Phytomedicine 18 (2011) 344–348

Contents lists available at ScienceDirect

Phytomedicine

journal homepage: www.elsevier.de/phymed

Isolation of substances with antiproliferative and apoptosis-inducing activities against leukemia cells from the leaves of *Zanthoxylum ailanthoides* Sieb. & Zucc

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ARTICLE INFO

Keywords:
Zanthoxylum ailanthoides Sieb. & Zucc.
Anti-leukemia effect
Cytotoxicity
Pheophorbide derivative
Chlorophyll derivatives

ABSTRACT

Extraction of the leaves of *Zanthoxylum ailanthoides* Sieb. & Zucc. affords extracts and four isolated compounds which exhibit activities against leukemia cells. The chloroform-soluble fraction (ZAC) of the crude extract of this plant showed cytotoxic activity against human promyelocytic leukemia (HL-60) and myelomonocytic leukemia (WEHI-3) cells with IC_{50} values of 73.06 and 42.22 μg/mL, respectively. The active ZAC was further separated to yield pheophorbide-a methyl ester (**1**), pheophorbide-b methyl ester (**2**), 13^2-hydroxyl (13^2-*S*) pheophorbide-a methyl ester (**3**) and 13^2-hydroxyl (13^2-*R*) pheophorbide-b methyl ester (**4**) whose structures were confirmed by spectroscopic methods. Compounds **2**–**4** showed cytotoxic activities against both leukemia cells with IC_{50} value in the range of 46.76–79.43 nM, whereas compound **1** exhibited only weak cytotoxic activity. The extracts and compounds **1**–**4** also induced apoptosis and DNA damage in leukemia cells after treatment. The results suggested that the *Z. ailanthoides* is biologically active against leukemia cells.

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Introduction

Cancer is the leading cause of death worldwide. Finding a cure for this disease is always an important objective for human endeavor. Natural products have long been considered as potential drug candidates for cancer prevention and treatment it turn out to be the sources from which many anticancer agents are made (Katz 2002). Epidemiologic studies have shown that dietary vegetables and fruits can prevent cancer from occurring in human beings (Stan et al. 2008). Plant of *Zanthoxylum ailanthoides* Sieb. & Zucc. is widely distributed at the high altitude in central of Taiwan. The tender leaves of *Z. ailanthoides* have been used as a substitute for the green onion in Chinese dishes for Taiwanese vegetarian because of religious reasons. *Z. ailanthoides* is also used as a folk medicine for the treatment of myocardium disorder attenuation, bone-injury alleviation and cold resistance in China. Our previous studies have demonstrated that the extract of the stems of *Z. ailanthoides* is safe in genotoxicity and acted as antioxidants (Chung et al. 2006; Chu et al. 2009). It is reported that flavonoids including rutin and hyperoside from the leaves of *Z. ailanthoides* have the protective effect on LDL oxidation and lipid accumulation in macrophage. Several alkaloids isolated from the root bark of *Z. ailanthoides* have been shown to have anti-HIV activity (Cheng et al. 2005). To the best of our knowledge, there is no literature data on the anti-leukemia activity of *Z. ailanthoides*. In this paper, we report the *in vitro* cytotoxic activities of the leaves of *Z. ailanthoides* against two leukemia cell lines including human promyelocytic leukemia (HL-60) and murine myelomonocytic leukemia (WEHI-3). Furthermore, as well as the identification and cytotoxicity of four pheophorbide derivatives from the most active chloroform-soluble fraction guided by cytotoxicity assay. In addition, the cytotoxicity of tested extract and compounds were compared with that of retinoic acid (RA). RA is able to induce a terminal differentiation of leukemic cell lines, such as HL-60 and U937 (Breitman et al. 1981; Chomienne et al. 1986) and now is available for the treatment of acute promyelocytic leukemia (Degos and Wang, 2001).

Materials and methods

Chemical and reagents

4′-6-Diamidino-2-phenylindole (DAPI), dimethyl sulfoxide (DMSO), eithidium bromide (EtBr), 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT), potassium phosphates,

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doi:10.1016/j.phymed.2010.08.018

propidium iodide (PI) and retinoic acid (RA) were obtained from Sigma Chemical Co. (St. Louis, MO, USA). DMEM medium with 2 mM L-glutamine, RPMI 1640 medium with 2 mM L-glutamine, fetal bovine serum (FBS), penicillin–streptomycin and trypsin–EDTA were obtained from HyClone (USA). All reagents used were of analytical grade or purer.

Plant material and preparation of extracts

The leaves of *Z. ailanthoides* Sieb. & Zucc. were collected from the mountain area of Sinshe Township, Taichung, Taiwan. The air-dried leaves of of *Z. ailanthoides* Sieb. & Zucc. (3.6 kg) were pulverized and extracted under reflux for 6 h with methanol six times. After removal of methanol under reduced pressure, the MeOH extract (789.1 g) was suspended into H_2O and then extracted with $CHCl_3$ and *n*-BuOH to give $CHCl_3$ (ZAC, 209.3 g), *n*-BuOH (ZAB) and H_2O-soluble part (ZAW), respectively. ZAC was subjected to chromatography over a silica gel column using *n*-hexane-ethyl acetate (9:1 to 1:1) as eluent to give13 fractions (F-1 to F-13), and the more active fractions, F-7 to F-10, were isolated by chromatography to afford **1** (597.4 mg), **2** (13.6 mg), **3** (7.2 mg) and **4** (13.2 mg).

For the present experiments, tested samples were dissolved in 0.1% DMSO (final concentration) before being added to cell cultures.

Nuclear magnetic resonance

The NMR spectra, including ^{1}H, ^{13}C, COSY, NOESY, HMBC and HMQC experiments, were recorded on Bruker AMX-400 and -300, using tetramethylsilane (TMS) as internal standard. All chemical shifts are reported in parts per million (ppm, δ).

Cell lines and culture conditions

Human colon adenocarcinoma cell line (colo 205), human hepatoma cell line (Hep G2), human murine leukemia cell line (WEHI-3), mouse melanoma cell line (B16–F1), human melanoma cell line (A375 S2) and promyelomonocytic cells line (HL-60) were obtained from the Food Industry Research and Development Institute (Shinchu, Taiwan). The colo 205, WEHI-3 and HL-60 cells were cultured in RPMI 1640 medium, to the Hep G2, B16-F1 and A375 S2 cells were cultured in Dulbecco's minimal essential (DMEM) medium supplemented with 10% fetal bovine serum, 2 mM L-glutamine, and 2% penicillin–streptomycin (10,000 U/ml penicillin and 10 mg/ml streptomycin). The cultures were maintained in a humidified 5% CO_2 incubator at 37 °C and the cells were subcultured every 3–4 days to maintain logarithmic growth and were allowed to grow for 24 h before use.

Cell viability analysis

Each of different cancer cells was plated in a 12-well plate at a density of 5×10^4 cells/ml and grown over 48 h. Different concentrations of ZAW, ZAB and ZAC were then added to cells for final concentrations of 0, 6.25, 12.5, 25.0, 50.0 and 100.0 μg/ml (adding DMSO solvent only for the control regimen) and grown at 37 °C, 5% CO_2 and 95% air over 48 h.

Leukemia cells were treated with different concentration of fractions F-1 to F-13 (0, 0.625, 1.25, 2.5, 5.0 and 10.0 μg/ml), compounds **1**–**4** (0, 5.0, 10.0, 20.0, 40.0 and 80 nM) and RA (0, 10.0, 20.0, 40.0, 60.0 and 80 μM). Following 24 or 48 h incubation, cell viability was determined using MTT assay (Sladowski et al. 1993) and IC_{50} value was calculated.

Cell cycle and apoptosis analysis

About 5×10^4 cells/ml (leukemia cells, HL-60 or WEHI-3 cells) in a 12-well plate were treated separately with 100.0 μg/ml of ZAC, 5 μg/ml of fraction extracts (F-7 to F-10), 80 nM of **1**, 50 nM of **2**, 80 nM of **3** or 50 nM of **4** and then were incubated in an incubator for 48 h. Cells were harvested by centrifugation, fixed gently (drop by drop) with 70% ethanol (in PBS) at 4 °C overnight and then resuspended in PBS containing 40 μg/ml PI and 0.1 mg/ml RNase and 0.1% Triton X-100 in dark room. After 30 min at 37 °C, the cells were analyzed with a flow-cytometry (Becton–Dickinson, San Jose, CA) equipped with an argon ion laser at 488 nm wavelength. Annexin V-FITC and PI double staining kit (PharMingen, San Diego, CA) were used for quantification of apoptotic cells (Su et al. 2006a,b).

DAPI staining assay

Approximately 5×10^4 cells/ml (leukemia cells, HL-60 or WEHI-3 cells) in a 12-well plate were treated with ZAC (100.0 μg/ml), fraction extracts (5 μg/ml) or the compounds **1**–**4** (50–80 nM) and then were incubated for 48 h. Then cells were washed by PBS and then were stained by DAPI before being photographed by a fluorescence microscope (Lin et al. 2006).

Single cell gel electrophoresis (comet assay)

Approximately 5×10^4 cells/ml (leukemia cells, HL-60 or WEHI-3 cells) in a 6 cm dish were treated with ZAC (100.0 μg/ml), fraction extracts (5 μg/ml) or the compounds **1**–**4** (50–80 nM) for 48 h. The resultant cells were then isolated to extract DNA for DNA gel electrophoresis according to the method described previously (Su et al. 2006a,b).

Statistical analysis

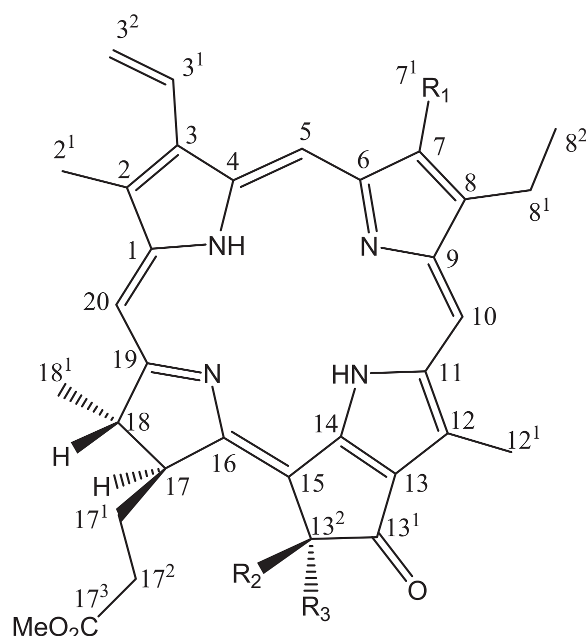
All data were expressed as mean ± standard deviation. Analysis of variance was performed by the ANOVA procedures. Duncan's new multiple-range test was used to determine the difference of means, and $P < 0.05$ was considered to be statistically significant.

Results and discussion

Z. ailanthoides has been used for dietary supplement in Taiwanese population for a long time but the anti-leukemia activities of its crude extract and active compounds are lacking. Here the cytotoxic activities of the water, chloroform and *n*-butanol-soluble fractions and compounds **1**–**4**, all of which were sourced from *Z. ailanthoides*, are presented.

Effects of the extracts of the leaves from Z. ailanthoides on cell viability of human cancer cell lines

In a bioassay-guided fractionation approach, the water (ZAW), chloroform (ZAC) and *n*-butanol (ZAB)-soluble fractions of the leaves of *Z. ailanthoides* were tested for their cytotoxic activities using cultured carcinoma cells including A375 S2, B16-F1, colo 205, Hep G2, HL-60 and WEHI-3 cells. As for the cytotoxic activity, each cell line responded differently to the treatment with the tested extract and only ZAC showed high cytotoxicity on leukemia cells including HL-60 and WEHI-3 cells. The growth inhibitory effect of ZAC was observed in a concentration-dependent manner (data were not shown). The 50 μg/ml ZAC treatments over 48 h decreased the cell viabilities of HL-60 and WEHI-3 by 39 and 55%, respectively. The IC_{50} values of ZAC were 73.06 μg/ml for HL-60 cells and 42.22 μg/mL for WEHI-3 cells, respectively. The results have suggested that the chloroform extract from the leaves of *Z. ailanthoides*



1	$R_1=CH_3$	$R_2=H$	$R_3=CO_2CH_3$
2	$R_1=CHO$	$R_2=H$	$R_3=CO_2CH_3$
3	$R_1=CH_3$	$R_2=OH$	$R_3=CO_2CH_3$
4	$R_1=CHO$	$R_2=CO_2CH_3$	$R_3=OH$

Fig. 1. The structures of **1**–**4** isolated from the leaves of *Z. ailanthoides*.

has the most antiproliferative activity on leukemia cells. Further fractionation and isolation of ZAC yieled to bioassay-directed chromatographic fractionation, resulting in the isolation of the most anti-leukemia active compounds.

Isolation of compounds from the leaves of Z. ailanthoides

During the course of screening the cytotoxic activities of different polar extracts of the leaves from *Z. ailanthoides*, the chloroform extracts (ZAC) was found to have the most potent activity on leukemia cells, and led to the isolation of pheophorbide-a methyl ester **(1)**, pheophorbide-b methyl ester **(2)**, 13^2-hydroxyl (13^2-*S*) pheophorbide-a methyl ester **(3)** and 13^2-hydroxyl (13^2-*R*) pheophorbide-b methyl ester **(4)** (Fig. 1) (Kenner et al., 1973; Nakatani et al. 1981).

Effects of these fractions and isolated compounds **1**–**4** on the growth of leukemia cells, HL-60 and WEHI-3, were investigated by the MTT method. As indicated in Table 1, F-7 to F-10 were the most active against both of the leukemia cell lines with the IC_{50} values in the range of 20.35–38.52 μg/ml for HL-60 cells and 2.28–14.34 μg/ml for WEHI-3 cells, respectively. Compounds **2**–**4** showed cytotoxic activities on both the leukemia cell lines with IC_{50} in the range of 46.76–79.43 nM, whereas compound **1** exhibited only weak cytotoxic activity (Table 1). Over a period of 48 hours, the maximal inhibitive effect on proliferation was observed with **4** at concentration of 80 nM, which inhibited proliferation of HL-60 and WEHI-3 cell line by 80.9 and 70.7%, respectively. On the basis of the IC_{50} values (Table 1), RA showed less cytotoxic against the HL-60 and WEHI-3 cells than the isolated active compounds **1**–**4**. RA, due to its differentiative potential, has been widely used in both cancer therapy and cancer prevention (Altucci and Gronemeyer, 2001; Sun and Lotan, 2002). It is therefore clear that the isolated

Table 1
Cytotoxic activity of the fraction extracts and **1**–**4** from the leaves of *Z. ailanthoides* toward two leukemia cell lines, HL-60 and WEHI-3.

	HL-60 cells		WEHI-3 cells	
	24 h	48 h	24 h	48 h
IC_{50} (μg/ml)[a]				
F-1	>50	>50	>50	>50
F-2	>50	>50	>50	>50
F-3	>50	>50	>50	>50
F-4	>50	>50	39.33	25.38
F-5	26.3	13.39	>50	40.35
F-6	>50	32.16	34.93	20.22
F-7	17.57	20.35	18.45	14.34
F-8	20.44	39.52	19.48	5.88
F-9	>50	21.86	14.19	3.06
F-10	>50	26.01	10.04	2.28
F-11	>50	>50	23.88	9.16
F-12	>50	>50	>50	>50
F-13	>50	>50	>50	17.65
IC_{50} (μM)				
1	>80	>80	>80	>80
2	>80	49.82	71.3	52.96
3	>80	73.53	73.08	79.43
4	73.09	46.83	54.5	46.76
IC_{50}(μM)				
Retinoic acid	>80	>80	>80	38.03

[a] IC_{50} is defined as the concentration that resulted in a 50% decrease in cell number.

active compounds **1**–**4** from *Z. ailanthoides* are worthy of further studies as a potential therapeutic application.

Effects of the extracts and isolated compounds of the leaves from Z. ailanthoides on cell cycle and apoptosis of leukemia cells

The effects of the extracts and the constituents of the leaves from *Z. ailanthoides* on the cell cycle arrest of leukemia cells were examined. The results demonstrated that ZAC, F-8, F-10, **3** and **4** induced G_2/M phase arrest, and F-9, **1** and **2** induced *S* phase arrest in HL-60 cells. However, all tested extracts and isolated compounds induced G_0/G_1 phase arrest (Table 2). They also induced apoptosis in HL-60 and WEHI-3 cells. To further confirm that the extracts and isolated compounds induced apoptosis, we isolated cells after 48 h of reaction. Those cells were then stained using DAPI and photographed by fluorescence microscope. As shown in Fig. 2, apparently the per-

Table 2
The effects of the extracts from the leaves of *Z. ailanthoides* and **1**–**4** on cell cycle distribution and apoptosis (%) in leukemia cells.

		G_0G_1 (%)	*S* (%)	G_2M (%)	Apoptosis (%)
HL-60	C	58.4 ± 3.1[a]	34.8 ± 2.9	6.8 ± 0.2	2.4 ± 3.4
	ZAC[b]	45.1 ± 4.7	7.3 ± 0.8	46.7 ± 3.2	15.8 ± 3.4*
	F-8	52.5 ± 7.5	36.0 ± 2.9	20.1 ± 4.1	63.6 ± 2.8
	F-9	53.9 ± 2.2	35.8 ± 3.3	10.4 ± 1.1	26.8 ± 4.2*
	F-10	43.9 ± 3.0	43.1 ± 1.1	12.9 ± 1.4	11.1 ± 0.4*
	1	43.9 ± 1.6	47.5 ± 0.5	8.6 ± 2.1	34.2 ± 1.4*
	2	50.2 ± 0.6	39.2 ± 4.5	10.6 ± 4.1	11.8 ± 4.8*
	3	52.3 ± 3.3	34.9 ± 3.8	12.8 ± 0.5	43.1 ± 1.5*
	4	62.9 ± 0.4	13.2 ± 1.2	2.9 ± 6.8	29.3 ± 0.9*
WEHI-3	C	30.3 ± 0.2	62.4 ± 0.7	7.3 ± 0.6	0.9 ± 0.2
	ZAC	63.6 ± 3.2	27.7 ± 3.5	8.5 ± 0.3	4.6 ± 3.8
	F-8	47.2 ± 1.2	38.1 ± 2.3	14.7 ± 3.3	38.8 ± 1.4*
	F-9	45.0 ± 1.7	37.8 ± 2.2	17.3 ± 1.2	32.6 ± 7.2*
	F-10	55.7 ± 9.4	36.7 ± 4.8	7.6 ± 5.3	40.1 ± 5.7*
	1	76.0 ± 0.4	5.7 ± 5.4	18.3 ± 5.0	33.2 ± 0.9*
	2	64.6 ± 3.3	29.1 ± 3.2	6.6 ± 2.4	11.0 ± 2.3*
	3	73.2 ± 1.1	4.2 ± 1.6	22.8 ± 3.0	8.5 ± 0.2*
	4	62.6 ± 0.9	17.6 ± 1.7	19.5 ± 1.14	10.8 ± 0.7*

[a] Data represents mean ± S.D. of three experiments.
[b] ZAC: the chloroform-soluble extract of the leaves from *Z. ailanthoides*.
* $p < 0.05$: significantly different compared with the control.

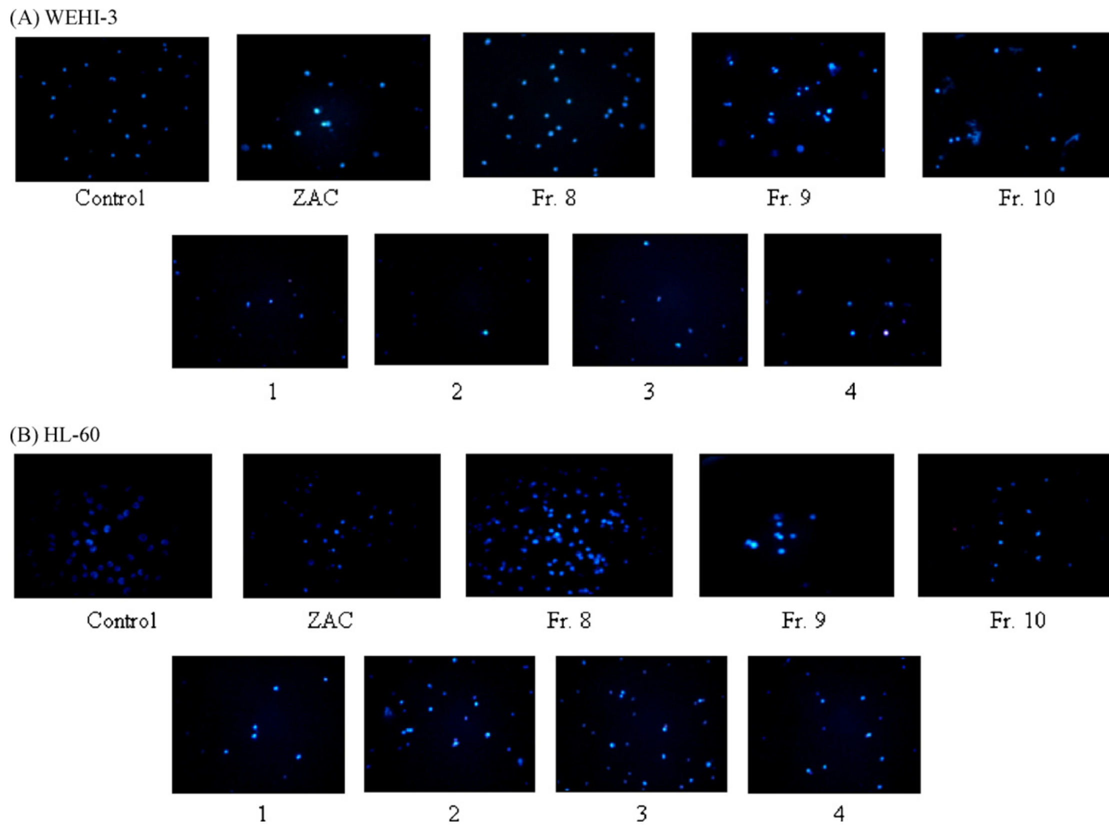


Fig. 2. The induction of apoptosis of HL-60 (A) and WEHI-3 (B) cells after treatment with the chloroform extract of the leaves of ZAC and **1**–**4** was examined by DAPI staining. The leukemia cells were incubated with tested samples over 48 h and apoptosis was determined by DAPI staining and fluorescence microscope (100×).

centage of cells stained by DAPI was significantly different between the groups consisting of tested extracts/isolated compounds and the control groups. DNA was isolated from leukemia cells after 48 h exposure to tested extracts/isolated compounds, and then examined and photographed for DNA damage by comet assay and fluorescence microscope, respectively. The comet assay is an extremely sensitive DNA damage assay. As shown in Fig. 3, all tested extracts and isolated compounds induced DNA damage.

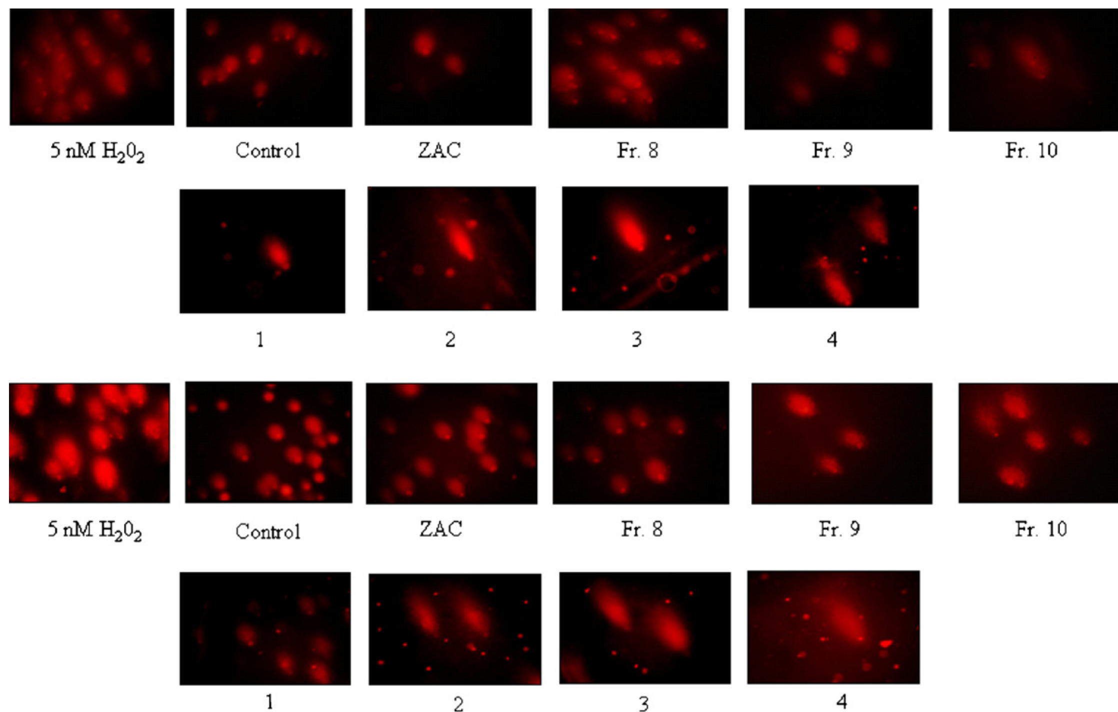


Fig. 3. DNA damage in HL-60 (A) and WEHI-3 (B) cells induced by the chloroform extract of the leaves of ZAC and **1**–**4** were examined by comet assay. Leukemia cells were incubated with tested sample over 48 h and DNA damage was determined by comet assay and fluorescence microscopy (100×).

When normal cells are out of control, apoptosis occurs and may cause diseases such as autoimmunity, immunodeficiency, and cancers, etc. It is well known that apoptosis plays a variety of important roles under normal physiological conditions (Alison and Sarraf 1995). It is also reported that impaired apoptosis is a crucial step in the process of cancer development (Johnstone et al. 2002). The majority of anticancer drugs in clinical trials exerted their effects on cancer cells via cell cycle arrest and induced apoptosis. Many studies have reported that plants and/or natural products like (−)-epigallocatechin 3-gallate (EGCG) and (−)-epigallocatechin (EGC) which are common polyphenols in green tea (Han and Kim 2009), curcumin which is a natural product present in turmeric (Angelo and Kurzrock 2009), *Isatis indigotica* (Hsuan et al. 2009), *Prunus mume* Sieb. et Zucc. (Masakazu et al. 2007) and Baizhu (*Atractylodes macrocephala* Koidz) (Huang et al. 2005) which are traditional herbal medicines are a potential inhibitor of tumor cell proliferation, carcinogen-induced carcinogenesis and apoptotic inducers in leukemia cells. In fact, many derivatives from plants have been used as anticancer agents in clinical patients (Katz 2002). *Z. ailanthoides* has been used as a dietary supplement in the Taiwanese population for many years but there is no information on the crude extract of *Z. ailanthoides* and its active principle in having anti-leukemia activity. *Z. ailanthoides* has been used as a dietary supplement in Taiwan for many years but its anti-leukemia activity has not been reported.

The current study demonstrated the anti-leukemia capability of *Z. ailanthoides* and the isolated active components (**1**–**4**) by decreasing cell viability and inducing cell cycle arrest/apoptosis in vitro. The DAPI staining (Fig. 2) and comet assay (Fig. 3) results also indicate that ZAC and **1**–**4** induced DNA damage in the leukemia cells, which may provide a basis for the potential therapeutic application of *Z. ailanthoides* and its active components. Further studies are needed to elucidate the precise mechanism of anti-leukemia activity and confirm the activities in animal models.

In conclusion, the present study has demonstrated an antitumor effect on leukemia cells of the leaves of *Z. ailanthoides*, and isolated active compounds **1**–**4**. These results may provide a basis for the potential therapeutic application of *Z. ailanthoides* and the active components to cancer therapy.

Declaration of conflicting interests

The authors declared no conflicts of interest with respect to the authorship and/or publication of this article.

Acknowledgments

The authors were greatful for financial support from the National Science Council of Republic of China awarded to S.-T. Chou (NSC96-2313-B-126-002-MY2) and T.-S. Wu. This study was supported in part by Taiwan Department of Health Cancer Research Center of Excellence (DOH99-TD-C-111-005).

References

Alison, M.R., Sarraf, C.E., 1995. Apoptosis: regulation and relevance to toxicology. Hum. Exp. Toxicol. 14, 234–247.

Altucci, L., Gronemeyer, H., 2001. The promise of retinoids to fight against cancer. Nat. Rev. Cancer 1, 181–193.

Angelo, L.S., Kurzrock, R., 2009. Turmeric and green tea: a recipe for the treatment of B-chronic lymphocytic leukemia. Clin. Cancer Res. 15, 1123–1125.

Breitman, T.R., Collins, S.J., Keene, B.R., 1981. Terminal differentiation of human promyelocytic leukemic cells in primary culture in response to retinoic acid. Blood 57, 1000–1004.

Cheng, M.J., Lee, K.H., Tsai, I.L., Chen, I.S., 2005. Two new sesquiterpenoids and anti-HIV principles from the root bark of *Zanthoxylum ailanthoides*. Bioorg. Med. Chem. 13, 5915–5920.

Chomienne, C., Balitrand, N., Abita, J.P., 1986. Inefficacy of the synthetic aromatic retinoid etretinate and of its free acid on the in vitro differentiation of leukemic cells. Leuk. Res. 10, 1079–1081.

Chu, C.Y., Lee, H.J., Chu, C.Y., Yin, Y.F., Tseng, T.H., 2009. Protective effects of leaf extract of *Zanthoxylum ailanthoides* on oxidation of low-density lipoprotein and accumulation of lipid in differentiated THP-1 cells. Food Chem. Toxicol. 47, 1265–1271.

Chung, Y.C., Chang, C.T., Teng, K.Y., Chou, S.T., 2006. Antioxidative and mutagenic properties of *Zanthoxylum ailanthoides* Sieb. & Zucc. Food Chem. 97, 418–425.

Degos, L., Wang, Z.Y., 2001. All trans retinoic acid in acute promyelocytic leukemia. Oncogene 20, 7140–7145.

Han, D.H., Kim, J.H., 2009. Difference in growth suppression and apoptosis induction of EGCG and EGC on human promyelocytic leukemia HL-60 cells. Arch. Pharm. Res. 32, 543–547.

Hsuan, S.L., Chang, S.C., Wang, S.Y., Liao, T.L., Jong, T.T., Chien, M.S., Lee, W.C., Chen, S.S., Liao, J.W., 2009. The cytotoxicity to leukemia cells and antiviral effects of *Isatis indigotica* extracts on pseudorabies virus. J. Ethnopharmacol. 123, 61–67.

Huang, H.L., Chen, C.C., Yeh, C.Y., Huang, R.L., 2005. Reactive oxygen species mediation of Baizhu-induced apoptosis in human leukemia cells. J. Ethnopharmacol. 97, 21–29.

Johnstone, R.W., Ruefli, A.A., Lowe, S.W., 2002. Apoptosis: a link between cancer genetics and chemotherapy. Cell 108, 153–164.

Katz, S., 2002. Beneficial uses of plant pathogens: anticancer and drug agents from plant pathogens. Can. J. Plant Pathol. 24, 10–13.

Kenner, G.W., McCombie, S.W., Smith, K.M., 1973. Pyrroles and related compounds. Part XXIV. Separation and oxidative degradation of chlorophyll derivatives. J. Chem. Soc., Perkin Trans. 1, 2517–2523.

Lin, C.C., Kao, S.T., Chen, G.W., Ho, H.C., Chung, J.G., 2006. Apoptosis of human leukemia HL-60 cells and murine leukemia WEHI-3 cell, induced by berberine through the activation of caspase-3. Anticancer Res. 26, 227–242.

Masakazu, A., Yoshihiko, S., Toshinobu, M., Tatsushi, O., Taro, A., Kazuhisa, O., Keiji, S., Kenji, S., Yoko, A., Kazuo, M., Miwa, U., Takashi, O., Mario, C., 2007. The "*Prunus mume* Sieb. et Zucc." (Ume) is a rich natural source of novel anti-cancer substance. Int. J. Food Properties 10, 375–384.

Nakatani, Y., Ourisoon, G., Beck, J.P., 1981. Chemistry and biochemistry of chinese drugs. VII. Cytostatic phenphytins from silkworm excreta, and derived photocytotoxic pheophorbides. Chem. Pharm. Bull. 29, 2261–2269.

Sladowski, D., Steer, S.J., Clothier, R.H., Balls, M., 1993. An improved MTT assay. J. Immunol. Methods 157, 203–207.

Stan, S.D., Kar, S., Stoner, G.D., Singh, S.V., 2008. Bioactive food components and cancer risk reduction. J. Cell Biochem. 104, 339–356.

Su, C.C., Chen, G.W., Tan, T.W., Lin, J.G., Chung, J.G., 2006a. Crude extract of garlic induced caspase-3 gene expression leading to apoptosis in human colon cancer cells. In Vivo 20, 85–90.

Su, C.C., Chen, G.W., Lin, J.G., Wu, L.T., Chung, J.G., 2006b. Curcumin inhibits cell migration of human colon cancer colo 205 cells through the inhibition of nuclear factor kappa B/p65 and down-regulates cyclooxygenase-2 and matrix metalloproteinase-2 expressions. Anticancer Res. 26, 1281–1288.

Sun, S.Y., Lotan, R., 2002. Retinoids and their receptors in cancer development and chemoprevention. Crit. Rev. Oncol. Hematol. 41, 41–55.