Flavonoid Glycosides and Their Derivatives from the Herbs of *Scorzonera austriaca* Wild

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Abstract: Five flavonoid glycosides and two derivatives were isolated from the herbs of *Scorzonera austriaca* Wild by silica gel column chromatography and preparative HPLC. Their structures were identified, using chemical and spectroscopic methods, as 5,7,4'1-trihydroxyflavone 6-C-(2'-O-β-D-glucopyranosyl β-D-glucopyranoside) (1), 5,7,3',41-tetrahydroxyflavone 6-C-(2'-O-β-D-glucopyranosyl β-D-glucopyranoside) (2), quercetin 3-O-rutinoside (3), 5,7,4'-trihydroxyflavone 6-C-β-D-glucopyranoside (4), 3'-methoxy-5,7,4'-trihydroxyflavone 6-C-β-D-glucopyranoside (5), 5,7,4'-trihydroxyflavone 8-C-(6'-O-trans-cafeoyl β-D-glucopyranoside) (6), and 5,7,3',41-tetrahydroxyflavone 8-C-(6'-O-trans-cafeoyl β-D-glucopyranoside) (7). Compounds 6 and 7 are new flavonoid glycoside derivatives, and compounds 1–5 were isolated from the herbs of *Scorzonera austriaca* for the first time. Compounds 6 and 7 were also assayed for their hepatoprotective activities with rat hepatocytes in vitro.

Keywords: *Scorzonera austriaca*; flavonoids; hepatoprotective activities

1. Introduction

*Scorzonera austriaca* Wild, a perennial herb of Compositae, is widely distributed in the northeast and northwest regions in China, especially abundant in Jilin Province, and has been widely used for curing fever, carbuncle, and mastitis as a traditional herbal medicine [1]. Since *S. austriaca* is used to treat hepatitis B as a folk medicine in our district, we have investigated its bioactivities and found that the total flavonoids from *S. austriaca* have hepatoprotective effects and inhibitory effects on hepatitis B virus [2–4]. Chronic infection with hepatitis B virus (HBV) often leads to the development of liver cancer and cirrhosis, creating immense sociological, clinical, and economic burdens worldwide [5]. Globally, 240 million people are infected with the HBV [6], and 650,000 people die every year from HBV-related cirrhosis or hepatocellular carcinoma [7]. Currently, seven drugs have been approved by FDA for the treatment of HBV infection: two interferons (standard and pegylated) and five nucleoside/nucleotide analogues (lamivudine, adefovir, telbivudine, entecavir, and tenofovir) [8]. Although interferons can reconstitute the host immune system, and have finite duration and no risk of drug resistance, the need for parenteral administration, the poor long-term response, and the high frequency of adverse side effects make them not ideal [8]. Nucleoside/nucleotide analogues are less expensive and orally available, have minimal side-effects comparing to interferons and can be used for decompensated cirrhosis and after liver transplantation [9]. However, because they have to be taken on a long-term basis, in general, drug resistance may evolve, and there is also a significant risk of HBV reactivation and sometimes HBV flare up after withdrawal of the antiviral agents [10]. Moreover, all of these drugs have low percentage of HBV e antigen seroconversion rate and HBV surface antigen loss, and none of them are able to clear chronic HBV infection [9,11,12]. Thus, new efforts are being directed to develop new and
more effective anti-HBV therapeutics. In order to screen the new drug candidate for treating hepatitis B, the isolation and structure identification of flavonoid glycosides and their derivatives from the herbs of *S. austriaca* have been carried out, and we report the isolation and identification of two new flavonoid glycoside derivatives (6 and 7), together with five flavonoid glycosides 1–5 in the present study (Figure 1).

![Chemical structures of compounds 1-7.](image)

**Figure 1.** Chemical structures of compounds 1–7.

### 2. Results and Discussion

The air-dried *S. austriaca* herbs were extracted with 70% aqueous ethanol solution (*v/v*), the extract was subjected to D101 poly porous resin column chromatography eluted with water and 60% aqueous ethanol solution (*v/v*), and the crude flavonoid extracts were obtained from 60% aqueous ethanol eluate. The crude flavonoid extracts were loaded on D4020 poly porous resin column eluting successively with water and different aqueous ethanol solutions, and four fractions were obtained. The four fractions were further separated and purified by silica gel column chromatography and semi-preparative RP-HPLC to afford compounds 1–7, including two new flavonoid glycoside derivatives (6 and 7).

The five known compounds were identified as 5,7,4'-trihydroxyflavone 6-C-(2"-O-β-D-glucopyranosyl β-D-glucopyranoside) (1) [13], 5,7,3',4'-tetrahydroxyflavone 6-C-(2"-O-β-D-glucopyranosyl β-D-glucopyranoside) (2) [14], quercetin 3-O-rutinoside (3) [15], 5,7,4'-trihydroxyflavone 6-C-β-D-glucopyranoside (4) [16], and 3'-methoxy-5,7,4'-trihydroxyflavone 6-C-β-D-glucopyranoside (5) [17], by comparison of various data with the reported compounds.
Compound 6 was obtained as yellow amorphous powders. Its HRESIMS displayed a [M + H]+ ion peak at m/z 595.1437 (calcd for C30H27O15, 595.1446), indicating the molecular formula C30H26O15. The 1H- and 13C-NMR spectra showed eleven aromatic or unsaturated proton signals, twenty-four unsaturated carbon signals, and six saturated carbons signals, among which a typical three-proton ABX aromatic spin system at δH 6.70 (1H, d, J = 7.0 Hz), 6.91 (1H, d, J = 7.0 Hz), 6.80 (1H, br. s), a trans-disubstituted double bond conjugated with a carbonyl group at δH 7.40 (1H, d, J = 15.7 Hz), 6.15 (1H, d, J = 15.7 Hz), and nine unsaturated carbon signals at δC 166.7 (C), 148.4 (C), 145.5 (C), 145.4 (CH), 125.3 (C), 120.5 (CH) 115.7 (CH), 115.3 (CH), and 113.6 (CH) were deduced to arise from a trans-caffeoyl moiety based on the analysis of 1H-1H COSY, HMQC, and HMB of 6 and the comparison of 1H- and 13C-NMR spectra of 6 with the reported values [18]. The 1H- and 13C-NMR spectra of 6 were very similar to those of the reported compound, 5,7,4′-trihydroxyflavone 8-C-β-D-glucopyranoside (6′) [19], except for a set of signals of the above trans-caffeoyl moiety. Comparison of 1H- and 13C-NMR spectra data of 6 and 6′ showed that the trans-caffeoyl moiety is attached to C-6′ of glucose on the basis of the acylation effects at the OH of C-6′, which are the downfield shift of the C-6′ protons (+0.66 and +0.73 ppm) and of the C-6′ carbon (+2.7 ppm) and the upfield shift of the C-5′ carbon (−3.5 ppm) [20], which was also confirmed by the correlation of the C-6′ protons at δH 4.17 and 4.48 with the carboxy carbon at δC 166.7 in HMBC of 6. The glucosyl anomeric proton observed at δH 4.77 as a doublet with J = 9.9 Hz was indicative of a β-configuration for the glucose. The full assignments of all protons and carbons were performed through the correlations in 2D-NMR spectra (1H-1H COSY, HMQC and HMB) of 6. For all of the data of 1H-, 13C-, and HMB-NMR of compound 6 see Table 1, and key correlations and the structure of compound 6, see Figure 2. Based on the above evidence, the structure of 6 was determined to be 5,7,4′-trihydroxyflavone 8-C-(6′-O-trans-caffeoyl β-D-glucopyranosyl)

Table 1. 1H-NMR (DMSO-d6, 400 MHz), 13C-NMR (DMSO-d6, 100 MHz), and HMB data of compounds 6 and 7 (TMS as the internal standard, δ in ppm, J in Hz).

| No. | 6             | 6′            | 7               | 7′               |
|-----|---------------|---------------|-----------------|-----------------|
|     | δC (Hz)       | δH (Hz)       | HMBC (H→C)      | δC (Hz)         | δH (Hz)       | HMBC (H→C)    | δC (Hz)         | δH (Hz)       |
|     | C–glycone moiety |                |                 | Sugar moiety    |                 |               |                 |                |
| 2   | 163.6         | 163.9         | 163.9           | 119.0, 150.0, 163.9 | 114.2          | 7.48 (Hz)     |
| 3   | 102.3         | 102.4         | 102.4           | 119.0, 150.0, 163.9 | 114.2          | 7.48 (Hz)     |
| 4   | 182.0         | 182.0         | 182.0           | 115.6           | 146.0          | 145.9         |
| 5   | 161.7         | 161.7         | 161.7           | 149.7           | 149.7          |
| 6   | 98.2          | 98.1          | 98.1            | 115.8           | 156.0          | 156.1         |
| 7   | 163.0         | 162.7         | 162.6           | 150.0           | 150.0          | 150.0         |
| 8   | 104.2         | 104.6         | 104.6           | 119.0           | 119.0          | 119.0         |
| 9   | 160.5         | 160.4         | 156.0           | 119.5           | 119.5          | 119.5         |
| 10  | 103.9         | 103.9         | 104.1           | 122.1           |                |
| 1′  | 121.2         | 121.6         | 121.8           |                |                |
| 2′  | 126.8         | 128.9         | 128.9           |                |                |
| 3′  | 116.0         | 115.8         | 146.0           |                |                |
| 4′  | 156.0         | 156.0         | 150.0           |                |                |
| 5′  | 116.0         | 115.8         | 115.6           |                |                |
| 6′  | 126.6         | 128.9         | 119.0           |                |                |

| 1″  | 73.4          | 73.4          | 73.6            | 70.6, 78.5, 104.1, 150.0, 162.6 | 73.5          | 4.68 (Hz)     |
| 2″  | 70.7          | 70.8          | 70.6            | 70.9             | 3.84 (Hz)     |
| 3″  | 78.4          | 78.6          | 78.5            | 78.8             | 3.31 (Hz)     |
| 4″  | 70.5          | 70.5          | 70.5            | 70.8             | 3.53 (Hz)     |
| 5″  | 78.3          | 81.8          | 78.5            | 82.0             | 3.54 (Hz)     |
| 6″  | 64.0          | 61.3          | 64.3            | 66.8             | 3.79 (Hz)     |

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Compound 7 was obtained as yellow amorphous powders. Its HRESIMS displayed a [M + H]+ ion peak at m/z 611.1408 (calcld for C30H27O14, 611.1395), indicating the molecular formula C30H26O14. The 1H- and 13C-NMR spectra of 7 were very similar to those of the reported compound, 5,7,3′,4′-tetrahydroxylavone 8-C-β-D-glucopyranoside (7′) [21], except for a set of signals indicative of a trans-caffeoyl moiety: δH 7.40 (1H, d, J = 15.8 Hz, H-7′′′), 6.23 (1H, d, J = 15.8 Hz, H-8′′′), 6.94 (1H, d, J = 7.0 Hz, H-5′′′), 6.73 (1H, d, J = 8.0 Hz, H-6′′′), and 6.85 (1H, d, J = 8.0 Hz, H-6′′′), and δC 166.8 (C-9′′′), 148.3 (C-4′′′), 145.3 (C-3′′′, 7′′′), 125.4 (C-1′′′), 120.6 (C-6′′′) 115.8 (C-5′′′), 115.6 (C-2′′′) and 113.7 (C-8′′′). The attachment of the trans-caffeoyl moiety to the 6′-position of the glucosyl part was also deduced from the acylation effects at the OH of C-6′, which are the downfield shift of the C-6′ protons (+0.59 and +0.75 ppm) and of the C-6′′ carbon (+2.6 ppm) and the upfield shift of the C-5′′ carbon (−3.5 ppm) [19], which was further confirmed by the correlation of the C-6′ protons at δH 4.15 and 4.54 with the carboxyl carbon at δC 166.8 in HMBC of 7. The glucosyl anomic proton observed at δH 4.75 as a doublet with J = 9.9 Hz was indicative of a β-configuration for the glucosyl part. Full assignments of all protons and carbons were preformed through the correlations in 2D-NMR spectra (1H-1H COSY, HMQC, and HMBC) of 7. For all of the data of 1H-, 13C-, and HMBC-NMR of compound 7 see Table 1, and key correlations and the structure of compound 7, see Figure 2. Based on the above evidence, the structure of 7 was determined to be 5,7,3′,4′-tetrahydroxylavone 8-C-(6′′-O-trans-caffeoyl β-D-glucopyranoside).

The hepatoprotective activities of compounds 6 and 7 were assessed by measuring the content of alanine aminotransferase (ALT) of the cultures of rat hepatocytes treated with CCl4. Compounds 6 and 7 exhibited hepatoprotective activities with values of 71.2% and 81.2%, respectively, at a concentration of 100 μM, comparable to that of silibinin [22], which was used as a positive control (68.3% at 50 μM).

Table 1. Cont.

| No.  | δC (ppm) | δH (Hz) | HMBC | δC (ppm) | δH (Hz) | HMBC | δC (ppm) | δH (Hz) |
|------|----------|---------|------|----------|---------|------|----------|---------|
| 6′   | 145.4    | 148.4   |      | 115.6    | 6.94    |      | 145.3    | 148.3   |
| 5′   | 120.5    | 115.5   | 145.4| 6.85     | 115.6   | 148.3| 15.8     | 125.4   |
| 4′   | 115.7    | 6.70    | 125.3| 6.73     | 124.5   | 145.5| 8.0      | 150.6   |
| 3′   | 145.4    | 7.40    | 115.3| 7.40     | 115.6   | 120.6| 8.0      | 150.6   |
| 2′   | 120.5    | 6.91    | 115.4| 6.85     | 115.6   | 148.3| 8.0      | 150.6   |
| 1′   | 113.6    | 6.15    | 125.3| 6.23     | 125.4   | 15.8| 113.7    | 15.8    |
| 9′   | 166.7    |         |      |          |         |      |          |         |

Note: the assignments were based on DEPT, HMQC, 1H-1H COSY, and HMBC experiments.

Figure 2. The key HMBC correlation of compounds 6 and 7 (arrows point from proton to carbon).
Therefore, the flavonoid glycoside derivatives 6 and 7 are considered to be two of the hepatoprotective principles in this plant.

Table 2. Effects of compounds 6 and 7 on CCl₄-induced toxicity of rat hepatocytes.

| Group       | Dose  | ALT (IU/L) | Relative Protection(%) |
|-------------|-------|------------|------------------------|
| Control     |       | 12.6 ± 2.4 | 100                    |
| CCl₄-treated|       | 101.5 ± 4.5* | 0                      |
| Silibinin   | 50 µM | 40.8 ± 2.9** | 68.3                   |
| 6           | 25 µM | 86.7 ± 3.6#  | 16.5                   |
|             | 50 µM | 54.5 ± 3.7** | 52.9                   |
|             | 100 µM| 38.2 ± 3.8** | 71.2                   |
| 7           | 25 µM | 76.6 ± 3.5** | 28.1                   |
|             | 50 µM | 42.5 ± 2.4** | 66.4                   |
|             | 100 µM| 28.8 ± 3.5** | 81.2                   |

All data were analyzed using SPSS version 20.0 (International Business Machines Corporation, Armonk, NY, USA); the each value represents the mean ± SD (n = 3); the % of protection is calculated as 100 – (value of CCl₄ – value of sample)/(value of CCl₄ – value of control); * p < 0.01, compared with control group; # p < 0.05, ## p < 0.01, compared with

3. Experimental Section

3.1. General Information

NMR spectra were recorded on a Bruker AV-400 spectrometer (Bruke Corporation, Faellanden, Switzerland). UV spectra were recorded on a Shimadzu UV-2401A spectrometer (Shimadzu Corporation, Kyoto, Japan). HR-ESI-MS were recorded on a Bruker microTOF-Q II mass spectrometer (Bruke Corporation, Bremen, Germany). HPLC was performed Shimadzu LC-10A with a SPD-10A detector (Shimadzu Corporation, Kyoto, Japan) and Gemini 5µ C18 110A column (250 mm x 10.00 mm, 5 µm, flow rate: 3.0 mL min⁻¹, Phenomenex, Torrance, CA, USA). Column chromatography was performed on silica gel (200–300 mesh, Qingdao Marine Chemical Inc., Qingdao, China), and D101 polyporous resin (Tianjin Pesticide Co., LTD., Resin Branch, Tianjin, China). Thin layer Chromatography was performed on glass precoated silica gel GF₂₅₄ plates (Qingdao Haiyang Chemical Co., Ltd, Qingdao, China), detection under UV light or by spraying with 10% H₂SO₄ in 95% EtOH followed by heating. Distilled water was purchased from Hangzhou Wahaha Group Co., Ltd. (Hongzhou, China). Acetonitrile of chromatographic grade for HPLC was purchased from Fisher Scientific (Fair Lawn, NJ, USA). Other chemicals and reagents of analytical grade were from Beijing Chemical Works (Beijing, China).

The bioactivities were measured on a DNM-9602 enzyme immunoassay spectrophotometer (Beijing, China). Enzyme-linked immunosorbert assay kits for alanine transferase (ALT) were purchased from Jiancheng Institute of Biotechnology (Nanjing, China), collagenase IV from Sigma (St. Louis, MO, USA), 1640 medium from HyClone (Logan, UT, USA), PBS from Gibco company (Carlsbad, CA, USA), fetal bovine serum (FBS) from Zhejiang Tianhang Biotechnology Co., Ltd. (Hangzhou, China), and silibinin from the Chinese National Institute for the Control of Pharmaceutical and Biological Products (Beijing, China).

Female Wistar rats (150–180g) were purchased from the Experimental Animal Center, Jilin University (Changchun, China).

The herbs of S. austriaca were collected in Si-ping District in Jilin Province, China. They were identified by Professor Jing-Min Zhang of the School of Pharmaceutical Sciences, Jilin University (Changchun, China).
3.2. Extraction and Isolation

Two kilograms of air-dried whole *S. austriaca* herbs were extracted twice with 20 L of 70% aqueous ethanol solution (v/v) at room temperature. The extraction solution was concentrated under reduced pressure to remove ethanol, and the water concentrate was filtered and then passed through a D101 polyporous resin column eluting successively with water and 60% aqueous ethanol solution (v/v). The crude flavonoid extracts were obtained from 60% aqueous ethanol eluate by vacuum distillation recovery and used for the next experiments. The crude flavonoid extracts were loaded on D4020 polyporous resin column eluting successively with water, 15%, 20%, 25%, 30%, and 40% aqueous ethanol solutions (v/v), and six fractions (Fractions 1–6) were obtained. Fraction 2 was chromatographed over silica gel eluting with CHCl₃-MeOH–EtOAc–H₂O (2:2:4:1, v/v, lower layer) to afford Fraction 2a, Fraction 4 with CHCl₃-MeOH–EtOAc–H₂O (2:1:4:1, v/v, lower layer) to afford Fraction 4a, and Fraction 6 with CHCl₃-MeOH–EtOAc–H₂O (2:1:4:1, v/v, lower layer) to afford Fraction 6a. They were further isolated by semi-preparative RP-HPLC using acetonitrile and 0.1% formic acid solution in water as the mobile phase and the eluate was monitored at 337 nm. Compound 1 (30 mg), 2 (30 mg), and 3 (20 mg) were obtained from Fraction 2a with gradient elution (10%–11% acetonitrile from 0.00–20.00 min, 11%–18% acetonitrile from 20.00–40.00 min), compound 4 (30 mg) and 5 (20 mg) from Fraction 4a by using 15% acetonitrile, and compound 6 (60 mg) and 7 (200 mg) from Fraction 6a with gradient elution (20%–22% acetonitrile from 0.00–45.00 min).

Compound 1: Yellow amorphous powder, yielded a positive reaction to FeCl₃ reagent, mp 201–203 °C; UV (MeOH), λ_max 265, 339 nm; HRESIMS m/z 595.1665 [M + H]⁺ (calcld for C₂₇H₃₁O₁₅, 595.1657); ¹H-NMR (DMSO-d₆, 400 MHz) δ: 7.93 (2H, d, J = 8.4 Hz, H-2',6'), 6.93 (2H, d, J = 8.4 Hz, H-3',5'), 6.78 (1H, s, H-3), 6.49 (1H, s, H-8), 4.65 (1H, d, J = 9.6 Hz, Glc-H-1), 4.44 (1H, m, Glc-H-2), 3.41 (1H, m, Glc-H-3), 3.16 (1H, m, Glc-H-4), 3.16 (1H, m, Glc-H-5), 3.33 (1H, m, Glc-H-6), 3.67 (1H, m, Glc-H-6), 4.18 (1H, d, J = 7.6 Hz, Glc'-H-1), 2.85 (1H, m, Glc'-H-2), 3.03 (1H, m, Glc'-H-3), 3.01 (1H, m, Glc'-H-4), 2.64 (1H, m, Glc'-H-5), 2.94 (1H, m, Glc'-H-6), 3.17 (1H, m, Glc'-H-6); ¹³C-NMR (DMSO-d₆, 100 MHz) δ: 163.4 (C-2), 102.7 (C-3), 182.0 (C-4), 161.1 (C-5), 107.9 (C-6), 161.1 (C-7), 93.3 (C-8), 154.6 (C-9), 103.3 (C-10), 121.1 (C-1'), 128.4 (C-2',6'), 116.0 (C-3',5'), 161.1 (C-4'), 71.1 (Glc-1), 81.0 (Glc-2), 78.4 (Glc-3), 70.4 (Glc-4), 81.6 (Glc-5), 61.4 (Glc-6), 105.3 (Glc'-1), 74.7 (Glc'-2), 76.4 (Glc'-3), 69.3 (Glc'-4), 76.4 (Glc'-5), 60.5 (Glc'-6).

Compound 2: Yellow amorphous powder, yielded a positive reaction to FeCl₃ reagent, mp 205–207 °C; UV (MeOH), λ_max 258, 366 nm; HRESIMS m/z 611.1623 [M + H]⁺ (calcld for C₂₇H₃₁O₁₆, 611.1607); ¹H-NMR (DMSO-d₆, 400 MHz) δ: 7.41 (1H, d, J = 8.1 Hz, H-6'), 7.40 (H, s, H-2'), 6.89 (1H, d, J = 8.1 Hz, H-5'), 6.65 (1H, s, H-3), 6.45 (1H, s, H-8), 4.66 (1H, d, J = 9.8 Hz, Glc-H-1), 4.44 (1H, m, Glc-H-2), 3.45 (1H, m, Glc-H-3), 3.17 (1H, m, Glc-H-4), 3.17 (1H, m, Glc-H-5), 3.41 (1H, m, Glc-H-6), 3.68 (1H, m, Glc-H-6), 4.19 (1H, d, J = 7.6 Hz, Glc'-H-1), 2.86 (1H, m, Glc'-H-2), 3.06 (1H, m, Glc'-H-3), 3.03 (1H, m, Glc'-H-4), 2.65 (1H, m, Glc'-H-5), 2.99 (1H, m, Glc'-H-6), 3.17 (1H, m, Glc'-H-6); ¹³C-NMR (DMSO-d₆, 100 MHz) δ: 163.5 (C-2), 102.7 (C-3), 181.9 (C-4), 160.6 (C-5), 107.9 (C-6), 163.0 (C-7), 93.3 (C-8), 154.6 (C-9), 103.3 (C-10), 121.4 (C-1'), 113.2 (C-2'), 145.7 (C-3'), 149.7 (C-4'), 116.0 (C-5'), 118.9 (C-6'), 71.2 (Glc-1), 80.9 (Glc-2), 78.4 (Glc-3), 70.4 (Glc-4), 81.6 (Glc-5), 61.4 (Glc-6), 105.5 (Glc'-1), 74.7 (Glc'-2), 76.2 (Glc'-3'), 69.3 (Glc'-4), 76.2 (Glc'-5), 60.6 (Glc'-6).

Compound 3: Yellow amorphous powder, yielded a positive reaction to FeCl₃ reagent, mp 185–187 °C; UV (MeOH), λ_max 259, 359 nm; HRESIMS m/z 611.1620 [M + H]⁺ (calcld for C₂₇H₃₁O₁₆, 611.1607). ¹H-NMR (DMSO-d₆, 400MHz) δ: 7.54 (1H, d, J = 8.1, H-6'), 7.53 (1H, s H-2'), 6.83 (1H, d, J = 8.1 Hz, H-5'), 6.36 (1H, s, H-8), 6.17 (1H, s, H-6), 5.33 (1H, d, J = 6.7 Hz, Glc-H-1), 4.39 (1H, s, Rha-H-1), 0.99 (3H, d, J = 6.1 Hz, Rha-H-6); ¹³C-NMR (DMSO-d₆, 100 MHz) δ: 156.4 (C-2), 133.2 (C-3), 177.2 (C-4), 161.1 (C-5), 98.8 (C-6), 164.7 (C-7), 93.6 (C-8), 156.4 (C-9), 103.6 (C-10), 121.0 (C-1'), 115.2 (C-2'), 144.8
Compound 4: Yellow amorphous powder, yielded a positive reaction to FeCl₃ reagent, mp 238–240 °C; UV (MeOH), λ_max 260, 351 nm; HRESIMS m/z 433.1157 [M + H]^+ (calcd for C_{21}H_{21}O_{10}, 433.1129); ¹H-NMR (DMSO-d₆, 400 MHz) δ: 7.90 (2H, d, J = 8.4 Hz, H-2',6'), 6.97 (2H, d, J = 8.4 Hz, H-3',5'), 6.74 (1H, s, H-3), 6.64 (1H, s, H-8), 4.58 (1H, d, J = 9.6Hz, Glc-H-1), 4.07 (1H, t, J = 8.8Hz, Glc-H-2), 3.20 (1H, m, Glc-H-3), 3.15 (1H, m, Glc-H-4), 3.15 (1H, m, Glc-H-5), 3.43 (1H, m, Glc-H-6), 3.67 (1H, d, J = 15.6 Hz, Glc-H-6); ¹³C-NMR (DMSO-d₆, 100 MHz) δ: 163.4 (C-2), 102.6 (C-3), 181.4 (C-4), 160.5 (C-5), 108.9 (C-6), 164.0 (C-7), 93.8 (C-8), 156.2 (C-9), 103.1 (C-10), 121.0 (C-1'), 128.3 (C-2',6'), 116.1 (C-3',5'), 161.4 (C-4'), 73.0 (Glc-1), 70.1 (Glc-2), 79.0 (Glc-3), 70.5 (Glc-4), 81.5 (Glc-5), 61.3 (Glc-6).

Compound 5: Yellow amorphous powder, yielded a positive reaction to FeCl₃ reagent, mp 208–210 °C; UV (MeOH), λ_max 261, 348 nm; HRESIMS m/z 463.1253 [M + H]^+ (calcd for C_{22}H_{23}O_{11}, 463.1235); ¹H-NMR (DMSO-d₆, 400 MHz) δ: 7.33 (1H, d, J = 8.4 Hz, H-6'), 7.33 (1H, s, H-2'), 6.82 (1H, d, J = 8.4 Hz, H-5'), 6.50 (1H, s, H-3), 6.37 (1H, s, H-8), 4.66 (1H, d, J = 9.8 Hz, Glc-H-1), 3.25 (3H, s, C-3'-OCH₃); ¹³C-NMR (DMSO-d₆, 100 MHz) δ: 164.1 (C-2), 102.3 (C-3), 182.0 (C-4), 160.1 (C-5), 107.3 (C-6), 163.4 (C-7), 93.5 (C-8), 156.8 (C-9), 103.2 (C-10), 121.6 (C-1'), 108.6 (C-2'), 147.6 (C-3'), 150.3 (C-4'), 115.1 (C-5'), 120.0 (C-6'), 73.5 (Glc-1), 69.8 (Glc-2), 78.2 (Glc-3), 70.7 (Glc-4), 80.7 (Glc-5), 61.1 (Glc-6).

Compound 6: Yellow amorphous powder, yielded a positive reaction to FeCl₃ reagent, mp 208–210 °C; UV (MeOH), λ_max 268, 339 nm; HRESIMS m/z 595.1437 [M + H]^+ (calcd for C_{30}H_{27}O_{13}, 595.1446); ¹H-NMR (DMSO-d₆, 400 MHz) and ¹³C-NMR (DMSO-d₆, 100 MHz) see Table 1.

Compound 7: Yellow amorphous powder, yielded a positive reaction to FeCl₃ reagent, mp 204–206 °C; UV (MeOH), λ_max 269, 350 nm; HRESIMS m/z 611.1408 [M + H]^+ (calcd for C_{30}H_{27}O_{14}, 611.1395); ¹H-NMR (DMSO-d₆, 400 MHz) and ¹³C-NMR (DMSO-d₆, 100 MHz) see Table 1.

3.3. In Vitro Hepatoprotective Activity

Isolated rat hepatocytes from female Wistar rats were prepared by the collagenase perfusion technique with minor modifications [23]. Culture medium was composed of RPMI 1640 medium supplemented with 10% fetal bovine serum and 1% Pen/Strep (100 IU·mL⁻¹ penicillin and 100 mg·mL⁻¹ streptomycin). The isolated cells were diluted to 8 × 10⁶ / mL using the culture medium, and every 8 × 10⁵ cells (0.1 mL) were seeded into a 48-well plate and incubated at 37 °C in a humidified atmosphere containing 5% CO₂. After preincubation for four hours, the medium was replaced with fresh medium containing CCl₄ (15 mM) and test specimens at various concentrations, and four hours later, the medium was taken to measure ALT. Enzyme-linked immunosorbent assay kits and DNM-9602 enzyme immunoassay spectrophotometer were used to measure ALT. For the results, see the Table 2.

4. Conclusions

Compounds 6 and 7 are new flavonoid glycoside derivatives. Compounds 1–5 were isolated from S. austriaca Wild for the first time. Compounds 6 and 7 were also assayed for hepatoprotective activities with rat hepatocytes, and the data proved that compounds 6 and 7 exhibited hepatoprotective activities. Therefore, the new flavonoid glycoside derivatives 6 and 7 are considered to be two of the hepatoprotective properties in this plant.

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**Sample Availability:** Samples of the compounds 1–7 are available from the authors.