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Compounds from the Aerial Parts of *Aceriphyllum rossii* and Their Cytotoxic Activity

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Abstract – The purification of the MeOH extracts from the aerial parts of *Aceriphyllum rossii* Engler (Saxifragaceae) using column chromatography furnished fourteen compounds (**1 - 14**). The chemical structures of the isolated compounds were determined using mainly nuclear magnetic resonance spectra and mass spectrometry. All the isolated compounds were tested for their cytotoxic activity against LH-60, MCF-7 and HeLa cancer cells *in vitro* using a MTT assay method. Compounds possessing an olean-triterpenoid skeleton entirely exhibit dose dependent cytotoxic activity. These findings partially confirmed the anticancer effect of this medicinal plant, suggesting a further study on the anticancer potential of the triterpenoid structure in compounds from this plant.

Keywords – *Aceriphyllum rossii*, Saxifragaceae, Cancer cells, Cytotoxic activity

Introduction

In a preliminary study, a methanol extract of the aerial parts of *Aceriphyllum rossii* Engler. (Saxifragaceae) had inhibitory effect against several cancer cell lines. *A. rossii*, an endemic species in Korea, is a perennial herb that grows on damp rocks along the valleys in the central northern parts of Korea. The whole plant of *A. rossii* contain oleanane-type triterpenoids that exhibited potential cytotoxic activity against MCF-7, K562 and LLC cancer cells.[1,2] The triterpenoids showed acyl-CoA-cholesterol, *O*-acyltransferase inhibition, antibacterial and anti-complementary activities.[3-6] Inhibition of the protein tyrosine phosphatase 1B activity by triterpenes isolated from the seeds of *A. rossii* has also been reported.[7] In addition, the flavonoids isolated from the aerial parts exhibited antioxidant activity.[8] In the present study, activity-guided fractionation led to the isolation of fourteen compounds (**1 - 14**) (Fig. 1). The present paper reports the isolation and structural elucidation of these compounds along with their cytotoxic activities.

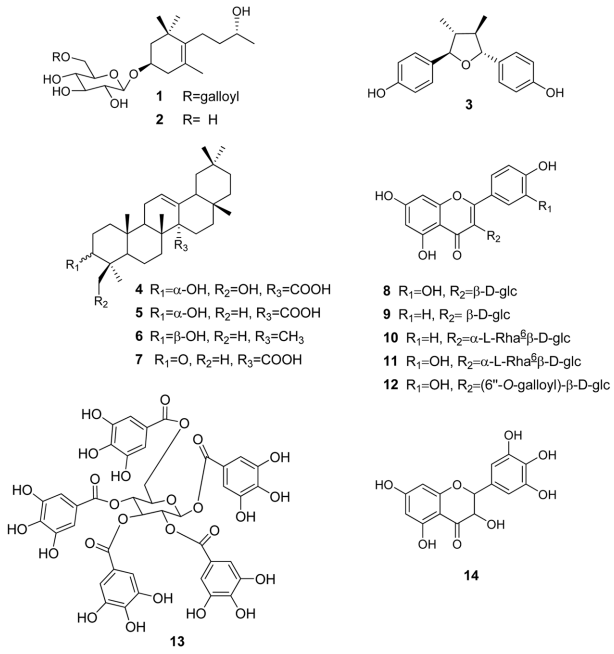

Fig. 1. Chemical structure of isolated compounds (**1 - 14**) from *Aceriphyllum rossii.*

Experimental

Chemicals and reagents – Solvents were purchased from Samchun Chemicals Company (Korea). The cancer cell lines LH-60, MCF-7 and HeLa were purchased from the Korean Cell Line Bank (KCLB, Seoul, Korea). DMEM, FBS, PBS, penicillin, streptomycin were bought from GIBCO (Inc., NY, USA).

Instruments – Optical rotations were measured with a JASCO DIP-370 polarimeter. UV spectra were measured

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with a Thermo 9423AQA2200E UV spectrometer. IR spectra were measured with a Bruker Equinox 55 FT-IR spectrometer. A Waters HPLC was used for purication and isolation with a YMC-Pack ODS-A HPLC column. NMR spectra were recorded on Varian Unity Inova 400 (^{1}H, 400 MHz; ^{13}C, 100 MHz) spectrometers. Conventional pulse sequences were used for COSY, HMBC and HMQC. All chemical shifts are given in ppm units with reference to tetramethylsilane (TMS) as an internal standard and the coupling constants (*J*) are in Hz. HRESIMS was measured on a JMS-700 Mstation mass spectrometer. TLC was carried out on precoated silica gel 60 F254 (Merck). Chromatography suppliers were used for isolation: silica-gel (Kieselgel 60, 40 - 63 μm, Merck) or reverse phase silica-gel (LiChroprep® RP-18, 40 - 63 μm, Merck). Optical density (OD) values in the cytotoxic activity by 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) assays were read on a TECAN-ELISA Microplate Reader.

Plant material – The aerial parts of *A. rossii* were collected at Jeongbong-san, Kangwondo, Korea, in June 2007 and identified by Dr. Hyeong Kyu Lee. A voucher specimen (PB-1636) was deposited at the herbarium of the Korea Research Institute of Bioscience and Biotechnology, Korea.

Materials and methods – The aerial parts (3.0 kg) were extracted reflux with MeOH (5 L × 3 times). After evaporation of the solvent under reduced pressure, the crude MeOH extract (290 g) was obtained and suspended in hot water and partitioned with *n*-hexane, $CHCl_3$, EtOAc and BuOH, successively, to afford *n*-hexane-(39.5 g), $CHCl_3$-(3.2 g), EtOAc-(53.8 g), BuOH-(67.2 g) soluble fractions, respectively. By the activity-guided fractionation, the $CHCl_3$ and EtOAc soluble fractions were choose to further study. The $CHCl_3$ soluble fraction (3.2 g) was chromatographed over a silica gel column (6 × 60 cm, 40 - 63 μm particle size, Merck) and eluted with *n*–hexane-EtOAc (20 : 1 → 1 : 1, each 1 L) yielded eight fractions (F1 - F8). Fraction F4 (430.0 mg) was further subjected to a silica gel column (4 × 50 cm, 40 - 63 μm particle size, Merck), eluted with $CHCl_3$-EtOAc (15 : 1 → 2 : 1, each 0.5 L) to obtain compounds **3** (3.4 mg) and **7** (50.0 mg). Fraction F6 (280 mg) was applied to a silica gel column (3 × 40 cm, 40 - 63 μm particle size, Merck), eluted with $CHCl_3$-EtOAc (25 : 1 → 5 : 1, each 0.5 L) to afford six sub-fractions (F6.1 - F6.6). The sub-fraction F6.2 (55.6 mg) was chromatographed over a silica gel column (2 × 40 cm, 40 - 63 μm particle size) and eluted with $CHCl_3$-MeOH (30 : 1 → 5 : 1, each 0.4 L) to obtain compounds **4** (7.0 mg) and **5** (7.2 mg). Compound **6** (7.1 mg) was crystallized from sub-fraction F6.4 (18 mg) with $CHCl_3$-MeOH (10 : 1). The EtOAc-soluble fraction (53.8 g) was also subjected to a silica gel column (12 × 60 cm, 40 - 63 μm particle size), eluted with $CHCl_3$-MeOH-H_2O (9 : 1 : 0.1 → 1 : 1 : 0.02, each 2 L) to obtain nine fractions (E1 - E9). Fraction E3 (3.5 g) was further applied to RP-18 silica gel column (6 × 60 cm, 40 - 63 μm particle size), eluted with MeOH-H_2O (1 : 4 → 1 : 1) to yield ten sub-fractions (E3.1 - E3.10). Sub-fraction E3.4 (72.0 mg) was applied to RP-18 silica gel column (2 × 60 cm, 40 - 63 μm particle size) and eluted with ACN-H_2O (1 : 8 → 2 : 3, each 1 L) to afford compounds **1** (15.0 mg) and **8** (25.0 mg). Sub-fraction E3.6 (85.0 mg) was purified by HPLC [eluting with MeOH-H_2O (75 : 25 → 55 : 45) over 90 min; flow rate: 5 mL/min; UV detection at 210 nm; column YMC-Pack ODS-A (20 × 250 mm, 5 μm)] to obtain compounds **9** (8.0 mg, t_R = 29.8 min), **10** (3.4 mg, t_R = 42.6 min) and **11** (7.1 mg, t_R = 51.3 min). Fraction E8 (2.2 g) was also subjected to RP-18 silica gel column (6 × 60 cm, 40 - 63 μm particle size), eluted with ACN-H_2O (1 : 5 → 2 : 3, each 1.2 L) to afford eight sub-fractions (E8.1 - E8.8). Sub-fraction E8.4 (65.0 mg) was further applied to RP-18 silica gel column (2 × 40 cm, 40 - 63 μm particle size) using MeOH-H_2O (1 : 4 → 2 : 3, each 0.6 L) to obtain compounds **12** (20.0 mg) and **14** (10.8 mg). Compound **2** (30.0 mg) was crystallized from sub-fraction E8.6 (45.0 mg). Sub-fraction E8.5 (48.0 mg) was chromatogaphed over RP-18 silica gel column (2 × 40 cm, 40 - 63 μm particle size), eluted with ACN-H_2O (1 : 5 → 1 : 2, each 0.5 L) to afford compound **13** (32.0 mg).

Galloyl linarionoside A (1) – White amorphous powder; $[\alpha]_D^{25}$ = 0.0° (*c* 0.1, MeOH); UV (MeOH) λ_{max}: 277, 216 nm; IR (KBr) ν_{max}: 3328, 2943, 2831, 2527, 2356, 1748, 1449, 1418, 1116, 1024 cm^{-1}; ^{1}H NMR (400 MHz, CD_3OD) δ: 7.10 (2H, s, H-2", H-6"), 4.48 (2H, d, *J* = 4.8 Hz, H-6'), 4.42 (1H, d, *J* = 7.6 Hz, H-1'), 3.93 (1H, dddd, *J* = 2.4, 6.0, 8.4, 12.0 Hz, H-3), 3.68 (1H, sext, *J* = 6.4 Hz, H-9), 3.58 (1H, m, H-5'), 3.38 (1H, m, H-3'), 3.34 (1H, m, H-4'), 3.19 (1H, dd, *J* = 2.0, 7.6 Hz, H-2'), 2.26 (1H, br dd, *J* = 4.8, 16.0, H_{eq}-4), 2.14 (1H, td, *J* = 6.4, 11.2 Hz, H-7b), 1.99 (1H, dd, *J* = 9.6, 16.0 Hz, H_{ax}-4), 1.86 (1H, td, *J* = 6.4, 11.2, H-7a), 1.77 (1H, br d, *J* = 13.2 Hz, H_{eq}-2), 1.45 (1H, m, H- 8a), 1.43 (1H, m, H-8b), 1.41 (1H, m, H_{ax}-2), 1.59 (3H, s, H-13), 1.15 (3H, d, *J* = 6.4 Hz, H-10), 0.93 (3H, s, H-12), 0.88 (3H, s, H-11); ^{13}C NMR (100 MHz, CD_3OD) δ: 38.9 (C-1), 47.7 (C-2), 74.5 (C-3), 39.9 (C-4), 125.0 (C-5), 138.7 (C-6), 25.6 (C-7), 40.7 (C-8), 69.3 (C-9), 23.3 (C-10), 28.8 (C-11), 30.2 (C-12), 20.1 (C-13), 103.1 (C-1'), 75.5 (C-2'), 78.1 (C-3'),

72.2 (C-4'), 75.3 (C-5'), 65.1 (C-6'), 121.5 (C-1"), 110.4 (C-2", C-6"), 139.9 (C-3", C-5"), 146.7 (C-4"), 168.4 (C = O); FAB-MS *m/z* 549.23 $[M + Na]^+$ (calcd. for $C_{26}H_{38}O_{11}Na$, 549.23).

Rhododendroside (2) – White amorphous powder; $[\alpha]_D^{25} = 0.0^o$ (*c* 0.1, MeOH); UV (MeOH) λ_{max}: 211, 213 nm; IR (KBr) ν_{max}: 3326, 2997, 2844, 2832, 1448, 1418, 1116, 1024 cm^{-1}; ^1H NMR (400 MHz, CD_3OD) δ: 4.45 (1H, d, *J* = 8.0 Hz, H-1'), 4.08 (1H, m, H-3), 3.86 (1H, dd, *J* = 1.2, 15.6 Hz, Hb-6'), 3.68 (1H, dd, *J* = 4.8, 15.6 Hz, H_a-6'), 3.75 (1H, m, H-9), 3.37 (1H, m, H_a-8), 3.34 (1H, m, H_b-8), 3.17 (1H, dd, *J* = 1.2, 8 Hz, H-2'), 2.35 (1H, brdd, *J* = 4.4, 16.4 Hz, H_{eq}-4), 2.23 (1H, td, *J* = 4.8, 9.6 Hz, H_a-7), 2.04 (1H, brdd, *J* = 6.4, 16.0 Hz, H_{ax}-4), 1.93 (1H, td, *J* = 6.0, 16.8 Hz, H_b-7), 1.86 (1H, ddd, *J* = 1.2, 3.2, 12.0 Hz, H_{eq}-2), 1.67 (3H, s, H-13), 1.54 (2H, m, H-8), 1.49 (1H, t, *J* = 7.6 Hz, H_{ax}-2), 1.19 (3H, d, *J* = 6.4 Hz, H-10), 1.08 (3H, s, H-12), 1.07 (3H, s, H-11); ^{13}C NMR (100 MHz, CD_3OD) δ: 38.9 (C-1), 47.6 (C-2), 73.4 (C-3), 39.9 (C-4), 125.2 (C-5), 138.6 (C-6), 25.8 (C-7), 40.8 (C-8), 69.3 (C-9), 23.4 (C-10), 28.9 (C-11), 30.4 (C-12), 20.2 (C-13), 102.4 (C-1'), 75.3 (C-2'), 78.2 (C-3'), 71.8 (C-4'), 77.9 (C-5'), 62.9 (C-6'); FAB-MS *m/z* 397.23 $[M + Na]^+$ (calcd. for $C_{19}H_{34}O_7Na$, 397.23).

(*2,3-trans*-3,4-*trans*-4,5-*trans*-2,5-*bis*-(4-hydroxyphenyl)-3,4-dimethyltetrahydrofuran) (3) – Colorless needles; mp. 230 – 232 °C; UV (MeOH) λ_{max}: 229, 276 nm; ^1H NMR (400 MHz, CD_3OD) δ: 7.28 (4H, d, *J* = 8.4 Hz, H-2', -6', -2", -6"), 6.80 (4H, d, *J* = 8.4 Hz, H-3', -5', -3", -5"), 4.43 (2H, d, *J* = 6.8 Hz, H-2, -5), 2.31 (2H, m, H-3, -4), 1.01 (6H, d, *J* = 6.8 Hz, 3,4-Me); ^{13}C NMR (100 MHz, CD_3OD) δ: 89.2 (C-2, C-5), 45.9 (C-3, C-4), 133.9 (C-1', C-1"), 129.1 (C-2', C-6', C-2", C-6"), 116.3 (C-3', C-5', C-3", C-5"), 158.4 (C-4', C-4"), 13.1 (3,4-Me); FAB-MS *m/z* 307.14 $[M + Na]^+$ (calcd. for $C_{18}H_{20}O_3Na$, 307.14).

3α,24-dihydroxyolean-12-en-27-oic acid (4) – White amorphous powder; mp. 245 – 247 °C; $[\alpha]_D^{25} = +110.0^o$ (*c* 1.1, $CHCl_3$); ^1H NMR (400 MHz, $CDCl_3$) δ: 5.67 (1H, br s, H-12), 3.68 (1H, br s, H-3), 3.56 (1H, d, *J* = 11.6Hz, H-24a), 3.34 (1H, d, *J* = 11.6 Hz, H-24b), 2.30 (1H, dd, *J* = 6.4, 11.6 Hz, H-9), 2.03 (1H, m, H-18), 1.04 (3H, s, H-26), 0.99 (3H, s, H-25), 0.86 (6H, s, H-28 and H-29), 0.83 (3H, s, H-30), 0.67 (3H, s, H-23); ^{13}C NMR (100 MHz, $CDCl_3$) δ: 36.9 (C-1), 27.8 (C-2), 70.9 (C-3), 44.2 (C-4), 49.3 (C-5), 18.4 (C-6), 33.1 (C-7), 36.1 (C-8), 47.2 (C-9), 37.1 (C-10), 23.1 (C-11), 126.3 (C-12), 138.0 (C-13), 56.2 (C-14), 22.3 (C-15), 28.5 (C-16), 31.3 (C-17), 47.6 (C-18), 42.5 (C-19), 33.6 (C-20), 34.6 (C-21), 37.1 (C-22), 25.8 (C-23), 64.5 (C-24), 16.7 (C-25), 17.9 (C-26), 179.7 (C-27), 28.3 (C-28), 33.1 (C-29), 23.9 (C-30); ESI-MS *m/z* 473 $[M + H]^+$, 495 $[M + Na]^+$; EI-MS *m/z* 472.35 $[M]^+$ (calcd. for $C_{30}H_{48}O_4$, 472.35).

3α-hydroxyolean-12-en-27-oic acid (5) – White crystals; ^1H NMR (400 MHz, $CDCl_3$) δ: 5.67 (1H, br s, H-12), 3.40 (1H, brs, H-3), 1.23 (3H, s H-26) 1.01 (3H, s, H-23), 0.94 (3H, s, H-25), 0.90 (3H, s, H-24), 0.83 (3H, s, H-28), 0.82 (3H, s, H-29), 0.81 (3H, s, H-30); ^{13}C NMR (100 MHz, $CDCl_3$) δ: 37.4 (C-1), 27.8 (C-2), 76.4 (C-3), 40.3 (C-4), 49.5 (C-5), 18.2 (C-6), 33.3 (C-7), 37.6 (C-8), 48.9 (C-9), 36.9 (C-10), 23.9 (C-11), 126.7 (C-12), 138.4 (C-13), 56.4 (C-14), 22.5 (C-15), 28.5 (C-16), 33.0 (C-17), 47.6 (C-18), 44.6 (C-19), 31.3 (C-20), 33.5 (C-21), 36.3 (C-22), 28.5 (C-23), 22.5 (C-24), 16.3 (C-25), 18.4 (C-26), 177.8 (C-27), 29.9 (C-28), 34.5 (C-29), 23.1 (C-30); EI-MS *m/z* 456.35 $[M]^+$ (calcd. for $C_{30}H_{48}O_3$, 456.35).

β-Amyrin (6) – White amorphous powder; ^1H NMR (400 MHz, $CDCl_3$) δ: 5.69 (1H, brs, H-12), 3.21 (1H, dd, *J* = 4.8, 11.2 Hz, H-3), 1.30 (3H, s, H-30), 1.04 (3H, s, H-27), 0.98 (3H, s, H-25), 0.97 (3H, s, H-24), 0.86 (3H, s, H-26), 0.85 (3H, s, H-23), 0.84 (3H, s, H-28), 0.78 (3H,s, H-29); ^{13}C NMR (100 MHz, $CDCl_3$) δ: 36.8 (C-1), 27.7 (C-2), 79.3 (C-3), 40.1 (C-4), 55.4 (C-5), 18.2 (C-6), 33.5 (C-7), 37.3 (C-8), 49.2 (C-9), 38.9 (C-10), 23.1 (C-11), 126.4 (C-12), 138.4 (C-13), 56.2 (C-14), 22.5 (C-15), 27.3 (C-16), 33.1 (C-17), 47.7 (C-18), 44.5 (C-19), 31.4 (C-20), 34.5 (C-21), 36.4 (C-22), 28.3 (C-23), 15.7 (C-24), 16.6 (C-25), 18.4 (C-26), 38.8 (C-27), 28.5 (C-28), 29.9 (C-29), 23.9 (C-30); EI-MS *m/z* 426.39 $[M]^+$ (calcd. for $C_{30}H_{50}O$, 426.39).

3-oxoolean-12-en-27-oic acid (7) – White crystals; ^1H NMR (400 MHz, $CDCl_3$) δ: 5.70 (1H, t, *J* = 3.6 Hz, H-12), 1.06 (3H, s, H-26), 1.05 (3H, s, H-23), 1.04 (3H, s, H-24), 1.03 (3H, s, H-25), 0.86 (3H, s, H-28), 0.83 (3H, s, H-30), 0.80 (3H, s, H-29); ^{13}C NMR (100 MHz, $CDCl_3$) δ: 39.1 (C-1), 34.2 (C-2), 218.1 (C-3), 47.3 (C-4), 55.9 (C-5), 19.8 (C-6), 33.9 (C-7), 40.2 (C-8), 49.1 (C-9), 36.7 (C-10), 23.1 (C-11), 126.0 (C-12), 137.7 (C-13), 54.7 (C-14), 22.4 (C-15), 27.7 (C-16), 33.2 (C-17), 46.3 (C-18), 44.2 (C-19), 31.3 (C-20), 36.0 (C-21), 36.9 (C-22), 27.2 (C-23), 21.6 (C-24), 16.5 (C-25), 18.2 (C-26), 182.3 (C-27), 28.4 (C-28), 34.5 (C-29), 23.7 (C-30); EI-MS *m/z* 454.34 $[M]^+$ (calcd. for $C_{30}H_{46}O_3$, 454.34).

Isoquercitrin (8) – Yellow amorphous powder; m.p. 230 – 232 °C; $[\alpha]_D^{25} -12.5^o$ (*c* 0.9, MeOH); ^1H NMR (400 MHz, CD_3OD) δ: 7.67 (1H, d, *J* = 2.4 Hz, H-2'), 7.53 (1H, dd, *J* = 2.4, 8.4 Hz, H-6'), 6.82 (1H, d, *J* = 8.4 Hz, H-5'), 6.31 (1H, d, *J* = 2.0 Hz, H-8), 6.13 (1H, d, *J* = 2.0 Hz, H-6), 5.19 (1H, d, *J* = 7.6 Hz), 3.69 (1H, dd, *J* = 2, 11.6 Hz, H-6a"), 3.55 (1H, dd, *J* = 5.2, 11.6 Hz, H-

6b"), 3.21 ~ 3.48 (4H, m, H- 2", 3", 4", 5"); ^{13}C NMR (100 MHz, CD_3OD) δ: 179.5 (C-4), 166.1 (C-7), 163.1 (C-5), 159.1 (C-9), 158.5 (C-2), 149.6 (C-4'), 146.0 (C-3'), 135.8 (C-3), 123.1 (C-1'), 123.2 (C-6'), 117.7 (C-5'), 116.1 (C-2'), 105.8 (C-10), 104.5 (C-1"), 100.0 (C-6), 94.9 (C-8), 78.4 (C-3"), 78.2 (C-5"), 75.9 (C-2"), 71.3 (C-4"), 62.7 (C-6"); EI-MS *m/z* 464.10 $[M]^+$ (calcd. for $C_{21}H_{20}O_{12}$, 464.10).

Astragalin (9) – Yellow amorphous powder; mp. 178 – 180 °C; $[\alpha]_D^{25}$ + 16.0° (*c* 1.1, MeOH); IR (KBr) ν_{max}: 3348, 2925, 2360, 1655, 1500 cm^{-1}; ^{1}H NMR (400 MHz, CD_3OD) δ: 8.06 (2H, d, *J* = 8.8 Hz, H-2', 6'), 6.90 (2H, d, *J* = 8.8 Hz, H-3', 5'), 6.39 (1H, s, H-8), 6.20 (1H, s, H-6), 5.24 (1H, d, *J* = 6.8 Hz, H-1"), 3.21~ 3.48 (4H, m, H-2", 3", 4", 5"), 3.70 (1H, dd, *J* = 2.2, 12.0 Hz, H-6"a), 3.55 (1H, dd, *J* = 5.4, 12.0 Hz, H-6"b); ^{13}C NMR (100 MHz, CD_3OD) δ: 179.4 (C-4), 168.3 (C-7), 163.1 (C-5), 161.8 (C-4'), 159.0 (C-9), 158.8 (C-2), 135.6 (C-3), 132.4 (C-2', 6'), 123.0 (C-1'), 116.2 (C-3', 5'), 105.3 (C-10), 104.5 (C-1"), 100.7 (C-6), 95.4 (C-8), 78.6 (C-3"), 78.2 (C-5"), 75.9 (C-2"), 71.5 (C-4"), 62.8 (C-6"); EI-MS *m/z* 448.18 $[M]^+$ (calcd. for $C_{21}H_{20}O_{11}$, 448.18).

Kaempferol 3-*O*-α-L-rhamnopyranosyl (1 → 6)-β-D-glucopyranoside) (10) – Yellow amorphous powder; mp. 168 – 170 °C; $[\alpha]_D^{25}$ = –14.9° (*c* 1.3, MeOH); IR (KBr) ν_{max}: 3364, 2935, 2362, 1654, 1510 cm^{-1}; ^{1}H NMR (400 MHz, CD_3OD) δ: 8.06 (2H, d, *J* = 8.8 Hz, H-2', 6'), 6.89 (2H, d, *J* = 8.8 Hz, H-3', 5'), 6.39 (1H, s, H-8), 6.20 (1H, s, H-6), 5.12 (1H, d, *J* = 7.2 Hz, H-1"), 4.52 (1H, s, H-1'"), 3.25 ~ 3.81 (10H, m, H-2", 2'", 3", 3'", 4", 4'", 5", 5'", 6"), 1.12 (3H, d, *J* = 6.4 Hz, H-6'"); ^{13}C NMR (100 MHz, CD_3OD) δ: 179.5 (C-4), 166.1 (C-7), 163.1 (C-5), 161.6 (C-4'), 159.5 (C-9), 158.6 (C-2), 135.7 (C-3), 132.5 (C-2', 6'), 122.9 (C-1'), 116.3 (C-3', 5'), 105.8 (C-10), 104.8 (C-1"), 102.6 (C-1'"), 100.1 (C-6), 95.1 (C-8), 78.3 (C-5"), 77.3 (C-3"), 75.9 (C-2"), 74.0 (C-4"), 72.4 (C-3'"), 72.2 (C-2'"), 71.6 (C-4'"), 69.9 (C-5'"), 68.7 (C-6"), 18.0 (C-6'"); EI-MS *m/z* 594.15 $[M]^+$ (calcd. for $C_{27}H_{30}O_{15}$, 594.15).

Rutin (11) – Yellow amorphous power; $[\alpha]_D^{25}$ = –8.8° (*c* 1.4, MeOH); IR (KBr) ν_{max}: 3410, 2935, 1658, 1560, 1502 cm^{-1}; ^{1}H NMR (400 MHz, DMSO-d_6) δ: 7.55 (1H, dd, *J* = 8.4, 2.0 Hz, H-6'), 7.53 (1H, d, *J* = 2.0 Hz, H-2'), 6.83 (1H, d, *J* = 8.0 Hz, H-5'), 6.38 (1H, d, *J* = 2.0 Hz, H-8), 6.19 (1H, d, *J* = 2.0 Hz, H-6), 5.34 (1H, d, *J* = 7.6 Hz, H-1"), 4.38 (1H, d, *J* = 0.8 Hz, H-1'"), 3.07 ~ 3.70 (10H, m, H-2", H-2'", H- 3", H- 3'", H- 4", H-4'", H-5", H-5'", H-6"), 0.99 (3H, d, *J* = 6.4 Hz, H-6'"); ^{13}C NMR (100 MHz, DMSO-d_6) δ: 177.4 (C-4), 164.2 (C-7), 161.2 (C-5), 156.6 (C-9), 156.4 (C-2), 148.5 (C-4'), 144.8 (C-3'), 133.3 (C-3), 121.6 (C-1'), 121.2 (C-6'), 116.3 (C-5'), 115.2 (C-2'), 104.0 (C-10), 101.2 (C-1"), 100.8 (C-1'"), 98.7 (C-6), 93.6 (C-8), 76.5 (C-5"), 75.9 (C-5'"), 74.1 (C-2"), 71.9 (C-4"), 70.6 (C-3'"), 70.4 (C-2'"), 70.2 (C-4'"), 68.3 (C-5'"), 67.0 (C-6"), 17.8 (C-6'"); EI-MS *m/z* 610.15 $[M]^+$ (calcd. for $C_{27}H_{30}O_{16}$, 610.15).

Quercetin 3-*O*-(6''-*O*-galloyl)-β-glucopyranoside) (12) – Yellow amorphous powder; UV (MeOH) λ_{max}: 258, 352 nm; ^{1}H NMR (400 MHz, CD_3OD) δ: 7.56 (1H, d, *J* = 2.0 Hz, H-2'), 7.59 (1H, dd, *J* = 2.0, 8.0 Hz, H-6'), 6.94 (2H, s, H-2'", H- 6"), 6.72 (1H, d, *J* = 8.0 Hz, H-5'), 6.36 (1H, d, *J* = 2.0 Hz, H-8), 6.18 (1H, d, *J* = 2.0 Hz, H-6), 5.20 (1H, d, *J* = 7.6 Hz, H- 1"), 4.33 (1H, dd, *J* = 1.6, 12.0 Hz, H-6"a), 4.29 (1H, dd, *J* = 4.8, 12.0 Hz, H-6"b), 3.35 ~ 3.47 (4H, m, H-2", H-3", H-4", H-5"); ^{13}C NMR (100 MHz, CD_3OD) δ: 179.5 (C-4), 168.3 (C = O), 166.0 (C-7), 162.9 (C-5), 159.5 (C-2), 158.5 (C-9), 149.8 (C-4'), 146.4 (C-3'", C-5'"), 145.9 (C-3'), 139.8 (C-4'"), 135.4 (C-3), 123.7 (C-1'), 123.2 (C-6'), 121.4 (C-1'"), 117.3 (C-5'), 116.0 (C-2'), 110.3 (C-2'", C-6'"), 105.7 (C-10), 104.4 (C-1"), 100.1 (C-6), 95.0 (C-8), 78.2 (C-3"), 76.0 (C-5"), 75.8 (C-2"), 71.6 (C-4"), 64.4 (C-6"); TOF-MS *m/z* 617.10 $[M + H]^+$ (calcd. for $C_{28}H_{24}O_{16}$, 616.10).

1,2,3,4,6-penta-*O*-galloyl-β-D-glucopyranoside (13) – Pale yellow amorphous powder; $[\alpha]_D^{25}$ = +23.0° (*c* 0.2, MeOH); ^{1}H NMR (400 MHz, CD_3OD) δ: 7.13 (2H, s, Gal^5 H-2, H-6), 7.07 (2H, s, Gal^4 H-2, H-6), 7.00 (2H, s, Gal^3 H-2, H-6), 6.99 (2H, s, Gal^2 H-2, H-6), 6.97 (2H, s, Gal^1 H-2, H-6), 6.25 (1H, d, *J* = 8.0 Hz, Glc H-1), 5.92 (1H, t, *J* = 10.0 Hz, Glc H-3), 5.63 (1H, t, *J* = 10.0 Hz, Glc H-4), 5.61 (1H, dd, *J* = 8.5, 10.0 Hz, Glc H-2), 4.54 (1H, dd, *J* = 2.0, 10.0 Hz, Glc H-6b), 4.44 (1H, ddd, *J* = 2.0, 4.5, 10.0 Hz, Glc H-5), 4.38 (1H, dd, *J* = 4.5, 10.0 Hz, Glc H-6a); ^{13}C NMR (100 MHz, CD_3OD) δ: 168.0, 167.4, 167.1, 167.0, 166.3 (5C, Gal C = O), 146.6, 146.5, 146.5, 146.4, 146.3 (10C, Gal C-3, C-5), 140.9, 140.5, 140.4, 140.2, 140.1 (5C, Gal, C-4), 121.2, 1120.5, 120.4, 120.3, 119.8 (5C, Gal C-1), 110.7, 110.5, 110.5, 110.5, 110.4 (10C, Gal C-2, C-6), 93.9 (Glc, C-1), 74.5 (Glc, C-5), 74.2 (Glc, C-3), 72.3 (Glc, C-2), 69.9 (Glc, C-4), 63.2 (Glc, C-6); FAB-MS *m/z* 963.20 $[M + Na]^+$ (calcd. for $C_{41}H_{32}O_{26}Na$, 963.20).

Dihydromyricetin (14) – Yellow amorphous powder; ^{1}H NMR (400 MHz, CD_3OD) δ: 6.55 (2H, s, H-2', 6'), 5.93 (1H, d, *J* = 2.0 Hz, H-8), 5.90 (1H, d, *J* = 2.0 Hz, H-6), 4.84 (1H, d, *J* = 11.6 Hz, H-2), 4.47 (1H, d, *J* = 11.6 Hz, H-3); ^{13}C NMR (100 MHz, CD_3OD) δ: 198.5 (C-4), 168.9 (C-7), 165.5 (C-5), 164.5 (C-9), 147.0 (C-3', 5'), 135.1 (C-1'), 129.2 (C-4'), 108.2 (C-2', 6'), 102.0 (C-10), 97.4 (C-6), 96.4 (C-8), 85.4 (C-2), 73.8 (C-3); EI-MS *m/z* 320.05 $[M]^+$ (calcd. for $C_{15}H_{12}O_8$, 320.05).

Results and Discussion

Preliminary results showed that the $CHCl_3$ soluble fraction of the aerial parts of *A. rossii* is capable of inhibiting the HL-60 cancer cell line (data not shown). To characterize the active compounds, bioassay-guided isolation was applied to the $CHCl_3$ and EtOAc fractions, which yielded fourteen compounds (**1** - **14**). The compounds were identified as galloyl linarionoside A (**1**),[9] rhododendroside (**2**),[10] 2,3-*trans*-3,4-*trans*-4,5-*trans*-2,5-*bis*-(4-hydroxyphenyl)-3,4-dimethyltetrahydrofuran (**3**),[11] 3α,24-dihydroxyolean-12-en-27-oic acid (**4**),[12] 3α-hydroxyolean-12-en-27-oic acid (**5**),[2] β-amyrin (**6**),[13] 3-oxoolean-12-en-27-oic acid (**7**),[2] isoquercitrin (**8**),[11] astragalin (**9**),[11] kaempferol-3-*O*-α-L-rhamnopyranosyl-(1 → 6)-β-D-glucopyranoside (**10**),[11] rutin (**11**),[11] quercetin-3-*O*-(6'-galloyl)-β-D-glucopyranoside (**12**),[9] 1,2,3,4,6-penta-*O*-galloyl-β-D-glucopyranoside (**13**, PGG)[14] and dihydromyricetin (**14**)[9] based on spectroscopic analysis, chemical evidence and literature data. All the isolated compounds were assayed for their cytotoxic activity against cancer cell lines, such as HL-60, MCF-7 and HeLa, using an MTT [3-4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide] method.[1] The results are presented in Table 1. Adriamycine, a known anti-cancer agent used as a positive control, exhibited cytotoxic activity against HL-60, MCF-7 and HeLa with IC_{50} values of 0.55, 6.4 and 4.0 μM, respectively.[1] Of the compounds tested, the olean-type triterpenoids (**4** - **7**) exhibited potential cytotoxic activity with IC_{50} values ranging from 7.5 to 24.2 μM. Among the active compounds, 3α,24-dihydroxyolean-12-en-27-oic acid (**4**) showed the strongest cytotoxic activity against HL-60, MCF-7 and HeLa with IC_{50} values of 7.5, 7.9 and 7.7 μM, respectively, followed by 3α-hydroxyolean-12-en-27-oic acid (**5**), other 3α-hydroxy and 12-en-27-oic functional linkages in the structure, with IC_{50} values of 7.9 μM (HL-60), 8.7 μM (MCF-7) and 12.9 μM (HeLa). Compound **6**, β-amyrin, is a well known olean-type triterpene with a 3β-hydroxy connection to the main skeleton but without a carbonyl group, showed some weak inhibitory with IC_{50} values of 12.8 μM (HL-60), 15.2 μM (MCF-7) and 19.9 μM (HeLa). Interestingly, 3-oxoolean-12-en-27-oic acid (**7**) still showed significant cytotoxic effects, particularly to HL-60 cells with an IC_{50} value of 9.1 μM. The other compounds (**1** - **3**, **8** - **12** and **14**) showed no inhibitory activity. Interestingly, PGG (**13**) exhibited cytotoxic activity against HeLa cancer cells with IC_{50} values of 84.8 μM, even though its effect was some-what weaker than the active compounds.

In addition to the cytotoxic activity of PGG (**13**), this compound had protective effects against cisplatin-induced cytotoxic activity and apoptosis in normal human primary renal epithelial cells (HRCs) while showing a synergistic effect against cisplatin-induced cell death in human Caki-2 renal cancer cells. This significantly blocked the cisplatin-mediated cytotoxic activity, reduced cisplatin-induced sub-G1 accumulation in the HRCs and reduced the apoptotic cell populations according to TdT-mediated dUTP nick end labeling (TUNEL) and Live/Dead assays in cisplatin-treated HRCs. Furthermore, it also suppressed the PARP cleavage and caspase-3 activation, cytochrome c release, up-regulation of bax and p53 in the cisplatin-treated HRCs.[15] In another report, PGG from the root of *Paeonia suffruticosa* exhibited an *in vitro* growth-inhibiting effect on a human hepatocellular carcinoma cell line, SK-HEP-1 cells, by G_0/G_1 phase arrest and suppressed the activation of nuclear factor-kappa B.[16] In addition, the related gallotannins with PGG from *Terminalia* fruits showed the enhancement of PPARα and/or PPARγ signaling, increased PPARα and PPARγ protein expression, and enhanced the insulin-stimulated glucose uptake into HepG2 cells.[17]

In previous studies of this plant, several triterpenoids were isolated and evaluated for their cytotoxic, antibacterial and anticomplementary activities.[3-6] Recently, assay-guided

Table 1. Cytotoxic activity of isolated compounds (**1** - **14**) against cancer cell lines

Compounds	IC_{50} (μM)[a]		
	HL-60	MCF-7	Hela
1	> 100	> 100	> 100
2	> 100	> 100	> 100
3	> 100	> 100	> 100
4	7.5	7.9	7.7
5	7.9	8.7	12.9
6	12.8	15.2	19.9
7	9.1	10.9	24.2
8	> 100	> 100	> 100
9	> 100	> 100	> 100
10	> 100	> 100	> 100
11	> 100	> 100	> 100
12	> 100	> 100	> 100
13	> 100	> 100	84.8
14	> 100	> 100	> 100
Adriamycine[b]	0.55	6.4	4.0

[a]The inhibitory effects are represented as the molar concentration (μM) giving 50% inhibition (IC_{50}) relative to the vehicle control. These data represent the average values of three repeated experiments.
[b]Positive control.

fractionation led to the isolation of several new oleanane-type triterpenoids along with serial known compounds. Some compound showed weak cytotoxic activity but some of them exhibited inhibitory strong activity with IC_{50} values less than 10 μM, particularly in the inhibition of LLC cell lines. Those results suggest that the olean-12-en-27-oic acid showed stronger cytotoxic activity than olean-12-en-28-oic acid against the MCF-7 and LLC cancer cell lines.[1] Overall, the apoptotic activity of the other olean-type triterpene, 3α,23-isopropylidenedioxyolean-12-en-27-oic acid, and the molecular mechanism of its action in human cervical cancer HeLa cells were investigated.[18] Another study also reported the effects of 3-oxoolean-12-en-27-oic acid on the viability and apoptosis of HL-60 human promyelocytic leukemia cells. The results suggested that 3-oxoolean-12-en-27-oic acid induces apoptosis by activating caspase-8 via FasL-stimulated death receptor signaling.[19] In accordance with previous reports, the potential cytotoxic activity of active compounds in this study suggested that the anticancer activity of this plant was due to the olean-type triterpenoid with a 27-oic acid functional group. Although the structure-activity relationships of triterpenoids of olean-27-carboxylic acid have not been studied thoroughly, these findings suggest that a carboxyl group at the C-27 position is important for the cytotoxic activity against some cancer cell lines. Further studies on potential cancer therapy will be needed.

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