was used for mass spectrometric measurements. The ESI–MS^n spectra were acquired in both positive and negative ion modes. The mass spectrometry detector (MSD) parameters were

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as follows: in (+) ESI, spray voltage, 3500 V (sheath gas, 15 arb; auxiliary gas, 5 arb; purge gas, 0 arb); capillary temperature, 275 °C; capillary voltage, 10 V; lens voltage, 80 V; in (−) ESI, spray voltage, 5000 V (sheath gas, 15 arb; auxiliary gas, 5 arb; purge gas, 0 arb); capillary temperature, 275 °C; capillary voltage, −10 V; lens voltage, −100V. Tandem mass spectrometry (MS/MS) was collected with data-dependent mode, and the three highest intensity peaks in full-scan spectra were acquired for MS/MS analysis. Helium was used as collision gas (collision energy, 35 eV). The full-scan range was from 100 to 1000 *m*/*z*.

2.3. Animals, Drug Administration, and Blood Sampling. Ten male Sprague-Dawley (SD) rats (160–220 g) were obtained from Chengdu Dashuo Laboratory Animal Co., Ltd. (Sichuan, China). The animals were acclimatized to the facilities for 5 days, and then fasted, with free access to water for 12 h prior to the experiment. All procedures were in accordance with the Guidelines on the Care and Use of Animals for Scientific Purposes 2004.

2.4. Sample Preparation

2.4.1. Preparation of SGJY Extract Samples. SGJY capsule was ground into fine powder. A total of 100 mg was accurately weighed, and 10 mL distilled water was added. Each extract of medicinal material contained in SGJY was 50 mg accurately weighed and dissolved in 10 mL distilled water. All the samples were ultrasonically extracted for 10 min and then filtered through a syringe filter (0.45 μm). Filtrate (10 μL) was subjected to UPLC–ESI–MS/MS analysis.

2.4.2. Preparation of Plasma Samples. Capsule contents of SGJY were dispersed with distilled water as stock solution (0.5 g/mL). The above suspension was orally administered to five rats (1.0 mL/100 g body weight). An equal volume of distilled water was orally administered to the other five rats as control; 60 min after drug administration, the animals were anesthetized by the injection of 7% chloral hydrate. The blood was collected from the abdominal aorta and then centrifuged at 10,000 rpm for 10 min at 4 °C. The supernatant (0.5 mL) was added into polypropylene test tube, and 1.5 mL methanol was added. The mixture was vortexed for 60 s and then centrifuged at 10,000 rpm for 10 min. Supernatant was collected and dried under nitrogen gas at 25 °C. The residues were redissolved in 200 μL of methanol and centrifuged at 10,000 rpm for 10 min, and an aliquot of supernatant was subjected to UPLC–ESI–MS/MS analysis.

3. Results and Discussions

3.1. UPLC–MS/MS Analysis and Identification the Constituents of SGJY. Figures 1 and 2 show the ion chromatogram of SGJY in both positive and negative ion modes. A total of 73 peaks were identified or tentatively characterized including 14 organic acids, 37 flavonoids, 8 prenylated phloroglucinols, 2 naphthodianthrones, 5 lignans, 3 phenylpropanoids, and 4 other compounds, on the basis of the ultraviolet (UV) spectra, MS spectra, and MS/MS spectra with fragmentation patterns of reference standards or literature data. All the detailed data are shown in Table 1.

3.1.1. Identification of Components by Standards. Compounds **5**, **9**, **13**, **20**, **29**, **30**, **31**, **59**, **71**, and **72** were respectively attributed to chlorogenic acid, (6*S*,7*E*,9*R*)-roseoside, epicatechin, eleutheroside E, rutin, isofraxidin, hyperoside, quercetin, hypericin, and hyperforin, by comparison with the retention times and mass spectral data of the reference standards.

3.1.2. Identification of Components through Investigating Literatures

3.1.2.1. Organic acids identification. Organic acids are vital compounds found in both *H. perforatum* L. and *A. senticosus* Harms. Chlorogenic acid (**5**) was identified for certain by comparison with the reference standards. Chlorogenic acid, one of the main organic acids in SGJY, could be used to characterize the fragmentation pathways. It gave diagnostic ions at *m*/*z* 372 $[M + NH_4]^+$, 355 $[M + H]^+$, and 163 $[M + H - 192]^+$ in positive mode and at *m*/*z* 353 $[M - H]^-$ and 191 $[M - H - Caffe]^-$ in negative mode. Based on these fragmentation patterns, compounds **1**–**4**, **6**, **7**, **11**, **15**, **16**, **19**, **22**, **37**, and **43** were identified.

Compounds **1** and **7** gave precursor ions at *m*/*z* 355 $[M + H]^+$ and 353 $[M - H]^-$ and fragment ions at *m*/*z* 163 in positive mode and 191 in negative mode, which, with the same diagnostic ions of chlorogenic acid (**5**), were assigned as neochlorogenic acid and 1-*O*-caffeoylquinic acid [1]. Compounds **4**, **11**, and **19** gave diagnostic ions 16 Da less than **5**, were assigned as *p*-coumaroylquinic acid [1]. Compounds **6** and **16** gave diagnostic ions 14 Da more than **5** and were assigned as feruloylquinic acid [1]. Compound **2** gave precursor ions at 529 $[M - H]^-$, and fragment ions at 367 and 191 in negative mode; through investigating references, compound **2** was identified as 3-(4-*O*-glucosylferuloyl) quinic acid [2]. Compounds **3**, **15**, **37**, and **43** with the same MS spectra gave diagnostic ions 162 Da more than **5** and were characterized as di-*O*-caffeoylquinic acid [1]. Compound **22** gave

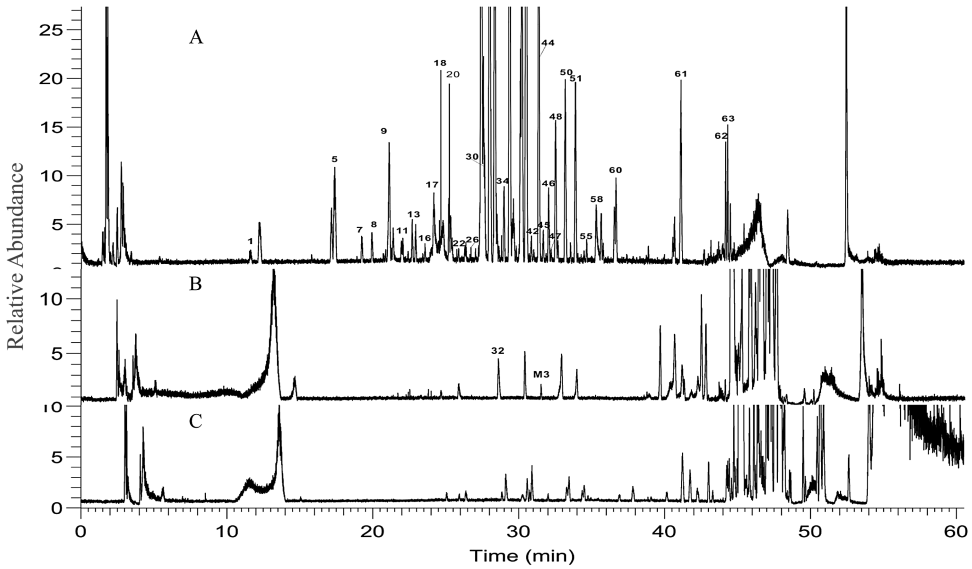


Figure 1. Base peak intensity chromatogram of SGJY (A), dosed plasma (B), and control plasma (C) in positive mode

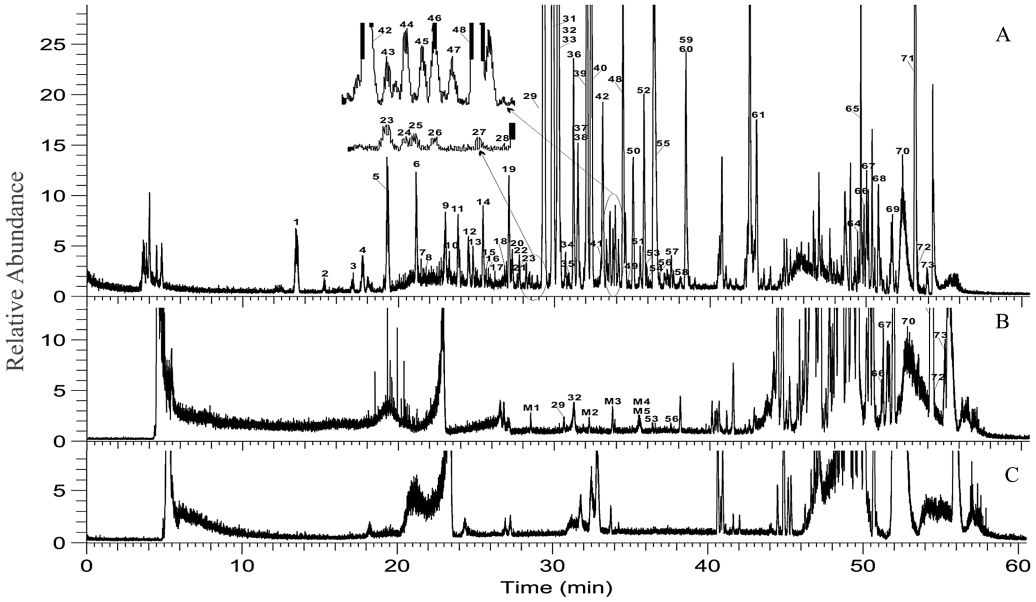


Figure 2. Base peak intensity chromatogram of SGJY (A), dosed plasma (B), and control plasma (C) in negative mode

Table 1 Characterization of compounds in SGJY by UPLC–ESI–MS/MS (MS in *m/z*, R_t in min)

Peak	Name	Origin	R_t	MW	MS (+) MS/MS	MS (−) MS/MS
1	Neochlorogenic acid	A, H	11.67	354	355 $[M + H]^+$ 163	353 $[M - H]^-$ 191 $[M - H - Caffe]^-$ 179 $[M - H - Quinic]^-$
2	3-(4-*O*-Glucosylferuloyl) quinic acid	A	13.35	530		529 $[M - H]^-$ 367 $[M - H - Glu]^-$ 191 $[M - H - Glu - Ferul]^-$
3	1,3-Di-*O*-caffeoylquinic acid	A, H	14.91	516		515 $[M - H]^-$ 353 $[M - H - Caffe]^-$ 191 $[M - H - 2Caffe]^-$
4	3-*O*-*p*-Coumaroylquinic acid	H	15.82	338	339 $[M + H]^+$ 147	337 $[M - H]^-$ 163 $[M - H - Quinic]^-$ 191 $[M - H - Couma]^-$
5[a]	Chlorogenic acid	A, H	17.39	354	355 $[M + H]^+$ 163 372 $[M + NH_4]^+$ 355, 163	353 $[M - H]^-$ 191 $[M - H - Caffe]^-$
6	5-Feruloylquinic acid	H	18.51	368		367 $[M - H]^-$ 193 $[M - H - Quinic]^-$
7	1-*O*-Caffeoylquinic acid	A, H	19.26	354	355 $[M + H]^+$ 163	353 $[M - H]^-$ 179 $[M - H - Quinic]^-$ 191 $[M - H - Caffe]^-$
8	Eleutheroside B1	A	19.97	384	402 $[M + NH_4]^+$ 223 $[M + H - Glu]^+$ 223 $[M + H - Glu]^+$ 208, 163, 135, 107	429 $[M + HCOOH - H]^-$ 221 $[M - H - Glu]^-$
9[a]	Roseoside	H	21.14	386	387 $[M + H]^+$ 207 $[M + H - Glu - H_2O]^+$ 225 $[M + H - Glu]^+$ 189, 369	431 $[M + HCOOH - H]^-$ 385 $[M - H]^-$ 223 $[M - H - Glu]^-$ 205 $[M - H - Glu - H_2O]^-$
10	Savinin	A	21.41	352		351 $[M - H]^-$ 249, 267, 333
11	5-*O*-*p*-Coumaroylquinic acid	H	21.98	338	339 $[M + H]^+$ 147	337 $[M - H]^-$ 191 $[M - H - Couma]^-$ 163 $[M - H - Quinic]^-$
12	5-Methoxylariciresinol-4-*O*-glucoside	A	22.44	552		551 $[M - H]^-$ 389 $[M - H - Glu]^-$ 341, 193
13[a]	Epicatechin	H	22.66	290	291 $[M + H]^+$ 123, 139, 165, 273	335 $[M + HCOOH - H]^-$ 289 $[M - H]^-$ 289 $[M - H]^-$ 245, 205, 271, 179
14	Quercetin 3,7-diglucoside	H	23.44	626		625 $[M - H]^-$ 463 $[M - H - Glu]^-$ 301 $[M - H - 2Glu]^-$
15	3,4-Di-*O*-caffeoylquinic acid	A, H	23.57	516		515 $[M - H]^-$ 353 $[M - H - Caffe]^-$ 335 $[M - H - Caffe - H_2O]^-$ 179 $[M - H - Caffe - Quinic]^-$

(*Continued*)

Table 1 *(contd.)*

Peak	Name	Origin	R_t	MW	MS (+) MS/MS	MS (−) MS/MS
16	4-Feruloylquinic acid	H	23.78	368	369 $[M + H]^+$ 177	367 $[M - H]^-$ 191 $[M - H - Ferul]^-$
17	Quercetin 3,4′-diglucoside	H	24.08	626		625 $[M - H]^-$ 463 $[M - H - Glu]^-$ 301 $[M - H - 2Glu]^-$
18	Quercetin 4′,7-diglucoside	H	24.57	626		625 $[M - H]^-$ 463 $[M - H - Glu]^-$ 301 $[M - H - 2Glu]^-$
19	4-*O*-*p*-Coumaroylquinic acid	H	24.94	338		337 $[M - H]^-$ 191 $[M - H - Couma]^-$ 163 $[M - H - Quinic]^-$
20[a]	Eleutheroside E	A	25.23	742	760 $[M + NH_4]^+$ 597	787 $[M + HCOOH - H]^-$ 579 $[M - H - Glu]^-$ 417 $[M - H - 2Glu]^-$
21	Secoisolariciresinol-9′-*O*-glucoside	A	25.77	524		741 $[M - H]^-$ 523 $[M - H]^-$ 361 $[M - H - Glu]^-$
22	3-*O*-*p*-Coumaroyl-4-*O*-caffeoylquinic acid	H	25.89	500	501 $[M + H]^+$ 193 $[M + H - Caffe]^+$ 355 $[M + H - Couma]^+$	545 $[M + HCOOH - H]^-$ 499 $[M - H]^-$
23	Ochroside	H	26.12	596		595 $[M - H]^-$ 463 $[M - H - Ara]^-$ 433 $[M - H - Glu]^-$ 301 $[M - H - Ara - Glu]^-$
24	Astragaline	H	26.49	448		447 $[M - H]^-$ 285 $[M - H - Glu]^-$
25	Quercetin 3-*O*-glucoside-7-*O*-arabionoside	H	26.58	596		595 $[M - H]^-$ 463 $[M - H - Ara]^-$ 433 $[M - H - Glu]^-$ 301 $[M - H - Ara - Glu]^-$
26	Quercetin 3-*O*-glucoside-7-*O*-rhamnoside	H	26.70	610	611 $[M + H]^+$ 303 $[M + H - Glu - Rha]^+$ 449 $[M + H - Glu]^+$ 465 $[M + H - Rha]^+$	609 $[M - H]^-$ 447 $[M - H - Glu]^-$ 463 $[M - H - Rha]^-$ 301 $[M - H - Glu - Rha]^-$
27	Multinoside A	H	27.07	610		609 $[M - H]^-$ 447 $[M - H - Glu]^-$ 301 $[M - H - Rutino]^-$
28	Quercetin 3-*O*-rhamnoside-7-*O*-glucoside	H	27.25	596		595 $[M - H]^-$ 433 $[M - H - Glu]^-$ 463 $[M - H - Ara]^-$ 301 $[M - H - Ara - Glu]^-$
29[a]	Rutin	H	27.44	610	611 $[M + H]^+$ 303 $[M + H - Rutino]^+$ 465 $[M + H - Glu]^+$	609 $[M - H]^-$ 301 $[M - H - Rutino]^-$
30[a]	Isofraxidin	A	27.69	222	223 $[M + H]^+$ 208, 163, 107, 135	
31[a]	Hyperoside	H	28.01	464	465 $[M + H]^+$ 303 $[M + H - Glu]^+$	463 $[M - H]^-$ 301 $[M - H - Glu]^-$
32	Quercetin 3-*O*-glucuronide	H	28.36	478		477 $[M - H]^-$ 301 $[M - H - GluA]^-$
33	Isoquercitrin	H	28.36	464	465 $[M + H]^+$ 303 $[M + H - Glu]^+$	463 $[M - H]^-$ 301 $[M - H - Glu]^-$
34	Sinapaldehyde 4-*O*-glucoside	A	29.08	370	371 $[M + H]^+$ 209 $[M + H - Glu]^+$ 133, 191, 353	
35	Quercetin 3 (2-glucosylrhamnoside)	H	29.22	610		609 $[M - H]^-$ 301 $[M - H - Glu - Rha]^-$ 447 $[M - H - Glu]^-$
36	Nicotiflorin	H	29.41	594		593 $[M - H]^-$ 285 $[M - H - Rutino]^-$
37	3,5-Di-*O*-caffeoylquinic acid	H	29.64	516		515 $[M - H]^-$ 353 $[M - H - Caffe]^-$ 335 $[M - H - Caffe - H_2O]^-$
38	Quercetin 3-*O*-arabinoside	H	29.64	434	435 $[M + H]^+$ 303 $[M + H - Ara]^+$	433 $[M - H]^-$ 301 $[M - H - Ara]^-$
39	Guaijaverin	A	30.22	434	435 $[M + H]^+$ 303 $[M + H - Ara]^+$	433 $[M - H]^-$ 301 $[M - H - Ara]^-$
40	Quercitrin	H	30.49	448	449 $[M + H]^+$ 303 $[M + H - Rha]^+$	447 $[M - H]^-$ 301 $[M - H - Rha]^-$
41	Quercetin 3-*O*-(6-acetylglucoside)	H	30.72	506		505 $[M - H]^-$ 301 $[M - H - Acetylglu]^-$
42	Apigenin 4′-*O*-glucuronide	H	31.03	446	447 $[M + H]^+$ 271 $[M + H - GluA]^+$	
43	4,5-Di-*O*-caffeoylquinic acid	H	31.34	516		515 $[M - H]^-$ 353 $[M - H - Caffe]^-$
44	Quercetin 3-*O*-(6-acetylgalactoside)	H	31.69	506	507 $[M + H]^+$ 303 $[M + H - Acetylgal]^+$	505 $[M - H]^-$ 301 $[M - H - Acetylgal]^-$ 463 $[M - H - Acetyl]^-$

(*Continued*)

Table 1 *(contd.)*

Peak	Name	Origin	R_t	MW	MS (+) MS/MS	MS (−) MS/MS
45	Quercetin 3-*O*-(2-acetylglucoside)	H	31.90	506	507 $[M + H]^+$ 303 $[M + H - Acetylglu]^+$	
46	Quercimetrin	H	32.04	464	465 $[M + H]^+$ 303 $[M + H - Glu]^+$	463 $[M - H]^-$ 301 $[M - H - Glu]^-$
47	Juglanin	H	32.37	418	419 $[M + H]^+$ 287 $[M + H - Ara]^+$	417 $[M - H]^-$ 285 $[M - H - Ara]^-$
48	Quercetin 3-*O*-(2-acetylgalactoside)	H	32.54	506	507 $[M + H]^+$ 303 $[M + H - Acetylgal]^+$ 205, 187	505 $[M - H]^-$ 301 $[M - H - Acetylgal]^-$ 463 $[M - H - Acetyl]^-$
49	Cynaroside	H	32.96	448		447 $[M - H]^-$ 285 $[M - H - Glu]^-$
50	Scutellarin A	H	33.20	446	447 $[M + H]^+$ 271	445 $[M - H]^-$ 269 $[M - H - GluA]^-$ 269 251, 241
51	Cedrurin	H	33.90	346	347 $[M + H]^+$ 332, 314	345 $[M - H]^-$ 330 $[M - H - Me]^-$
52	Kaempferol 3-*O*-(6-acetylglucoside)	H	34.07	490		489 $[M - H]^-$ 285 $[M - H - Acetylglu]^-$ 429, 447
53	Licochalcone A	H	34.29	338		337 $[M - H]^-$ 322 $[M - H - Me]^-$ 257
54	Vincetoxicoside B	H	34.49	448		447 $[M - H]^-$ 301 $[M - H - Rha]^-$
55	Linarin	H	34.68	592	593 $[M + H]^+$ 447 $[M + H - Rha]^+$ 285 $[M + H - Glu]^+$	637 $[M + HCOOH - H]^-$ 591 $[M - H]^-$ 283 $[M - H - Rha - Glu]^-$
56	Corylifolinin	H	34.91	324		323 $[M - H]^-$ 243
57	Acacetin 7-*O*-glucuronide	H	35.66	460	461 $[M + H]^+$ 285 $[M + H - GluA]^+$	459 $[M - H]^-$ 283 $[M - H - GluA]^-$ 283 268 $[M - H - GluA - Me]^-$
58	Tilianin	H	36.37	446	447 $[M + H]^+$ 285 $[M + H - Glu]^+$	491 $[M + HCOOH - H]^-$ 283 $[M - H - Glu]^-$ 329
59[a]	Quercetin	H	36.57	302		301 $[M - H]^-$ 179, 151, 257, 273
60	Glychionide B	H	36.68	460	461 $[M + H]^+$ 285 $[M + H - GluA]^+$	459 $[M - H]^-$ 283 283 $[M - H - GluA]^-$ 268 $[M - H - GluA - Me]^-$
61	Apigenin	H	41.12	270	271 $[M + H]^+$ 253, 225, 167, 123	269 $[M - H]^-$ 251 $[M - H - H_2O]^-$
62	Acacetin	H	44.20	284	285 $[M + H]^+$ 270 $[M + H - Me]^+$	
63	Wogonin	H	44.51	284	285 $[M + H]^+$ 270 $[M + H - Me]^+$	
64	Garsubellin E	H	47.36	498		497 $[M - H]^-$ 428, 357, 399
65	17*R*,18-Dihydroxyfurohyperforin	H	47.44	586		585 $[M - H]^-$ 445, 516, 399, 291
66	Pseudohypericin	H	47.91	520		519 $[M - H]^-$ 503
67	Furohyperforin	H	48.17	552		551 $[M - H]^-$ 482, 413, 383, 315
68	Hyperfirin	H	48.86	468		467 $[M - H]^-$ 398
69	Adhyperfirin	H	49.66	482		481 $[M - H]^-$ 412
70	Oxedhyperforin	H	50.56	554		553 $[M - H]^-$ 484, 401, 415, 333
71[a]	Hyperforin	H	51.16	504		503 $[M - H]^-$ 459
72[a]	Hypericin	H	51.39	536		535 $[M - H]^-$ 466, 383, 397, 315
73	Adhyperforin	H	52.14	550		549 $[M - H]^-$ 480, 411, 397, 329

[a]The compounds have been identified by reference standards; A indicates *Acanthopanax senticosus* Harms; H, *Hypericum perforatum* L.; Me, methyl; Glu, glucosyl; Ara, arabionosyl; Gla, galactosyl; Rha, rhamnosyl; GluA, glucuronosyl; Caffe, caffeoyl; Quinic, quinic acid; Couma, coumaroyl.

diagnostic ions 16 Da less than **3** and were identified as 3-*O*-*p*-coumaroyl-4-*O*-caffeoylquinic acid [3].

3.1.2.2. Flavonoids identification. Flavonoids are abundant in *H. perforatum* L. Rutin (**29**), hyperoside (**31**), and quercetin (**59**) were identified for certain by comparison with the reference standards. Hyperoside, one of the most abundant and well responded flavonoids in SGJY, could be used to characterize the fragmentation pathways. It gave diagnostic ions at *m*/*z* 465 $[M + H]^+$ and 303 $[M + H - Glu]^+$ in positive mode, and at *m*/*z* 463 $[M - H]^-$ and 301 $[M - H - Glu]^-$ in negative mode. Based on these fragmentation patterns, compounds **14**, **17**, **18**, **23**–**28**, **32**, **33**, **35**, **36**, **38**–**42**, **44**–**50**, **52**, **54**, **55**, **57**, **58**, and **60**–**63** were identified.

Compounds **14**, **17**, **18**, **23**, **25**–**28**, **32**–**33**, **35**, **38**, **40**, **41**, **44**–**46**, **48**, and **54** gave diagnostic ions at 303 in positive mode and/or 301 in negative mode, which suggested that these compounds

should be quercetin (**59**) derivatives. Compounds **38** and **39** gave diagnostic ions 132 Da more than **59** and were identified as quercetin 3-*O*-arabinoside and guaijaverin [4, 5]. Compounds **40** and **54** showed diagnostic ions 146 Da more than **59** and were characterized as quercetin-*O*-rhamnoside [6, 7]. Compounds **33** and **46** with the same MS spectra were assigned as isoquercitrin and quercimetrin, respectively, for their diagnostic ions 162 Da more than **59** [6, 8]. Compound **32** gave diagnostic ions 176 Da more than **60** and were identified as quercetin 3-*O*-glucuronide [9]. With the same approach, compounds **26**, **27**, **28**, and **35** were assigned as quercetin-*O*-glucoside-rhamnoside [10–13]; compounds **23** and **25** were assigned as quercetin-*O*-glucoside-arabionoside [14, 15]; compounds **41**, **44**, **45**, and **48** were assigned as quercetin-*O*-acetylglucoside [16–19]; and compounds **14**, **17**, and **18** were assigned as quercetin diglucoside [20–22].

Compounds **24**, **36**, **47**, **49**, and **52** gave diagnostic ions at 287 in positive mode and/or 285 in negative mode, which suggested that these compounds should be kaempferol or luteolin derivatives. Compound **47** gave diagnostic ions 132 Da more than kaempferol and were identified as kaempferol 3-*O*-arabinoside [23]. Compounds **24** and **49** showed diagnostic ions 162 Da more than kaempferol or luteolin and were characterized as astragaline and cynaroside [24, 25]. Compounds **52** and **36** showed diagnostic ions 204 Da and 308 Da more than kaempferol, respectively, and were characterized as kaempferol 3-*O*-(6-acetylglucoside) and kaempferol 3-*O*-rutinoside [16, 24].

Compounds **42** and **50** with the same MS spectra showed precursor ion at *m/z* 447 $[M + H]^+$ and 445 $[M - H]^-$, which produced prominent ions as compound **61** at *m/z* 271 in positive mode and 269 in negative mode, owing to loss of a glucuronyl. By comparing with reference, these compounds were characterized as scutellarin A, apigenin 4′-*O*-glucuronide, and apigenin [26–28].

Compounds **62** and **63** with the same MS spectra showed precursor ion at *m/z* 285 $[M + H]^+$, and gave the fragment ion at 270; through investigating references, these two compounds were identified as acacetin and wogonin [29, 30]. Compounds **58** and **60** with the same MS spectra showed diagnostic ions 162 Da more than **62** and **63** and were characterized as tilianin and glychionide B [31]. Compound **55** gave diagnostic ions 308 Da more than **62** and were identified as linarin [32]. Compound **57** gave diagnostic ions 176 Da more than **62** and were identified as acacetin 7-*O*-glucuronide [33].

3.1.2.3. Prenylated phloroglucinols identification. Prenylated phloroglucinols are one of the most famous natural herbal ingredients which are abundant in *H. perforatum* L. Hyperforin (**71**) was identified for certain by comparison with the reference standards. Hyperforin, one of the most abundant and well responded prenylated phloroglucinols in SGJY, could be used to characterize the fragmentation pathways. It gave diagnostic ions at *m/z* 535 $[M - H]^-$, 466, 383, 397, and 315 in negative mode. Based on these fragmentation patterns, compounds **64**, **65**, **67**–**70**, and **73** were identified.

Compounds **64**, **65**, **67**–**70**, and **73** gave the characteristic loss of 69 or 68 Da, the same with hyperforin (**72**). By investigating reference data, these compounds were identified as garsubellin E (**64**) [34], 17*R*,18-dihydroxyfurohyperforin (**65**) [35], furohyperforin (**67**) [36], hyperfirin (**68**) [9], adhyperfirin (**69**) [9], oxedhyperforin (**70**) [37], and adhyperforin (**73**) [9].

3.1.2.4. Naphthodianthrones identification. Naphthodianthrones are another one of the most famous natural herbal ingredients which are abundant in *H. perforatum* L. Hypericin (**72**) was identified for certain by comparison with the reference standards. Compound **66** showed precursor ion at *m/z* 519 $[M - H]^-$ and fragment ions at *m/z* 503, and was identified as pseudohypericin through investigating references [9].

3.1.2.5. Lignans identification. Lignans are one of the main ingredients which are abundant in *A. senticosus* Harms. Eleutheroside E (**20**) was identified for certain by comparison with the reference standards. Eleutheroside E, which responded well in SGJY, could be used to characterize the fragmentation pathways. It gave diagnostic ions at *m/z* 787 $[M + HCOOH - H]^-$, 741 $[M - H]^-$, 579 $[M - H - Glu]^-$, and 417 $[M - H - 2Glu]^-$ in negative mode. Based on these fragmentation patterns, compounds **10**, **12**, **21**, and **51** were identified.

Compound **10** produced a precursor ion at *m/z* 351 $[M - H]^-$ and fragment ions at *m/z* 249, 267, 333. By investigating reference data, it was identified as savinin [38].

Compound **12** showed precursor ion at *m/z* 551 $[M - H]^-$ and fragment ions at *m/z* 389 $[M - H - Glu]^-$, 341, and was identified as 5-methoxylariciresinol-4-*O*-glucoside through investigating references [39].

Compound **21** produced a precursor ion at *m/z* 523 $[M - H]^-$, and fragment ions at *m/z* 361 $[M - H - Glu]^-$. By investigating reference data, it was identified as secoisolariciresinol-9′-*O*-glucoside [40].

Compound **51** produced precursor ions at *m/z* 347 $[M + H]^+$ and 345 $[M - H]^-$, and fragment ions at *m/z* 332 $[M + H - Me]^+$ and 330 $[M - H - Me]^-$. By investigating reference data, it was identified as cedrurin [41].

3.1.2.6. Phenylpropanoids identification. Isofraxidin (**30**), identified for certain by comparison with the reference standard, was the main constituent of *A. senticosus* Harms. It gave diagnostic ions at *m/z* 223 $[M + H]^+$, 208, 163, 107, and 135 in positive mode. Based on these fragmentation patterns, compounds **8** and **34** were identified.

Compound **8** showed precursor ion at *m/z* 402 $[M + NH_4]^+$, which produced prominent ions as compound **30** at *m/z* 223 in positive mode, owing to loss of a glucuronyl. By comparing with referenced, compound **8** was characterized as eleutheroside B1 [2].

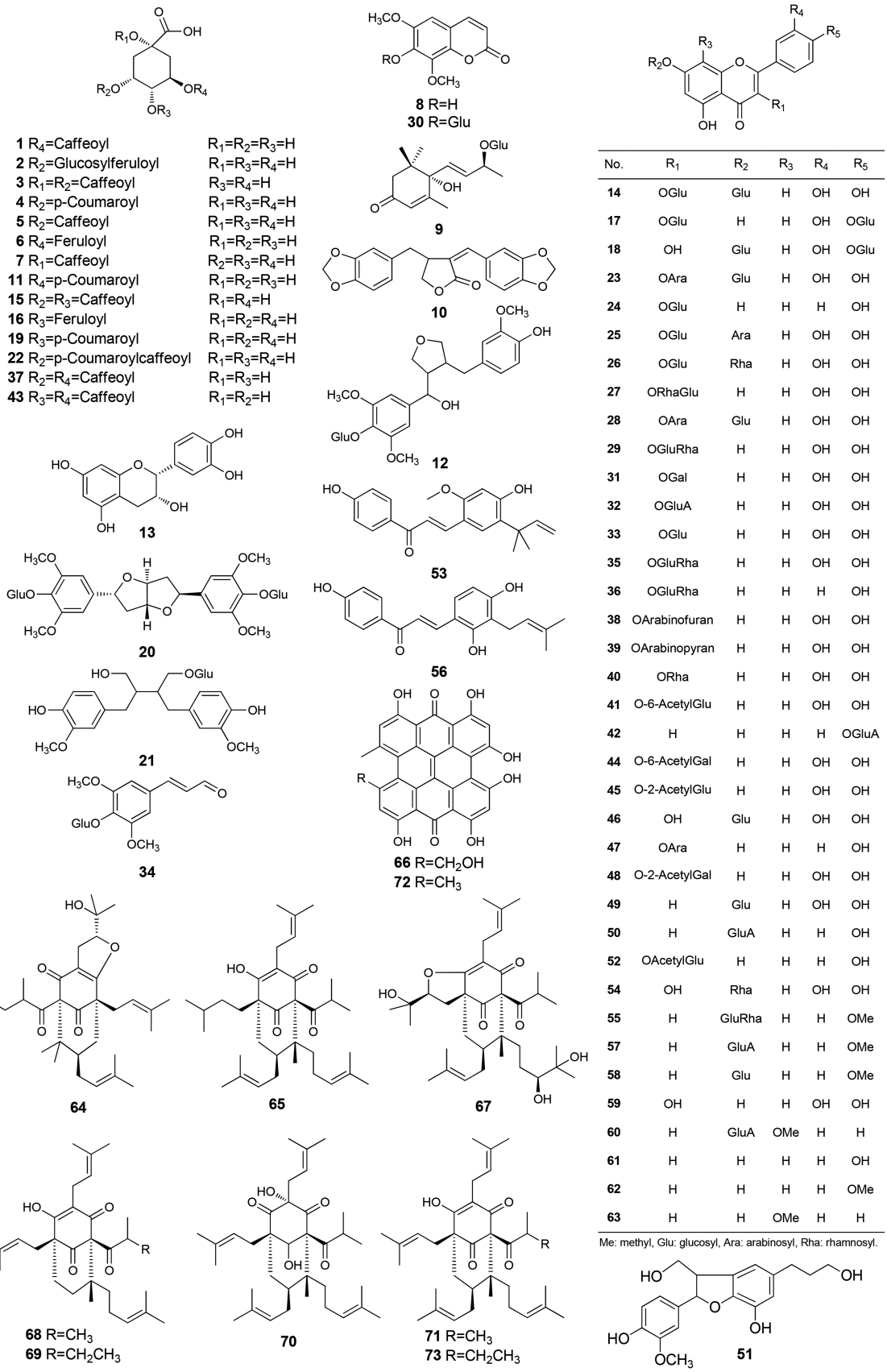
Table 2. Characterization of compounds in SGJY treated rat plasma by UPLC–ESI–MS/MS

No.	Name	R_t (min)	MW	MS (−)	MS/MS (−)
29	Rutin	27.47	610	609 $[M - H]^-$	301
32	Quercetin 3-*O*-glucuronide	28.28	478	477 $[M - H]^-$	301
53	Licochalcone A	34.44	338	337 $[M - H]^-$	322, 257
56	Corylifolinin	34.99	324	323 $[M - H]^-$	243
66	Pseudohypericin	48.01	520	519 $[M - H]^-$	503
67	Furohyperforin	48.01	552	551 $[M - H]^-$	482, 413, 383, 315
70	Oxedhyperforin	50.56	554	553 $[M - H]^-$	484, 415, 401, 333
71	Hyperforin	51.26	536	535 $[M - H]^-$	466, 397, 383, 315, 313
72	Hypericin	51.16	504	503 $[M - H]^-$	459, 327
73	Adhyperforin	52.14	550	549 $[M - H]^-$	480, 397, 329, 465, 313
M1	Quercetin bisglucuronide	25.59	654	653 $[M - H]^-$	477, 301
M2	Kaempferol 3-*O*-glucuronide	30.39	462	461 $[M - H]^-$	285, 175
M3	Isorhamnetin glucuronide	31.01	492	491 $[M - H]^-$	315, 300
M4	Tamarixetin glucuronide	31.85	492	491 $[M - H]^-$	315, 300
M5	Epicatechin glucuronide	31.94	466	465 $[M - H]^-$	289

Compound **34** showed precursor ion at *m/z* 371 $[M + H]^+$ and fragment ions at *m/z* 209 $[M + H - Glu]^+$, and was identified as sinapaldehyde 4-*O*-glucoside through investigating references [2].

3.1.2.7. Other compounds identification. Compound **53** gave precursor ions at *m/z* 337 $[M - H]^-$ and fragment ions at *m/z* 322 $[M - H - Me]^-$, 247 in negative mode. By comparing with referenced, compound **53** was characterized as licochalcone A [42]. Compounds **56** showed diagnostic ions 14 Da less than **53** and were characterized as corylifolinin [43].

3.2. UPLC–MS/MS Analysis and Identification the Constituents of SGJY in Rat Plasma. To clarify the active constituents responsible for the pharmacological action, it is



No.	R_1	R_2	R_3	R_4	R_5
14	OGlu	Glu	H	OH	OH
17	OGlu	H	H	OH	OGlu
18	OH	Glu	H	OH	OGlu
23	OAra	Glu	H	OH	OH
24	OGlu	H	H	H	OH
25	OGlu	Ara	H	OH	OH
26	OGlu	Rha	H	OH	OH
27	ORhaGlu	H	H	OH	OH
28	OAra	Glu	H	OH	OH
29	OGluRha	H	H	OH	OH
31	OGal	H	H	OH	OH
32	OGluA	H	H	OH	OH
33	OGlu	H	H	OH	OH
35	OGluRha	H	H	OH	OH
36	OGluRha	H	H	H	OH
38	OArabinofuran	H	H	OH	OH
39	OArabinopyran	H	H	OH	OH
40	ORha	H	H	OH	OH
41	O-6-AcetylGlu	H	H	OH	OH
42	H	H	H	H	OGluA
44	O-6-AcetylGal	H	H	OH	OH
45	O-2-AcetylGlu	H	H	OH	OH
46	OH	Glu	H	OH	OH
47	OAra	H	H	H	OH
48	O-2-AcetylGal	H	H	OH	OH
49	H	Glu	H	OH	OH
50	H	GluA	H	H	OH
52	OAcetylGlu	H	H	H	OH
54	OH	Rha	H	OH	OH
55	H	GluRha	H	H	OMe
57	H	GluA	H	H	OMe
58	H	Glu	H	H	OMe
59	OH	H	H	OH	OH
60	H	GluA	OMe	H	H
61	H	H	H	H	OH
62	H	H	H	H	OMe
63	H	H	OMe	H	H

Me: methyl, Glu: glucosyl, Ara: arabinosyl, Rha: rhamnosyl.

Figure 3. Chemical constituents of SGJY capsule

necessary to analyze the chemical constituent profile in vivo. Therefore, the rat plasma after oral administration of SGJY capsule was analyzed by the same UHPLC–ESI–MS/MS method used above. By comparing the retention time and mass chromatography of dosed rat plasma with control plasma and SGJY, 15 compounds were observed in dosed rat plasma which did not appear in control plasma. Among them, 10 compounds (**29**, **32**, **53**, **56**, **66**, **67**, **70**, **71**, **72**, and **73**) were indicated as original constituents of SGJY, compounds **M1–5** were tentatively predicted to be metabolites of SGJY. Ion chromatograms of dosed and controlled rat plasma are shown in Figure 2. The MS spectra and retention behavior of 15 peaks for original constituents and metabolites are summarized in Table 2.

3.2.1. Identification of Original Constituents in Rat Plasma. Ten compounds were indicated to be original constituents of SGJY. They were identified as rutin, quercetin 3-*O*-glucuronide, licochalcone A, corylifolinin, pseudohypericin, furohyperforin, oxedhyperforin, hypericin, hyperforin, and adhyperforin, respectively.

3.2.2. Identification of Metabolites of SGJY in Rat Plasma. To identify the metabolites accurately, probable structures were first assumed in accordance with the rules of drug metabolism in vivo. Flavones were the main constituents of SGJY and showed as mentioned above. The main metabolic pathways of flavones were glucuronidation, sulfation, and methylation. In this study, the constituents of SGJY identified as mentioned above may provide guidance for investigating the metabolites of SGJY in rat plasma. The loss of 176 Da could be assigned as a glucuronate in the structure (Figure 3).

The metabolite **M1** gave a precursor ion at *m*/*z* 653 $[M - H]^-$ and product ions at *m*/*z* 477 and 301 in negative mode; the loss of 176 Da and 176 Da could be assigned as two glucuronate in the structure. By investigating reference data, it was identified as quercetin di-*O*-glucuronide [44].

The metabolites **M3** and **M4** produced same precursor ion at *m*/*z* 491 $[M - H]^-$, eluted at 30.39 and 31.01 min, respectively. They exhibited the same product ions at *m*/*z* 315 and 300 in negative mode, and were identified as isorhamnetin glucuronide and tamarixetin glucuronide through investigating references [45]. The metabolites **M2** and **M5** also gave the same MS fragmentation patterns [44] (Table 2).

4. Conclusion

UPLC–ESI–MS/MS was proved to be an effective method for the characterization and identification of major components of SGJY capsule. A total of 73 constituents were successfully separated and identified by this method. In vivo, the absorption and metabolism of SGJY capsule were explored. As a result, a total of 15 compounds were identified from rat plasma after oral administration of SGJY, including 10 of the original constituents and 5 of the metabolites. In addition, this study demonstrated that UHPLC–ESI–MS/MS would be a useful tool to investigate the potential effective constituents in SGJY capsule.

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