Isolation of Limonoids and Alkaloids from *Phellodendron amurense* and Their Multidrug Resistance (MDR) Reversal Activity

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Three limonoids and five alkaloids were isolated from the chloroform layer of the MeOH extract of the bark of *Phellodendron amurense* (Rutaceae). The structures of the compounds isolated were determined to be obacunone (1), limonin (2), 12α-hydroxylimonin (3), γ-fagarine (4), oxyberberine (5), canthin-6-one (6), 4-methoxy-N-methyl-2-quinolone (7) and oxypalmatine (8) based on the physicochemical and spectroscopic data. Compounds 3, 5, 7, and 8 were first isolated from the *Phellodendron amurense*. The isolated compounds were then tested for their cytotoxicity against five human tumor cell lines in vitro using the SRB method. Compound 5 showed significant cytotoxicity against the five tumor cell lines with ED$_{50}$ values ranging from 0.30 to 3.0 μg/mL. The marginal or noncytotoxic compounds (1, 2, 3, 4, and 7) were examined for their P-gp related MDR reversal activities. Compound 1 showed significant P-gp MDR inhibition activity in MES-SA/DX5 and HCT15 cells with an ED$_{50}$ value of 0.028 μg/mL and 0.0011 μg/mL, respectively.

Key words: *Phellodendron amurense*, Rutaceae, Limonoid, Alkaloid, Multidrug resistance

INTRODUCTION

In traditional Chinese Medicine, Phellodendri Cortex (The stem bark of *Phellodendron amurense* Rupr., Rutaceae) has been used to treat dysentery, jaundice, yellow thick foul leukorrhagia, swelling pain in the knees and feet, urinary tract infections, and infections on the body surfaces (Yan *et al.*, 1999). Isoquinoline alkaloids, phenolic compounds, butenolides and limonoids from the bark of this plant have been reported (Kondo *et al.*, 1985; Wada *et al.*, 1990; Miyaki *et al.*, 1992; Kishi *et al.*, 1992; Ida *et al.*, 1994). Indolopyridoquinazoline alkaloids, furoquinoline alkaloids and isoquinoline alkaloids were also extracted from the callus tissues of bark of this plant (Ikuta *et al.*, 1995, 1998a, 1998b).

As part of an ongoing search for multidrug resistance (MDR) reversal compounds from Korean medicinal plants, the present study examined Phellodendri Cortex because the MeOH extract was found to show P-gp mediated MDR reversal activity in human cancer cells.
(Waters Co.). The nuclear magnetic resonance (NMR) spectra were recorded on Varian VXR-500 and JNM-LA400. The EI-MS data was obtained using a JMS700 spectrometer (Jeol Co.). The LC-ESI-MS/MS data were obtained using a Quattro micro (Waters Co.). The prep-HPLC was performed using a Prep Nova-Pak HR C18 (6 μm, 19×300 mm) column with a PDA detector (Waters Co., model 2996) and RI detector (Waters Co., model 2414). Silica gel 60 (0.063-0.200 mm, Merck Co.) was used for column chromatography. Kiesel gel 60F2~4 precoated plates (Merck Co.) were used for thin layer chromatography (TLC). The packing material used for molecular sieve column chromatography was Sephadex LH-20 (Pharmacia Co.).

The following analytical conditions were used for HPLC: detector = PDA; column = RP18, 5 μm, 4.6×150 mm; eluent = gradient 20% MeOH -->90% MeOH (15 min.) →90% MeOH (10 min.).

Plant material

The bark of *Phellodendron amurense* was purchased at the Kyungdong herbal market, in March, 2003, Seoul, Korea and a voucher specimen was deposited in the College of Pharmacy at Sungkyunkwan University.

Extraction and isolation

The dried, chopped bark (2.5 kg) was extracted three times with 80% MeOH (6 L × 3) at room temperature. The resulting extracts (150 g) were suspended with distilled water (3 L), followed by fractionation with n-hexane and chloroform. The chloroform layer (40 g) was subjected to molecular sieve chromatography using a solvent system (n-hexane : ethylacetate : methanol = 10 : 10 : 0.3) to give two fractions (C-22), 84.66 (C-4), 78.67 (C-17), 65.74 (C-14), 58.03 (C-5), 54.02 (C-15), 53.65 (C-8), 49.92 (C-9), 43.83 (C-10), 40.60 (C-6), 38.14 (C-13), 33.47 (C-12), 32.73 (CH3), 27.51 (CH3), 21.83 (CH3), 20.16 (CH3), 17.69 (C-11), 17.14 (CH3).

Limonin (2)

Colorless crystal; mp 287-293°C (CH2Cl2/MeOH); [α]D 20° + 127.7° (c 0.2, CHCl3); IR (neat) νmax cm⁻¹: 2967, 1747, 1714, 1503, 1460, 1285, 1025; EI-MS m/z (rel. int.): 470 (M⁺, 0.6), 454 (4), 412 (13), 347 (100), 329 (15), 135 (22); 1H-NMR (500 MHz, CDCl3): δ 7.41 (1H, m, H-21), 7.40 (1H, t, J = 1.8 Hz, H-23), 6.51 (1H, dd, J = 11.7 Hz, H-2), 5.46 (1H, s, H-17), 3.65 (1H, s, H-15), 2.98 (1H, t, J = 14.1 Hz, H-6δ), 2.60 (1H, dd, J = 14.1, 5.0 Hz, H-5), 2.28 (1H, dd, J = 14.1, 5.0 Hz, H-6δ), 2.14 (1H, br.d, J = 8.5, 3.5 Hz, H-9), 1.50 (6H, s, H-29 and H-30), 1.45 (3H, s, H-28), 1.24 (3H, s, H-19), 1.12 (3H, s, H-18); 13C-NMR (100 MHz, CDCl3): δ 127.7 (C=O, 2), 110.44 (C-22), 84.66 (C-4), 78.67 (C-17), 65.74 (C-14), 58.03 (C-5), 54.02 (C-15), 53.65 (C-8), 49.92 (C-9), 43.83 (C-10), 40.60 (C-6), 38.14 (C-13), 33.47 (C-12), 32.73 (CH3), 27.51 (CH3), 21.83 (CH3), 20.16 (CH3), 17.69 (C-11), 17.14 (CH3).

12α-Hydroxylimonin (3)

White powder; [α]D 20° + 141.7° (c 0.02, CHCl3); IR (neat) νmax cm⁻¹: 3526, 2969, 1742, 1505, 1459, 1281, 1022; EI-MS m/z (rel. int.): 486 (M⁺, 3), 471 (3), 440 (19), 429 (14), 363 (100), 345 (11); 1H-NMR (500 MHz, CDCl3): δ 7.52 (1H,