Inhibition of DNA Topoisomerases I and II and Cytotoxicity of Compounds from *Ulmus davidiana* var. *japonica*

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Twenty five compounds including ten triterpenes (1-3, 5-11), six flavonoids (12-15, 24, 25), five lignans (17, 18, 21-23), two butenyl clohexnone glycosides (19-20), one fructofuranoside (16) and one fatty acid (4) were isolated from the roots of *Ulmus davidiana* var. *japonica*. The structures of those compounds were identified by comparing their physicochemical and spectral data with those of published in literatures. All the compounds were evaluated for DNA topoisomerase inhibitory activities and cytotoxicities. Among the purified compounds, 4 and 19 showed more potent inhibitory activities (IC₅₀: 39 and 19 μM, respectively) than camptothecin, as the positive control (IC₅₀: 46 μM) against topoisomerase I. Compounds, 4, 10, 12, 19, 24 and 25 showed strong inhibitory activities toward DNA topoisomerase II (IC₅₀: 0.1, 0.52, 0.47, 0.42, 0.17 μM and 17 nM, respectively), which were more potent than that of etoposide as positive control (IC₅₀: 20 μM). In A549 cell line, 5 and 6 showed cytotoxicities (IC₅₀: 4 μM and 3 μM, respectively, with IC₅₀ of camptothecin as positive control: 10.3 μM). In the HepG2 cell line, 3, 5 and 7 showed cytotoxicity (IC₅₀: 3 and 4 μM, respectively, with IC₅₀ of camptothecin: 0.3 μM). Compounds 6, 12 and 23 showed cytotoxicities in the HT-29 cell line (IC₅₀: 19, 19 and 15 μM, respectively, with IC₅₀ of camptothecin: 2 μM).

**Key words:** *Ulmus davidiana* var. *japonica*, Topoisomerase, Cytotoxicity

**INTRODUCTION**

DNA topoisomerases are enzymes involved in the relaxation of DNA during a number of critical cellular processes, including replication, recombination and transcription, by transiently breaking one or two strands of DNA, passing a single- or double-stranded DNA through the break, and finally resealing the break (D’Arpa and Liu, 1989; Pommier, 1993; Wang, 1996). Currently, only the camptothecin (CPT) family of compounds, as DNA topoisomerase I-directed drugs has been introduced into clinical practice (Slichenmyer et al., 1993; Potmesil, 1994), although topoisomerase II-directed drugs have been in clinical use for many years (Chen and Liu, 1994). DNA topoisomerases have been established as important molecular targets for anticancer drugs (Liu, 1989). In the course of screening tests for topoisomerase I and II inhibitors and cytotoxicity of 100 species of Korean traditional anticancer plants, we found that the MeOH extract of *Ulmus davidiana* var. *japonica* showed cytotoxicity against A549, SK-OV-3, HepG2 and HT-29 cell lines, as well as DNA topoisomerase I and II inhibitory activities.

*Ulmus davidiana* var. *japonica* (Ulmaceae) is a deciduous tree, which is widely distributed in Korea, China and Japan. The barks of the stem and root of this plant have been used in the treatment of oedema, mastitis, gastric cancer, and inflammation (Jun et al.,...
1998). Some scientific reports on biological actions have already been reported, for example, anticancer, antiviral, antibacterial, and anti-inflammatory properties (Jun et al., 1998; Jin et al., 2006, 2008; Kang et al., 2006; Suh et al., 2007). Recently, 75% MeOH extract of *U. davidiana* has been reported that inhibitory effect of topoisomerase I (Lee et al., 2001a). In this study, we have isolated twenty-five compounds (1-25) by column chromatographies over silica gel, RP-18, Sephadex LH20 and HPLC, and investigated their DNA topoisomerase I and II inhibitory activities and cytotoxicities.

**MATERIALS AND METHODS**

**General experimental procedures**

Optical rotations were measured using a JASCO DIP-1000 automatic digital polarimeter. The NMR spectra were recorded on a Bruker 250 MHz (DMX 250) spectrometer using Bruker's standard pulse program. Samples were dissolved in CDCl₃-d₆, pyridine-d₅ or CD₂OD, with chemical shifts reported in ppm downfield from TMS. FABMS was obtained on a JEOL JMS700 spectrometer (JEOL). The stationary phases used for column chromatography (Silica gel 60, 70-230 and 230-400 mesh, Lichroprep RP-18 gel, 40-63 µm, LiChroprep RP-18) with MeOH-H₂O (gradient from 80:20 to 100%) were purchased from Merck KGaA. Spots were detected under UV radiation and by spraying with 10% H₂SO₄, followed by heating. The HPLC system consisted of a Shimadzu LC-20AD pump and SPD-20A (Shimadzu) UV/VIS detector. Camptothecin (CPT, purity 95%) and etoposide (purity 98%) were purchased from MBI Fermentas, Inc. and one unit of the enzyme completely relaxes 1 µg of pBR322 DNA in 30 min at 37ºC. The human topoisomerase II was purchased from TopoGEN, Inc. and one unit of the enzyme completely relaxes 1 µg of pBR322 DNA in 30 min at 37ºC. All other chemicals and solvents were of analytical grade, and used without further purification.

**Plant material**

The root barks of *U. davidiana* were purchased in February 2007 at a folk medicine market, “Yak-ryong-si” in Daegu, Republic of Korea. These materials were confirmed taxonomically by Professor Ki-Hwan Bae, Chungnam National University, Daejeon, Republic of Korea. A voucher specimen (YNUD-2007) has been deposited at the College of Pharmacy, Yeungnam University.

**Extraction and isolation**

Dried root barks (10 kg) of *U. davidiana* were extracted three times with 13 L of 70% methanol (MeOH) by reflux. The dried MeOH extract (1.1 kg) was suspended with distilled 1.4 L water and the solution was partitioned with n-hexane (1.4 L × 3), ethyl acetate (EtOAc, 1.4 L × 3) and n-butanol (n-BuOH, 1.4 L × 3), successively. After drying, four solvent extracts of n-hexane (67.6 g), EtOAc (70.5 g), n-BuOH (320 g) and H₂O (555 g) were obtained. The n-hexane extract (67 g) was applied to a silica gel column (60 × 11 cm, No. 9385, 230-400 mesh) and the column was eluted by stepwise gradient modes with n-hexane-EtOAc (from 100% n-hexane to 100% EtOAc) and EtOAc-MeOH (from 100% EtOAc to 100% MeOH). Fractions were combined based on TLC analysis and 34 fractions (UDH1-34) were obtained. Fractions, UDH1, UDH3, UDH6, UDH9, UDH11, UDH22, UDH31 and UDH34 afforded compounds 1 (80 mg), 2 (120 mg), 3 (100 mg), 4 (20 mg), 5 (1 g), 6 (20 mg), 11 (33 mg) and 7 (1.5 g), respectively. Fraction UDH27 (1.0 g) was further separated into 5 fractions (UDH27-1-UDH27-5) by a Sephadex LH-20 column (3 × 90 cm, 1 L) and eluted with CHCl₃:MeOH (4:6). Compound 10 (8 mg) was obtained from UDH27-2 with a reverse-phase column (4 × 50 cm, LiChroprep RP-18) by the elution with MeOH-H₂O (gradient from 80:20 to 100% MeOH). Fraction UDH23 (300 mg) was applied into a Sephadex LH-20 column (3 × 90 cm, 1 L) and eluted with 100% MeOH to give 8 (30 mg). Compound 9 (27 mg) was obtained from UDH30 by eluting through a Sephadex LH-20 column (3 × 90 cm, 0.5 L) with MeOH and a reverse-phase column (4 × 50 cm, LiChroprep RP-18) with MeOH-H₂O (gradient from 80:20 to 100% MeOH), successively.

The EtOAc extracts (65 g) was applied to a silica gel column (9 × 60 cm, NO. 9385, 230-400 mesh), and eluted with methylene chloride (CH₂Cl₂)-MeOH gradient elution (from CH₂Cl₂ 100% to 100% MeOH). The eluates were combined based on TLC, giving 29 fractions (UDE1-29). Compound 12 (30 mg) was obtained from UDE20 by Sephadex LH20 columns (3 × 90 cm, 0.5 L) with isotropic elution (100% MeOH). Fraction UDE26 was further chromatographed by a Sephadex LH20 columns (3 × 90 cm, 1 L) with isotropic elution (100% MeOH) and obtained 13 (500 mg) and fractions UDE26-1-UDE26-3. Fraction UDE26-3 purified by a reverse-phase column (4 × 50 cm, LiChroprep RP-18) with MeOH-H₂O gradient elution (from 10 to 100% MeOH) to afford compounds 14 (32 mg) and 15 (25 mg).

*n*-BuOH extract (150 g) was applied into a column packed with silica gel (9 × 60 cm, No. 9385, 230-400 mesh).