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Effect of Isosecotanapartholide Isolated from Artemisia princeps Pampanini on Interleukin-33 Production and signal transducer and activator of transcription-1 Activation in HaCaT Keratinocytes

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Abstract

To investigate the anti-inflammatory effect and mechanism of action of Isosecotanapartholide, isolated from Artemisia princeps Pampanini extract. We assessed the effects of isosecotanapartholide and A. princeps Pampanini extract on the proliferation of human keratinocytes after tumor necrosis factor-alpha/interferon-γ induction. Isosecotanapartholide and A. princeps Pampanini extract downregulated the expression of signal transducer and activator of transcription 1, as well as decreased interleukin-33 production in HaCaT cells stimulated with tumor necrosis factor-alpha/interferon-γ. Isosecotanapartholide and A. princeps Pampanini extract inhibited the induction of thymus- and activation regulated chemokine mRNA in a dose-dependent manner. Immunoblot assay showed that isosecotanapartholide and A. princeps Pampanini extract dose-dependently inhibited intercellular adhesion molecule 1 and phosphorylation of signal transducer and activator of transcription 1 protein. Isosecotanapartholide and A. princeps Pampanini extract significantly inhibited tumor necrosis factor-alpha/interferon-γ-induced interleukin-33 release under inflammatory conditions. These results indicate that isosecotanapartholide inhibits thymus- and activation regulated chemokine production in human epidermal keratinocytes via the signal transducer and activator of transcription 1 pathway and may be related to the inhibition of interleukin-33 production. This suggests that isosecotanapartholide is an active component in A. princeps Pampanini extract and may have therapeutic potential in inflammatory skin disorders.

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Effect of Isosecotanapartholide Isolated from *Artemisia princeps* Pampanini on Interleukin-33 Production and signal transducer and activator of transcription-1 Activation in HaCaT Keratinocytes

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ABSTRACT

To investigate the anti-inflammatory effect and mechanism of action of Isosecotanapartholide, isolated from *Artemisia princeps* Pampanini extract. We assessed the effects of isosecotanapartholide and *A. princeps* Pampanini extract on the proliferation of human keratinocytes after tumor necrosis factor-alpha/interferon-γ induction. Isosecotanapartholide and *A. princeps* Pampanini extract downregulated the expression of signal transducer and activator of transcription 1, as well as decreased interleukin-33 production in HaCaT cells stimulated with tumor necrosis factor-alpha/interferon-γ. Isosecotanapartholide and *A. princeps* Pampanini extract inhibited the induction of thymus- and activation regulated chemokine mRNA in a dose-dependent manner. Immunoblot assay showed that isosecotanapartholide and *A. princeps* Pampanini extract dose-dependently inhibited intercellular adhesion molecule 1 and phosphorylation of signal transducer and activator of transcription 1 protein. Isosecotanapartholide and *A. princeps* Pampanini extract significantly inhibited tumor necrosis factor-alpha/interferon-γ-induced interleukin-33 release under inflammatory conditions. These results indicate that isosecotanapartholide inhibits thymus- and activation regulated chemokine production in human epidermal keratinocytes via the signal transducer and activator of transcription 1 pathway and may be related to the inhibition of interleukin-33 production. This suggests that isosecotanapartholide is an active component in *A. princeps* Pampanini extract and may have therapeutic potential in inflammatory skin disorders.

Key words: Isosecotanapartholide; IL-33; STAT1; anti-inflammation

INTRODUCTION

Cytokines and chemokines are involved in the development of many inflammatory skin disorders (1). Abnormal and dysregulated expression of inflammatory mediators in keratinocytes are related to the pathogenesis of chronic inflammatory skin diseases (2). Upon stimulation by inflammatory cytokines such as tumor necrosis factor alpha (TNF-α) and interferon-γ (IFN-γ), epidermal keratinocytes express adhesion molecules such as intracellular adhesion molecule 1 (ICAM-1) (3). Previous studies have suggested that the serum levels of ICAM-1 correlate with the disease progression of Alzheimer's disease (AD) (4). Modulation of ICAM-1 expression in epidermal keratinocyte provides a rationale for the development of therapeutic agents for various inflammatory skin diseases (5). In addition, exposure of keratinocytes to TNF-α and IFN-γ leads to dysregulated expression of cytokines and chemokines and increase infiltration of monocytes/T cells into the site of inflammation on the skin (6). Thymus and activation-regulated chemokine (TARC/CCL17) is constitutively expressed in the thymus and is produced by dendritic cells (DC), endothelial cells, keratinocytes (KC), and fibroblasts (7). Further, keratinocytes increase CCL17 in the lesional skin of AD (8). Therefore, modulation of keratinocyte CCL17 production may lead to the pathologic processes of inflammatory skin diseases such as AD.

Artemisia princeps Pampanini (AP) is an herbaceous plant that is widely used in traditional medicine in Asia (9). Various species of Artemisia have shown functional properties, including immunostimulatory effect (10), anti-cancerous (11), anti-inflammatory (12), and antibacterial effect (13). The constituents of AP have been reported by Ryu et al. (14), and include flavonoids such as eupatilin and jaceosidin. Isosecotanapartholide (ISTP), a sesquiterpene lactone isolated from *A. rutifolia* and *A. iwayomogi*, has anti-inflammatory and anti-cancer properties. It also inhibits nitric oxide

synthase (15). Nevertheless, there is limited clinical evidence to support the anti-inflammatory effects of ISTP. Therefore, the present study investigated the anti-inflammatory effects of ISTP isolated from ethanol extracts of AP.

Interleukin-33 (IL-33) is closely associated with type 2 immune responses (16) and is important in the pathogenesis of several type 2 helper cell (Th2)-biased inflammatory conditions and allergic reactions (17). Natural helper cells and nuocytes produce abundant Th2 cytokines when stimulated by IL-33 (18). The mature form of IL-33 is released into the cytoplasm and stimulates keratinocyte, T cells, and mast cells. Subsequently, IL-33 can act as a transcription factor by trafficking into the nucleus, where it regulates several inflammatory responses (19).

In the present study, we investigated the effect of ISTP on the production of TARC and IL-33 in TNF-α- and IFN-γ-stimulated HaCaT keratinocytes. We also determined ISTP mechanism of action.

MATERIALS AND METHODS

Extraction and isolation of active components

AP was purchased from a herb shop at Gyeongdong medicinal herb market. Methanol purchased from Duksan Chemical Co. LTD. (Ansan, Korea) was of high-performance liquid chromatography (HPLC) grade for HPLC analysis. Ethanol, ethyl acetate, and dichloromethane were purchased from Duksan Chemical Co. LTD. (Ansan, Korea). DMSO-d6, a common solvent used in nuclear magnetic resonance (NMR) spectroscopy, was purchased from Sigma-Aldrich Co. LLC (USA).

Dried AP (5 kg) was extracted with ethanol (95%) for 3 or 4 days at room temperature. After filtration through a 400-mesh filter, the filtrate was filtered again through filter

paper (Whatman Grade No. 5) and concentrated under reduced pressure by rotary evaporation (EYELA N-1000; Japan). The ethanol extract of AP (279.4 g) was suspended in H_2O and extracted with ethyl acetate to obtain an ethyl acetate soluble layer (97.8 g). The ethyl acetate soluble layer was applied to silica column (4.5 × 40 cm) chromatography using gradient elution with methanol in dichloromethane (80 : 1, 50 : 1, 30 : 1, 20 : 1, 15 : 1, 5 : 1, 1 : 1). Six sub-fractions (APEA-1～ APEA-6) were collected, and APEA-3 was purified on a Shim-Pack Prep-ODS (20 mm × 250 mm, 5 μm) column using the mobile phase of methanol-H_2O (50 : 50) as the eluent at a flow rate of 10 mL min^{-1} to obtain pure isosecotanapartholide (111 mg). This active component was identified by ^{1}H-NMR and ^{13}C-NMR.

Isosecotanapartholide : syrup,
^{1}H-NMR(DMSO-*d6*,400MHz):δ1.70(1H,m,H-8),1.85(1H,m,H-8’),2.05(3H,s,H-14),2.08 (1H,d,H-2),2.11(3H,s,H-15),2.45(2H,m,H-9),2.66(1H,dd,J=18.2,6.2Hz,H-2’),3.05(1H, m,H-7),4.55(1H,s,H-6),5.02(1H,d,J=5.6Hz,H-3),5.61(1H,brs,-OH),5.73(1H,d,J=2.5Hz, H-13),6.11(1H,d,J=2.9Hz,H-13’)
^{13}C-NMR(DMSO-*d6*,100MHz):δ13.4(C-15),26.8(C-8),29.7(C-14),39.1(C-9),41.3(C-7), 44.3(C-2),69.7(C-3),75.4(C-6),121.6(C-13),135.7(C-5),139.1(C-11),169.6(C-12),175.0(C-4),203.4(C-1),207.6(C-10).

HPLC analysis

A modular Shimadzu LC-20A System was utilized. A Capcell Pak C-18 Column (250 mm × 4.6 mm i.d. × 5 μm; Shiseido Co., Ltd., Japan) was employed at 30°C. Isocratic elution (mobile phase, solvent mixture of methanol (15%)) was performed for 1 h at a

flow rate of 1 mL min^{-1} with an injection volume of 20 µl. UV detector was set at 220 nm.

Human keratinocyte cultures

HaCaT was cultured in Dulbecco's modified Eagle's medium (DMEM) supplemented with 10% fetal bovine serum and 100 U/mL penicillin/streptomycin at 37°C (5% CO_2). Cells were pre-treated with ISTP and APE at the indicated concentrations for 30 min and then incubated with TNF-α (10 ng/mL) and IFN-γ (10 ng/mL). For IL-33 induction, HaCaT cells were treated with TNF-α (20 ng/mL) and IFN-γ (20 ng/mL).

CCK-8 assay

Human keratinocytes (2.5×10^4 cells/well) were plated in 96-well plates, and their proliferation was measured using a cholecystokinin (CCK)-8 assay (Dojindo, Rockville, MD, USA). Cells were treated with various concentrations of APE (125, 250, and 500 µg/ml) and ISTP (2.5, 5, and 10 µg/ml) for 24 and 48 h. CCK-8 solution (10 µL) was added to the cells in 1 mL DMEM. The cells were then incubated for 2 h at 37°C. Absorbance was measured at 450 nm using a microplate reader (SpectraMax 340; Molecular Devices, Sunnyvale, CA USA).

Western blot analysis

Western blots were performed as described previously (20). Proteins were quantified using a Bio-Rad DC Protein Assay Kit II (Bio-Rad, Hercules, CA, USA). Equal amounts of protein were separated by sodium dodecyl sulfate-polyacrylamide gel electrophoresis, transferred to polyvinylidene fluoride membranes, and incubated with

total and phosphorylated protein-specific antibodies: tyrosine 701 (pY-STAT1) (1:1000, 9167; Cell Signaling Technology, Danvers, MA, USA), serine 727 (pS-STAT1) (1:1000, 9177; Cell Signaling Technology), total STAT1 (1:1000, 9172; Cell Signaling Technology), ICAM-1 (1:1000, ab2213; Abcam, Cambridge, MA, USA), β-actin (1:1000, 4967; Cell Signaling Technology), and IL-33 (1:1000, sc-98660; Santa Cruz Biotechnology, CA, USA).

Measurement of chemokines

The concentration of six cytokines and chemokines (IL-1b, IL-6, Monocyte chemoattractant protein-1 (MCP-1)/CCL2, TARC, soluble intercellular adhesion molecule-1 (sICAM), and IL-33) were measured in the cell supernatant samples, using human enzyme-linked immunosorbent assay (ELISA) kits (eBioscience, San Diego, CA, USA). ELISA was performed according to the manufacturer's protocol.

RNA extraction and gene expression

RNA was isolated from keratinocytes using RNeasy Plus Mini Kit (Qiagen**,** Valencia, CA) according to the protocol of the manufacturer. The primers for reverse transcription PCR (RT-PCR) were as follows: *ICAM-1*, sense 5’ CAC CCT AGA GCC AAG GTG AC 3’, and antisense 5’ CAT TGG AGT CTG CTG GGA AT 3’; *TARC*, sense 5’ CTT CTC TGC AGC ACA TCC 3’ and antisense 5’ AAG ACC TCT CAA GGC TTTG 3’; *IL-33*, sense 5’ AGC CTT GTG TTT CAA GCT GG 3’ and antisense 5’ ATG GAG CTC CAC AGA GTG TTC 3’. The general PCR conditions were 30–35 cycles at 94°C for 2–10 min, 94°C for 30 s to 3 min, 50–58°C for 30 s to 1 min, 72°C for 30 s to 1 min, and 72°C for 4–7 min. The digitized gel images were analyzed using Quantity One®

1-D Analysis software (Bio-Rad, CA, USA)

Immunocytochemistry

HaCaT cells (1.5×10^4 cells/well) were seeded on a 4-well chamber slide and treated with ISTP and APE for 24 h. After fixation, permeabilization, blocking, and incubation, the mode of cell death of primary antibodies was assessed, by incubating overnight with anti-IL-33 (1:100, sc-98660; Santa Cruz Biotechnology, CA, USA) or FITC-labeled goat anti-rabbit IgG (1:1000, NB730-F; Novus Biologicals, CO, USA) at 4°C. DAPI (4, 6-diamidino-2-phenylindole) was used to counterstain the nuclei. Immunostained cells were visualized using a confocal microscope (Olympus FluoView FV10i).

Statistical analysis.

Data are presented as mean ± SD (n = 3). Statistical significance was calculated by one-way ANOVA followed by Duncan's multiple range test. $P < 0.05$ (*), $p < 0.01$ (**), and $p < 0.001$ (***) were statistically significant.

RESULTS

Effect of isosecotanapartholide on viability of HaCaT cell

The structure of isosecotanapartholide is shown in Fig. 1A. Isosecotanapartholide, isolated from *A. rutifolia* and *A. iwayomogi*, has a sesquiterpene lactone structure and has been shown to inhibit nitric oxide synthase. We investigated whether AP extract contained other major compounds such as 1) isosecotanapartholide, 2) eupatilin (21) and 3) jaceosidin (22). Compounds were extracted from AP by HPLC analysis and several other peaks were detected (Fig. 1B). To exclude the possibility that the

cytotoxicity of ISTP might contribute to its suppressive effects of TARC expression, cell viability was determined using CCK-8 assay. HaCaT cells were stimulated with TNF-α and/or IFN-γ in the absence or presence of ISTP and APE. As shown in Fig. 2, ISTP and APE showed no significant cytotoxic effect on HaCaT cells at the concentrations tested.

ISTP and APE suppress TNF-α/IFN-γ-induced TARC/CCL17 production and ICAM-1/STAT1 activation in human keratinocytes

ISTP mechanism of action on the inhibition of chemokines and cytokines released from TNF-α/IFN-γ-stimulated HaCaT were examined. Previous studies reported that TNF-α/IFN-γ-stimulation activates signaling molecules such as STAT, extracellular signal-regulated kinase (ERK), c-Jun N-terminal kinase (JNK), p38 mitogen-activated protein (MAP) kinases, and nuclear factor (NF)-κB in HaCaT cells (23). Thus, we evaluated whether ISTP affects the STAT pathway in TNF-α/IFN-γ-stimulated HaCaT cells using Western blot analysis. HaCaT cells were pre-treated with APE and ISTP, followed by incubation with TNF-α and IFN-γ and analyzed for phosphorylation of STAT1 and ICAM-1. Treatment with ISTP and APE downregulated ICAM-1 expression and decreased STAT1 protein phosphorylation in a dose-dependent manner (Fig. 3A). In addition, we examined ISTP fractions ability to inhibit ICAM-1 and TARC mRNA expression. RT-PCR showed that the expression of TARC and ICAM-1 mRNA was upregulated by TNF-α/IFN-γ. We also quantified the decreased expression levels of TARC and ICAM-1 mRNA by ISTP and APE (Fig. 3B). ELISA was performed to determine the inhibitory effects of ISTP and APE on TARC production. ISTP and APE significantly inhibited TNF-α/IFN-γ-induced TARC production in HaCaT cells in a dose-dependent manner (Fig. 3C). These results indicate that ISTP inhibits

TNF-α/IFN-γ-induced TARC expression by suppression of ICAM-1 and STAT1 activation.

Effects of ISTP and APE on TNF-α/IFN-γ-induced chemokines/cytokines production in HaCaT cells

The extent to which pro-inflammatory cytokine gene expression was affected by ISTP and APE was investigated. Subsequently, we evaluated whether ISTP inhibits inflammatory cytokine and chemokine production in TNF-α/IFN-γ-stimulated HaCaT cells. The STAT family plays an important role in cytokine production. The production of most cytokines and chemokines are primarily regulated at the transcription level, through activation of specific sets of transcription factors controlled by NF-κB and MAPKs. As a result, pre-treatment with ISTP and APE inhibited the induction of (A) IL-1β, (B) IL-6, (C) MCP-1/CCL-2, and (D) sICAM-1 levels dose-dependently in supernatants from cultured HaCaT cells (Fig. 4). These results indicate that ISTP effectively inhibits MCP-1/CCL2, IL-1β, IL-6, and sICAM as well as regulate pro-inflammatory cytokine release.

ISTP and APE markedly suppress IL-33 production

It has been reported that IL-33 is upregulated when keratinocytes are exposed to pro-inflammatory stimuli such as TNF-α/IFN-γ and may therefore be important in the pathogenesis of chronic inflammatory skin disorders such as atopic dermatitis and psoriasis. Therefore, we evaluated the effect of ISTP on IL-33 activation in TNF-α/IFN-γ-treated HaCaT keratinocytes and detected a high level of IL-33 at 20 ng/ml TNF-α/IFN-γ. The effects of ISTP and APE on protein and mRNA expression

levels of IL-33 were also investigated. As shown in Fig. 5A, protein and mRNA levels of IL-33 were enhanced after TNF-α/IFN-γ stimulation. Conversely, ISTP and APE markedly suppressed IL-33 expression in a dose-dependent manner (Fig. 5A). Also, pre-treatment with ISTP and APE inhibited the induction of IL-33 production dose-dependently in supernatants from cultured HaCaT cells (Fig. 5B). The immunocytochemistry results indicated that stimulation of HaCaT keratinocytes with TNF-α /IFN-γ led to an increase in IL-33. However, IL-33 was detected weakly after pre-treatment of HaCaT cells with 10 μg/mL and 500 μg/mL, respectively (Fig. 5C). These results indicate that ISTP inhibit IL-33 production and could possibly be important in the crosstalk between pro-inflammatory cytokines.

DISCUSSION AND CONCLUSION

In recent years, some herbal medicines have been considered as potential novel anti-inflammatory drugs (24). Natural products have been used extensively in the treatment of chronic skin diseases such as atopic dermatitis and psoriasis (25). Anti-inflammatory drugs developed from natural resources have attracted considerable attention worldwide. There are more than 400 classes of Artemisia identified (26). One of them, AP demonstrated various biological activities *in vitro* (27) and *in vivo (28)*. ISTP, an active component of APE suppressed LPS-induced nitric oxide production in murine macrophage RAW 264.7 cells (15). However, the anti-atopic activity and mechanism of action of ISTP have yet been elucidated. Therefore, in the present study, the anti-inflammatory properties of APE and ISTP were investigated. The inhibitory role of ISTP on AD-related factors was also examined. We proposed that ISTP's specific inhibition of new cytokines could be an alternative approach for the treatment of AD.

TARC/CCL17 is an extremely useful clinical biomarker for AD treatment (7). Also, TARC correlates with AD immunopathology, TNF-α, and IFN-γ (29). Therefore, we investigated the inhibitory activity of ISTP on inflammatory chemokine TARC. ISTP and APE inhibited the expression of TARC mRNA dose-dependently and showed no cytotoxicity on HaCaT cells (Fig. 2). Consequently, we examined the effect of ISTP and APE on TNF-α/IFN-γ signaling in HaCaT cells. STAT1 regulates the expression of numerous genes underlying cellular processes such as the immune response, antiviral protection, and apoptosis (30). Several plant extracts and compounds have been shown to inhibit the activities of inflammatory chemokines via the regulation of signaling pathways stimulated by TNF-α and IFN-γ, including STAT1 (31), (32). Thus, several reports have implicated inflammatory signaling in the regulation of STAT1 in inflammatory processes. The present study showed that treatment of HaCaT cells with ISTP and APE significantly inhibited ICAM-1 expression and subsequent STAT1 phosphorylation (Fig. 3).

Many reports have identified a panel of pro-inflammatory cytokines with important roles in the induction and maintenance of chronic skin inflammation (33). In this study, ISTP significantly inhibited MCP-1/CCL2, IL-1β, IL-6, and sICAM and also regulated pro-inflammatory cytokine production (Fig. 4). IL-1 promotes expression of adhesion molecules on keratinocytes and endothelial cells, providing infiltration of inflammatory factors (34). IL-1 and IL-33 may function as both a pro-inflammatory cytokine and an intracellular nuclear factor involved in transcriptional regulation. In humans, IL-33 mRNA levels are induced almost 10-fold in the skin of AD patients compared to healthy skin (19). According to reports, TNF-α and IFN-γ play a key role in type 1 immune responses and induce expression of IL-33, which could promote type 2 immune

responses in keratinocytes (35). Also, IL-33 levels were relatively higher in the presence of both TNF-α and IFN-γ. Pre-treatment with ISTP and APE inhibited dose-dependently TNF-α /IFN-γ-induced IL-33 production (Fig. 5). However, this study was limited to HaCaT keratinocytes and further studies are required to confirm ISTP effects on other cell types, such as human primary keratinocytes from AD patients or in an AD-like animal model. Taken together, our findings suggest that ISTP is an active component in APE that may regulate the recruitment of Th2-type cells into AD lesions by suppressing the expression of inflammatory chemokines related to AD.

In this study, we demonstrated that ISTP isolated from APE strongly suppressed TARC and IL-33 production in HaCaT human keratinocytes. Additionally, ISTP particularly inhibited the activation of ICAM-1/STAT1 induced by TNF-α/IFN-γ. These data provides new evidence regarding the anti-chemokine and anti-inflammatory functions of ISTP. Moreover, our findings suggest potential therapeutic applications of ISTP for atopic dermatitis and other inflammatory skin diseases.

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CONFLICT OF INTEREST

The authors have no conflicts of interest to declare.

FIGURE LEGENDS

Figure 1. Isolation of ISLP. (A) Isolation of the natural compound ISTP. (B) The

chemical structure of ISTP (Black arrow) and analytical HPLC chromatograph of a dried ethanol extract from *Artemisia princeps* Pampanini at a 220-nm wavelength.

Figure 2. Effects of ISTP and APE on HaCaT human keratinocyte viability. The cytotoxic effects of ISTP and AE on HaCaT cells were measured using CCK-8 assays. Cells (2×10^4/well) were plated in 96-well microplates and treated with APE (62.5, 125, 250, 500 μg/ml) and ISTP (1.25, 2.5, 5, and 10 μg/ml) for 24 h (solid line). Cells (2×10^4/wells) were also incubated with TNF-α (20 ng/ml) and IFN-γ (20 ng/ml) for 30 min after treatment (hatched line). Values represent the mean ± SD (n = 3).

Figure 3. Effects of ISTP and APE on TNF-α/IFN-γ-induced ICAM-1/STAT1 activation and TARC production in HaCaT cells. (A) Cells were pretreated with APE (125, 250, and 500 μg/ml) and ISTP (2.5, 5, and 10 μg/ml) for 30 min and then incubated with TNF-α (10 ng/ml) and IFN-γ (10 ng/ml) for 24 h. Cell lysates were subjected to Western blotting for pY-STAT1, pS-STAT1, ICAM-1, and β-actin. (B) The mRNA levels of ICAM-1 and TARC were analyzed by RT-PCR. (C) Production of TARC was measured by enzyme-linked immunosorbent assays of cell supernatants. *P < 0.05; **P < 0.01; ***P < 0.001 versus control. A: *Artemisia princeps* Pampanini ethanol extract, I: isosecotanapartholide.

Figure 4. Effects of ISTP and APE on TNF-α/IFN-γ-induced chemokines/cytokines production in HaCaT cells. Pretreated with APE (125, 250, and 500 μg/ml) and ISTP (2.5, 5, and 10 μg/ml) for 30 min and then incubated with TNF-α (10 ng/ml) and IFN-γ (10 ng/ml) for 24 h. Production of (A) IL-1β, (B) IL-6, (C) MCP-1/CCL2, and (D)

sICAM were measured by ELISA. Values represent the mean ± SD (n = 3). Significant differences from the control group are *P < 0.05; **P < 0.01; ***P < 0.001 versus TNF-α/IFN-γ-induced treatment alone. A: *Artemisia princeps* Pampanini ethanol extract, I: isosecotanapartholide.

Figure 5. Effects of ISTP and APE on TNF-α/IFN-γ-induced IL-33 production in HaCaT cells. (A) Cells were pretreated with APE (125, 250, and 500 μg/ml) and ISTP (2.5, 5, and 10 μg/ml) for 30 min followed by the addition of TNF-α (20 ng/ml) and IFN-γ (20 ng/ml) for 24 h and then subjected to Western blot analysis and RT-PCR. (B) Productions of IL-33 were measured by ELISA of cell supernatants. (C) Representative images of immunocytochemical staining for IL-33 in HaCaT cells treated with the indicated concentrations of APE and ISTP for 24 h. Immunocytochemical staining with anti-IL-33 (green). Cells were located by counterstaining with DAPI (blue). A: *Artemisia princeps* Pampanini ethanol extract, I: isosecotanapartholide.

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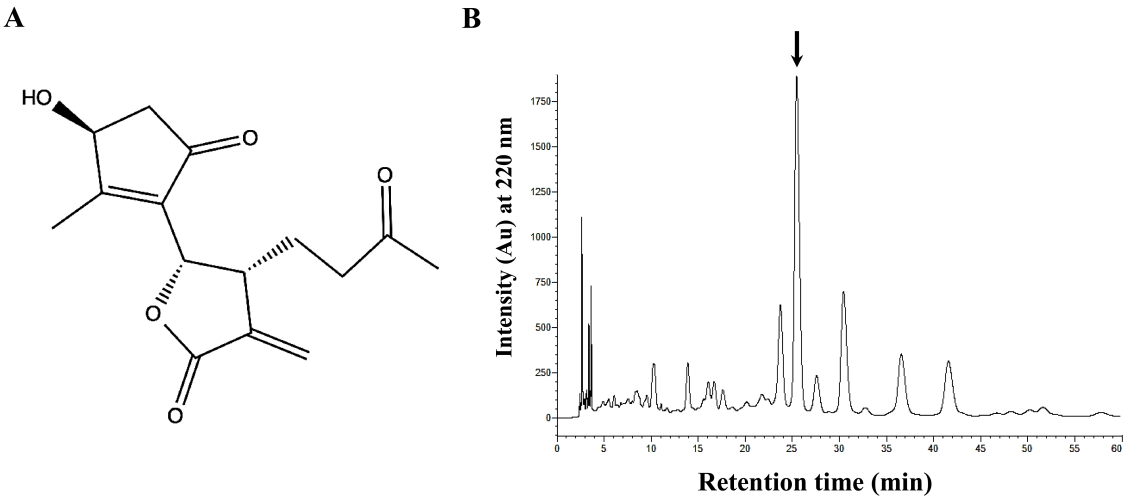

Figure 1. Isolation of ISLP.

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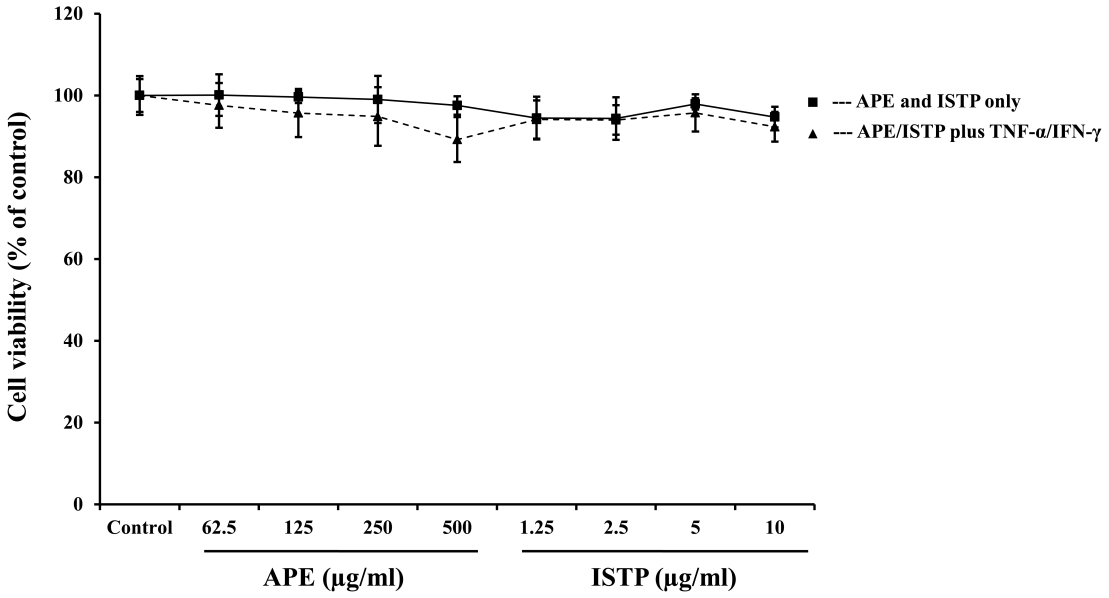

Figure 2. Effects of ISTP and APE on HaCaT human keratinocyte viability.

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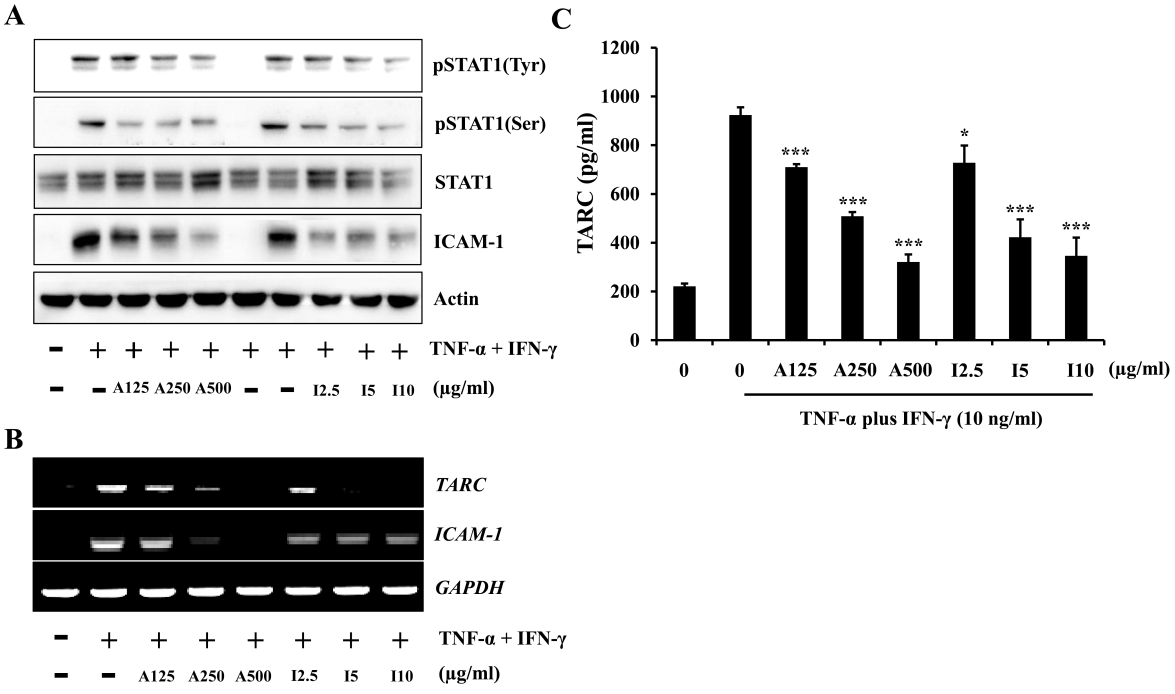

Figure 3. Effects of ISTP and APE on TNF-α/IFN-γ-induced ICAM-1/STAT1 activation and TARC production in HaCaT cells.

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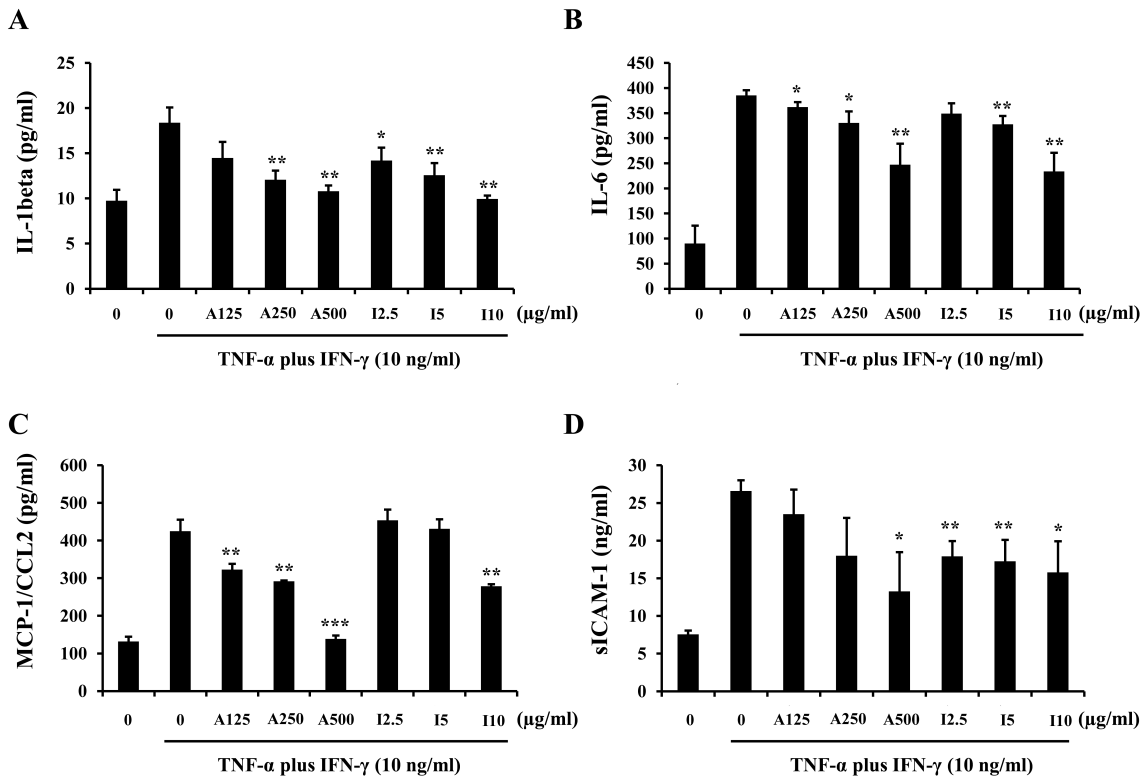

Figure 4. Effects of ISTP and APE on TNF-α/IFN-γ-induced chemokines/cytokines production in HaCaT cells.

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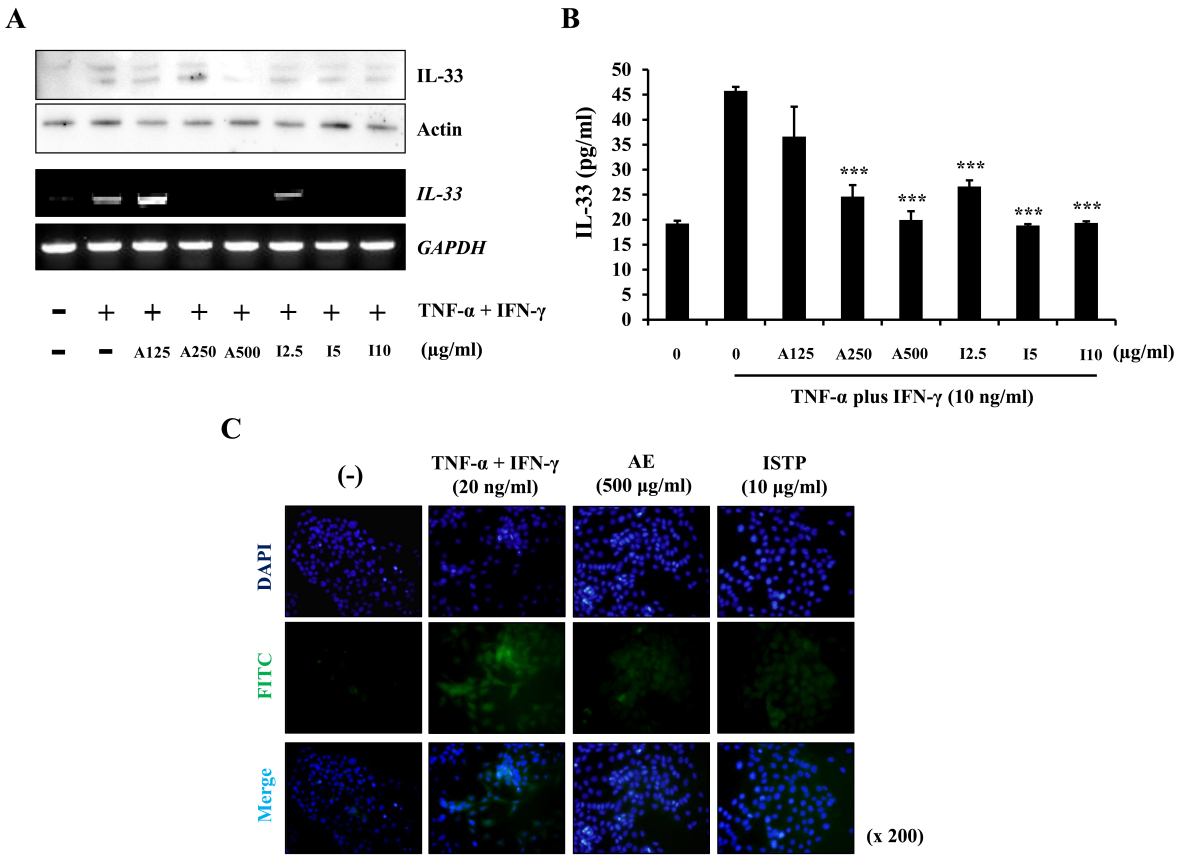

Figure 5. Effects of ISTP and APE on TNF-α/IFN-γ-induced IL-33 production in HaCaT cells.