Journal of Medicinal Plants Research Vol. 6(24), pp. 4091-4095, 28 June, 2012
Available online at http://www.academicjournals.org/JMPR
DOI: 10.5897/JMPR11.1757
ISSN 1996-0875 ©2012 Academic Journals

Full Length Research Paper

Anti-leukemic and topoisomerase I inhibitory effect of Mansonone E isolated from *Ulmus davidiana*

Keyong Ho Lee[1], Choa Hyoung Cho[2] and Ki - Hyeong Rhee[3]*

[1]Korea Prime Pharm Company, Suwon City, 443-270, Korea.
[2]College of Environmental and Biosciences, Chonbuk National University, Iksan 570-752, Korea.
[3]College of Industrial Sciences, Kongju National University, Yesan 340-702, Korea.

Accepted 16 February, 2012

A compound has been isolated from *Ulmus davidiana*, which has been traditionally used as the folk medicine material in Asia, and was determined to be sesquiterpene ortho-naphthoquionone (Mansonone E) from spectral data such as UV, IR, MS and NMR spectrometry. This compound showed an inhibitory effect against topoisomerase I and anti-tumor activities at various leukemia cell lines, U937, K562, HL60 and THP-1 using hollow fiber assay.

Key words: *Ulmus davidiana*, topoisomerase I, hollow fiber assay, leukemia.

INTRODUCTION

Ulmus davidiana is a small deciduous tree widely distributed across Korea, China and Japan, where it is found in wetlands along streams at high elevation areas. The stem and root bark of this species have been used in traditional Korean medicine for treatment of gastric erosion, ulcers and cancer (Lee et al., 1995; Lee et al., 2004). In China, this treatment has also been used in traditional Chinese medicine (TCM) for edema, mastitis, gastric cancer, and inflammation (Wang et al., 2004). The biological effects of *U. davidiana* have been determined as antioxidant, anti-inflammatory, matrix-metalloproteinase and antitumor activities, and the species of targeted diseases were asthma, sepsis, osteoarthritis and cancer (Choi et al., 2010; Kim et al., 2010; Lee et al., 1995; Lee and Lim, 2007a, b; Lee et al., 2010; Song et al., 2007; Zheng et al., 2011). Major compounds isolated from *U. davidiana* were sesquiterpene ortho-naphthoquinones, such as davidianones A, B and C, and mansonones E, F, H and I (Kim et al., 1996). Through these studies, the biological activity of *U. davidiana* was attributed to those sesquiterpene ortho-naphthoquinones and to glycoprotein of which is unclear to the chemical structure (Ko and Lim, 2006; Oh et al., 2006).

For antitumor activity, an active compound of mansonone E was separated from 60% methanol extract of the root bark of *U. davidiana* through chromatographic work, and the cytotoxic and topoisomerase I inhibitory effects of mansonone E isolated methanol extract of *U. davidiana* on human leukemia cell lines U937, K562, HL60 and THP-1 were investigated.

*Corresponding author. E-mail: howard@kongju.ac.kr. Tel: + 82-41-330-1626. Fax: + 82-41-330-1629.

MATERIALS AND METHODS

Root bark samples of *U. davidiana* were collected from the Naju traditional market in the Chonnam province of South Korea. Fresh root bark samples were dried in a dark, well-ventilated place, and a voucher specimen was deposited at the College of Industrial Sciences, Kongju National University. Polyvinylidene fluoride (PVDF) hollow fibers (500,000 Da molecular weight cut-off and 1.0 mm ID) were purchased from Spectrum Laboratories, Inc, USA.

Cell lines

The human leukemia cell lines used in this study were as follows: human leukemic monocyte lymphoma U937, human myeloid leukemia K562, human promyelocytic leukemia HL60 and human acute monocytic leukemia THP-1. The cell lines were maintained in an RPMI 1640 medium, supplemented with 10% fetal bovine serum (FBS, JRH Bioscience).

Drug treatment

Isolated mansonone E and camptothecin as positive control were dissolved in dimethyl sulfoxide as a solvent control (DMSO) to make stock solutions; these were then diluted in cell culture medium at different concentrations, and used immediately. In all assays, the final concentrations of DMSO in the medium were less than 0.1%.

Extraction and Isolation

The dried root bark samples of *U. davidiana* (5 kg) were extracted with 60% methanol under reflux. The dried methanol (MeOH) extract was partitioned with CH_2Cl_2. The CH_2Cl_2 layer was subjected to silica gel column chromatography eluted with an *n*-hexane / ethyl acetate mixture by stepwise gradient elution. The fractions which were collected and monitored with analytical TLC were separated by a Sephadex LH-20 column (MeOH), and then followed by semipreparative high-performance liquid chromatography (HPLC) (70% aqueous MeOH) to yield 24 mg of the compound. The compound was identified as monsonone E by comparing the spectra data with that in a published paper (Kim et al., 1996). The purity of this compound was assessed by HPLC.

Animals

Five weeks old BALB/C nu/nu mice (male), purchased from Japan SLC, Inc., were acclimatized under controlled standard conditions (temperature of 23 ± 2°C, relative humidity of 50 ± 5% and illumination cycle for 12/12 h light/darkness, respectively), and housed in polycarbonate cages for a week prior to the experiment. Mice were maintained according to accredited procedures in our facility, and fed irradiated Orient bio (Korea) chow and UV sterilized water *ad libitum*.

Hollow fiber assay

For the assay, capsules were prepared following the procedures outlined in previous research (Casciari et al., 1994; Sadar et al., 2002). Prior to filling with cells, each fiber was individually rinsed with ice-cold fresh RPMI 1,640, containing 20% fetal bovine serum (FBS). The cell suspension was drawn into a 5 ml syringe, and the fibers were filled with the cell suspension via a 20-gauge needle. After filling, the ends of the fibers were heat-sealed, with individual fibers filled with cells prepared by heat-sealing the fibers at 2 cm intervals. Heat sealing was accomplished by clamping the fibers with hot smooth-jawed needle holders. Prior to implantation of capsules into the mice, the capsules were incubated overnight at 37°C in a 5% CO_2 atmosphere. For subcutaneous (SC) implantation, a small skin incision was made at the nape of the neck to allow insertion of an 11-gauge tumor implant trocar. The trocar containing the hollow fiber capsules was inserted through the subcutaneous tissue. Generally, each mouse received four hollow fiber capsules, each containing four different cell lines. Sample treatment was carried out for two weeks with a double injection per week, and then the experiment was terminated in a further two weeks. Therefore, the total evaluation term was four weeks. The anti-tumor activity was evaluated using the MTT assay. The fibers were placed into 2 ml of fresh, pre-warmed (37°C) culture medium/35 mm dish, and allowed to equilibrate for 30 min at 37°C. The fibers were then stained with MTT solution (MTT 1 mg/ml) and washed twice with phosphate buffered saline (PBS) containing 2.5% protamine sulfate. The formazan extracted from the fiber was dissolved in DMSO, transferred to individual wells in 96-well plates, and then accessed for optical density at a 540 nm.

DNA topoisomerase I assay

Relaxation activity of deoxyribonucleic acid (DNA) topoisomerase I was determined by using topoisomerase I assay kit (TopoGen, Inc. Florida, USA). The principle of the assay is to measure the conversion of supercoiled pBR322 plasmid DNA to its relaxed form. In brief, 20 µl of reaction mixture containing 0.25 µg plasmid pBR322 DNA in relaxation buffer (10 mM Tris, pH 7.5, 1 mM EDTA, 150 mM NaCl, 0.1% Bovine Serum Albumin, 0.1 mM Spermidine, 5% glycerol) was incubated with 0.2 U calf thymus Topoisomerase I in the absence or in the presence of samples for 30 min at 37°C. After the termination, the sample was analyzed using a 1% agarose gel in 40 mM Tris-acetate (pH 8.0), 1 mM EDTA (TAE buffer) at 1 V/cm. After electrophoresis the gels were stained with ethidium bromide (1 mg/ml), photographed under ultraviolet (UV) light and band distribution was analyzed with a gel analysis system. The rate of formation of the newly formed bands was used as a measure of enzyme activity. Camptothecin as positive control of topoisomerase I inhibitor was used as the reference drug (Wu et al., 2010).

RESULTS AND DISCUSSION

Identification of Mansonone E

Identification of mansonone E from *U. davidiana* was carried out via the spectra analysis of UV, infrared (IR), electron ionization mass spectrometry (EI-MS) and nuclear magnetic resonance spectroscopy (NMR); then the spectra data was compared with that in a published paper (Kim et al., 1996) (Figure 1). Analytic results for mansonone E were as follows; orange needles (MeOH), mp 148°C, UV λ_{max} (MeOH) nm (log ε): 445 (3.05), 370 (3.03), 264 (4.21), 220 (3.74); IR λ_{max} (KBr) cm^{-1}: 1695, 1645, 1615, 1580; EI-MS: *m*/*z* 244 $[M+2]^+$, 242 $[M]^+$. 214$[M\text{-}CO]^+$, 199$[M\text{-}CO\text{-}Me]^+$; ^{1}H-NMR (300 MHz, $CDCl_3$): δ 1.37 (3H, d, J = 7.1 Hz, 3-Me), 1.96 (3H, s, 9-Me), 2.61 (3H, s, 6-Me), 3.09 (1H, m, 3-H), 4.23 (1H, dd, J = 10.7, 5.1 Hz, 2-H), 4.41 (1H, dd, J = 10.7, 4.0 Hz, 2-H), 7.26 (1H, d, J = 6.7 Hz, 5-H), 7.35 (1H, d, J = 8.0 Hz, 4-H); ^{13}C-NMR (75 MHz, $CDCl_3$): δ 7.78 (9-Me), 17.82 (3-Me), 22.33 (6-Me), 30.86 (C-3), 70.81 (C-2), 117.15 (C-9), 126.71 (C-9b), 127.04 (C-6a), 132.64 (C-4), 134.62 (C-5), 136.71 (C-3a), 142.64 (C-6), 162.42, (C-9a), 180.25 (C-8), 182.28 (C-7).

Inhibition of DNA topoisomerase I

DNA topoisomerase I activities in cell-free systems were evaluated by the relaxation assay (Wu et al., 2010). The relaxation assay utilizes supercoiled plasmid as substrate and has been used by many researchers to screen DNA topoisomerase inhibitors such as topoisomerase I, II and IV. The supercoiled substrate and its relaxed product can easily be distinguished by the difference of migration

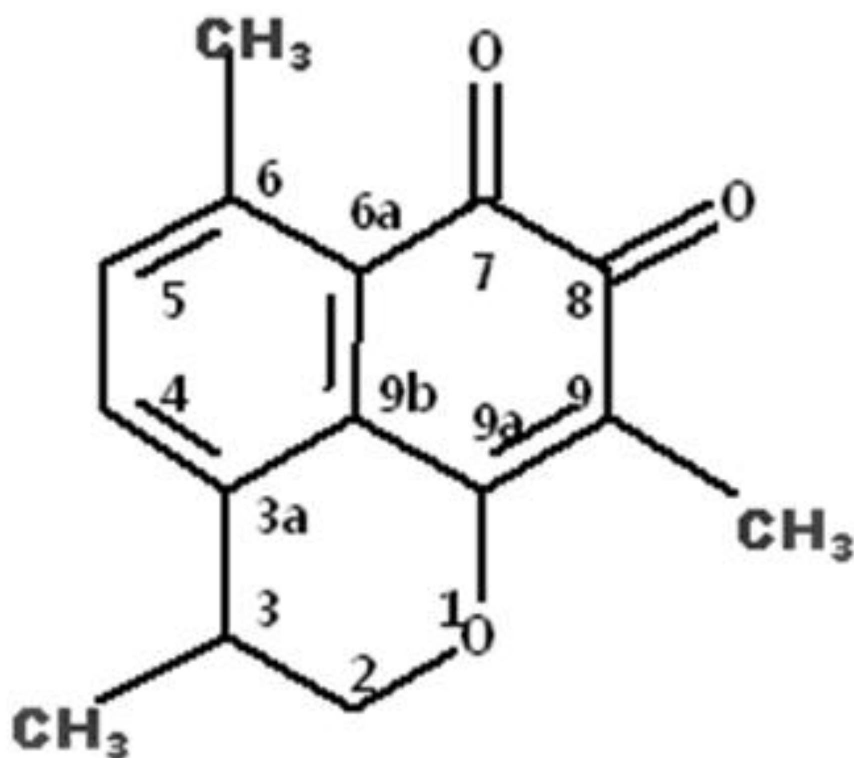

Figure 1. Chemical structure of mansonone E isolated from *Ulmus davidiana*.

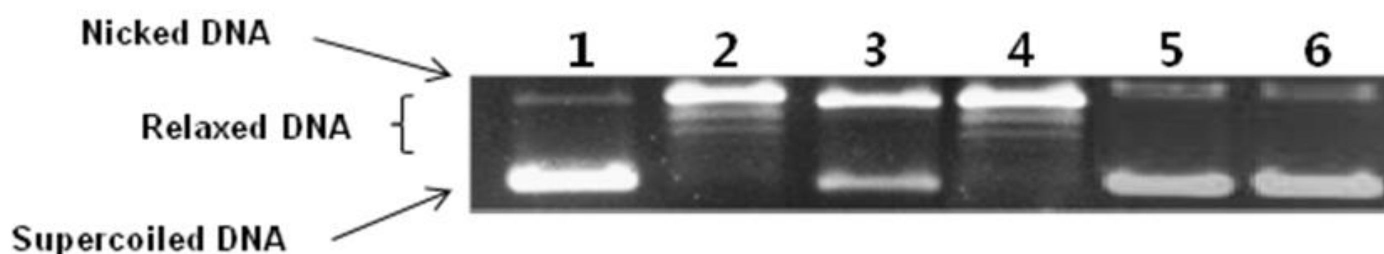

Figure 2. Topoisomerase I inhibitory effect of mansonone E. Lane 1: Supercoiled DNA; Lane 2: Supercoiled DNA + topoisomerase I; Lane 3: Supercoiled DNA + topoisomerase I + camptothecin (100 µM); Lane 4: Supercoiled DNA + topoisomerase I + mansonone E (10 µM); Lane 5: Supercoiled DNA + topoisomerase I + mansonone E (100 µM); Lane 6: Supercoiled DNA + topoisomerase I + mansonone E (200 µM).

between relaxed forms and supercoiled forms in agarose gel containing ethidium bromide because the relaxed isomers migrate more slowly than the supercoiled isomer (Barrett et al., 1990). In three concentrations (10, 100 and 200 µM) of mansonone E, two doses of 100 and 200 µM clearly showed the potential inhibition of topoisomerase I similar to that of camptothecin of 100 µM. In contrast, mansonone E of 10 µM was very weakly active. Therefore, according to these results for cell-free system using the relaxation assay, mansonone E has been shown to have a potential effect against DNA topoisomerase inhibitor camptothecin (Figure 2).

Anti-leukemic activity in hollow fiber assay

The anti-leukemic activities of mansonone E, shown against various leukemia cell lines using hollow fiber assay (Table 1). For the sensitivity test of mansonone E, we used four leukemia cell lines that had a different kind of character which can incur drug resistance against anti-cancer agents. Anti-tumor activity in a dose dependent manner was shown with the treatment of mansonone E for all tested cell lines. There was a similar level of inhibition in the tested concentrations of between 25 and 125 mg/kg; the inhibition rate was 57 and 68% in

Table 1. Anti-leukemic activity of mansonone E on human leukemia cell lines that was cultivated in hollow fibers in mice.

Sample	Conc. (mg/kg)	Schedule	Inhibition rate (%)			
			HL60	K562	THP-1	U937
Mansonone E	5	2 times per week for 2 weeks	24 ± 5	34 ± 3	23 ± 3	14 ± 3
	25		57 ± 2[a]	51 ± 4[a]	65 ± 5[a]	50 ± 3[a]
	125		68 ± 5[a]	75 ± 6[a]	78 ± 5[a]	88 ± 6[a]
Camptothecin	25		54 ± 3[a]	23 ± 2	65 ± 3[a]	49 ± 3

Each value represents the mean (mean ± SD) of three independent measurements. *Sensitive, that is, % inhibition ≥ 50.

HL60, 51 and 75% in K562, 65 and 78% in THP-1 and 50 and 88% in U937. In the case of camptothecin as positive control, there was a sensitive effect in HL60 (inhibitory rate, 54%) and THP-1 (inhibitory rate, 65%), only, in this assay. According to several other research papers, camptothecin inhibits topoisomerase I activity by stabilizing the cleavable enzyme-DNA complex, which leads to DNA breakage (Hsiang et al., 1985). In low concentrations (~ 60 nM), camptothecin increases the capacity of differentiation in cells by increasing the levels of the differentiation-related CD IIb, CD IIc, and vimentin mRNAs (Aller et al., 1992). Therefore, camptothecin has the effect of growth inhibition in low concentration. On the other hand, our results show that only two cell lines, HL60 and THP-1, have drug sensitivity when there is a concentration of 25 mg/kg camptothecin. At present, it is difficult to establish a reason for this discrepancy, leading to difference of effects for camptothecin on K562 and U937 cells, which remains to be investigated. It has been proposed that the strong resistance of K562 cells to apoptosis induction is related to bcr-ab1 tyrosine kinase, produced by specific chromosome translocation (Hamblin 1995; Zhu et al., 1999). It was reported that mansonone E, a major active component of *U. davidiana*, induced cell death by modulating the balance of Bcl-2 family proteins and signals to caspases that partially induces apoptosis (Wang et al., 2004). From these studies, mansonone E might have other cell death activity besides the mechanism of apoptosis. In the case of mansonone F, one paper reported that a series of mansonone F derivatives were found to be strong inhibitors for topoisomerase I and II (Wu et al., 2011).

According to our results, it was found that mansonone E, which has been known for anti-tumor activity by apoptosis, has a potential inhibition activity against topoisomerase I, and also shows anti-tumor effects on human leukemia cell lines such as HL60, K562, THP-1 and U937 in hollow fiber assay. The topoisomerase activity of mansonone E has never been reported before, so it needs to be studied further in regards to various topoisomerase activities on the derivatives of mansonone E as on optimum pharmaceutical candidate.

REFERENCES

Aller P, Rius C, Mata F, Zorrilla A, Cabañas C, Bellón T, Bernabeu C (1992). Camptothecin induces differentiation and stimulates the expression of differentiation-related genes in U-937 human promonocytic leukemia cells. Cancer Res., 52(5): 1245-1251.

Barrett JF, Sutcliffe JA, Gootz TD (1990). *In vitro* assays used to measure the activity of topoisomerases. Antimicrob. Agents Chemother., 34: 1–7.

Casciari JJ, Hollingshead MG, Alley MC, Mayo JG, Malspeis L, Miyauchi S, Grever MR, Weinstein JN (1994). Growth and chemotherapeutic response of cells in a hollow fiber *in vitro* solid tumor model. J. Natl. Cancer Inst., 86: 1846-1852.

Choi SY, Lee S, Choi WH, Lee Y, Jo YO, Ha TY (2010). Isolation and anti-inflammatory activity of Bakuchiol from *Ulmus davidiana* var. japonica. J. Med. Food, 13(4): 1019-1023.

Hamblin TJ (1995). Disappointments in treating acute leukemia in the elderly. N. Engl. J. Med., 332: 1712-1713.

Hsiang YH, Hertzberg R, Hech S, Liu L (1985). Camptothecin induces protein-linked DNA breaks via mammalian DNA topoisomerase I. J. Biol. Chem., 260: 14873-14878.

Kim EJ, Jang MK, Yoon EH, Jung CY, Nam DW, Lee SD, Kim KS (2010). Efficacy of pharmacopuncture using root bark of *Ulmus davidiana* Planch in patients with knee osteoarthritis: a double-blind randomized controlled trial. J. Acupunct. Meridian. Stud., 3(1): 16-23.

Kim JP, Kim WG, Koshino H, Jung J, Yoo ID (1996). Sesquiterpene O-naphthoquinones from the root bark of *Ulmus davidiana*. Phytochemistry, 43(2): 425-430.

Ko JH, Lim KT (2006). Glycoprotein isolated from *Ulmus davidiana* NAKAI protects against carbon tetrachloride-induced liver injury in the mouse. J. Pharmacol. Sci., 101(3): 205-213.

Lee EB, Kim OK, Jung CS, Jung KW (1995). The influence of methanol extract of *Ulmus davidiana* var. japonica cortex on gastric erosion and ulcer, and paw edema in rats. Yakhak Hoeji, 39(6): 671-675.

Lee KH, Cho CH, Yoon WH (2004). In vivo antitumor activity of mansonone E isolated from *Ulmus davidiana* var. japonica NAKAI. Korean J. Pharmacogn., 35(3): 199-202.

Lee MY, Seo CS, Ha H, Jung D, Lee H, Lee NH, Lee JA, Kim JH, Lee YK, Son JK, Shin HK (2010). Protective effects of *Ulmus davidiana* var. japonica against OVA-induced murine asthma model via upregulation of heme oxygenase-1. J. Ethnopharmacol., 130(1): 61-69.

Lee SJ, Lim KT (2007a). UDN glycoprotein inhibits activator protein-1 and matrix metalloproteinase-9 via blocking of oxygen radicals in HT-29 cells. Mol. Cell Biochem., 304(1-2): 13-23.

Lee SJ, Lim KT (2007b). Glycoprotein isolated from *Ulmus davidiana* Nakai modulates inflammatory related factors in mouse colonic tissues. Environ. Toxicol. Pharmacol., 23(2): 234-241..

Oh PS, Lee SJ, Lim KT (2006). Glycoprotein (116 kD) isolated from *Ulmus davidiana* Nakai protects from injury of 12-O-tetradecanoylphorbol 13-acetate (TPA)-treated BNL CL.2 cells. Pharmacol. Rep., 58(1): 67-74.

Sadar MD, Akopian VA, Beraldi E (2002). Characterization of a new *in vivo* hollow fiber assay model for the study of progression of prostate cancer to androgen independence. Mol. Cancer Ther., 1: 629-637.

Song IK, Kim KS, Suh SJ, Kim MS, Kwon DY, Kim SL, Kim CH (2007). Anti-inflammatory effect of *Ulmus davidiana* Planch (Ulmaceae) on collagen-induced inflammation in rats. Environ. Toxicol. Pharmacol., 23(1): 102-110.

Wang D, Xia M, Cui Z, Tashiro S, Onodera S, Ikejima T (2004). Cytotoxic effects of mansonone E and F isolated from *Ulmus pumila*. Biol. Pharm. Bull., 27(7): 1025-1030.

Wu N, Wu XW, Agama K, Pommier Y, Du J, Li D, Gu LQ, Huang ZS, An LK (2010). A novel DNA topoisomerase I inhibitor with different mechanism from camptothecin induces G2/M phase cell cycle arrest to K562 cells. Biochemistry, 49(47): 10131-10136.

Wu WB, Ou JB, Huang ZH, Chen SB, Ou TM, Tan JH, Li D, Shen LL, Huang SL, Gu LQ, Huang ZS (2011). Synthesis and evaluation of mansonone F derivatives as topoisomerase inhibitors. Eur. J. Med. Chem., 46(8): 3339-3347.

Zheng MS, Li G, Li Y, Seo CS, Lee YK, Jung JS, Song DK, Bae HB, Kwak SH, Chang HW, Kim JR, Son JK (2011). Protective constituents against sepsis in mice from the root barks of *Ulmus davidiana* var. japonica. Arch. Pharm. Res., 34(9): 1443-1450.

Zhu XH, Shen YL, Jing YK, Cai X, Jia PM, Huang Y, Tang W, Shi GY, Sun YP, Dai J, Wang ZY, Chen SJ, Zhang TD, Waxman S, Chen Z, Chen GQ (1999). Apoptosis and growth inhibition in malignant lymphocytes after treatment with arsenic trioxide at clinically achievable concentrations. J. Natl. Cancer Inst., 91: 743-745.