Supporting information

Rec. Nat. Prod. 7:2 (2013) 129-132

Chemical constituents of *Canarium subulatum* and their anti-herpetic and DPPH free radical scavenging properties

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Experimental

Instruments

Mass spectra were recorded on a Micromass LCT mass spectrometer (ESI-MS) or a GCMS-QP5050A (EI-MS). NMR spectra were recorded on a Bruker Avance DPX-300 FT-NMR spectrometer or a Varian Unity INOVA-500 NMR spectrometer. Microtiter plate reading was performed on a Perkin-Elmer Victor3™ 1420 multilabel counter. Vaccuum-liquid chromatography (VLC) and column chromatography (CC) were performed on silica gel 60 (Merck, Kieselgel 60, 70-320 mesh) and silica gel 60 (Merck, Kieselgel 60, 230-400 mesh), respectively. Size-exclusion chromatography was conducted on Sephadex LH-20 (25-100 μm, Pharmacia Fine Chemical Co. Ltd.).

Identification of compounds

Compound 1 (*β*-amyrin)

White powder, $C_{30}H_{50}O$, EI-MS: 426 (M^+ 12), 218 (86), 203 (40), 69 (80). ^{1}H NMR (500 MHz, $CDCl_3$) δ: 3.21 (1H, m, H-3), 5.16 (1H, br s, H-12), 0.97 (3H, s, H_3-23), 0.78 (3H, s, H_3-24), 0.93 (3H, s, H_3-25), 0.99 (3H, s, H_3-26), 1.11 (3H, s, H_3-27), 0.81 (3H, s, H_3-28), 0.85 (6H, s, H_3-29, H_3-30). ^{13}C NMR (125 MHz, $CDCl_3$) δ: 38.7 (C-1), 27.3 (C-2), 79.0 (C-3), 38.8 (C-4), 55.2 (C-5), 18.4 (C-6), 32.7 (C-7), 38.7 (C-8), 47.7 (C-9), 37.1 (C-10), 23.5 (C-11), 121.7 (C-12), 145.1 (C-13), 41.7 (C-14), 26.2 (C-15), 27.0 (C-16), 32.5 (C-17), 47.3 (C-18), 46.8 (C-19), 31.1 (C-20), 34.7 (C-21), 37.0 (C-22), 28.1 (C-23), 15.5 (C-24), 15.6 (C-25), 16.9 (C-26), 26.0 (C-27), 28.4 (C-28), 33.3 (C-29), 23.7 (C-30).

Compound 2 ((–)-Cubebin**)**

This compound was a mixture of *α*-isomer and *β*-isomer. White powder, $C_{20}H_{20}O_6$, EI-MS: 356 (M^+ 32), 338 (12), 203 (15), 135 (100). ^{1}H NMR (500 MHz, $CDCl_3$) δ: 2.40-2.76 (5H, m, H_2-7, H-8, H_2-7′), 2.14 (1H, m, H-8′*α*), 1.99 (1H, m, H-8′*β*), 5.20 (1H, br s, H-9′*α*), 5.21 (1H, d, *J* = 4.8 Hz, H-9′*β*), 3.79 (1H, t, *J* = 8.4 Hz, H-9_a*α*), 3.57 (1H, t, *J* = 8.4 Hz, H-9_a*β*), 3.98 (1H, t, *J* = 8.4 Hz, H-9_b*α*), 4.08 (1H, t, *J* = 8.4 Hz, H-9_b*β*), 5.89 (2H, s, OCH_2O), 5.90 (2H, s, OCH_2O), 6.45-6.72 (6H, overlapped, aromatic protons). ^{13}C NMR (125 MHz, $CDCl_3$) δ**:** 134.1 (C-1*α*), 133.9 (C-1*β*), 108.1 (C-2*α*), 108.0 (C-2*β*), 147.5 (C-3*α*), 147.6 (C-3*β*), 145.9 (C-4*α*), 145.7 (C-4*β*), 108.9 (C-5*α*), 109.2 (C-5*β*), 121.4 (C-6*α*), 121.3 (C-6*β*), 38.4 (C-7*α*), 38.9 (C-7*β*), 45.9 (C-8*α*), 42.9 (C-8*β*), 72.2 (C-9*α*), 72.6 (C-9*β*), 133.3 (C-1′*α*), 134.5 (C-1′*β*), 108.2 (C-2′*α*), 108.1 (C-2′*β*), 147.5 (C-3′*α*), 147.6 (C-3′*β*), 145.9 (C-4′*α*),

145.7 (C-4′*β*), 109.3 (C-5′*α*), 109.2 (C-5′*β*), 121.7 (C-6′*α*), 121.6 (C-6′*β*), 39.2 (C-7′*α*), 33.6 (C-7′*β*), 53.1 (C-8′*α*), 52.0 (C-8′*β*), 103.4 (C-9′*α*), 98.8 (C-9′*β*), 100.8 (O<u>C</u>H_2O).

Compound 3 (Scopoletin)

White powder, $C_{10}H_8O_4$, EI-MS**:** 192 (M^+ 100), 177 (59), 164 (29), 149 (43). ^{1}H NMR (300 MHz, $CDCl_3$) δ**:** 6.26 (1H, d, *J* = 9.3 Hz, H-3), 7.60 (1H, d, *J* = 9.3 Hz, H-4), 6.82 (1H, s, H-5), 6.90 (1H, s, H-8), 3.93 (3H, s, MeO-6). ^{13}C NMR (75 MHz, $CDCl_3$) δ**:** 161.4 (C-2), 113.4 (C-3), 143.3 (C-4), 107.5 (C-5), 144.0 (C-6), 150.2 (C-7), 103.2 (C-8), 150.2 (C-9), 111.5 (C-10), 56.4 (MeO-6).

Compound 4 (3,4-Dihydroxybenzoic acid)

White powder, $C_7H_6O_4$, EI-MS: 154 (M^+ 100), 137 (84), 109 (19). ^{1}H NMR (300 MHz, acetone-d_6) δ: 7.53 (1H, br s, H-2), 6.90 (1H, d, *J* = 8.1 Hz, H-5), 7.48 (1H, br d, *J* = 8.1 Hz, H-6). ^{13}C NMR (75 MHz, acetone-d_6) δ: 122.7 (C-1), 115.2 (C-2), 145.2 (C-3), 150.8 (C-4), 117.0 (C-5), 123.5 (C-6), 168.0 (<u>C</u>OOH).

Compound 5 (3,3′-di-*O*-methylellagic acid-4′-*O*-*α*-L-rhamnopyranoside)

White powder, $C_{22}H_{20}O_{12}$, ESI-MS *m/z* 499 $[M+Na]^+$. ^{1}H NMR (500 MHz, DMSO-d_6) δ: 7.49 (1H, s, H-5), 7.76 (1H, s, H-5′), 4.04 (3H, s, MeO-3), 4.05 (3H, s, MeO-3′), 5.55 (1H, d, *J* = 1.5 Hz, Rha-H-1), 3.95 (1H, dd, *J* = 1.5, 3.5 Hz, Rha-H-2), 3.70 (1H, dd, *J* = 3.5, 9.0 Hz, Rha-H-3), 3.33 (1H, t, *J* = 9.0 Hz, Rha-H-4), 3.51 (1H, dd, *J* = 6.5, 9.0 Hz, Rha-H-5), 1.12 (3H, d, *J* = 6.5 Hz, Rha-H_3-6). ^{13}C NMR (125 MHz, DMSO-d_6) δ: 110.7 (C-1), 141.5 (C-2), 140.2 (C-3), 153.2 (C-4), 111.7 (C-5), 112.6 (C-6), 158.2 (C-7), 114.1 (C-1′), 140.9 (C-2′), 141.8 (C-3′), 150.2 (C-4′), 111.6 (C-5′), 111.8 (C-6′), 158.4 (C-7′), 61.6 (MeO-3), 60.9 (MeO-3′), 99.8 (Rha-C-1), 70.0 (Rha-C-2), 70.4 (Rha-C-3), 71.5 (Rha-C-4), 70.3 (Rha-C-5), 17.9 (Rha-C-6).

Compound 6 (3,3′-di-*O*-methylellagic acid-4′-*O*-*β*-D-glucopyranoside)

White powder, $C_{22}H_{20}O_{13}$, ESI-MS *m/z* 515 $[M+Na]^+$. ^{1}H NMR (300 MHz, DMSO-d_6) δ: 7.50 (1H, s, H-5), 7.80 (1H, s, H-5′), 4.04 (3H, s, MeO-3), 4.07 (3H, s, MeO-3′), 5.14 (1H, d, *J* = 6.9 Hz, Glc-H-1), 3.10-3.70 (6H, overlapped, Glc-H-2-H-6). ^{13}C NMR (75 MHz, DMSO-d_6) δ: 111.9 (C-1), 140.8 (C-2), 140.5 (C-3), 151.3 (C-4), 112.0 (C-5), 112.8 (C-6),

158.5 (C-7), 114.3 (C-1′), 141.7 (C-2′), 141.8 (C-3′), 154.2 (C-4′), 112.8 (C-5′), 112.9 (C-6′), 158.5 (C-7′), 60.8 (MeO-3), 61.6 (MeO-3′), 101.3 (Glc-C-1), 73.4 (Glc-C-2), 77.3 (Glc-C-3), 69.6 (Glc-C-4), 76.5 (Glc-C-5), 60.6 (Glc-C-6).

Compound 7 (3-*O*-methylellagic acid-4′-*O*-*α*-L-arabinofuranoside)

White powder, $C_{20}H_{16}O_{12}$, HR-ESI-MS *m/z* 471.0533 $[M+Na]^+$ (calcd. for $C_{20}H_{16}O_{12}Na$ 471.0539). ^{1}H NMR (500 MHz, DMSO-d_6) δ: 7.51 (1H, s, H-5), 7.65 (1H, s, H-5′), 4.02 (3H, s, MeO-3), 5.59 (1H, d, *J* = 1.5 Hz, Ara-H-1), 4.27 (1H, dd, *J* = 1.5, 4.0 Hz, Ara-H-2), 3.82 (1H, dd, *J* = 4.0, 6.0 Hz, Ara-H-3), 3.95 (1H, dt, *J* = 3.0, 6.0, 7.0 Hz, Ara-H-4), 3.46 (1H, dd, *J* = 7.0, 11.0 Hz, Ara-H_a-5), 3.56 (1H, dd, *J* = 3.0, 11.0 Hz, Ara-H_b-5). ^{13}C NMR (125 MHz, DMSO-d_6) δ: 111.7 (C-1), 142.0 (C-2), 140.0 (C-3), 152.5 (C-4), 111.2 (C-5), 113.2 (C-6), 158.8 (C-7), 114.6 (C-1′), 137.0 (C-2′), 136.3 (C-3′), 146.9 (C-4′), 112.7 (C-5′), 113.2 (C-6′), 159.0 (C-7′), 60.9 (MeO-3), 107.7 (Ara-C-1), 81.3 (Ara-C-2), 76.6 (Ara-C-3), 86.1 (Ara-C-4), 61.1 (Ara-C-5).

Compound 8 (Scopolin)

White powder, $C_{16}H_{18}O_9$, ESI-MS *m/z* 355 $[M+H]^+$. ^{1}H NMR (300 MHz, MeOH-d_4) δ: 6.31 (1H, d, *J* = 9.6 Hz, H-3), 7.90 (1H, d, *J* = 9.6 Hz, H-4), 7.20 (1H, s, H-5), 7.17 (1H, s, H-8), 5.07 (1H, d, *J* = 6.9 Hz, Glc-H-1), 3.37-3.92 (6H, overlapped, Glc-H-2-H-6). ^{13}C NMR (75 MHz, MeOH-d_4) δ: 163.5 (C-2), 110.8 (C-3), 145.6 (C-4), 115.6 (C-5), 148.3 (C-6), 151.7 (C-7), 105.3 (C-8), 150.7 (C-9), 109.9 (C-10), 56.7 (MeO-6), 102.1 (Glc-C-1), 74.7 (Glc-C-2), 78.4 (Glc-C-3), 71.2 (Glc-C-4), 77.8 (Glc-C-5), 62.4 (Glc-C-6).

Compound 9 (3-*O*-methylellagic acid-4′- *O*-*β*-D-xylopyranoside)

White powder, $C_{20}H_{16}O_{12}$, ESI-MS *m/z* 449 $[M+H]^+$. ^{1}H NMR (300 MHz, DMSO-d_6) δ**:** 7.51 (1H, s, H-5), 7.63 (1H, s, H-5′), 4.90 (1H, d, *J* = 7.2 Hz, Xly-H-1), 3.30-3.84 (5H, overlapped, Xyl-H-2-H-5). ^{13}C NMR (75 MHz, DMSO-d_6) δ**:** 114.9 (C-1), 140.0 (C-2), 135.9 (C-3), 147.5 (C-4), 111.5 (C-5), 111.6 (C-6), 158.8 (C-7), 113.3 (C-1′), 142.0 (C-2′), 141.0 (C-3′), 152.6 (C-4′), 111.3 (C-5′), 111.6 (C-6′), 158.9 (C-7′), 103.4 (Xyl-C-1), 73.1 (Xyl-C-2), 75.8 (Xyl-C-3), 69.3 (Xyl-C-4), 66.0 (Xyl-C-5).

Assay of biological activities

Anti-herpetic activity

Antiviral activity against HSV-1 (Strain KOS) was determined using the plaque reduction method. Briefly, virus (30 PFU/25 µL) was mixed with complete medium (25 µL) containing various concentrations of test compound and then incubated at 37 ºC for 1 h. After incubation, the mixtures were added to Vero cells (6×10^5 cells/well) in 96-well microtiter plates and incubated at 37 ºC for 2 h. The overlay medium containing the various concentrations of test compound was added to the Vero cells and incubated at 37 ºC in humidified CO_2 incubator for 2 days. Then, virus growth inhibition was evaluated by counting the virus plaque forming on Vero cells compared with the controls. The cells also were stained with 1% crystal violet in 10% formalin for 1 h. The percent plaque inhibition was determined. Acyclovir was used as positive control.

DPPH radical scavenging method

The free radical scavenging effect of the samples was assessed by measuring their ability to decolor a methanolic solution of 1,1,-diphenyl-2-picrylhydrazyl radical (DPPH, Sigma). Briefly, test samples were initially prepared as a solution in EtOH (1000 µg/ml). Each compound was first tested at the concentration of 100 µg/ml. An IC_{50} value was determined if the compound showed more than 50% inhibition. For IC_{50} analysis, two fold serial dilutions were performed to give seven concentrations. The test was done by addition of the sample solution (20 µl) to the solution of 50 µM DPPH in EtOH (180 µl) in a 96-well microtiter plate. The reaction mixture was incubated at room temperature for 30 min, and then its absorbance at 510 nm was measured with a microplate reader. Quercetin (Sigma) was used as positive control.