

FLAVONOIDS FROM LEAVES AND TWIGS OF *Stachyurus himalaicus* VAR. *himalaicus*

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Stachyuraceae comprises only the genus *Stachyurus*, which is distributed from the Himalayas to Japan. *Stachyurus himalaicus* var. *himalaicus* is a shrub growing in southwestern China and is known as “Tong-Cao” in Chinese folklore. It has been used as a galactopoietic, and diuretic, and for the treatment of dropsy and gonorrhoea for a long time [1]. To the best of our knowledge, no work has been reported on the active constituents of *Stachyurus himalaicus* var. *himalaicus*. Our previous research on this plant resulted in the isolation of two new polyoxygenated triterpenoids [2]. In this study, the isolation and identification of seven flavonoids **1–7** are reported for the first time from the genus *Stachyurus*.

The powdered leaves and twigs of *S. himalaicus* var. *himalaicus* (33 kg) were repeatedly extracted with 90% EtOH at room temperature. The extract was then concentrated under reduced pressure to give a brown syrup, which was diluted with water and extracted successively with petroleum ether and EtOAc. The EtOAc extracts (700 g) were subjected to silica gel column chromatography eluting with PE–EtOAc (20:1–1:1), EtOAc, EtOAc–MeOH (10:1–1:1), and MeOH. The resulting fractions were resubmitted to silica gel column chromatography, Sephadex LH-20, and RP-18 to yield compounds **1–7**.

The structures of compounds **1–7** were elucidated on the basis of their MS, ^1H NMR, and ^{13}C NMR spectra. All experimental data were in agreement with the respective data in the literature.

Ampelopsin (1): $\text{C}_{15}\text{H}_{12}\text{O}_8$, yellow powder. FAB[–]-MS (Gly, m/z , %): 319 ($[\text{M} - 1]^-$, 100), 222 (115), 157 (5); ^1H NMR (500 MHz, CD_3OD , δ , ppm, J/Hz): 5.88 (1H, d, J = 2.1, H-6), 5.91 (1H, d, J = 2.1, H-8), 4.46 (1H, d, J = 11.3, H-2), 4.84 (1H, d, J = 11.3, H-3), 6.52 (2H, d, J = 1.9, H-2', 6'); ^{13}C NMR (125 MHz, CD_3OD , δ , ppm): 85.7 (d, C-2), 74.1 (d, C-3), 197.8 (s, C-4), 165.7 (s, C-5), 97.7 (d, C-6), 169.1 (s, C-7), 96.7 (d, C-8), 164.9 (s, C-9), 102.2 (s, C-10), 129.5 (s, C-1'), 108.4 (d, C-2'), 147.3 (s, C-3'), 135.3 (s, C-4'), 147.3 (s, C-5'), 108.4 (d, C-6').

Kaempferol (2): $\text{C}_{15}\text{H}_{10}\text{O}_6$, yellow needles, mp 280°C. EI-MS m/z : 286 $[\text{M}]^+$, 258, 241, 229, 121, 69.

Quercetin-3-O- β -D-6'-acetylglucopyranoside (3): $\text{C}_{23}\text{H}_{22}\text{O}_{13}$, yellow powder. FAB-MS (%) m/z : 505 ($[\text{M} - 1]^-$, 100), 339 (20), 325 (15), 301 (30), 265 (5); ^1H NMR (500 MHz, $\text{DMSO}-d_6$, δ , ppm, J/Hz): 6.20 (1H, d, J = 2.0, H-6), 6.41 (1H, d, J = 2.0, H-8), 6.83 (1H, d, J = 6.3, H-5'), 7.54 (1H, dd, J = 2.1, 6.3, H-6'), 7.53 (1H, d, J = 2.1, H-2'), 12.62 (1H, s, 5-OH), 10.87 (1H, s, 7-OH), 9.65 (1H, s, 3'-OH), 9.27 (1H, s, 4'-OH), 1.73 (3H, 6''-COCH₃), 5.37 (1H, d, J = 7.5, H_{Glc}-1); ^{13}C NMR (125 MHz, $\text{DMSO}-d_6$, δ , ppm): 155.8 (s, C-2), 132.6 (s, C-3), 176.9 (s, C-4), 160.7 (s, C-5), 98.2 (d, C-6), 163.7 (s, C-7), 93.4 (d, C-8), 156.0 (s, C-9), 103.4 (s, C-10), 120.5 (s, C-1'), 115.6 (d, C-2'), 144.3 (s, C-3'), 148.0 (s, C-4'), 115.8 (d, C-5'), 121.0 (d, C-6'), 100.5 (d, C-1''), 75.7 (d, C-2''), 76.5 (d, C-3''), 69.3 (d, C-4''), 73.5 (d, C-5''), 62.3 (t, C-6''), 19.6 (q, 6''-COCH₃), 169.7 (s, 6''-COCH₃) [3].

Kaempferol-3-O- β -D-6'-acetylglucopyranoside (4): $\text{C}_{23}\text{H}_{22}\text{O}_{12}$, yellow powder. FAB-MS (%) m/z : 491 ($[\text{M} + 1]^+$, 100), 449 (20), 401 (25), 387 (8), 268 (5). ^1H NMR (500 MHz, $\text{DMSO}-d_6$, δ , ppm, J/Hz): 6.22 (1H, d, J = 1.8, H-6), 6.45 (1H, d, J = 1.8, H-8), 6.88 (2H, d, J = 8.8, H-2', 6'), 8.01 (2H, d, J = 8.8, H-3', 5'), 12.58 (1H, s, 5-OH), 10.89 (1H, s, 7-OH), 10.19 (1H, s, 4'-OH), 5.37 (1H, d, J = 7.1, H_{Glc}-1''), 1.74 (3H, s, CH₃CO-); ^{13}C NMR (125 MHz, $\text{DMSO}-d_6$, δ , ppm): 156.7 (s, C-2), 133.4 (s, C-3), 177.7 (s, C-4), 161.5 (s, C-5), 99.1 (d, C-6), 164.5 (s, C-7), 94.0 (d, C-8), 156.9 (s, C-9), 104.2 (s, C-10), 121.1 (s, C-1'), 131.2 (d, C-2', 6'), 115.4 (d, C-3', 5'), 160.4 (s, C-4'), 101.4 (d, C-1''), 74.2 (d, C-2''), 76.5 (d, C-3''), 70.1 (d, C-4''), 74.4 (d, C-5''), 63.1 (d, C-6''), 20.5 (q, 6''-COCH₃), 169.7 (s, 6''-COCH₃) [4].

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Epicatechin (5): C₁₅H₁₄O₆, white needles. EI-MS *m/z*: 290 [M]⁺, 152, 139, 123, 77, 69. ¹H NMR (500 MHz, CDCl₃, δ, ppm, J/Hz): 4.74 (1H, d, J = 7.4, H-2), 4.04 (1H, m, H-3), 2.71 (1H, dd, J = 16.4, 4.4, H-4ax), 2.50 (1H, dd, J = 16.4, 3.5, H-4eq), 5.92 (1H, d, J = 2.3, H-6), 5.75 (1H, d, J = 2.3, H-8), 6.91 (1H, s, H-2'), 6.68 (1H, s, H-5'), 6.68 (1H, s, H-6'), 4.56 (1H, d, J = 4.7, 3-OH), 9.10 (1H, s, 5-OH), 8.89 (1H, s, 7-OH), 8.79 (1H, s, 4'-OH), 8.71 (1H, s, 3'-OH). ¹³C NMR (125 MHz, CDCl₃, δ, ppm): 78.2 (C-2), 65.0 (C-3), 28.3 (C-4), 156.5 (C-5), 95.2 (C-6), 156.4 (C-7), 94.1 (C-8), 155.8 (C-9), 98.7 (C-10), 130.7 (C-1'), 114.9 (C-2'), 144.4 (C-3'), 144.5 (C-4'), 114.8 (C-5'), 118.0 (C-6') [5].

Kaempferol 3-O-β-D-glucopyranoside (6): C₂₁H₂₀O₁₁, yellow powder. FAB-MS *m/z* (%): 447 ([M - 1]⁻, 100), 382 (2), 157 (4). ¹H NMR (500 MHz, CDCl₃, δ, ppm, J/Hz): 6.25 (1H, d, J = 1.9, H-6), 6.49 (1H, d, J = 1.9, H-8), 8.04 (2H, d, J = 9.0, H-3', 5'), 6.92 (2H, d, J = 9.0, H-2', 6'), 12.56 (1H, s, 5-OH), 10.84 (1H, s, 7-OH), 10.17 (1H, s, 4'-OH), 5.45 (1H, d, J = 7.1, H_{Glc}-1''). ¹³C NMR (125 MHz, DMSO-d₆, δ, ppm): 156.6 (C-2), 133.5 (C-3), 177.8 (C-4), 161.5 (C-5), 99.2 (C-6), 164.8 (C-7), 94.1 (C-8), 156.7 (C-9), 104.2 (C-10), 121.4 (C-1'), 131.2 (C-2'), 115.5 (C-3'), 160.4 (C-4'), 115.5 (C-5'), 131.2 (C-6'), 101.2 (C-1''), 74.5 (C-2''), 76.8 (C-3''), 70.2 (C-4''), 77.8 (C-5''), 61.1 (C-6''). The compound is directly compared with authentic samples.

Quercetin-3-O-β-D-glucopyranoside (7): C₂₁H₂₀O₁₂, yellow amorphous powder. FAB⁻-MS *m/z* (%): 463 ([M - 1]⁻, 100), 367 (10), 336 (9), 255 (30), 181 (100). ¹H NMR (500 MHz, CDCl₃, δ, ppm, J/Hz): 6.19 (1H, d, J = 1.9, H-6), 6.40 (1H, d, J = 1.9, H-8), 6.84 (1H, d, J = 5.8, H-5'), 7.58 (1H, dd, J = 1.9, 5.8, H-6'), 7.57 (1H, d, J = 1.9, H-2'), 12.65 (1H, s, 5-OH), 10.85 (1H, s, 7-OH), 9.72 (1H, s, 3'-OH), 9.21 (1H, s, 4'-OH), 5.46 (1H, d, J = 7.3, H_{Glc}-1). ¹³C NMR (125 MHz, DMSO-d₆, δ, ppm): 156.3 (s, C-2), 133.4 (s, C-3), 177.5 (s, C-4), 161.3 (s, C-5), 98.6 (d, C-6), 164.1 (s, C-7), 93.5 (d, C-8), 156.1 (s, C-9), 104.0 (s, C-10), 121.2 (s, C-1'), 116.2 (d, C-2'), 144.8 (s, C-3'), 148.4 (s, C-4'), 115.2 (d, C-5'), 121.6 (d, C-6'), 100.9 (d, C-1''), 76.5 (d, C-2''), 77.5 (d, C-3''), 70.0 (d, C-4''), 74.1 (d, C-5''), 61.0 (t, C-6''). The compound is directly compared with authentic samples.

All flavonoids were isolated from *Stachyurus* for the first time. Ampelopsin (**1**) was reported to show potent anti-HIV-1 effects *in vitro*, and could strengthen the immune system *in vivo* [6]. Compounds **6** and **7** are well-known flavonoid glycosides; **3** and **4** are acetylated flavonol glucosides of **6** and **7**. Compound **7** was found to possess strong antioxidant properties, inhibiting lipid oxidation by 79.7% at 50 μg/mL [7].

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