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RESEARCH ARTICLE

Inhibitory effects of oleanane-type triterpenes and saponins from the stem bark of *Kalopanax pictus* on LPS-stimulated pro-inflammatory cytokine production in bone marrow-derived dendritic cells

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Abstract *Kalopanax pictus* (Araliaceae) is a deciduous tree distributed in Korea, Japan, and China. The stem bark of *K. pictus* has been functionally used as a traditional crude drug for the treatment of various inflammatory diseases. In the present study, we describe the inhibitory effects of oleanane-type triterpenes and saponins isolated from the stem bark of *K. pictus* on production of pro-inflammatory cytokines in LPS-stimulated bone marrow-derived dendritic cells. Of the compounds tested, 16,23,29-trihydroxy-3-oxo-olean-12-en-28-oic acid (**1**), 4,23,29-trihydroxy-3,4-*seco*-olean-12-en-3-oate-28-oic acid (**2**), 3*β*,6*β*,23-trihydroxyolean-12-en-28-oic acid 28-*O*-*β*-D-glucopyranoside (**3**), nipponogenin E (**6**), 3*β*,6*β*,23-trihydroxyolean-12-en-28-oic acid (**7**), and caulophyllogenin (**19**) significantly inhibited the production of IL-12 p40 and IL-6 with IC_{50} values ranging from 3.3 to 9.1 μM. Compounds **2**, **3**, **7**, and **19** significantly suppressed the secretion of TNF-α with IC_{50} ranging from 8.8 to 20.0 μM. These data provide scientific support for the use of *K. pictus* stem bark and its triterpene and saponin components in the inhibition of pro-inflammatory cytokine secretion, including IL-12 p40, IL-6, and TNF-α, and for prevention and treatment of inflammatory diseases.

Keywords *Kalopanax pictus* · Araliaceae · Oleanane-type triterpene · IL-12 p40 · IL-6 · TNF-α · LPS-stimulated BMDC

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Introduction

Inflammation is mediated by a variety of soluble factors, including a group of secreted polypeptides known as cytokines, which play a key role in the modulation of immune responses. In the immune system, cytokine networks regulate lymphocyte turnover, differentiation, and activation. In inflammatory diseases, these networks are imbalanced. Interleukin-12 (IL-12), a pro-inflammatory cytokine, is produced by activated antigen-presenting cells, dendritic cells, monocytes/macrophages and B cells in response to bacterial products and immune signals (Trinchieri 1995). The biologically active IL-12 is a 70-kDa heterodimeric protein composed of disulfide-linked p35 and p40 subunits expressed by two distinct genes (Gubler et al. 1991; Kobayashi et al. 1989; Wolf et al. 1991). Whereas the p35 subunit is constitutively expressed, the p40 subunit is induced only after macrophage activation. IL-12 produced early during an infection acts as a pro-inflammatory stimulus, inducing the activation of natural killer (NK) cells and the production of interferon-γ (IFN-γ),

which activates phagocytes and primes them for enhanced production of other pro-inflammatory cytokines, including IL-12 (Trinchieri 1998; Wolf et al. 1991). IL-12 then, directly and through the induced IFN-γ, acts on antigen-specific T-cells, determining the differentiation and generation of Th1 cells (Manetti et al. 1993; Trinchieri 1993). Therefore, IL-12 plays a key role in promoting Th1 immune responses, as demonstrated both in vitro (Manetti et al. 1993) and in vivo (Sypek et al. 1993). The primary targets for the effects of IL-12 are T-cells, both $CD4^+$ and $CD8^+$ subtypes, and NK cells. Through these target cells, IL-12 mediates such biological effects as induction of cytokine production and promotion of proliferative effects (Trinchieri 1998). IL-12 may also induce proliferation of B cells (Jelinek and Braaten 1995). The IL-12 family of cytokines, including IL-12 and IL-23, plays an important role in bridging innate and adaptive immune responses via the induction and maintenance of Th1-mediated inflammation. Consequently, it follows that regulation of IL-12 and IL-23 function affects the pathophysiology of immune-mediated inflammatory diseases, including psoriasis (Hong et al. 1999), multiple sclerosis (Comabella et al. 1998; Soldan 2004), and Crohn's disease (Gately et al. 1998; Podolsky 2002). These two cytokines share some functions, likely via their common subunit IL-12 p40, such as the ability to induce IFN-γ and to drive Th1 differentiation. Therefore, blockage of IL-12 p40 can inhibit either cytokine and inhibition of IL-12/IL-23 p40 in multiple immune-mediated inflammatory disorders is a growing field of research, with several anti-IL-12 agents in clinical development (Barrie and Plevy 2005). Interleukin 6 (IL-6), which was originally identified as a B cell differentiation factor, is now known to be a multifunctional cytokine that participates in a broad spectrum of biological events, including immune responses, hematopoiesis and acute-phase reactions (Kishimoto 2010). In addition to the stimulation of acute phase protein synthesis by the liver, IL-6 acts as a growth factor for mature B cells, inducing their differentiation into mature antibody-producing plasma cells. It is also involved in T cell activation, and participates in the induction of IL-2 and IL-2 receptor expression. Some of the regulatory effects of IL-6 involve inhibition of tumor necrosis factor (TNF) production, providing negative feedback for limiting the acute inflammatory response (Hirano 1992a; Van Snick 1990). Upregulation of IL-6 production has been observed in a variety of chronic inflammatory and autoimmune disorders such as thyroiditis, type I diabetes, rheumatoid arthritis (Hirano 1992b; Tan et al. 1990), systemic sclerosis (Feghali et al. 1992), mesangial proliferative glomerulonephritis, psoriasis, and neoplasms such as cardiac myxoma, renal cell carcinoma, multiple myeloma, lymphoma, and leukemia (Hirano 1992b). TNF-α, a pro-inflammatory cytokine, is produced by many cell types, including macrophages, lymphocytes, fibroblasts, and keratinocytes, in response to inflammation, infection, and certain environmental stresses. The binding of TNF-α to its two receptors, TNFR1 and TNFR2, results in the recruitment of signal transducers that activate at least three distinct effectors. These effectors activate caspases and two transcription factors, activation protein-1 and NF-κB through complex signaling cascades and networks (Smith et al. 1994). Upon assembly of this submembranous complex, two major downstream signaling pathways are activated: The Jun NH_2 terminal kinase-activating protein-1 and IκB kinase (IKK)-NF-κB pathways (Hsu et al. 1996; Raingeaud et al. 1995). TNF induces the activation of NF-κB via the phosphorylation-dependent degradation of IκB proteins, which allows the translocation of activated NF-κB to the nucleus, and induction of target gene expression (Tian et al. 2005). The activation of NF-κB is involved in many diseases, including inflammatory disorders and cancer (Baldwin 2001; Pande and Ramos 2005). Therefore, inhibition of the expression and production of powerful mediators, including IL-12 p40, IL-6, and TNF-α by anti-inflammatory components might represent a possible preventive or therapeutic target and may be used to develop anti-inflammatory agents for health promotion and disease prevention.

The stem bark of *Kalopanax pictus* (Araliaceae), a deciduous tree that grows in East Asian countries, has been used in traditional medicine to treat rheumatic arthritis, neurotic pain, and diabetes mellitus (Kim 1996). Previous studies on the stem bark have demonstrated the presence of hederagenin glycosides, syringin, liriodendrin, and coniferylaldehyde glucosides (Sano et al. 1991; Shao et al. 1990). The stem bark of *K. pictus* has been functionally used as a traditional crude drug for the treatment of various inflammations, and several reports have described the influence of *K. pictus* extracts on inflammation (Kim et al. 2004; Kim et al. 2002; Lee et al. 2001; Li et al. 2002; Park et al. 2005). Some previous studies on the isolation of anti-inflammatory compounds from extracts of *K. pictus* bark suggest that kalopanaxsaponin A and I and hederagenin monodesmosides, have anti-inflammatory effects on RAW 264.7 murine macrophage cells stimulated with bacterial endotoxic lipopolysaccharide (Kim et al. 2002) and in rats during a response to Freund's complete adjuvant (Choi et al. 2002; Li et al. 2002). Our previous investigation on the chemical components of the stem bark of *K. pictus* resulted in the isolation of 25 oleanane-type compounds, including six triterpenes and 19 saponins (Quang et al. 2011a; Quang et al. 2011b; Quang et al. 2012). These compounds were identified as 16,23,29-trihydroxy-3-oxo-olean-12-en-28-oic acid **(1)**, 4,23,29-trihydroxy-3,4-*seco*-olean-12-en-3-oate-28-oic acid **(2)**, 3*β*,6*β*,23-trihydroxyolean-12-en-28-oic acid 28-*O*-*β*-D-glucopyranoside **(3)**, 3-*O*-[2,3-di-*O*-acetyl-α-L-arabinopyranosyl]hederagenin 28-*O*-α-L-rhamnopyranosyl-(1 → 4)-*β*-D-glucopyranosyl-(1 → 6)-*β*-D-glucopyranoside **(4)**, 3-*O*-[3,4-di-*O*-acetyl-α-L-arabinopyranosyl]hederagenin 28-*O*-α-L-

rhamnopyranosyl-(1 → 4)-*β*-D-glucopyranosyl-(1 → 6)-*β*-D-glucopyranoside (**5**), nipponogenin E (**6**), 3*β*,6*β*,23-trihydroxyolean-12-en-28-oic acid (**7**), kalopanaxsaponin A (**8**), kalopanaxsaponin B (**9**), kalopanaxsaponin C (**10**), sieboldianoside A (**11**), hederagenin 28-*O*-*β*-D-glucopyranosyl ester (**12**), kalopanaxsaponin L (**13**), cauloside D (**14**), hederagenin (**15**), 6*β*,16α-dihydroxy-hederagenin 3-*O*-*β*-D-glucuronopyranoside (**16**), 3-*O*-*β*-D-glucuronopyranosyl-28-*O*-*β*-D-glucopyranosyl-6*β*,16α-dihydroxy-oleanolic acid (**17**), and 3-*O*-*β*-D-galactopyranosyl(1 → 3)-α-L-arabinopyranosyl hederagenin 28-*O*-*β*-D-glucopyranosyl-(1 → 6)-*β*-D-glucopyranosyl ester (**18**), caulophyllogenin (**19**), hederagenin 3-*O*-α-L-arabinopyranoside (**20**), dipsacussaponin A (**21**), cussonoside B (**22**), sapindoside B (**23**), 3*β*,6*β*,23-trihydroxyolean-12-en-28-oic acid 3-*O*-α-L-arabinopyranoside (**24**), and cussonoside A (**25**) (Fig. 1). Thirteen of these compounds inhibited TNF-α-induced NF-κB transcriptional activity and decreased COX-2 and iNOS gene expression in HepG2 cells (Quang et al. 2011a). In the present study, we examined the inhibitory capacity of the oleanane-type triterpenes and saponins isolated from the stem bark of *K. pictus* on LPS-induced expression of the pro-inflammaroty cytokines IL-12 p40, IL-6, and TNF-α in bone marrow-derived dendritic cells (BMDCs).

Materials and methods

Cell culture and measurement of cytokine production

Bone marrow-derived dendritic cells were grown from wild-type C57BL/6 mice (Orient Bio Inc., South Korea) as previously described (Koo et al. 2012). All animal procedures were approved by and performed according to the guidelines of the Institutional Animal Care and Use Committee of Jeju National University (#2010-0028). Briefly, the mouse tibia and femur was obtained by flushing with Dulbecco's modified Eagle medium to yield bone marrow cells. The cells were cultured in RPMI 1640 medium containing 10 % heat-inactivated fetal bovine serum (FBS; Gibco), 50 μM β-mercaptoethanol, and 2 mM glutamine supplemented with 3 % J558L hybridoma cell culture supernatant containing granulocyte–macrophage colony-stimulating factor (GM-CSF). The culture medium was replaced with fresh medium every other day. At day 6 of culture, non-adherent cells and loosely adherent DC aggregates were harvested, washed, and resuspended in RPMI 1640 supplemented with 5 % FBS. The BMDCs were incubated in 48-well plates in 0.5 mL containing 1×10^5 cells per well, and then treated with the isolated compounds at different concentrations for 1 h before stimulation with 10 ng/mL LPS from *Salmonella minnesota* (Alexis). Supernatants were harvested 16 h after stimulation. Concentrations of murine TNF-α, IL-6, and IL-12 p40 in the culture supernatant were determined by ELISA (BD PharMingen) according to the manufacture's instructions. The data are presented as mean ± S.D. of at least three independent experiments performed in triplicate.

Results

Screening effects of compounds **1–25** on the production of IL-12 p40

To evaluate the effects of compounds **1–25** on the secretion of cytokines, we initially screened the inhibitory potential of the compounds on the production of IL-12 p40 at 25 μM. BMDCs were exposed to LPS in the presence or absence of compounds **1–25**, and the level of IL-12p40, a pro-inflammatory protein produced during the inflammatory process, was measured in the medium. The result indicated that the production of IL-12 p40 was decreased significantly by the presence of **1**–**8**, **12**, **15**, **17**, and **19–25** (Fig. 2A). To inspect these inhibitory effects, we next examined the cytotoxicity of compounds **1–25** toward BMDCs.

Effects of compounds **1–25** on cell viability

The cytotoxicity of compounds **1–25** toward BMDCs was evaluated using the MTT colorimetric assay at the concentration of 25 μM. Among the compounds tested, compounds **8**, **12**, **20**, **22**, and **23** showed strong cytotoxicity toward BMDCs. Other compounds displayed no notable cytotoxicity against BMDCs (Fig. 2B).

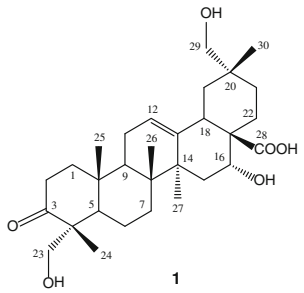
Effects of compounds **1–3, 6, 7, and 19** on the production of IL-12 p40, IL-6, and TNF-α

Since compounds **1–3**, **6**, **7**, and **19** inhibited noticeably the production of IL-12 p40, we next examined the effects of these compounds on the production of the pro-inflammatory cytokines, IL-12 p40, IL-6, and TNF-α at various concentrations. BMDCs were incubated with compounds **1–3**, **6**, **7**, and **19** in the presence of LPS for 16 h, and then TNF-α and IL-6 levels were measured in the culture supernatants. The results indicated that compounds **1–3**, **6**, **7**, and **19** significantly inhibited the secretion of IL-12 p40 and IL-6. Compounds **2**, **3**, and **7** significantly inhibited the production of TNF-α, while compounds **1** and **6** displayed weak activities (Table 1).

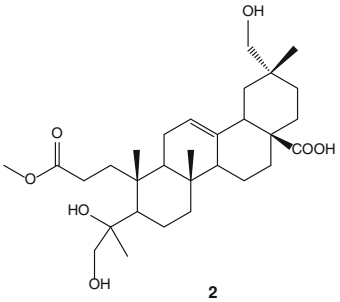
Discussion

During an inflammatory response, mediators, including the pro-inflammatory cytokines, IL-1, TNF, INF-γ, IL-6,

Fig. 1 Structure of oleanane-type triterpenes and saponins from the stem bark of *K. pictus*



1



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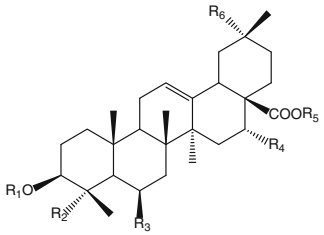

	R_1	R_2	R_3	R_4	R_5	R_6
3	H	CH_2OH	OH	H	—Glc	CH_3
4	—Ara^3(Ac^2)—Ac	CH_2OH	H	H	—Glc^6—Glc^4—Rha	CH_3
5	—Ara^4(Ac^3)—Ac	CH_2OH	H	H	—Glc^6—Glc^4—Rha	CH_3
6	H	CH_2OH	H	H	H	CH_2OH
7	H	CH_2OH	OH	H	H	CH_3
8	—Ara^2—Rha	CH_2OH	H	H	H	CH_3
9	—Ara^2—Rha	CH_2OH	H	H	—Glc^6—Glc^4—Rha	CH_3
10	—Ara^3(Rha^2)—Glc	CH_2OH	H	H	—Glc^6—Glc^4—Rha	CH_3
11	—Ara^2—Rha^3—Xyl	CH_2OH	H	H	—Glc^6—Glc^4—Rha	CH_3
12	H	CH_2OH	H	H	—Glc	CH_3
13	—GlcA^2—Gal	CH_2OH	H	H	—Glc^6—Glc^4—Rha	CH_3
14	—Ara	CH_2OH	H	H	—Glc^6—Glc^4—Rha	CH_3
15	H	CH_2OH	H	H	H	CH_3
16	—GlcA	CH_2OH	OH	OH	H	CH_3
17	—GlcA	CH_3	OH	OH	—Glc	CH_3
18	—Ara^3—Gal	CH_2OH	H	H	—Glc^6—Glc	CH_3
19	H	CH_2OH	H	OH	H	CH_3
20	—Ara	CH_2OH	H	H	H	CH_3
21	H	CH_2OH	H	H	—Glc^6—Glc	CH_3
22	H	CH_3	H	H	—Glc^6—Glc^4—Rha	CH_3
23	—Ara^2—Rha^3—Xyl	CH_2OH	H	H	H	CH_3
24	—Ara	CH_2OH	OH	H	H	CH_3
25	H	CH_2OH	H	H	—Glc^6—Glc^4—Rha	CH_3

Ara: α-L-arabinopyranosyl

Gal: β-D-galactopyranosyl

Glc: β-D-glucopyranosyl

GlcA: β-D-glucuronopyranosyl

Rha : α-L-rhamnopyranosyl

Xyl : β-D-xylopyranosyl

IL-12, and IL-18, and the granulocyte–macrophage colony-stimulating factor, are released; this response is antagonized by anti-inflammatory cytokines, including IL-4, IL-10, IL-13, and IFN-γ, and by transforming growth factor. The nuclear factor-κB (NF-κB), also plays an important role in the inflammatory response by regulating the expression of various genes encoding pro-inflammatory cytokines, adhesion molecules, chemokines, growth factors, and inducible enzymes such as cyclooxygenase-2 (COX-2) (Hanada and Yoshimura 2002; Makarov 2000); inducible nitric oxide synthase (iNOS) and COX-2 both stimulate the production of large amounts of pro-inflammatory mediators. Moderate levels of these inflammatory mediators are important for host survival from infection, and are also required for the repair of tissue injury. However, overproduction of these inflammatory mediators may be hazardous to healthy tissue and are involved in the development of many inflammatory diseases. For example, extensive studies have demonstrated the efficacy of TNF-α and IL-12 blocking therapies in various inflammatory and autoimmune diseases such as rheumatoid arthritis and Crohn's disease (Barrie and Plevy 2005; Tracey and Cerami 1994). Therefore, suppression of the production of these pro-inflammatory cytokines by bioactive compounds is becoming a therapeutic target for the prevention and treatment of various inflammatory diseases.

BMDCs are vital cellular components of the innate immune system (Efron et al. 2005). In these cells, recognition of pathogen-associated molecular patterns (PAMPs) by the Toll-like receptor (TLR) triggers activation of downstream signaling cascades including, the NF-κB and mitogen-activated protein kinases (MAPKs) pathways,

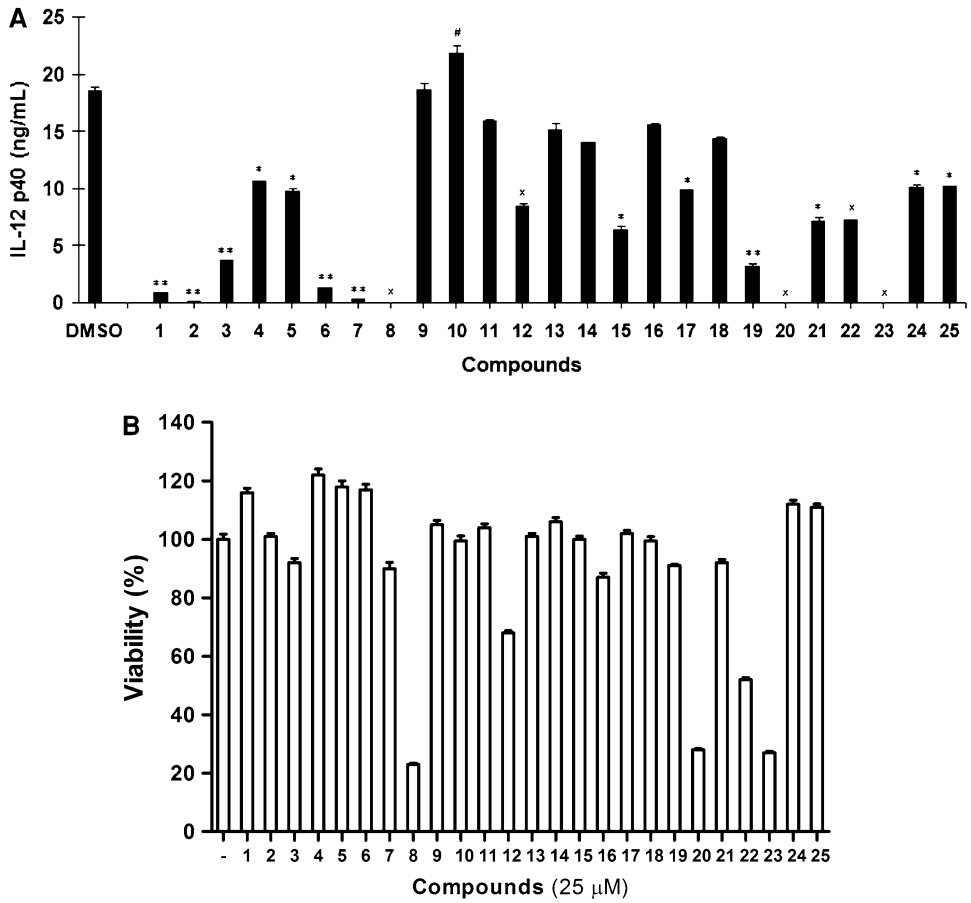

Fig. 2 **A**. Effects of compounds **1–25** on IL-12 p40 production in LPS-stimulated BMDCs. BMDCs were treated with the compounds (25 μM) for 1 h before stimulation with LPS (10 ng/mL). Supernatants were harvested 16 h after stimulation. Concentration of murine IL-12 p40 in the culture supernatants were determined by ELISA. Candidate compound showed marginal activity (*), strong activity (**), and immunostimulatory activity ($^{\#}$). (^{x}) Probably due to cytotoxicity. Figure 2**B** Cytotoxic effects of compounds **1–25** toward BMDCs in the presence of LPS were measured by MTT assay

Table 1 Inhibitory effects of compounds **1–3**, **6**, **7**, and **19** on the production of IL-12 p40, IL-6, and TNF-α

Compound	IC_{50} (μM)[a]		
	IL-12 p40	IL-6	TNF-α
1	9.1 ± 0.6	5.4 ± 0.8	40.7 ± 1.6
2	7.6 ± 0.5	4.1 ± 0.3	20.0 ± 1.1
3	7.5 ± 0.7	3.3 ± 0.2	14.3 ± 0.6
6	5.5 ± 0.3	3.5 ± 0.3	37.4 ± 1.4
7	7.8 ± 0.4	6.0 ± 0.4	12.3 ± 0.9
19	7.3 ± 0.5	3.5 ± 0.2	8.8 ± 0.4
SB203580[b]	6.1 ± 0.4	3.2 ± 0.1	8.1 ± 0.3

[a] IC_{50} values for selected compounds are given in column IL-12 p40, IL-6, and TNF-α

[b] Positive control

leading to production of pro-inflammatory cytokines and induction of adaptive immune response (Koh 2011; Medzhitov 2001; Takeuchi and Akira 2010; Yuk and Jo 2011). Activated BMDCs perform crucial functions in immune and inflammatory responses via the PAMPs-stimulated production of pro-inflammatory cytokines such as IL-12 p40, IL-6, and TNF-α (Kawai and Akira 2010). These pro-inflammatory cytokines play a crucial role in host defense and inflammatory response.

IL-12 is an inducible, heterodimeric disulfide-linked cytokine composed of p35 and p40 subunits (Murphy et al. 1995; Wolf et al. 1991). Expression of the p35 subunit of IL-12 is constitutive and ubiquitous. Therefore, the biological activity of IL-12 is regulated mainly by induction of the p40 subunit and is regulated primarily at the level of transcription (Murphy et al. 1995). Since IL-12 is a key cytokine in Th1-mediated autoimmune responses, down-regulation of IL-12 production by the oleanane-type triterpenes and saponins from *K. pictus* may ameliorate autoimmune diseases (Bao et al. 2002; Plevy et al. 1997).

In this study, we used LPS-stimulated BMDCs as a model for testing the inhibitory effects of the isolated compounds on the secretion of pro-inflammatory cytokines IL-12 p40, IL-6, and TNF-α. We initially screened effects of the compounds on the secretion of IL-12 p40 at a concentration of 25 μM. BMDCs (1×10^5 cells) were seeded in 48-well plates at 37 °C, 5 % CO_2 for 1 h, and then treated for 1 h with 25 μM of each isolated compound, and then stimulated with LPS (10 ng/mL). The supernatants were harvested 16 h after stimulation and the secretion of IL-12 p40 was measured using ELISA. The results indicated that the production of IL-12 p40 was significantly decreased by compounds **1–8**, **12**, **15**, **17**, and **19–25**

(Fig. 2A). We then examined the cytotoxicity of the compounds (same concentration) toward the BMDCs using the MTT colorimetric assay (Fig. 2B). Of the compounds tested, compounds **8**, **12**, **20**, **22**, and **23** showed strong cytotoxicity against BMDCs; this cytotoxicity could itself be causing the inhibition of IL-12 p40 production by these specific compounds. The other compounds exhibited no cytotoxicity toward BMDCs at the indicated concentration. Since compounds **1–3**, **6**, **7**, and **19** noticeably suppressed the production of IL-12 p40 by 95.0, 99.5, 80.0, 92.4, 98.4, and 82.7 %, respectively, relative to the vehicle group, we selected these compounds for further experiments to evaluate their effects at various concentrations on the production of the pro-inflammatory cytokines IL-12 p40, IL-6, and TNF-α in LPS-stimulated BMDCs. Compounds **1–3**, **6**, **7**, and **19** significantly inhibited the secretion of IL-12 p40 with IC_{50} values ranging from 5.5 to 9.1 μM (Table 1). Of the compounds tested, compound **6**, a hederagenin triterpene with a hydroxyl group at C-29, showed the most inhibitory effect ($IC_{50} = 5.5$ μM); which was comparable to that of the positive control, SB203580 ($IC_{50} = 6.1$ μM).

IL-6 is a pro-inflammatory cytokine that promotes inflammatory events through the activation and proliferation of lymphocytes, differentiation of B cells, leukocyte recruitment and the induction of the acute-phase protein response in the liver (Pecoits-Filho et al. 2003). TNF-α is another cytokine that mediates many crucial events for the initiation of both acute and chronic inflammation by regulating the production of some other cytokines, upregulating adhesion molecule expression, and activating leukocyte-specific chemotactic cytokines (Beutler and Cerami 1989). IL-6 and TNF-α are also interlinked with the production of some small inflammatory mediators such as nitric oxide (NO) and prostaglandin (PGE_2), which contribute inflammatory response. Overexpression of these pro-inflammatory cytokines is related to the development of autoimmune, inflammatory, and immunopathological diseases. Therefore blockage of IL-6 and TNF-α and their respective signalling pathways is effective at prevention and treatment in models of inflammatory diseases. As a result, compounds **1–3**, **6**, **7**, and **19** considerably decreased the production of IL-6 in the LPS-stimulated BMDCs with IC_{50} values ranging from 3.3 to 6.0 μM (Table 1). Remarkably, the inhibitory effects of compounds **3**, **6**, and **19** were similar to that of the positive control ($IC_{50} = 3.2$ μM). In term of the effects on TNF-α in the LPS-stimulated BMDCs, compound **19** showed the best inhibition with an IC_{50} value of 8.8 μM, which was comparable to that of the positive control ($IC_{50} = 8.1$ μM). Compounds **2**, **3**, and **7** significantly inhibited the production of TNF-α, with IC_{50} values of 20, 14.3, and 12.3 μM, respectively, while compounds **1** and **6** exhibited weak activities (Table 1).

Previous studies revealed that some of oleanane-trype triterpenes and saponins from *K. pictus* showed the anti-inflammatory activity in different models. In RAW 264.7 cells, kalopanaxsaponin A and I inhibited the production of TNF-α and resulted in downregulation of iNOS and COX-2 expression during LPS stimulation. In addition, kalopanaxsaponin A inhibited the LPS-induced DNA-binding activity of NF-κB by blocking the degradation of IκBα (Kim et al. 2002). Using the NF-κB luciferase assay and RT-PCR, we reported the inhibitory effects of 15 oleanane-trype triterpenes and saponins isolated from *K. pictus* on a TNF-α-induced NF-κB luciferase reporter and the attenuation of TNF-α-induced pro-inflammatory gene (iNOS and COX-2) expression in HepG2 cells (Quang et al. 2011a). Of the compounds tested, compounds **1–5**, **7–12**, **14**, and **21** significantly inhibited TNF-α-induced NF-κB transcriptional activity and the induction of iNOS and COX-2 mRNA in a dose-dependent manner (Quang et al. 2011a). In the previous study on structure–activity relationship, we found that sugar moieties played an important role in the anti-inflammatory activities of the oleanane-type saponins isolated from *K. pictus* (Quang et al. 2011a). Some triterpenes, including compounds **1**, **2**, and **7**, also significantly inhibited TNF-α-induced NF-κB transcriptional activity and iNOS and COX-2 gene expression (Quang et al. 2011a). In the present study, we found that all the oleanane-type triterpenes, including compounds **1**, **2**, **6**, **7**, and **19** exhibited strong inhibition of IL-12 p40 production (Table 1); monodesmosidic saponins, including compounds **3**, **21**, **24**, and **25** significantly inhibited IL-12 p40 production in LPS-stimulated BMDCs, whereas the bisdesmosidic saponins, except compounds **4**, **5**, and **17**, were inactive at the concentration of 25 μM (Fig. 2A). These triterpenes also dramatically inhibited the production of IL-6 (Table 1). These findings indicate that oleanane-type triterpenes and monodesmosidic saponins play an important role in the inhibition of the secretion of pro-inflammatory cytokines, such as IL-12 p40 and IL-6 rather than bisdesmosidic saponins. The stem bark of *K. pictus* contains numerous saponins, including mono- and bisdesmosides. Although almost bisdesmosidic saponins showed no inhibitory effects on IL-12 p40 and IL-6 production in BMDCs in vitro, in an animal model, after oral intake, the glycosides of saponins are hydrolyzed by digestive enzymes and/or intestinal bacteria into low-sugar-saponins and aglycones, which are absorbed slowly in the gastrointestinal tract to exhibit inhibitory activity (Francis et al. 2002). Therefore, these results provide scientific support for the use of *K. pictus* stem bark and its triterpene and saponin components in the inhibition of pro-inflammatory cytokines secretion, including IL-12 p40, IL-6, and TNF-α, and for prevention and treatment of inflammatory diseases.

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