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Cytotoxic Activity of Parthenin, a Sesquiterpene Isolated from a *Crinum ensifolium*†

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Abstract – Phytochemical study on the ethanol extract of a *Crinum* species by bioassay-guided fractionation resulted in the isolation of an active principle, which was determined as parthenin (**1**) on the basis of physicochemical and spectroscopic analyses. This is the first report on the existence of a sesquiterpenoid from *Crinum ensifolium*. Compound **1** was found to show strong cytotoxic activity against some cancer cell lines and strongly inhibit NF-κB activity with the IC_{50} value of 1.82 μM.
Keywords – *Crinum ensifolium,* Amaryllidaceae, parthenin, cytotoxic activity, NF-κB inhibition

Introduction

Crinum is a large genus of the family Amaryllidaceae, comprising of about 160 species worldwide (Mabberly, 1990). These species have long been used in ethnomedicine all over the world to treat illness and diseases (Fennell and Staden, 2001; Nguyen *et al.*, 2002). Previous phytochemical studies have revealed some active constituents from these species, for example alkaloids (Fennell and Staden, 2001; Nguyen *et al.*, 2002; Ghosal *et al.*, 1985; Sun *et al.*, 2009), flavonoids (Nguyen *et al.*, 2002; Sun *et al.*, 2009; Nam *et al.*, 2004), phenolics (Sun *et al.*, 2009), triterpenoids and coumarins (Nam *et al.*, 2004). These species have also been shown various biological activities, such as analgesic, anticancer, anti-infective, antifulgal, anti-hypertensive, and tonic effects (Fennell and Staden, 2001; Nguyen *et al.*, 2002; Ghosal *et al.*, 1985). In Vietnam, these plants are reputed as anticancer drugs in ethnomedicine (Do, 2003; Do *et al.*, 2004). As part of a project to search for anticancer constituents from medicinal plants, we set out to investigate the active principle of a *Crinum* species collected in Hanoi, Vietnam. In the following we report the isolation, structure identification of parthenin, an active principle from this *Crinum* plant, and evaluation of its cytotoxic activity against some cancer cell lines.

Experimental

Plant materials – The plant material (aerial part) was collected at a botanical garden in Hanoi, Vietnam, in 2005. The sample was authenticated as a *Crinum ensifolium* Roxb. (Amaryllidaceae) by Dr. Tran Van Thanh, Department of Pharmacognosy, Hanoi University of Pharmacy.

General experimental procedure – Melting point determinations were performed using a Kofler microhotstage. Optical rotations were measured on a JASCO DIP-370 polarimeter. IR spectra (KBr) were obtained on a Brucker spectrometer. FAB-MS were registered using a JEOL JMS-DX 300 spectrometer. NMR spectra including ^{1}H- and ^{13}C-NMR, ^{1}H-^{1}H COSY (Homonuclear Correlation Spectroscopy), HMBC (Heteronuclear Multiple-Bond Connectivity), HMQC (^{1}H-detected Heteronuclear Multiple-Quantum Coherence), and NOESY (Nuclear Overhauser and Exchange Spectroscopy) were recorded on a Bruker DRX-300 or DRX-600 NMR spectrometers. Analytical TLC was performed on pre-coated silica gel 60 F_{254} plates (Merck) or RP-18 F_{254} (Merck). For column chromatography, silica gel (Kieselgel 60, 40 - 63 μm particle size, Merck) was used.

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Extraction and isolation – The air dried aerial parts of the plant (1 kg) were extracted with EtOH (5 L × 3 times) at room temperature for 1 week and repeated twice. The combined extracts was evaporated to yield a crude residue (74 g), which was resuspended in H_2O (0.5 L) and then partitioned with *n*-hexane (0.5 L × 3 times). The *n*-hexane fraction was concentrated under reduced pressure to afford a dry extract (23 g). This extract was subjected to column chromatography over silica gel (1 kg) using eluted solvent as *n*-hexane-EtOAc with increasing polarity from *n*-hexane to EtOAc. The fraction (235 mg) eluted with *n*-hexane-EtOAc (4 : 1 and 3 : 1, v/v). Purification by a silica gel column eluted with *n*-hexane-EtOAc (4 : 1, v/v) yielded compound **1** (37 mg).

Compound **1** (parthenin): white amorphous powder; mp 163 - 165 °C; $[\alpha]_D^{23}$ +3.5 (*c* 0.5, MeOH); IR (KBr) ν_{max} 3425 (OH), 1740 (C = O, ketone carbonyl), 1655 (C = O, lactone carbonyl), 1455 (C = C), 1165, 1120 (C-O); ^{1}H-NMR (300 MHz) and ^{13}C-NMR (75 MHz): see Table 1; FAB-MS *m/z* 285 $[M + Na]^+$, 263 $[M + H]^+$.

Cancer cell lines and cultures – Cancer cell lines used for experiment are Hep3B (human hepatoma cells), HeLa (human epithelial cervical cancer cells), MCF-7 (human breast adenocarcinoma cells), AGS (human gastric carcinoma cells), HL-60 (human promyelocytic leukemia cells), K-562 (human erythromyeloblastoid leukemia cells), B16F10 (murine melanoma cell line), and L-1210 (mouse lymphocytic leukemia cells). All cell lines were kindly supplied by a Cell Bank at Korea Research Institute of Bioscience and Biotechnoogy (KRIBB).

Cells were cultured in Dulbecco modified Eagle's medium (DMEM, Gibco BRL) supplemented with 10% fetal bovine serum (FBS) at 37 °C in a humidity atmosphere containing 5% CO_2.

Cytotoxic assay – Cytotoxic activity of isolated compound was tested against some tumor cell lines by Microculture Tetrazolium (MTT) assay method as prescribed previously (Cui *et al.*, 2008). In brief, cells undergoing exponential growth were suspended in fresh medium at a concentration of 2.5×10^4 cells/mL and plated in 96-well plates in a volume of 200 μL each well. Cells were allowed to grow for 24 h at 37 °C under 5% CO_2 humidified atmosphere, and 200 μL of new medium containing the test samples was added for each concentration. Tested samples prepared in 20 μL of DMEM supplemented with 5% FBS from stock solutions in dimethyl sulfoxide (DMSO). The final concentration of DMSO tested was less then 0.1%. After incubation for 48 h under the same conditions, the media were removed and 100 μL of 0.5 mg/mL MTT solution was added to each well, and the plates were further incubated for 4 h. The media were discarded, and then 100 μL of DMSO was treated to each well and mixed thoroughly to dissolve the formazan crystals. MTT reduction in living cells was read on a microplate reader (ELISA reader) at 540 nm, wells with untreated cells served as controls. Results are average values of at least three independent measurements. The IC_{50} value was defined as a sample's concentration that reduces absorbance by 50% in comparison with a control. Parthenolide and adriamycin were used as positive controls.

Table 1. ^{1}H-NMR (300 MHz) and ^{13}C-NMR (75 MHz) data of parthenin (**1**)[a]

No	δ_H (mult., *J* in Hz)	δ_C
1		84.9
2	7.54 (1H, d, 6.0)	163.3
3	6.19 (1H, d, 6.0)	132.3
4		210.9
5		59.5
6	5.02 (1H, d, 8.1)	79.1
7	3.52 (1H, m)	44.6
8	1.70 (1H, m) 2.36 (1H, m)	28.8
9	1.85 (1H, m) 2.23 (1H, m)	30.2
10	2.34 (1H, m)	41.1
11		171.1
12		140.8
13	5.61 (1H, d, 2.4) 6.29 (1H, d, 2.4)	122.1
14	1.13 (3H, d, 7.5)	17.9
15	1.30 (3H, s)	18.8

[a] Spectra were obtained in $CDCl_3$ with tetramethylsilane (TMS) as the internal standard.

NF-κB reporter assay – NF-κB activity of compound **1** was evaluated by a reporter assay in TNF-α stimulated HeLa cells. For the assay, the cells were transiently transfected with a plasmid containing eight copies of B elements linked to the SEAP (secreted alkaline phosphatase) gene, then the SEAP assay was performed as previously described (Dat *et al.*, 2009). Parthenolide was used as a reference compound.

Results and Discussion

A phytochemical investigation of the aerial parts of *C. ensifolium* resulted in the isolation of compound **1**, which was obtained as a white powder with mp 163-165 °C and positive optical rotation $[\alpha]^{23}$ +3.5 (*c* 0.5, MeOH). The IR

spectrum of **1** revealed the presence of hydroxyl (3425), ketone (1740), and lactone carbonyl (1655) groups. The ^{13}C-NMR spectrum of **1** exhibited resonances for 15 carbons in total, and ^{1}H-NMR displayed resonances for 17 protons. However, FAB-MS spectrum of **1** showed the molecular peaks at *m/z* 285 $[M + Na]^+$, 263 $[M + H]^+$, hence, compound **1** was assigned for a sesquiterpenoid having molecular formula $C_{15}H_{18}O_4$. Further analyzes spectral data of **1** indicated that this compound has one ketone carbon (δ_C 210.9, C-4), one lactone carbonyl carbon (δ_C 171.1, C-11), one double bond C/CH_2 [140.8 (C-12), 122.1 (C-13); δ_H 5.61 and 6.29 (each 1H, d, J = 2.4 Hz, H_2-13)], one double bond CH/CH [δ_C 163.3 (C-2), δ_H 7.54 (1H, d, J = 6.0 Hz, H-2); and 132.3 (C-3), δ_H 6.19 (1H, d, J = 6.0 Hz, H-3)], two quaternary carbons [δ_C 84.9 (C-1, oxygenated), and 59.5 (C-5)], five aliphatic groups [δ_C 79.1 (oxygenated), 44.6, 28.8, 30.2, 41.1], and two methyl groups [δ_C 17.9 (C-14), δ_H 1.13 (3H, d, J = 7.5 Hz, H-14); and δ_C 18.8 (C-15), δ_H 1.30 (3H, s, H-15)]. The IR and NMR indicated a lactone-ring moiety (ring C) in the structure of **1** that has a C-12/C-13 double bond. The downfield signals of olefinic carbons (δ_C 163.3 and 132.3) and protons (δ_H 7.54 and 6.19) demonstrated the C-2/C-3 double being present together with the ketone carbonyl carbon at C-4 in ring A. All these observations suggested that compound **1** might be parthenin, a major sesquiterpenoids isolated from the plant *Parthenium hysterophorus* (Herz *et al.*, 1962; Ramesh *et al.*, 2003). The comparision of physical (mp, $[\alpha]_D$) and spectral data to those published in literature (Chen *et al.*, 1991; Herz *et al.*, 1962; Ramesh *et al.*, 2003) led to the identification that **1** was parthenin. The HMQC, HMBC and COSY spectra of **1** (Fig. 1) supported the conclusion.

The cytotoxic activities of parthenin (**1**) were examined against some tumor cell lines using MTT method and the results are presented in Table 2. Compound **1** showed cytotoxic effect on eight cancer cell lines tested, exhibiting significant cytotoxic effects on five cell lines, including HeLa, HL-60, K-562, B16F10, and L-1210 with IC_{50} values of 10.3, 4.1, 2.7, 3.5, and 1.8 mM, respectively. Parthenin was found to strongly inhibit NF-κB activation in TNF-α stimulated HeLa cells with the IC_{50} value of 1.82 μM. This inhibitory effect was significant in comparison with that of parthenolide (IC_{50} value of 4.14 μM).

Previous investigations have reported the presence of alkaloids, chromones, coumarins, flavonoids, triterpenoids in the *Crinum* species. In this work, we report for the first time on the presence of parthenin, a sesquiterpene, in a species of the genus *Crinum*. Accordingly, parthenin is the major and characteristic constituent in the *Parthenium hysterophorus* (Herz *et al.*, 1962; Ramesh *et al.*, 2003; Biswanath *et al.*, 2007; Mew *et al.*, 1982), and is constituent of several other genus in the Asteraceae such as *Ambrosia spp.* (Chen *et al.*, 1991; Mew *et al.*, 1982; Salam *et al.*, 1984), and *Dichrocephala integrifolia* (Morikawa *et al.*, 2006). Hence, the first report on the presence of this compound from a *Crinum* species (Amaryllidaceae) in this study remarkably contributes to the phytochemical data of natural source. Studies have pointed out variety of pharmaceutical activities of parthenin, such as antitumor (Biswanath *et al.*, 2007; Mew *et al.*, 1982; Fernandes *et al.*, 2008), anti-allergic (Picman *et al.*, 1985), antimalaria (Hooper *et al.*, 1990), and potent angiotensin II binding (Chen *et al.*, 1991) activities. In this work, we examined and found the strong cytotoxic activities of parthenin against eight cancer cell lines. Furthermore, our result showed for the first time that parthenin strongly inhibited the activity of NF-κB, a transcriptional nuclear factor involving in the tumor development (Luqman and Pezzuto, 2010). Interestingly,

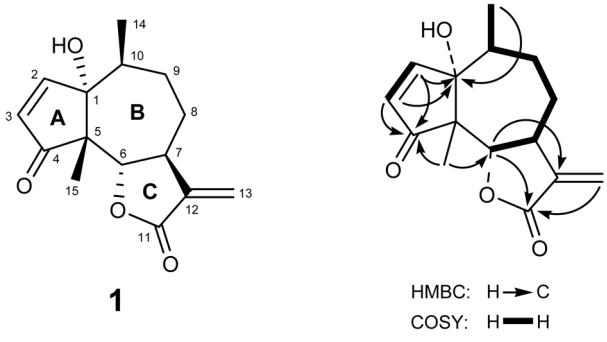

Fig. 1. Structure, key HMBC and COSY correlations of compound **1**.

Table 2. Cytotoxic activities[a] of parthenin against some tumor cell lines

Cell lines	Hep3B	HeLa	MCF-7	AGS	HL-60	K-562	B16F10	L-1210
Parthenin	13.2 ± 2.0	10.3 ± 1.5	64.7 ± 1.0	114.5 ± 2.5	4.1 ± 0.6	2.7 ± 0.8	3.5 ± 0.6	1.8 ± 0.5
Parthenolide[b]	5.81 ± 0.7	18.1 ± 2.3	37.4 ± 1.3	26.3 ± 0.9	NT	NT	NT	NT
Adriamycin[b]	NT	NT	NT	NT	2.6 ± 0.3	1.8 ± 0.4	1.3 ± 0.3	1.1 ± 0.3

[a] Data are means ± SD (in μM) from three separated experiments; [b] Parthenolide and adriamycin were used as positive controls; NT: not tested.

parthenin was more active than the known NF-κB inhibitor, parthenolide (Hehner *et al.*, 1999; Sohma *et al.*, 2011). This suggested that the cytotoxicity of parthenin may relate to the inhibition of NF-κB and supported the view on antitumor of this compound in the previous studies. Since parthenin and parthenolide structurally belong to the class of sesquiterpene lactone, parthenin could suppress NF-κB activity by a similar mechanism. Further studies are needed to clarify the antitumor property of parthenin.

In this study, we describe for the first time the isolation of parthenin from a *Crinum* species. The results showed that this compound displayed marked cytotoxic effects against some cancer cell lines and inhibit NF-κB activation, comparable to those of parthenolide. The finding may explain, in part only, the use of some *Crinum* species for the treatment of cancer in Vietnamese ethnomedicine.

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References

Biswanath, D., Reddy, V.S., Krishnaiah, M., Sharma, A.V.S., Kumar, K.R., Rao, J.V., and Sridhar, V., Acetylated pseudoguaianolides from *Parthenium hysterophorus* and their cytotoxic activity. *Phytochemistry* **68**, 2029-2034 (2007).

Chen, Y., Bean, M.F., Chambers, C., Francis, T., Huddleston, M.J., Offen, P., Westley, J.W., and Carté, B.K., Timmermann, B.N., Arrivacins, novel pseudoguaianolide esters with potent angiotensin II binding activity from *Ambrosia psilostachya*. *Tetrahedron* **47**, 4869-4878 (1991).

Cui, L., Thuong, P.T., Lee, H.S., Njamen, D., Mbafor, J.T., Fomum, Z.T., Lee, J., Kim, Y.H., and Oh, W.K., Four new chalcones from *Erythrina abyssinica*. *Planta Med.* **74**, 422-426 (2008).

Dat, N.T., Lee, J.H., Lee, K., Hong, Y.S., Kim, Y.H., and Lee, J.J., Phenolic constituents of *Amorpha fruticosa* that inhibit NF-κB activation and related gene expression. *J. Nat. Prod.* **71**, 1696-1700 (2008).

Do, H.B., Dang, Q.C., Bui, X.C., Nguyen, T.D., and Do, T.D., *The medicinal plants and animals of Vietnam*. Publishing House for Science and Technology, Hanoi, Vietnam. Vol 2, pp. 351-353, 1018-1020 (2004).

Do, T.L., *The Vietnamese medicinal plants and remedies*. Medicinal Publishing House, Eleventh edition, pp. 509-512 (2003).

Fennell, C.W. and Staden, J., *Crinum* species in traditional and modern medicine. *J. Ethnopharmacol.* **78**, 15-26 (2001).

Fernandes, M.B., Scotti, M.T., Ferreira, M.J.P., and Emerenciano, V.P., Use of self-organizing maps and molecular descriptors to predict the cytotoxic activity of sesquiterpene lactones. *Eur. J. Med. Chem.* **43**, 2197-2205 (2008).

Ghosal, S., Saini, K.S., and Razdan, S., *Crinum* alkaloids: their chemistry and biology. *Phytochemistry* **24**, 2141-2156 (1985).

Hehner, S.P., Hofmann, T.G., Dröge, W., and Schmitz, M.L., The antiinflammatory sesquiterpene lactone parthenolide inhibits NF-kappa B by targeting the I kappa B kinase complex. *J. Immunol.* **163**, 5617-5623 (1999).

Herz, W., Watanabe, H., Miyazaki, M., and Kishida, Y., The structure of parthenin and ambrosin. *J. Am. Chem. Soc.* **84**, 2601-2610 (1962).

Hooper, M., Kirby, G.C., Kulkarni, M.M., Kulkarni, S.N., Nagasampagi, B.A., O'Neill, M.J., Phillipson, J.D., Rojatkar, S.R., and Warhurst, D.C., Antimalarial activity of parthenin and its derivatives. *Eur. J. Med. Chem.* **25**, 717-723 (1990).

Luqman, S. and Pezzuto, J.M., NFkappaB: a promising target for natural products in cancer chemoprevention. *Phytother. Res.* **24**, 949-963 (2010).

Mabberly, D.J., 1990. The plant book. Cambridge University Press.

Mew, D., Balza, F., Towers, G.H., and Levy, J.G., Anti-tumour effects of the sesquiterpene lactone parthenin. *Planta Med.* **45**, 23-27 (1982).

Morikawa, T., Abdel-Halim, O.B., Matsuda, H., Ando, S., Muraoka, O., and Yoshikawa, M., Pseudoguaiane-type sesquiterpenes and inhibitors on nitric oxide production from *Dichrocephala integrifolia*. *Tetrahedron* **62**, 6435-6442 (2006).

Nam, N.H., Kim, Y., You, Y.J., Hong, D.H., Kim, H.M., and Ahn, B.Z., New constituents from *Crinum latifolium* with inhibitory effects against tube-like formation of human umbilical venous endothelial cells. *Nat. Prod. Res.* **18**, 485-491 (2004).

Nguyen, T.N.T., Titorenkova, T.V., Bankova, V.S., Handjieva, N.V., and Popov, S.S., *Crinum* L. (Amaryllidaceae). *Fitoterapia* **73**, 183-208 (2002).

Picman, J. and Picman, A.K., Treatment of dermatitis from parthenin. *Contact Dermatitis* **13**, 9-13 (1985).

Ramesh, C., Ravindranath, N., Biswanath, D., Prabhakar, A., Bharatam, J., Ravikumar, K., Kashinatham, A., and McMorris, T.C., Pseudoguaianolides from the flowers of *Parthenium hysterophorus*. *Phytochemistry* **64**, 841-844 (2003).

Salam, A.N.A., Mahmoud, Z.F., Ziesche, J., and Jakupovic, J., Sesquiterpene lactones from *Ambrosia maritima* (Damssissa). *Phytochemistry* **23**, 2851-2853 (1984).

Sohma, I., Fujiwara, Y., Sugita, Y., Yoshioka, A., Shirakawa, M., Moon, J.H., Takiguchi, S., Miyata, H., Yamasaki, M., Mori, M., and Doki, Y., Parthenolide, an NF-{kappa}B inhibitor, suppresses tumor growth and enhances response to chemotherapy in gastric cancer. *Cancer Genomics Proteomics* **8**, 39-47 (2011).

Sun, Q., Shen, Y.H., Tian, J.M., Tang, J., Su, J., Liu, R.H., Li, H.L., Xu, X.K., and Zhang, W.D., Chemical constituents of *Crinum asiaticum* L. var. *sinicum* Baker and their cytotoxic activities. *Chem. Biodiver.* **6**, 1751-1757 (2009).

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