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Structural Identification of Chemical Constituents from *Scutellaria baicalensis* by HPLC-ESI-MS/MS and NMR Spectroscopy

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Scutellariae Radix, the dried roots of *Scutellaria baicalensis* Georgi, which is one of the famous herb widely used in the treatment of pyrexia, jaundice and hypertension. To directly identify the compounds of the extract of the *S. baicalensis* was performed using HPLC-ESI-MS/MS. Chromatographic (analytical and preparative HPLC), chemical (hydrolysis) and spectroscopic (UV, ^{1}H and ^{13}C NMR, MS) techniques were applied for separation, isolation, purification and identification of new compounds. A systematic phytochemical investigantion of *S. baicalensis* led to the isolation of 7 new and 40 known chemical constituents. By HPLC-ESI-MS/MS and NMR spectral analyses, the new compounds were identified as 7-hydroxy-3,5,8-trimethoxy-3',4'-methylenedioxy flavone-7-*O*-β- glucopyranoside (**1**), 7-hydroxy-5,6,8-trimethoxy-3',4'-methylenedioxy flavone-7-*O*-β-glucopyranoside (**2**), patuletin-7-β-glucuronide (**3**), pinobankasin-6-C-glucopyranosyl-8-C-arabinopyranoside (**4**), chrysin-3-C-α-arabinopyranosyl-8-C-β-glucopyranoside (**11**), 6-sulfooxy-chrysin-7-glucoside (**12**) and 5-hydoxy-6,7,4'-trimethoxy-flavanone 5-sulfate (**24**).

Key Words: *Scutellaria baicalensis*; Structural elucidation, Chemical constituents, HPLC-MS/MS, NMR.

INTRODUCTION

Huangqin (*Radix scutellariae*) is an important traditional Chinese medicine prepared from the roots of *Scutellaria baicalensis* Georgi (Labiatae family). It is widely used in traditional Chinese medicine to treat inflammation, pyrexia, jaundice, hepatitis and hypertension[1]. In the clinic, it is widely applied to cure pneumonia, hypertension, jaundice, dysentery and intestinal catarrh, pyogenic infection, *etc*.,[2]. It acts as a key ingredient in a number of formulae such as Shuanghuanglian oral liquid, Huangqin compound recipes granule, Yinhuang table, *etc*. Flavonoids and their derivatives are the main components of the *S. baicalensis*. Including baicalin, baicalein, oroxylin A, wogonoside, wogonin, apigenin and scutellarein, *etc*.[3].

This paper describes 7 new and 38 known chemical consitituents, including 2 flavone sulfates representing the first report of natural flavone sulfates from this genus and 5 novel flavone glycosides, from the aerial parts of this plant. The molecular structures of these new compounds were elucidated based on ^{1}D and ^{2}D NMR spectra and comparisons with literature data.

EXPERIMENTAL

Optical rotations were measured using a Perkin-Elmer 241 MC polarimeter. IR spectra were recorded using a Perkin-Elmer 577 spectrometer. UV spectra were obtained on a Shimadzu UV-2401PC spectrophotometer. HR-TOF-MS was performed using a Waters Q-TOF Ultima. NMR experiments were run on a Varian Mercury-400 spectrometer (USA). Chemical shifts are given on a δ (ppm) scale using TMS as an internal standard. HPLC was performed using Davisil® ODS (35-60 μm, 60Å, Grace) on a BUCHI C-615 pump (UV-detector C-635 and C-660 fraction collector, BUCHI, Switzerland). Semi-preparative HPLC was carried out using EasySep TM-1010 instrument equipped with a Daisogel C_{18} column (12 μm, 20 mm × 250 mm).

HPLC conditions: An Agilent series 1100 HPLC instrument (Waldbronn, Germany) equipped with a quaternary pump, a UV detector and a column compatment was used for analyses. The samples were separated on a Apollo C_{18} column (5 μm, 4.6 × 250 mm, Grace), including an Easy Guard Kit C_{18} (4 × 2 mm, Dikma) guard column. A gradient elution of 0.5 % aqueous formic acid (A) and acetonitrile (B) was used

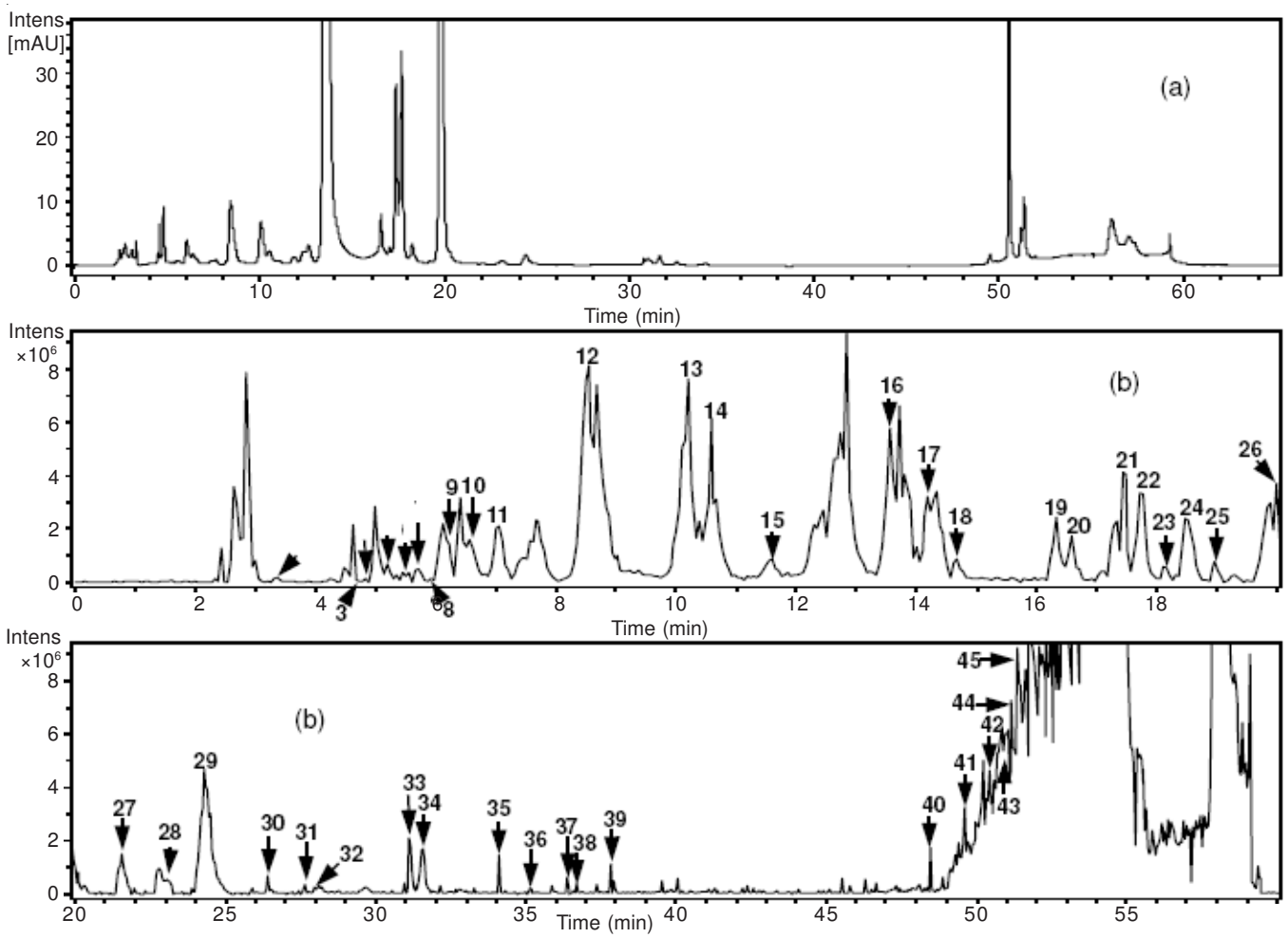

Fig. 1. A representative HPLC-ESI-MSn analysis of the extract of *S. baicalensis*. (a) HPLC-UV chromatogram at 280 nm (b) LC-negative ion ESI-MS total ion current (TIC) profile

as follows: 20 % B in the first 10 min, 20 %-25 % B at 10-11 min, then B held at 25 % for 14 min, linearly gradient to 35 % B at 26 min and hold for 15 min, 35 % - 55 % B at 41-45 min, linearly gradient to 100 % B at 50 min and hold for 5 min. The column was maintained at 25 ºC. Detection wavelengths were set at 280 nm. The flow rate was 0.8 mL/min (Fig. 1).

Mass spectrometric conditions: HPLC-MS experiments were performed with an Agilent 1100 series LC/MSD Trap mass spectrometer was connected to an Agilent 1100 HPLC instrument *via* an ESI source. The LC/MSD Trap software (5.3 version) was applied for system operation and data collection. The mass spectra were recorded in negative mode, drying gas flow rate 10 L/min, drying gas temperature 350 ºC, nebulizer 35 psi., capillary voltage 3500 V, mass range 50-1500 m/z.

The roots of Scutellaria baicalensis Georgi were collected from Xi'an, ShanXi province, China and authenticated by Professor Shen Jingui of Shanghai Institute of Materia Medica, Chinese Academy of Sciences. A voucher specimen (SIMM-HCG-HQ0905) was deposited in the Herbarium of Shanghai Institute of Materia Medica, Chinese Academy of Sciences.

Extraction and isolation: The roots of *S. baicalensis* (5 Kg) were extracted twice with 95 % ethanol at room temperature. The EtOH extracts were combined and evaporated under reduced pressure to give a residue (677 g). A protion of the extract (384 g) was suspended in distilled water and chromatographed using medium-pressure ODS column chromatography (column dimensions 100 mm × 460 mm) eluting followed by increasing concentrations of MeOH in H_2O (10, 20, 30, 50, 80 and 100 % MeOH; fraction volume 1200 mL). Give 48 fractions (355 g), which were combined into seven main groups (A-G). The fraction A (33.8 g) eluted with 10 % MeOH was rechromatographed using semi-preparative HPLC to give compound **1** (5.4 mg) and compound **2** (6.0 mg). Fraction B (26.4 g) eluted with 13 % MeOH was repeatedly chromatographed using semi-preparative HPLC to yield compound **3** (4.5 mg) and compound **4** (5.4 mg). The fraction C (11.4 g) eluted with 15 % MeOH was rechromatographed using semi-preparative HPLC to yield compound **11** (15.7 mg). The fraction D (93.9 g) eluted with 17 % MeOH was rechromatographed using semi-preparative HPLC to give compound **12** (21.1mg). The fraction F (73.9 g) eluted with 22 % MeOH was rechromatographed using semi-preparative HPLC to give compound **24** (6.8 mg).

Experimental data for the new flavonoid glycosides

7-Hydroxy-3,5,8-trimethoxy-3',4'-methylenedioxy flavone-7-O-β-glucopyranoside (1): Pale yellow needles crystals; m.p.: 284-285 ºC; UV (MeOH) λ_{max} (log ε): 256 (4.21), 273 (4.23), 350 (4.23); IR (KBr, ν_{max}, cm^{-1}): 3100 (OH), 1620 (C=O), 1605, 1590, 1495; ESI-MS [M-H]-*m/z*, 533; HR-

ESI-MS [M-H]- *m/z*, 533.1274 (calcd. for $C_{25}H_{26}O_{13}$, 533.1295); ^{1}H NMR (DMSO-d_6, 400 MHz) δ 7.69 (1H, dd, *J* = 8.5, 2.0 Hz, H-6'), 7.54 (1H, d, *J* = 2.0 Hz, H-2'), 7.13 (1H, d, *J* = 8.5 Hz, H-5'), 6.41 (1H, s, H-6), 6.13 (2H, s, -O-CH_2-O-), 5.11 (1H, d, *J* = 7.1 Hz, Glc-1), 3.90 (1H, m, Glc-6), 3.81 (3H, s, 5-OCH_3), 3.75 (3H, s, 8-OCH_3), 3.72 (3H, s, 3-OCH_3), 3.71 (1H, m, Glc-6), 3.46 (1H, m, Glc-5), 3.38 (1H, m, Glc-3), 3.35 (1H, m, Glc-4), 3.21 (1H, m, Glc-2); ^{13}C NMR (DMSO-d_6, 100 MHz) δ 174.8 (C-4), 157.1 (C-5), 155.4 (C-7), 152.0 (C-2), 151.3 (C-3'), 149.2 (C-9), 147.5 (C-4'), 140.9 (C-3), 131.0 (C-8), 124.8 (C-1'), 119.0 (C-6'), 109.3 (C-5'), 109.0 (C-10), 108.1 (C-2'), 103.1 (-O-CH_2-O-), 99.5 (Glc-1), 94.2 (C-6), 77.0 (Glc-5), 76.3 (Glc-3), 73.1 (Glc-2), 69.6 (Glc-4), 61.2 (8-OCH_3), 60.7 (Glc-6), 59.7 (3-OCH_3), 56.4 (5-OCH_3); ESI-MS/MS data (Table-1).

7-Hydroxy-5,6,8-trimethoxy-3',4'-methylenedioxy flavone-7-O-β-glucopyranoside (2): Pale yellow needles; UV (MeOH) λ_{max} (log ε): 257, 350 nm. IR (KBr, ν_{max}, cm^{-1}): 3100 (OH), 1620 (C=O), 1605, 1590, 1495; ESI-MS [M-H]-*m/z*, 533; HR-ESI-MS [M-H]-*m/z*, 533.1275 (calcd. for $C_{25}H_{26}O_{13}$, 533.1295); ^{1}H NMR (DMSO-d_6, 400 MHz) δ 7.69 (1H, dd, *J* = 8.8, 2.3 Hz, H-6'), 7.55 (1H, d, *J* = 2.3 Hz, H-2'), 7.14 (1H, d, *J* = 8.8 Hz, H-5'), 6.63 (1H, s, H-3), 6.11 (2H, s, -O-CH_2-O-), 5.08 (1H, d, *J* = 7.3 Hz, Glc-1), 3.92 (3H, s, 5-OCH_3), 3.90 (1H, m, Glc-6), 3.89 (3H, s, 8-OCH_3), 3.85 (3H, s, 6-OCH_3), 3.71 (1H, m, Glc-6), 3.46 (1H, m, Glc-5), 3.39 (1H, m, Glc-3), 3.35 (1H, m, Glc-4), 3.21 (1H, m, Glc-2); ^{13}C NMR (DMSO-d_6, 100 MHz) δ 175.1 (C-4), 160.6 (C-2), 151.5 (C-3'), 150.3 (C-6), 149.1 (C-8), 147.8 (C-4'), 147.6 (C-9), 144.0 (C-5), 137.1 (C-7), 124.3 (C-1'), 119.3 (C-6'), 110.1 (C-5'), 109.5 (C-10), 109.0 (C-2'), 106.3 (C-3), 103.2 (-O-CH2-O-), 99.9 (Glc-1), 77.1 (Glc-5), 76.4 (Glc-3), 73.1 (Glc-2), 69.5 (Glc-4), 61.5 (5-OCH_3), 61.2 (6-OCH_3), 61.0 (8-OCH_3, Glc-6); ESI-MS/MS data (Table-1).

Patuletin-7-β-glucuronide (3): Pale yellow crystals; UV (MeOH) λ_{max} (log ε): 255, 267sh, 336 nm; IR (KBr, ν_{max}, cm^{-1}): 3327, 1654, 1610, 1504, 1457, 881, 805; ESI-MS [M-H]-*m/z*, 507; HR-ESI-MS [M-H]-*m/z*, 507.0760 (calcd. for $C_{22}H_{20}O_{14}$, 507.0775); ^{1}H NMR (DMSO-d_6, 400 MHz) δ 12.31 (1H, s, 5-OH), 9.92 (1H, s, 3'-OH), 9.80 (1H, s, 3-OH), 9.40 (4'-OH), 7.67 (1H, d, *J* = 2.1 Hz, H-2'), 7.56 (1H, dd, *J* = 7.8, 2.1 Hz, H-6'), 6.89 (1H, d, *J* = 7.8 Hz, H-5'), 6.51 (1H, s, H-8), 5.17 (1H, d, *J* =7.4 Hz, GlcA-1), 4.15 (1H, d, *J* = 9.5 Hz, GlcA-5), 3.81 (3H, s, 6-OCH_3), 3.67 (1H, t, *J* = 9.5 Hz, GlcA-4), 3.59 (2H, m, GlcA-2, GlcA-3); ^{13}C NMR (DMSO-d_6, 100 MHz) δ 176.3 (C-4), 169.9 (GlcA-6), 157.3 (C-7), 151.5 (C-9), 151.4 (C-5), 147.5 (C-4'), 146.5 (C-2), 145.3 (C-3'), 135.1 (C-3), 130.9 (C-6), 122.1 (C-1'), 119.8 (C-6'), 115.5 (C-5'), 114.7 (C-2'), 103.8 (C-10), 101.2 (GlcA-1), 94.5 (C-8), 75.8 (GlcA-5), 75.3 (GlcA-3), 72.8 (GlcA-2), 71.2 (GlcA-4), 59.9 (6-OCH_3); ESI-MS/MS data (Table-1).

Pinobankasin-6-C-glucopyranosyl-8-C-arabinopyranoside (4): Colourless needles; m.p.: 164- 166 ºC; $[\alpha]^{25}_D$ = 10.1 (c 0.5, MeOH). UV (MeOH) λ_{max} (log ε): 380, 315 nm; IR (KBr, ν_{max}, cm^{-1}): 3460, 3120, 1650, 1620, 1480, 1285, 1178; ESI-MS [M-H]-*m/z*, 565; HR-ESI-MS [M-H]-*m/z*, 565.1563 (calcd. for $C_{26}H_{30}O_{14}$, 565.1558); ^{1}H NMR (CD_3OD, 400 MHz) δ 7.49 (2H, m, H-2', H-6'), 7.27 (1H, m, H-4'), 7.25 (2H, m, H-3', H-5'), 5.07 (1H, d, *J* = 12.8 Hz, H-3), 4.51 (1H, d, *J* = 12.8 Hz, H-2), 4.80 (1H, d, *J* = 9.6 Hz, Glc-1), 4.64 (1H, d, *J* = 9.0 Hz, Ara-1), 4.01 (1H, m, Ara-2), 3.93 (1H, m, Ara-5), 3.89 (1H, m, Glc-2), 3.88 (1H, m, Ara-4), 3.70 (1H, m, Glc-6), 3.69 (1H, m, Ara-5), 3.54 (1H, m, Glc-6), 3.53 (1H, m, Ara-3), 3.28 (3H, m, Glc-3, Glc-4, Glc-5); ^{13}C NMR (CD_3OD, 100 MHz) δ 195.6 (C-4), 165.1 (C-7), 161.5 (C-5), 160.5 (C-9), 136.3 (C-1'), 129.2 (C-3', C-5'), 128.7 (C-2', C-6'), 127.5 (C-4'), 106.9 (C-6), 105.4 (C-8), 96.8 (C-10), 83.6 (C-2), 77.7 (Glc-3, Glc-5), 75.2 (Ara-3), 74.2 (Ara-1), 73.2 (Glc-1), 72.7 (C-3), 71.1 (Glc-4), 70.8 (Glc-2), 69.6 (Ara-5), 69.1 (Ara-2), 68.6 (Ara-4), 62.2 (Glc-6); ESI-MS/MS data (Table-1).

Chrysin 3-C-a-arabinopyranosyl-8-C-β-glucopyranoside (11): Pale yellow needles crystals; m.p.: 274-276 ºC; UV (MeOH) λ_{max} (log ε): 269, 316 nm; IR (KBr, ν_{max}, cm^{-1}): 3264 (-OH), 1650 (-C=O), 1595, 1342, 1216, 845; ESI-MS [M-H]-*m/z*, 547; HR-ESI-MS [M-H]-*m/z*, 547.1435 (calcd. for $C_{26}H_{28}O_{13}$, 547.1452); ^{1}H NMR (DMSO-d_6, 400 MHz) δ 12.90 (1H, s, 5-OH), 10.51 (1H, s, 7-OH), 8.13 (2H, m, H-2', H-6'), 7.70 (1H, m, H-4'), 7.66 (2H, m, H-3', H-5'), 6.27 (1H, s, H-6), 4.80 (1H, d, *J* = 9.4 Hz, Ara-1), 4.76 (1H, d, *J* = 9.8 Hz, Glc-1), 4.00 (2H, m, Glc-2, Ara-2), 3.79 (1H, m, Ara-5), 3.77 (1H, m, Ara-4), 3.74 (1H, m, Glc-6), 3.57 (1H, m, Ara-5), 3.52 (1H, m, Glc-6), 3.42 (1H, m, Ara-3), 3.36 (1H, m, Glc-4), 3.30 (1H, m, Glc-3), 3.27 (1H, m, Glc-5); ^{13}C NMR (DMSO-d_6, 100 MHz) δ 178.3 (C-4), 161.0 (C-5, C-7), 160.1 (C-2), 154.3 (C-9), 131.9 (C-4'), 129.3 (C-1'), 128.7 (C-3', C-5'), 126.1 (C-2', C-6'), 101.3 (C-10), 115.3 (C-3), 105.1 (C-8), 95.8 (C-6), 81.5 (Glc-5), 79.1 (Glc-3), 74.6 (Ara-1), 74.5 (Glc-1), 73.9 (Ara-3), 71.5 (Glc-2), 70.8 (Glc-4), 70.1 (Ara-5), 68.9 (Ara-2), 68.4 (Ara-4), 60.9 (Glc-6); ESI-MS/MS data (Table-1).

6-Sulfooxy-chrysin-7-glucoside (12): Pale yellow needles crytals; UV λ_{max} (log ε): 267, 313nm; IR (KBr, ν_{max}, cm^{-1}): 3348, 1655, 1617, 1510, 1068, 854, 768, 686; ESI-MS [M-H]-*m/z*, 511; HR-ESI-MS [M-H]-*m/z*, 511.0562 (calcd. for $C_{21}H_{20}O_{13}S$, 511.0547); ^{1}H NMR (DMSO-d_6, 400 MHz) δ 12.63 (1H, s, 5-OH), 8.08 (2H, m, H-2', H-6'), 7.58 (3H, m, H-3', H-4', H-5'), 7.36 (1H, s, H-8), 7.11 (1H, s, H-3), 4.53 (1H, d, *J* = 6.8 Hz, Glc-1), 3.92 (1H, m, Glc-6), 3.71 (1H, m, Glc-6), 3.45 (1H, m, Glc-5), 3.37 (1H, m, Glc-3), 3.35 (1H, m, Glc-4), 3.20 (1H, m, Glc-2); ^{13}C NMR (DMSO-d_6, 100 MHz) δ 182.6 (C-4), 166.5 (C-7), 164.5 (C-2), 164.2 (C-5), 153.2 (C-9), 132.5 (C-4'), 130.4 (C-1'), 129.3 (C-3', C-5'), 126.7 (C-2', C-6'), 105.7 (C-3, C-10), 101.3 (Glc-1), 94.7 (C-6), 94.4 (C-8), 77.0 (Glc-5), 76.2 (Glc-3), 73.5 (Glc-2), 70.0 (Glc-4), 61.0 (Glc-6); ESI-MS/MS data (Table-1).

5-Hydroxy-6,7,4'-trimethoxy flavanone 5-sulfate (24): Pale yellow crytals; m.p.: 164-166 ºC; UV (MeOH) λ_{max} (log ε): 225, 287, 340nm; IR (KBr, ν_{max}, cm^{-1}): 3400, 2905, 1630, 1565, 1510, 1445, 1270, 1250, 1190, 1155, 1105, 1005, 965, 890 and 830; ESI-MS [M-H]-*m/z*, 409; HR-ESI-MS [M-H]-*m/z*, 409.0590 (calcd. for $C_{18}H_{18}O_9S$, 409.0594); ^{1}H NMR (DMSO-d_6, 400 MHz) δ 7.48 (2H, d, *J* = 8.8 Hz, H-2', H-6'), 6.95 (2H, d, *J* = 8.8 Hz, H-3', H-5'), 6.71 (1H, s, H-8), 5.57 (1H, dd, *J* = 8.8, 3.8 Hz, H-2), 3.85 (3H, s, 7-OCH_3), 3.80 (3H, s, 6-OCH_3), 3.78 (3H, s, 4'-OCH_3), 3.35 (1H, dd, *J* =

TABLE-1
CHARACTERIZATION OF COMPOUNDS BY HPLC-ESI-MS/MS FROM *S. baicalensis*

No.	Rt (min)	UV λ_{max} (nm)	ESI-MS/MS m/z (abundance)	Identification results
1	3.3	256,273,350	MS^2[533]: 516(1.2), 317(8.4), 353(27.3), 341(7.8), 329(0.6), 313(2.3)	7-Hydroxy-3,5,8-trimethoxy-3',4'-(methylene-dioxy) flavone-7-O-β-glucopyranoside
2	3.4	258,350	MS^2[533]: 516(1.2), 317(8.4), 353(27.3), 341(7.8), 313(2.3)	7-Hydroxy-5,6,8-trimethoxy-3',4'-(methylene-dioxy) flavone-7-O-β-glucopyranoside
3	4.8	255,267,336	MS^2[507]: 491(26.5), 399(99.3), 331(31.1)	Patuletin-7-β-glucuronide
4	4.9	280,315	MS^2[565]: 547(100.0), 487(50.7), 457(75.9), 445 (43.9), 367(86.0), 337(51.5)	Pinobankasin-6-C-glucopyranosyl-8-C-arabinopyranoside
5	5.2	255,370	MS^2[657]: 495(100.0), 333(0.2)	6,8-Dihydroxy quercetin diglucopyranoside
6	5.5	282,330	MS^2[671]: 509(100.0), 389(4.4), 347(50.4), 228(1.4)	3,5,7,8,3',4'-Hexahydroxy-6-methoxy flavone diglucopyranoside
7	5.7	271,338	MS^2[563]: 443(100.0), 545(15.0), 503(27.0), 486 (14.6), 473(67.1), 383(33.9), 353(73.1)	Apigenin-6-C-β-D-glucoside-8-C-?-L-arabinoside
8	5.9	271,338	MS^2[563]: 443(100.0), 545(28.5), 503(44.4), 485 (15.6), 473(67.7), 425(26.3), 383 (38.7), 353(34.8)	Apigenin-6-C-α-L-arabinoside-8-C-β-D-glucoside
9	6.4	222,388	MS^2[577]: 457(100.0), 559(8.2), 517(1.3), 487(45.4), 367(21.1), 337(39.8)	Chrysin-6,8-C-diglucoside
10	6.7	224,272,316	MS^2[547]: 487(100.0), 529(14.5), 457(79.3), 427 (79.3), 367(41.0), 337(85.9)	Chrysin-6-C-α-L-arabinoside-8-C-β-D-glucoside
11	7.1	269,316	MS^2[547]: 457(100.0), 529(22.4), 487(55.3), 427 (58.9), 367(48.2), 337(50.3)	Chrysin-3-C-α-arabinopyranosyl-8-C-β-glucopyranoside
12	8.6	267,313	MS^2[511]: 431(100.0), 349(10.6), 269(3.2), 241(1.2)	6-Sulfooxy-chrysin 7-glucoside
13	10.1	220,274,316	MS^2[547]: 457(100.0), 529(10.4), 487(38.1), 427 (22.6), 367(34.6)	Chrysin-6-C-β-D-glucoside-8-C-αL-arabinoside
14	10.6	279,317	MS^2[511]: 269(100.0), 431(20.5), 351(4.2), 349(3.1), 241(8.6)	Bicalein-7-O-β-D-glucopyranosyl sulfate conjugate
15	11.6	252,347	MS^2[475]: 299(100.0), 300(26.2), 284(5.4), 175(3.7)	Chrysoeriol-7-O-β-D-glucuronide
16	13.6	276,315	MS^2[431]: 269(100.0), 271(3.3), 270(16.5), 201(1.2), 196(1.0)	Baicalein-7-O-β-D-glucopyranoside
17	14.0	272,316	MS^2[445]: 430(100.0), 283(87.6), 268(82.4), 161 (1.2)	Wogonin-5-O-β-D-glucopyranoside
18	14.7	270,313	MS^2[415]: 295(100.0), 325(17.2), 296(14.2), 267 (3.2)	Chrysin-8-C-β-D-glucopyranoside
19	16.4	276,315	MS^2[445]: 269(100.0), 366(2.5), 341(2.2), 270(19.5), 223(3.3), 175(20.7)	Baicalein-7-O-glucuronide (baicalin)
20	16.6	263, 323	MS^2[475]: 299(100.0), 301(3.2), 300(19.4), 285(2.2), 284(37.1), 175(5.2)	5,7,6'-trihydroxy-2'-methoxy flavone-7-O-β-D-glucuronide
21	17.4	282,333	MS^2[445]: 269(100.0), 427(2.0), 385(1.0), 175(14.9), 137(2.9)	Apigenin-7-O-β-D-glucuronopyranoside (scutellarin A)
22	17.7	268,336	MS^2[445]: 269(100.0), 244(35.0), 216(1.4), 175(2.6)	2'-Hydroxychrysin-7-glucuronide
23	18.1	254,346	MS^2[463]: 161(100.0), 301(68.4), 283(3.4), 191(8.1), 162(12.7), 139(5.5)	Quercetin-7-O-β-D-glucopyranoside (quercimeritrin)
24	18.4	287,340	MS^2[409]: 329(100.0), 315(0.2), 314(0.9), 299(0.2)	5-Hydroxy-6,7,4'-trimethoxy flavanone-5-sulfate
25	18.9	272,312	MS^2[459]: 283(100.0), 268(8.6), 175(20.7)	Oroxylin A-7-O-β-D-glucuronide
26	19.3	274,349(sh)	MS^2[459]: 283(100.0), 269(5.0), 268(4.2), 176(1.3), 175(24.5)	Wogonin-7-O-β-D-glucuronopyranoside
27	21.5	276,320	MS^2[349]: 269(100.0), 332(0.1), 270(23.0), 225(0.3)	Baicalein-7-O-sulfate
28	23.1	288	MS^2[303]: 125(100.0), 285(3.1), 275(9.0), 259(8.7), 217(25.3), 177(30.9)	3,5,7,3',4'-pentahydroxy flavanone (taxifolin)
29	24.4	324,287	MS^2[303]: 125(100.0), 285(6.1), 259(15.6), 177 (12.9)	(2R,3R)-3,5,7,2',6'-pentahydroxy flavanone
30	26.4	290,330	MS^2[303]: 125(100.0), 285(4.4), 275(10.2), 259(6.1), 217(13.8), 177(27.6)	3,5,7,2',6'-Pentahydroxy flavanone
31	27.6	254,351	MS^2[301]: 161(100.0), 283(4.8), 273(3.8), 270(1.2), 139(44.0)	3,5,7,3',4'-Pentahydroxy flavone (quercetin)
32	28.1	253,302	MS^2[301]: 161(100.0), 283(7.5), 273(17.9), 269 (18.2), 139(86.4), 133(15.3)	5,7,2',6'-Tetrahydroxy flavonol (visciclulin I)
33	31.1	267,342	MS^2[345]: 330(100.0), 331(17.5), 329(4.9), 328(1.4), 315(5.1)	5,7,2',5'-Tetrahydroxy-8,6'-dimethoxy flavone (viscidulin III)
34	31.5	261,310	MS^2[285]: 125(100.0), 269(5.6), 241(1.0), 177(1.0), 161(28.7), 151(2.6),	5,7,2',6'-Tetrahydroxy flavone
35	34.2	275,340	MS^2[393]: 313(100.0), 331(0.9), 314(8.1), 298(0.7)	5,2'-Dihydroxy-7,8-dimethoxy flavone (panicolin)
36	35.2	272,313	MS^2[283]: 269(100.0), 175(4.1)	5,7-Dihydroxy-6-methoxy flavone (oroxylin A)
37	36.3	262,346	MS^2[285]: 241(11.0), 237(7.6), 227(9.9), 151(10.0)	3,5,7,4'-Tetrahydroxy flavone (kaempferol)
38	36.8	241,253,349	MS^2[285]: 241(6.8), 233(5.8), 231(2.4), 201(6.7), 199(1.2), 175(4.9), 151(13.7)	5,7,3',4'-Tetrahydroxy flavone (luteolin)
39	37.9	222,278,304	MS^2[285]: 267(10.0), 243(2.5), 223(3.5), 218(4.1), 201(9.1), 153(5.3), 133(9.1)	5,7,8,4'-tetrahydroxy flavone (isoscutellarein)
40	48.5	276,320	MS^2[285]: 268(18.5), 241(7.2), 213(18.7), 205(3.4), 149(1.3)	5,6,7,4'-Tetrahydroxy flavone (scutellarein)
41	49.6	260(sh),274,362	MS^2[331]: 331(100.0), 300(1.2), 273(10.7), 157(1.8)	5,7,3',4',5'-Pentahydroxy-3,6,8-trimethoxy flavone
42	50.4	274	MS^2[283]: 268(100.0), 283(4.2), 269(16.2), 137(5.4)	5,7-Dihydroxy-8-methoxy flavone (wogonin)
43	51.0	230,253,311(sh)	MS^2[253]: 181(100.0), 208(9.4), 164(20.9), 125 (47.7)	7,4'-Dihydroxy flavone
44	51.1	268,315	MS^2[253]: 153(100.0), 186(4.5), 171(3.9), 145(13.7)	5,7-Dihydroxy flavone (chrysin)
45	51.2	269,324	MS^2[283]: 267(100.0), 136(1.8)	5,7-Dihydroxy-4'-methoxy flavone (acacetin)

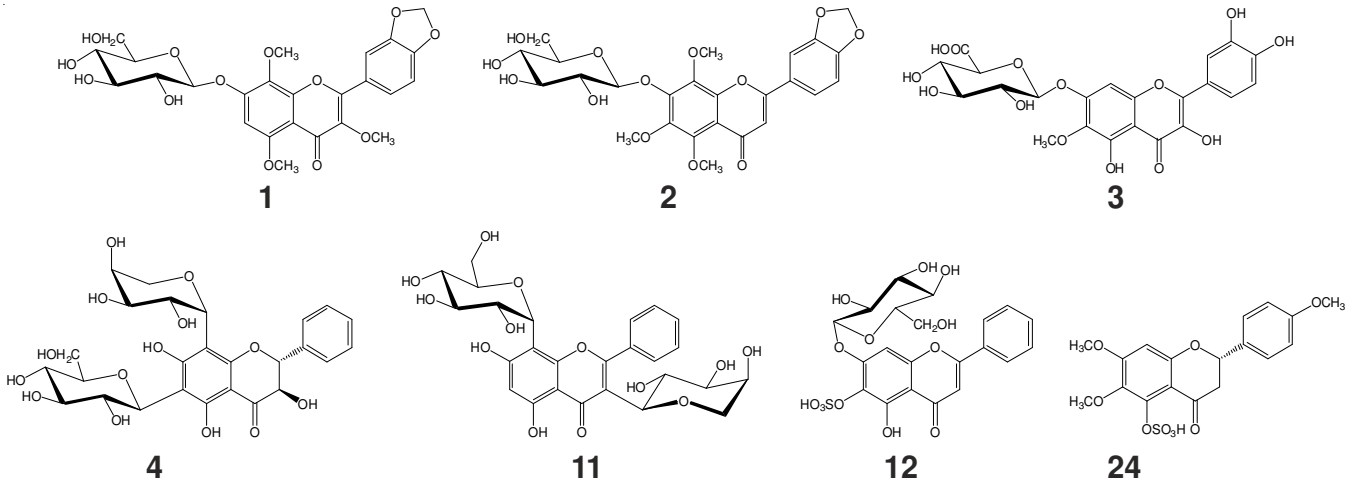

Fig. 2. Structures of the new components in the extract of *S. baicalensis*

12.0, 8.8 Hz, H-3), 2.81 (1H, dd, *J* = 12.0, 3.8 Hz, H-3); ^{13}C NMR (DMSO-d_6, 100 MHz) δ 197.5 (C-4), 159.7 (C-4'), 158.6 (C-7), 155.7 (C-5), 153.5 (C-9), 133.6 (C-6), 130.3 (C-1'), 128.4 (C-2', C-6'), 113.8 (C-3', C-5'), 106.2 (C-10), 90.5 (C-8), 78.9 (C-2), 59.9 (7-OCH_3), 58.2 (6-OCH_3), 55.1 (4'-OCH_3), 42.7 (C-3); ESI-MS/MS data (Table-1).

Supporting information: NMR spectra (^{1}H NMR, ^{13}C NMR, HMQC and HMBC), HR-ESI-MS of all new compounds and detailed extrction and isolation procedures are given as supporting information.

RESULTS AND DISCUSSION

Systematic phytochemical investigations were carried out to probe the chemical profile of the *S. baicalensis*, the successive purification of the EtOH extract of *S. baicalensis* by medium-pressure ODS column chromatography. Seven new and 38 known compounds were isolated and purified through medium-pressure ODS column, as well as repeated preparative using semi-preparative HPLC.

Compound **1**, obtained as pale yellow needles crystals. The molecular formula of **1** was determined to be $C_{25}H_{26}O_{13}$ on the basis of negative ion ESI-MS, ^{1}H and ^{13}C NMR spectral data. In the UV spectrum of **1**, the maximum bands are at 256, 273 and 350 nm. Its IR spectrum showed absorption bands due to hydroxy (3100 cm^{-1}), carbonyl (1620 cm^{-1}), aromatic (1605, 1590, 1495 cm^{-1}) groups. In the ^{1}H NMR spectrum (Table-1), three singlets (3H, each) were observed at δ_H = 3.72, 3.75 and 3.81, indicating three methoxyl groups. The ^{13}C NMR signals of **1** were assigned with the help of an HMQC experiment, establishing direct C-H bonding. The location of the three methoxyl groups were assigned to C-3, C-5 and C-8 based on the observed HMBC correlations from 3-OCH_3 (δ 3.72), 5-OCH_3 (δ 3.81) and 8-OCH_3 (δ 3.75) to C-3 (δ 140.9), C-5 (δ 157.1) and C-8 (δ 131.0), respectively. This experiment also clarified the site of glycosidation showing a long-range correlation between the anomeric proton of glucose at δ_H 5.11 (d, *J* = 7.7 Hz, H-1") and the oxygenated carbon atom at δ_C 155.4 (C-7). The ^{1}H and ^{13}C data of the aglycone were similar to those reported for 7-hydroxy-3,5,8-trimethoxy-3',4'-methylenedioxyflavone[4]. Finally, the structure of **1** was established as 7-hydroxy-3,5,8-trimethoxy-3',4'-methylenedioxy flavone-7-O-β-glucopyranoside.

Compound **2**, obtained as pale yellow needles with the molecular formula $C_{25}H_{26}O_{13}$ (negative ion ESI-MS, ^{1}H and ^{13}C NMR spectral data). The ^{1}H and ^{13}C NMR spectra of **2** were similar to those of **1**, which are different with site of methoxyl linkage. Comparison with the spectrum of compound **1** allowed an upfield shift for C-3 signal and a downfield shift for the C-6 signal, furthermore, signals of methoxyl groups could be assigned to C-6. The structure of aglycone was identified by comparison of its MS and ^{1}H and ^{13}C NMR data with those reported in the literature[5]. Based on these findings, the chemical structrue of **2** has been elucidated to be 7-hydroxy-5,6,8-trimethoxy-3',4'-methylenedioxy flavone-7-O-β-glucopyranoside.

Compound **3**, the ^{13}C NMR spectrum of **3** displayed the presence of one glucuronide unit in addition to 16 carbon signals for the aglycone. The chemical shifts of the aglycone carbons were similar to those reported for patuletin[6]. In the HMBC spectrum, long-rang correlations were observed from δ 5.17 (H-1 of GlcA) to δ 157.3 (C-7 of the aglycone). Accordingly, the structure of 3 was formulated as patuletin 7-β-glucuronide.

Compound **4** is optically active, $[\alpha]^{25}_D$ = 10.1 (c 0.5, MeOH) and its molecular formula was established as $C_{26}H_{30}O_{14}$ by high resolution ESIMS. Additionally, two anomeric proton resonances were observed at δ 4.64 (d, *J* = 9.0 Hz) and 4.80 (d, *J* = 9.6 Hz), indicating its diglycosidic structure. The sugar functionality was identified as a β-glucopyranose and arabinopyranoside by ^{1}H and ^{13}C NMR spectral data together with ESI-MS. The ^{13}C NMR spectrum of **4** exhibited 26 carbon resonances: 8 quaternary carbons (C), 16 methine (CH) and 2 methylene (CH_2). In the HMBC spectrum, the proton signal at δ 4.51(d, *J* = 12.8Hz, H-2) showed long-range correlations with the carbon signals at δ 160.5 (C-9), whereas the anomeric proton (δ 4.64) showed long-range correlation with C-8 (δ 105.4) and C-9 (δ 160.5), indicating that the arabinose moiety was located at C-8. In the HMBC spectrum, the long-rang correlation between the anomeric proton and C-3" (δ 77.7) of the glucose moiety, correlation to C-5 (δ 161.5)/C-6 (δ 106.9)/C-7 (δ 165.1), confirmed the attachment of glucose moiety to the carbon C-6. The structures of aglycone were identified by comparison of its MS and ^{1}H and ^{13}C NMR data with those reported in the literature[7]. Therefore, the structure

of **4** can be identified as pinobankasin-6-C-glucopyranosyl-8-C-arabinopyranoside.

Compound **11**, the ^{1}H and ^{13}C NMR spectral data together with ESI-MS supported the presence of diglycosidic. The structure of the sugar moieties could be entirely established on the basis of ^{1}H and ^{13}C NMR data. In HMBC, the anomeric proton (δ 4.64) showed long-range correlation with C-8 (δ 105.1), C-9 (δ 154.3) and C-3" (δ 79.1) indicating that the glucose moiety was located at C-8. Moreover, this experiment also clarified the site of glycosidation showing a long-range correlation between the anomeric proton of arabinose at δ_H 4.80 (d, J = 9.4 Hz) and the carbon atom at δ_C 178.3 (C-4). Thus, compound **11** was identified as chrysin 3-C-α-arabinopyranosyl-8-C-β-glucopyranoside by comparing its ^{1}H and ^{13}C NMR data with previously published data[8].

Compound **12**, obtained as a yellow amorphous powder. The molecular formula was determined to be $C_{21}H_{20}O_{1}3S$ on the basis of negative ion ESI-MS, ^{1}H and ^{13}C NMR spectral data. The UV spectrum exhibited maxima at 267 and 313 nm and IR spectrum suggested the presence of hydroxy groups (3348 cm^{-1}). The ^{1}H and ^{13}C data of the aglycone were similar to those reported for chrysin. The significant shifts of the carbon signals of the *ipso*, *ortho* and *para* positions caused by the sulphation at C-6 are consistent with literature data for flavonoid sulphates[9]. The β-glucopyranosyl unit is linked to C-7 of the aglycone from the chemical shift of this carbon at δ 166.5. Therefore, the structures of compound **12**, 6-sulfoxy-chrysin-7-glucoside, were identified on the basis of comparison of its spectroscopic (NMR, MS) data in comparison with literature values[10].

Compound **24** was isolated as an pale yellow crystal. The molecular formula was established as $C_{18}H_{18}O_{9}S$ by ESI-MS, ^{1}H and ^{13}C NMR spectral data. The IR spectrum showed absorption bands at 1630 (C=O), 1445 (aromatic), 1270 (-C-O-) and the UV spectrum exhibited maxima at 225, 287 and 340 nm. The signal at δ 6.71 related to two methylene protons showed long-range correlations with the signals for C-7, C-9, C-6 and C-10, whereas the methoxy protons at δ 3.80 and δ 3.85 showed long-range correlation with C-5 and C-6, repectively. Additional, the signal at δ_H 3.78 showed long-range correlations with the signals for C-3', C-5' and 4'-OCH_3 in the B ring. The significant shifts of the carbon signals of the *ipso, ortho* and *para* positions caused by the sulphation at C-5. Therefore, indicating the sulphation at C-5. Based on these findings, compound **25** were identified by comparing their physical and spectral data with the literature values: 5-hydroxy-6,7,4'- trimethoxy flavanone 5-sulfate[11].

By comparing their physical and spectroscopic data with published data, the structures of the other 38 known chemical constituents were elucidated as 6,8-dihydroxy quercetin diglucopyranoside (**5**)[12], 3,5,7,8,3',4'-hexahydroxy-6-methoxy flavone diglucopyranoside (**6**)[13], apigenin-6-C-β-D-glucoside-8-C-α-L-arabinoside (**7**)[14], apigenin-6-C-α-L-arabinoside-8-C-β-D-glucoside (**8**)[14], chrysin-6,8-C-diglucoside (**9**)[15], chrysin-6-C-α-L-arabinoside-8-C-β -D-glucoside (10)[16], chrysin-6-C-β-D-glucoside-8-C-α-L-arabinoside (**13**)[16], baicalein-7-O-β-D-glucopyranosyl sulfate conjugate (**14**)[17], chrysoeriol-7-O-β-D-glucuronide (**15**)[18], baicalein-7-O-β-D-glucopyranoside (**16**)[17], wogonin-5-O-β-D-glucopyranoside (**17**)[19], chrysin-8-C-β-D-glucopyranoside (**18**)[20], baicalein-7-O-glucuronide (baicalin) (**19**)[21], 5,7,6'-trihydroxy-2'-methoxy flavone-7-O-β-D-glucuronide (**20**)[22], apigenin7-O-β-D-glucuronopyranoside (scutellarin A) (**21**)[23], 2'-hydroxychrysin-7-glucuronide (**22**)[24], quercetin-7-O-β-D-glucopyranoside (quercimeritrin) (**23**)[25], oroxylin A-7-O-β-D-glucuronide (**25**)[26], wogonin-7-O-β-D-glucuronopyranoside (**26**), baicalein-7-O-sulfate (**27**)[27], 3,5,7,3',4'-pentahydroxy flavanone (taxifolin) (**28**)[28], (2R, 3R)-3,5,7,2',6'-pentahydroxy flavanone (**29**)[29], 3,5,7,2',6'-pentahydroxy flavanone (**30**)[30], 3,5,7,3',4'-pentahydroxy flavone (quercetin) (**31**), 5,7,2',6'-tetrahydroxy flavonol (visciclulin I) (**32**)[31], 5,7,2',5'-tetrahydroxy-8,6'-dimethoxy flavone (viscidulin III) (**33**)[32], 5,7,2',6'-tetrahydroxy flavone (**34**)[33], 5,2'-dihydroxy-7,8-dimethoxy flavone (panicolin) (**35**)[34], 5,7-dihydroxy-6-methoxy flavone (oroxylin A) (**36**)[35], 3,5,7,4'-tetrahydroxy flavone (kaempferol) (**37**)[36], 5,7,3',4'-tetrahydroxy flavone (luteolin) (**38**)[37], 5,7,8,4'-tetrahydroxy flavone (isoscutellarein) (**39**)[38], 5,6,7,4'-tetrahydroxy flavone (scutellarein) (**40**)[36], 5,7,3',4',5'-pentahydroxy-3,6,8-trimethoxy flavone (**41**)[39], 5,7-dihydroxy-8-methoxy flavone (wogonin) (**42**)[40], 7,4'-dihydroxy flavone (**43**)[41], 5,7-dihydroxy flavone (chrysin) (**44**)[42], 5,7-dihydroxy-4'-methoxy flavone (acacetin) (**45**)[43].

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