Chemical Composition and Antitumor Apoptogenic Activity of Methylene Chloride Extracts from the Leaves of *Zanthoxylum schinifolium*

Jun-Seok Kim, Do Youn Jun[1], Mi-Hee Woo[2], In-Koo Rhee[3] and Young Ho Kim*

Department of Microbiology, College of Natural Sciences, Kyungpook National University, Daegu 702-701
[1]Institute of Genetic Engineering, Kyungpook National University, Daegu 702-701
[2]Department of Pharmacy, College of Pharmacy, Catholic University of Daegu, Gyeongbuk, 712-702
[3]Department of Agricultural Chemistry, College of Agricultural Life Sciences, Kyungpook National University, Daegu 702-701

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To understand antitumor activity of *Zanthoxylum schinifolium*, which has been used as an aromatic and medicinal plant in Korea, the cytotoxic effect of various organic solvent extracts of its leaves on human tumor cells were investigated. Among these extracts such as methanol extract (SL-13), methylene chloride extract (SL-14), ethyl acetate extract (SL-15), n-butanol extract (SL-16), and residual fraction (SL-17), SL-14 appeared to contain the most cytotoxic activity against leukemia and breast cancer cells tested. The methylene chloride extract (SL-14) possessed an apoptogenic activity causing apoptotic DNA fragmentation of human acute leukemia Jurkat T cells via mitochondrial cytochrome c release into cytoplasm, subsequent activation of caspase-9 and caspase-3, and cleavage of PARP, which could be negatively regulated by antiapoptotic protein Bcl-xL. The GC-MS analysis of SL-14 revealed that the twenty-two ingredients of SL-14 were 9,19-cyclolanost-24-en-3-ol (15.1%), 2-a-methyl-17, b-hop-21-ene (15.1%), 15-methyl-2,3-dihydro-1H benzazepin (11.95%), phytol (10.38%), lupeol (9.92%), 12-methylbenzofuran (8.23%), hexadecanoic acid (5.96%), *cis,cis,cis*-9,12,15-octadecatrienoic acid-methyl-ester (5.49%), 9,12,15-octadecatrienoic acid-methylester (3.59%), 15-methyl-4-(1-methylethylidene)-2-(4-nitrophenyl) (3.36%), hexadecanoic acid methyl ester (1.93%), vitamine E (1.88%), beta-amyrin (0.96%), and auraptene (0.89%). These results demonstrate that the cytotoxicity of the methylene chloride extract of the leaves of *Z. schinifolium* toward Jurkat T cells is mainly attributable to apoptosis mediated by mitochondria-dependent caspase cascade regulated by Bcl-xL, and provide an insight into the mechanism underlying antitumor activity of the edible plant *Z. schinifolium*.

Key words – *Zanthoxylum schinifolium*, methylene chloride extract, antitumor cytotoxicity, mitochondria-dependent apoptosis, GC-MS analysis

Introduction

Zanthoxylum schinifolium belongs to the Rutaceae family is a plant widely distributed in Korea, China, and Japan. This plant is a large deciduous shrub or small multi-trunked tree with bright green pinnate leaves. The trunk and branches are well armed with sharp spines. Panicles of pale green flowers are produced in early summer, followed by clusters of aromatic pink fruit that splits to reveal the very aromatic black seeds in the fall. Pericarps and leaves of *Z. schinifolium* have been widely used as a favorite spice in Korea and other East Asian countries. In addition, fruits, seeds, leaves, and roots of *Z. schinifolium* are known to possess effects as traditional medicine[4,17]. In particular, the dried leaves and the essential oil obtained from pericarps of *Z. schinifolium* have been used as traditional oriental medicine to treat toothache, muscle pain, sthenia, ascaricide, and confusion. Phytochemical investigations on the components of the root bark of *Z. schinifolium* resulted in the isolation of cis-fagaramide[4], coumarin, peroxyterpenyl coumarin[3,17,18], schinifolin, 5'-acetoxyschinifolin[5], and others[7,11]. The bioactive ingredients from *Z. schinifolium* have been reported for their medicinal activities including anti-platelet aggregation [5,17], anti-oxidant activity[13,14], inhibition of the production of monoamine oxidase[7], and antimicrobial activity[12].

One of well-studied chemical components in the root bark of *Z. schinifolium* is the coumarin, which is known to exert cytotoxic effect on cancer cells[6]. However, little is known about the cellular mechanism responsible for this cytotoxicity. Recently it has been reported that the essential oils from *Z. schinifolium* pericarp, whose major components

*Corresponding author
Tel : +82-53-950-5378, Fax : +82-53-955-5522
E-mail : ykim@knu.ac.kr

are sabinene (15.40%), citronellal (15.75%) and geranyl acetate (29.8%), is able to induce apoptosis of human hepatoma HepG2 cells[2]. In the present study, we describe that the methylene chloride extract of the leaves of *Z. schinifolium* possesses a potent cytotoxic activity toward human acute leukemia Jurkat T cells. Since the methylene chloride extract can induce apoptotic cell death in Jurkat T cells, the mechanism underlying the induced apoptosis was further investigated in order to evaluate its potency as an antitumor agent and establish its safety as a condiment.

Materials and Methods

Reagents, antibodies, cells, and culture medium

The ECL Western blotting kit was purchased from Amersham (Arlington Heights, IL, USA), and Immobilon-P membrane was obtained from Millipore Corporation (Bedford, MA, USA). Anti-cytochrome *c* was purchased from Pharmingen (San Diego, CA, USA). Anti-PARP, and anti-β-actin were purchased from Santa Cruz Biotechnology (Santa Cruz, CA, USA). Human acute leukemia Jurkat T cell line E6.1, clone J/Neo infected with vector, and clone J/Bcl-xL infected with Bcl-xL gene were maintained in RPMI 1640 (Life Technologies, Gaithersburg, MD, USA) containing 10% fetal bovine serum, 20 mM HEPES (pH 7.0), 5 × 10^{-5} M β-mercaptoethanol, and 100 μg/ml gentamycin. For the culture of both J/Neo and JT/Bcl-xL, G418 was added to the RPMI 1640 medium at a concentration of 400 μg/ml. Both estrogen receptor-positive breast cancer MDA361 cells and estrogen receptor-negative breast cancer MDA438 cells were maintained in DMEM (Life Technologies, Gaithersburg, MD, USA) containing 10% fetal bovine serum, 20 mM HEPES (pH 7.0), 5 x 10^{-5} M β-mercaptoethanol, and 100 μg/ml gentamycin.

Isolation of a cytotoxic component from *Z. schinifolium*

The leaves of *Z. schinifolium* (Korean name; Sancho) were purchased in the fall of 2003 from Dong-A Garden located in nearby Daegu, South Korea. In order to extract the cytotoxic component in *Z. schinifolium*, the powder of dried leaves (3 kg) was extracted with 80% methanol. Sequentially, the methanol extract (SL-13) was evaporated, dissolved in water, and then sequentially extracted with methylene chloride (SL-14), ethyl acetate (SL-15), n-hexane (SL-16), and n-butanol (SL-17) as described in Fig. 1. A voucher specimen is preserved at the Department of Pharmacy, the Catholic University of Daegu, Gyongsan, Korea.

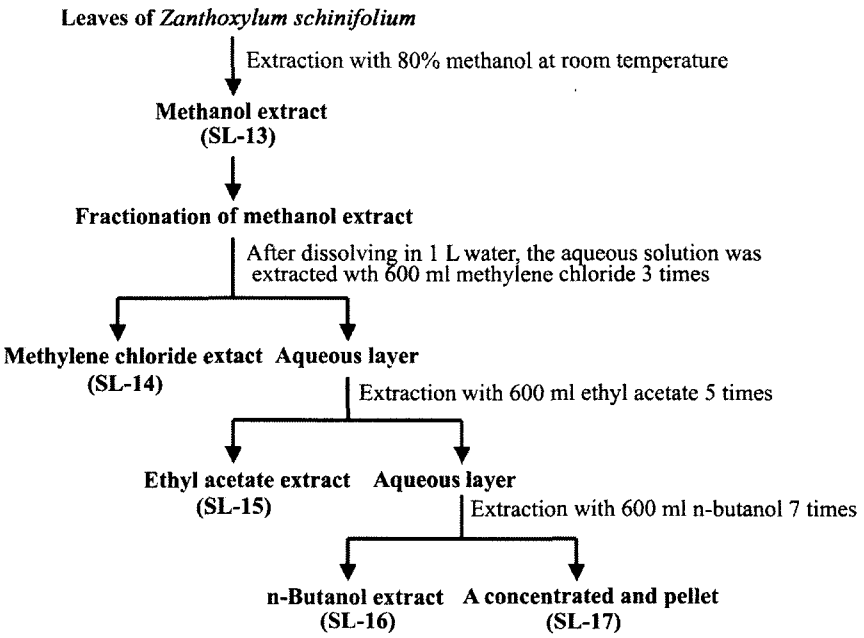

Fig. 1. The procedure for preparation of various organic solvent extracts of the leaves of *Zanthoxylum schinifolium*.

Cytotoxicity assay

The cytotoxic effect of the individual extract of *Z. schinifolium* on human tumor cells was analyzed by 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) assay reflecting cell viability as previously described[8]. For MTT assay, Jurkat T cells (4.0 x 10^4 cells/well), MDA361 cells (2.0 x 10^4 cells/well) or MDA438 cells (2.0 x 10^4 cells/well) were treated with the extract at various concentrations in a 96-well plate. After incubation for 44 h, 50 μl of MTT solution (1.1 mg/ml) was added to each well and incubated for an additional 4 h. The cells were processed to assess the colored formazan crystal produced from MTT as an index of cell viability.

Flow cytometric analysis

The cell cycle progression of Jurkat T cells following the SL-14 treatment was analyzed by flow cytometry as described elsewhere[9]. Approximately, 1 x 10^6 cells were suspended in 100 μl of PBS, and 200 μl of 95% ethanol were added while vortexing. The cells were incubated at 4℃ for 1 h, washed with PBS, and resuspended with 12.5 μg of RNase in 250 μl of 1.12% sodium citrate buffer (pH 8.45). Incubation was continued at 37℃ for 30 min before staining of the cellular DNA with 250 μl of propidium iodide (50 μg /ml) for 30 min at room temperature. The stained cells were analyzed on a flow cytometer for relative DNA content, based on increased red fluorescence.

DNA fragmentation analysis

Apoptotic DNA fragmentation induced in Jurkat T cells with the SL-14 treatment was determined as previously described[8]. Briefly, the cells were harvested by centrifugation and then treated with a lysis buffer (0.5% Triton X-100, 5 mM EDTA, 10 mM Tris-HCl, pH 7.4) for 20 min on ice. After centrifugation for 15 min at 14,000 rpm, the supernatant was collected and treated for 2 h at 50℃ with proteinase K and subsequently with RNase for 4 h at 37℃. After extraction with an equal volume of buffer-saturated phenol, the DNA fragments were precipitated with 2.5 volumes of ethanol in the presence of 0.5 M NaCl and visualized following electrophoresis on a 1.2% agarose gel.

Protein extraction and Western blot analysis

Cellular lysates were prepared by suspending Jurkat T cells (5 x 10^6 cells) in 300 μl of lysis buffer (137 mM NaCl, 15 mM EGTA, 1 mM sodium orthovanadate, 15 mM $MgCl_2$, 0.1% Triton X-100, 25 mM 3-(N-morpholino) propanesulfonic acid (MOPS), and 2.5 μg/ml proteinase inhibitor E-64, pH 7.2). The cells were disrupted by sonication and extracted at 4 ℃ for 30 min. An equivalent amount of protein lysate (20 μg) was denatured with SDS sample buffer, and subjected to electrophoresis on 4–12% SDS gradient polyacrylamide gel with MOPS buffer. For detection of caspase-3 activation and mitochondrial cytochrome *c* release, the protein lysates were electrophoresed on 10% SDS polyacrylamide gel with 2-(N-morpholino) ethane-sulfonic acid (MES) buffer. The proteins were electrotransferred to Immobilon-P membranes (Millipore Corporation, Bedford, MA, USA). Detection of each protein was carried out with an ECL Western blotting kit according to the manufacturer's instructions.

Detection of mitochondrial cytochrome c release in cytosolic protein extracts

To assess mitochondrial cytochrome *c* release in Jurkat T cells after treatment with SL-14, cytosolic protein extracts were obtained as previously described[8]. Briefly, approximately 5 x 10^6 cells treated with SL-14 were washed with cold PBS twice and then suspended in 0.25 ml lysis buffer (250 mM sucrose, 10 mM KCl, 1.5 mM $MgCl_2$, 1 mM DTT, 1 mM PMSF, 2.5 mg/ml E-64, and 20 mM HEPES, pH 7.2). The cells were allowed to swell on ice for 30 min and homogenized with Dounce homogenizer with 20 strokes. The homogenates were centrifuged at 3,500 rpm for 10 min at 4°C, and the supernatants were centrifuged at 13,700 rpm for 15 min at 4°C. The supernatants were harvested as cytosolic extracts free of mitochondria, and analyzed for mitochondrial cytochrome *c* release.

Analysis of the methylene chloride extract by gas chromatography-mass spectrometry (GC-MS)

The GC-MS analysis was conducted with Hewlett-Packard (HP) 6890 gas chromatography coupled with HP 5903N mass spectrometer. A HP-5MS capillary column filled with 5% Phenyl Methyl Siloxane was connected to the GC instrument. The analytical conditions were as follows: helium carrier gas flow rate, 0.7 ml/min; oven temperature program, 60℃ (hold for 15 min) rising to 280℃; split ratio, 30:1. The MS instrument was operated in the electron impact (EI) mode and used with ionization energy of 70 eV in an m/z range of 50-800 mass units. The constituents

were identified by comparison of their mass spectra with those of internal (computer) library, NIST/EPA/MSDC libraries.

Results and Discussion

Organic solvent extraction of antitumor component in the leaves of *Z. schinifolium*

When 3 kg of the powered dry leaves from *Z. schinifolium* was extracted with 80% methanol and the methanol extract was then evaporated, 648.6 g of the solid residue (SL-13) was obtained. To fractionate further the components in the methanol extract, the solid residue was dissolved in water and then sequentially extracted with various organic solvents, such as methylene chloride, ethyl acetate, and n-butanol. The yields of individual solvent extractions were 21.7 g for methylene chloride extraction (SL-14), 2.3 g for ethyl acetate extraction (SL-15), 37.6 g for n-butanol extraction (SL-16), and 144.8 g for residual water fraction (SL-17), respectively.

To examine whether each extract possesses antitumor activity, its cytotoxicity against human tumor cells was investigated by MTT assay. When human acute leukemia Jurkat T cells, estrogen receptor-positive breast cancer MDA361 cells, and estrogen receptor-negative breast cancer MDA438 cells were incubated with each extract at concentration of 50 μg/ml for 36 h, the viability of the tumor cells appeared to decline in the presence of the methylene chloride extract (SL-14)(Fig. 2). Under these conditions, the viability of the tumor cells, however, was not significantly altered in the presence of SL-13, SL-15, SL-16, or SL-17. These results demonstrate that the methylene chloride extract of the leaves of *Z. schinifolium* contains the most cytotoxic activity against human leukemia and breast cancer cells.

Apoptogenic activity of methylene chloride extract (SL-14) toward Jurkat T cells

To understand the mechanism underlying the cytotoxicity of the methylene chloride extract (SL-14), we investigated whether the apoptotic cell death is induced in Jurkat T cells after treatment with SL-14. In addition, because the anti-apoptotic role of Bcl-2 and Bcl-xL was known to center around their prevention of effector caspases including caspase-3 activation through blocking cytochrome c release from mitochondria[1,15,16], we decided to take advantage of this anti-apoptotic role of Bcl-xL to examine whether the mitochondrial cytochrome c release and the subsequent activation of caspase cascade are essential steps for SL-14-induced apoptosis. In this context, the cytotoxic effect of SL-14 was compared between Jurkat T cells transfected with Bcl-xL gene (J/Bcl-xL) and Jurkat T cells transfected with vector (J/Neo).

The cytotoxicity of SL-14, which increased in a dose-dependent manner at concentrations of 50~100 μg/ml in J/Neo cells, was significantly inhibited in J/Bcl-xL cells

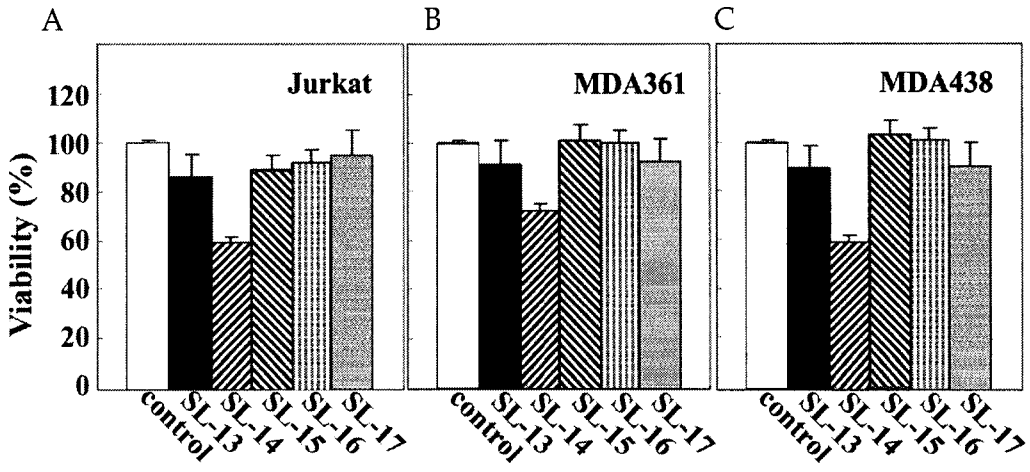

Fig. 2. Cytotoxic effect of the individual organic solvent extracts on human acute leukemia Jurkat T cells (A), estrogen receptor-positive breast cancer MDA361 cells (B), and estrogen receptor-negative breast cancer MDA438 cells (C). The cells (4×10^4 cells/well for Jurkat; 2×10^4 cells/well for the breast cancer cells) were incubated with the individual extracts at a concentration of 50 μg/ml in a 96-well plate for 48 h and the final 4 h was incubated with MTT.

overexpressing of Bcl-xL protein (Fig. 3A and 3B,). At the same time, the apoptotic DNA fragmentation was also induced by SL-14 and enhanced dose-dependently in J/Neo cells, whereas it was completely abrogated in J/Bcl-xL cells (Fig. 3C). These results demonstrate that the methylene chloride extract (SL-14) is able to induce apoptotic cell death of Jurkat T cells, which is negatively regulated by antiapoptoptic protein Bcl-xL. Although ectopic overexpression of Bcl-xL protein in Jurkat T cells appeared to completely rescue the cells from SL-14-induced apoptotic DNA fragmentation, it failed to completely protect J/Neo cells from the cytotoxicity of SL-14, in that the viability of J/Bcl-xL cells in the presence of 100 μg SL-14 was reduced to the level of 90% as compared to control cells untreated with SL-14. This indicates that the cytotoxicity of SL-14 might be mainly attributable to apoptotic cell death, and suggests that the growth-arrest might also contribute for the cytotoxic activity of SL-14. Subsequently, in order to investigate whether there is an apoptotic change in the cell cycle distribution of Jurkat T cells following exposure to SL-14, both J/Neo and J/Bcl-xL cells treated with SL-14 at various concentrations were analyzed by flow cytometry. When J/Neo cells were treated for 36 h in the presence of 25 μg/ml, 50 μg/ml or 100 μg/ml of SL-14, 8.3%, 19.6% or 50.1% of the cells were detected as the sub-G_1 phase representing apoptotic cells, respectively (Fig. 4A). However, the sub-G_1 peak was not detected in J/Bcl-xL cells (Fig. 4B). Under these conditions, the level of the G_1 cells appeared to slightly increase in J/Bcl-xL cells as compared to control cells untreated with SL-14. This flow cytometric data confirms that the apoptotic cell death of Jurkat T cells was induced dose-dependently by SL-14, and that the induced apoptosis was not accompanied by cell cycle-arrest at a specific stage of the cell cycle progression.

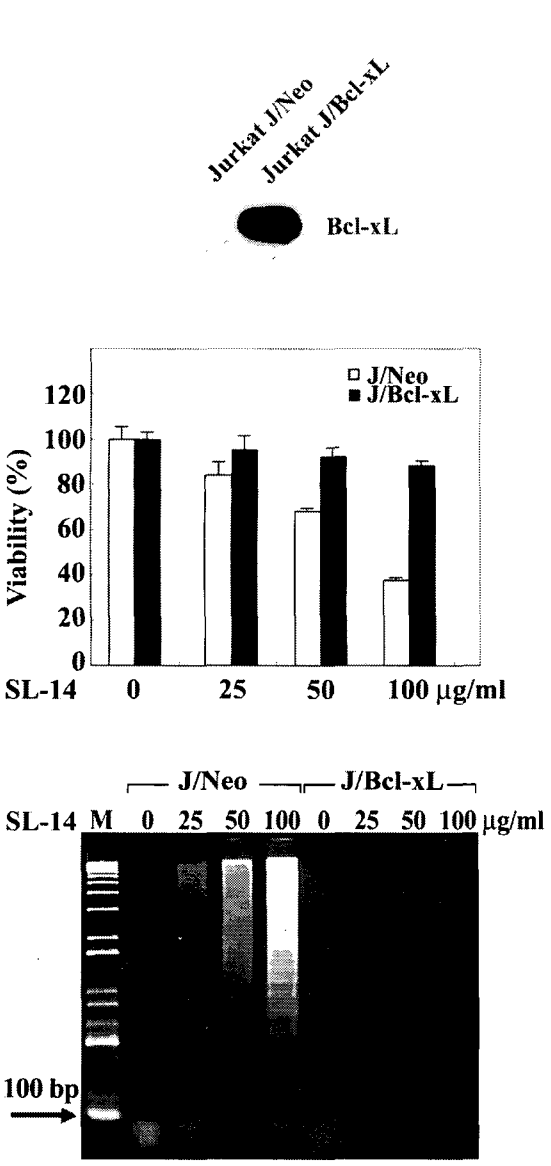

Fig. 3. Effect of the methylene chloride extract (SL-14) on on cell viability of Jurkat T cells transfected with vector (J/Neo) or Bcl-xL gene (J/Bcl-xL). Ectopic overexpression of Bcl-xL protein in Jurkat T cells transfected with Bcl-xL gene construct was confirmed by Western analysis (A). Jurkat T cells overexpressing Bcl-xL (J/Bcl-xL) or control cells (J/Neo) were incubated at a density of 4 x 10^4/well with various concentrations of SL-14 in 96-well plates for 40 h and the final 4 h were incubated with MTT to assess the colored formazan crystals produced from MTT as an index of cell viability (B). Equivalent cultures were processed to analyze apoptotic DNA fragmentation (C).

Involvement of mitochondrial cytochrome c-mediated activation of caspase cascade in SL-14-induced apoptosis

Previously, several reports have shown that mitochondrial cytochrome c release into cytoplasm is frequently involved in a chemical-induced apoptotic signaling pathway that activates caspase cascade including capase-9 and capase-3, leading to PARP degradation[1,15,16]. Since both DNA fragmentation and flow cytometric analysis indicate that SL-14-induced apoptosis in Jurkat T cells could be prevented by ectopic overexpression of Bcl-xL, it was likely that apoptotic cell death might be associated with mitochondrial cytochrome c release and resultant activation of caspase cascade. To examine this prediction, we decided to investigate whether SL-14-induced apoptosis accom-

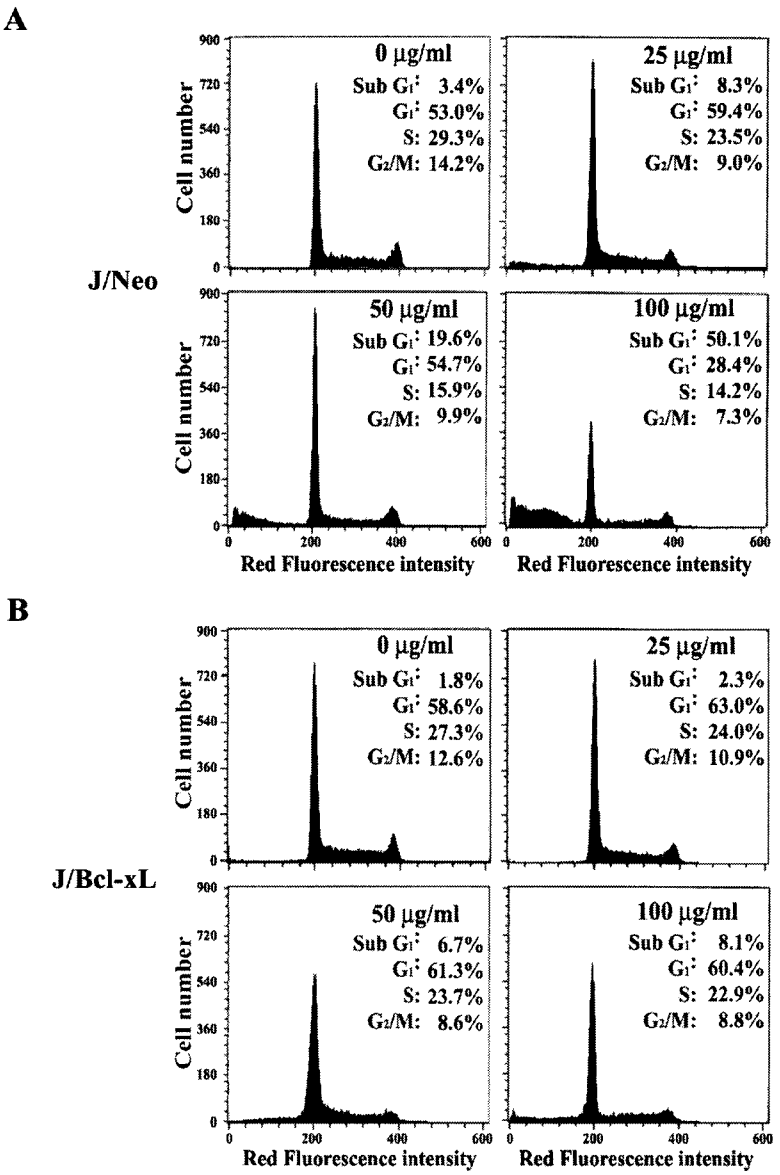

Fig. 4. Apoptotic change in the cell cycle distribution of Jurkat T cells transfected with vector (J/Neo) (A) or Bcl-xL gene (J/Bcl-xL) (B) after treatment with various concentrations of the methylene chloride extract (SL-14) for 48 h. After J/Neo and J/Bcl-xL were incubated in the presence of SL-14 under individual conditions, the cells were harvested. The analysis of cell cycle distribution was performed on an equal number of cells (5×10^5) by flow cytometry after staining of DNA by propidium iodide.

panies mitochondrial release of cytochrome c, and activation of caspase-9 and -3, and PARP degradation. As shown in Fig. 5A, although there was barely detectable or undetectable cytochrome c in the cytosolic fraction of continuously growing Jurkat T cells, the level of cytochrome c released from mitochondria increased dose-dependently in the presence of SL-14 ranging from 50 to 100 μg/ml. In accordance with mitochondrial cytochrome c release, caspase-9 activation that proceeds through proteolytic degradation of the inactive pro-enzyme (46 kDa) into the active form (35 kDa) was detected (Fig. 5B). The activation of caspase-3 through proteolytic degradation of a 32-kDa pro-enzyme into a 19-kDa activated form was also detected in a dose-dependent manner in the presence of 25 to 100 μg/ml SL-14. As a downstream target of active caspase-3 during induction of apoptosis, poly (ADP-ribose) polymerase

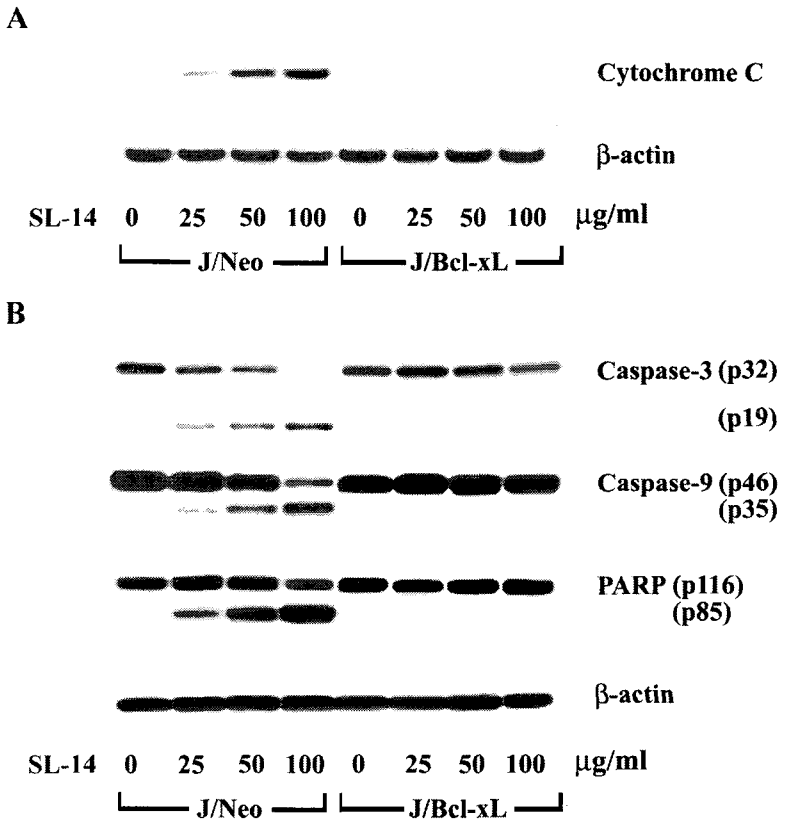

Fig. 5. Western blot analysis of mitochondrial cytochrome c release and β-actin (A), and caspase-3 activation, caspase-9 activation, cleavage of PARP and β-actin (B) in Jurkat T cells Jurkat T cells overexpressing J/Bcl-xL gene (J/Bcl-xL) or control cells (J/Neo) after treatment with the methylene chloride extract (SL-14) for 48 h. The cells (~5 x 10^6) were incubated at a concentration of 4 x 10^5/ml with indicated concentrations of SL-14 for 48 h and prepared for cell lysates. Equivalent amounts of cell lysates were electrophoresed on 4-12% SDS gradient polyacrylamide gels and electrotransferred to Immobilon-P membrane. Western analysis was performed as described in Materials and Methods using ECL Western blotting detection system.

(PARP) has been reported to be cleaved into two fragments[10]. This cleavage of PARP by active caspse-3 has been generally accepted as a marker of apoptosis in many experimental models. In J/Neo cells following the treatment of SL-14 ranging from 25 to 100 μg/ml, the cleavage of PARP was detected along with activation of caspase-3 in the presence of SL-14. However, these apoptotic celluar events were completely abrogated in J/Bcl-xL cells. These results indicate that SL-14-induced apoptosis involves mitochondrial cytochrome c-dependent activation of caspase cascade including caspase-9 and caspase-3 induction of apoptotic cell death.

Chemical composition of SL-14 obtained from the leaves of *Zanthoxylum schnifolium*

To examine the ingredients of the methylene chloride extract (SL-14) obtained from the leaves of *Z. schnifolium*, the extract was analyzed by GC-MS. As shown in Table 1, the SL-14 appeared to contain twenty-two ingredients, which correspond to 99.9% of total constituents detected in the extract. As the main components in SL-14 were 9,19-cyclolanost-24-en-3-ol (15.1%), 2-α-methyl-17, β-hop-21-ene (15.1%), 15-methyl-2,3-dihydro-1H benzazepin (11.95%), phytol (10.38%), lupeol (9.92%), 12- methyl-benzofuran (8.23%), hexadecanoic acid (5.96%), *cis,cis,cis*-9, 12,15-octadecatrienoic acid-methylester (5.49%), 9,12,15-octadecatrienoic acid-methylester (3.59%), 15-methyl-4-(1-methylethylidene)-2-(4-nitrophenyl) (3.36%), hexadecanoic acid methyl ester (1.93%), vitamine E (1.88%), beta-amyrin (0.96%), and auraptene (0.89%). This composition of the ingredients is significantly different from that of the essensial oil prepared from pericarps of *Z. schinifolium* which

Table 1. Chemical composition of the methylene chloride extract of the leaves of *Zanthoxylum schnifolium*

No.	Compounds	RT(min)[a]	Composition (%)[b]
1	2-ethyl-2,3-dihydro-1,3,2-benzoxaborole	12.581	0.39
2	germacrened	14.673	0.39
3	14-methoxy-2-methylcinnamic acid	20.775	0.31
4	hexadecanoic acid methyl ester	24.105	1.93
5	hexadecanoic acid	24.933	5.96
6	9,12-octadecadienoic acid	27.190	0.59
7	9,12,15-octadecatrienoic acid-methylester	27.325	3.59
8	phytol	27.530	10.38
9	*cis,cis,cis*-9,12,15-octadecatrienoic acid-methylester	28.162	5.49
10	15-methyl-4-(1-methylethylidene)-2-(4'-nitrophenyl)	31.884	3.36
11	auraptene	35.641	0.89
12	11-H-indole 1-methyl	36.133	0.31
13	I-indolize	36.377	0.14
14	12-methylbenzoforan	37.245	8.23
15	15-methyl-2,3-dihydro-1H Benzazepin	37.602	11.95
16	vitamine E	42.344	1.88
17	23-S-methylcholesterol	43.425	0.72
18	23-S-ethylcholest-5-en-3, beta-1	44.506	2.42
19	beta-amyrin	44.863	0.96
20	2-alpha-methyl-17, beta-hop-21-ene	45.159	15.10
21	9,19-cyclolanost-24-en-3-ol	45.164	15.10
22	lupeol	45.425	9.92

[a] Retention time (in minutes)
[b] Relative amount (%)

was able to induce apoptosis in HepG2 cells through increased production of reactive oxygen species[2].

In conclusion, these results demonstrate that the methylene chloride extract of leaves of *Zanthoxylum schinifolium* possesses an apoptogenic activity causing apoptotic DNA fragmentation of human acute leukemia Jurkat T cells via mitochondrial cytochrome c release into cytoplasm, and resultant activation of caspase-9 and caspase-3, and cleavage of PARP, which can be negatively regulated by anti-apoptotic protein Bcl-xL. Additional GC-MS analysis show that the methylene chloride extract (SL-14) is composed of 9,19-cyclolanost-24-en-3-ol (15.1%), 2-α-methyl-17, β-hop-21-ene (15.1%), 15-methyl-2,3-dihydro-1H benzazepin (11.95%), phytol (10.38%), lupeol (9.92%), 12-methylbenzofuran (8.23%), hexadecanoic acid (5.96%), *cis,cis,cis*-9,12,15-octadecatrienoic acid-methylester (5.49%), 9,12,15-octadecatrienoic acid-methylester (3.59%), 15-methyl-4-(1-methylethylidene) -2-(4-nitrophenyl) (3.36%), hexadecanoic acid methyl ester (1.93%), vitamine E (1.88%), beta-amyrin (0.96%), and auraptene (0.89%). These findings are useful for evaluating the apoptogenic activity in the edible plant *Zanthoxylum schinifolium*.

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초록 : *Zanthoxylum schinifolium* 잎의 methylene chloride 추출물의 화학적 조성 및 암세포에 대한 세포자살 유도활성과 그 작용기전

김준석 · 전도연[1] · 우미희[2] · 이인구[3] · 김영호*

(경북대학교 자연과학대학 미생물학과 면역생물학연구실, [1]경북대학교 유전공학 연구소, [2]약학과, 약학대학, 대구가톨릭대학교, [3]경북대학교 농업생명과학대학 응용생명과학부)

식용 및 약용으로 이용되는 산초 (*Zanthoxylum schinifolium*) 잎에 함유된 항암활성 성분을 분리하기 위하여 산초잎을 유기용매로 추출하여, 각 추출물의 암세포에 대한 독성 및 세포자살 유도 활성을 조사하였다. Methanol, methylene chloride, ethyl acetate, n-butanol로 추출한 각 추출물의 세포 독성을 인체 급성백혈병 Jurkat T 세포주, estrogen receptor-positive 유방암 세포주 MDA 361과 estrogen receptor-negative 세포주 MDA 438를 대상으로 조사한 결과, 이들 암세포주에 대한 세포독성이 methylene chloride 추출물 (SL-14)에서 주로 확인되었다. Methylene chloride 추출물 (SL-14)의 Jurkat T세포주에 대한 세포독성의 기전은 mitochondria로부터cytochrome c 방출, 이에 뒤이은caspase-9 및 caspase-3의 활성화, PARP 분해, internucleosomal DNAfragmentation등의 일련의 생화학적 과정을 통해 유도되며 또한 Bcl-xL의 ectopic overexpression에 의해서는 negative regulation되는 세포자살임을 확인하였다. 또한 SL-14를 GC-MS 분석하여, 9,19-cyclolanost-24-en-3-ol (15.1%), 2-a-methyl-17, b-hop-21-ene (15.1%), 15-methyl-2,3-dihydro-1H benzazepin (11.95%), phytol (10.38%), lupeol (9.92%), 12-methylbenzofuran (8.23%) 등을 포함한 22가지의 구성성분과 그 조성비를 확인하였다. 이상의 연구결과들은 식용 산초잎에 함유된 항암 활성으로서의 세포자살유도 활성의 규명과 이해에 유익하게 활용될 것으로 기대된다.