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Fatty Acid Components of Hardy Kiwifruit (*Actinidia arguta*) as IL-4 Production Inhibitor

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Abstract

The fruit of *Actinidia arguta* (AA) has been used mainly for the treatment of skin diseases, diuresis, diabetes mellitus and osteoporosis in Korean traditional medicine. It is known that AA (hardy kiwi) fruit extract has an effect on 2-chloro-1,3,5-trinitrobenzene-induced atopic dermatitis-like skin lesions in NC/Nga mice. Mode of action for it is associated with the modulation of biphasic Th1/Th2 cytokines. Furthermore, DA9102 containing AA is a herbal medicine currently under phase II clinical trial for atopic dermatitis in Korea. However, no active principles of AA on the decrease of Th2 cytokines including IL-4 and IL-10 have been identified. In this study, bioactivity-guided fractionation of an alcohol extract from the dried fruits of AA using ELISA assay for IL-4 production led to the isolation of α-linolenic acid (I), linoleic acid (II), ethyl linolenate (III), ethyl linoleate (IV) and ethyl stearate (V) as the major active components. These compounds showed the down-regulatory effects of IL-4 production in A23187-stimulated RBL-2H3 cells without cytotoxicity.

Key Words: *Actinidia arguta*, Fatty acid, IL-4 production

INTRODUCTION

The term ''atopy'' was introduced to designate phenomena of hypersensitivity in humans by Coca and Cooke in 1923. Atopy was adapted from the Greek word meaning ''out of place'' or ''strange disease'' (Coca and Cooke, 1923) Causes have been sought for the rise in the prevalence of allergic diseases, with investigations ranging from nutrition to general environmental changes (Laitinen *et al.*, 2006). Atopic dermatitis is a common, chronic fluctuating skin disease. Over the last 30 years, the prevalence of atopic dermatitis and other atopic allergic disorders has increased continuously (Ring *et al.*, 2001). So the importance of research regarding the treatment of atopic dermatitis is increasing.

Allergic diseases including atopic dermatitis are one of the representative chronic inflammatory diseases involved in the degranulation of immunoglobulin E (IgE) dependent mast cells and the accumulation of eosinophils at the sites of inflammation, which are characterized by uncontrolled overactivation of T_H2 responses and T_H2-mediated hyperproduction of IgE. The representative cytokines of T_H2 cells, such as interleukin-4 (IL-4), IL-5, IL-10, and IL-13, play important roles in these reactions. Furthermore, it was reported that T_H1-mediated cytokines, such as IL-12 and IFN-γ, produced by T_H1 cells act as an antagonist for these T_H2 cytokines (Cooffman and Carty, 1986; Abbas *et al.*, 1991; Vercelli, 2001).

Various natural products have been used as traditional herbal remedies of atopic diseases for a long time. Many natural products with inhibitory activities on atopic diseases are as followings; rice bran (Fujiwaki and Furusho, 1992), *Mallotus philippensis* (Daikonya *et al.*, 2002), purple bamboo salt (Shin *et al.*, 2004), *Rumex Japonicus* Houtt. (Lee *et al.*, 2006), *Mangifera indica* (Rivera *et al.*, 2006) and *Evodia rutaecarpa* (Shin *et al.*, 2007) have inhibitory activities on atopic diseases. However, these natural products have also been known to have side effects (Moto *et al.*, 2004; Thakur *et al.*, 2005; Rodeiro

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et al., 2006; Yang *et al.*, 2006). Efforts had been made to find more distinguished and non-toxic materials with anti-allergic activity than the existing known natural products. As a result, *Actinidia arguta* (AA) was used as a traditional treatment of skin diseases and its effect is more potent than any others.

Actinidia arguta (Sieb.Et Zucc.) Planch. ex Miq. var. *arguta* is a smooth-skinned grape-sized kiwifruit. AA mostly grows in northern China, Korea, Siberia and Japan. Several studies have been performed on the volatile aromatic and flavorous compounds of kiwifruit fruit. And it was reported that components of AA fruits were primarily a mixture of esters, aldehydes and alcohols, with various monoterpenes (Matich *et al.*, 2003). AA commonly known as hardy kiwifruit is being cultivated on limited acreage in western North America, and has been tested as a potential processed food product. In Korean traditional medicine, it has been known to have effects on allergic disorders, diuresis, diabetes mellitus, osteoporosis, cardiopathy, and gastroenteric trouble. Recently, it was reported that the PG102 fraction from AA controls IgE and selective T_H1 and T_H2 cytokines (Park *et al.*, 2005). And DA9102, isolated from AA, suppressed the mRNA expression of T_H2 cytokines including IL-4 and IL-10 in the Mg-deficiency induced dermatitis model of hairless rats (Choi *et al.*, 2007).

This study focuses on the isolation and elucidation of compounds with IL-4 inhibitory activities from AA using an activity-guided isolation method. The extract of *A.arguta* fruits prepared by extraction with water-*n*-BuOH-EtOH (1:1:1) mixed solvent exhibited significant biological activity in a preliminary *in vitro* screening against IL-4 production. In the present study, bioassay-fractionation of the extract led to the isolation of five fatty acid derivatives as IL-4 production inhibitors. All isolates-containing fractions were evaluated for their potential to modulate the production of IL-4. The structural elucidation of the isolated components and the biological evaluation of the isolates-containing fractions from AA are described herein.

MATERIALS AND METHODS

Plant material

Dried AA fruits from Shaan-xi (China) were purchased at a specialized market for traditional herbal medicine (Kyungdong herb market, Seoul) in Korea and their identity was kindly confirmed by Dr. Changsoo Yuk (specialist in plant classification).

Reagents

Extra pure grade solvents (Samchon Chemical Co., Korea) were used for extraction, partition and isolation. All solvents (Aldrich, Milwaukee, WI, U.S.A.; Fisher Scientific, Schwerte, Germany) used for instrumental analysis were guaranteed grade reagent. Minimum Essential Medium (MEM), fetal bovine serum (FBS) and penicillin-streptomycin (PS) were purchased from Gibco-BRL (Grand Island, NY, USA). A23187 and dexamethasone were purchased from Sigma (St. Louis, MO, USA).

Extraction and partition

Dried fruits (1.7 kg) of AA were extracted with a mixed solution containing water:*n*-BuOH:EtOH (1:1:1) at 60°C for 2 days and then filtered. The filtrate was concentrated by rotary evaporator. The extract was suspended with distilled water, and then successively partitioned with *n*-BuOH. The *n*-BuOH layer (120 g) was concentrated by rotary evaporation (EYELA, Tokyo, Japan) at 50°C.

Activity-guided isolation

The *n*-BuOH soluble fraction was applied to flash column chromatography system (HP20 75M cartridge) and the step-gradient elution using H_2O, H_2O:MeOH (1:1) and MeOH yielded three fractions, of which the MeOH soluble fraction showed higher antiallergic effects than other fractions. The MeOH soluble fraction (AAM, 11.9 g) was chromatographed with silica gel (70-230 mesh, Merck, Damstadt, Germany). The column was first eluted with a mixed solvent (CHCl3:EtOAc=20:1), stepwise-eluted with different compositions of mixed solvents (CHCl3:EtOAc=10:1→5:1→1:1→EtOAc), and then yielded nine fractions. The nine fractions were monitored by colorizing reaction with anisaldehyde-sulfuric acid on TLC plate as well as bioassay. AAM-2 (1.69 g) showed higher inhibitory activities of IL-4 production than the other eight fractions. The TLC chromatogram of AAM-6 (1.7 g) exhibited similar color bands in comparison with AAM-2. Concerning these fractions, vacuum flash column chromatography was performed respectively. The procedures for the isolation and purification of active compounds from AA are outlined in Fig. 1.

Isolation of compounds I and II

The isolation of AAM-6 (1.7 g) was performed as follows. AAM-6 was fractionated in C-18 vacuum flash column chromatography using a step gradient elution with mixed solvents of acetonitrile (AcCN) and water (AcCN:H_2O=2:1→4:1→6:1→AcCN) to give six fractions. Among the six fractions, AAM-6-II included major compounds of AAM-6.

AAM-6-II was applied to vacuum flash chromatography using ODS gel. The column was first eluted with AcCN:H_2O (3:1), and then stepwise-eluted with gradient condition (AcCN:H2O=4:1→AcCN) to yield six subfractions including compound I (AAM-6-II-b, 200 mg). AAM-6-II-d was further purified by using preparative HPLC equipped with a Luna 15 μ C18(2) column (250×20 mm, 15 micron, phenomenex®, Torrance, CA) and RI detector (Shodex, Japan) to obtain compound II (30 mg). The mobile phase was an isocratic solvent, AcCN in water (AcCN:H_2O=8:1) and the flow rate was set at 6 ml/min. Compound I and II showed higher inhibitory effects than other subfractions from AAM-6.

Isolation of compound III

AAM-2 (1.69 g) was fractionated in vacuum flash chromatography packed with ODS gel. The column was first eluted with AcCN in water (AcCN:H_2O=7:1), and then stepwise-eluted with each of different concentrations of the solvent mixture (AcCN:H_2O=8:1→9:1→10:1→15:1→AcCN). Finally five fractions (AAM-2-I - AAM-2-V) were obtained. Among the five fractions, AAM-2-I contained a major compound of this fraction, and the same method was used to purify the compound III. The purity of the compound III (AAM-2-I-c, 400 mg) was checked by TLC, NMR and EI-MS.

Isolation of compound IV

The separation of compound IV from AAM-2-II fraction was performed by prep-HPLC system equipped with a Luna 10 μ C18(2) column (250×10 mm, 10 micron, phenomenex®, Torrance, CA) and RI detector. The mobile phase was an isocratic solvent, AcCN in water (AcCN:H_2O=10:1) and flow rate was

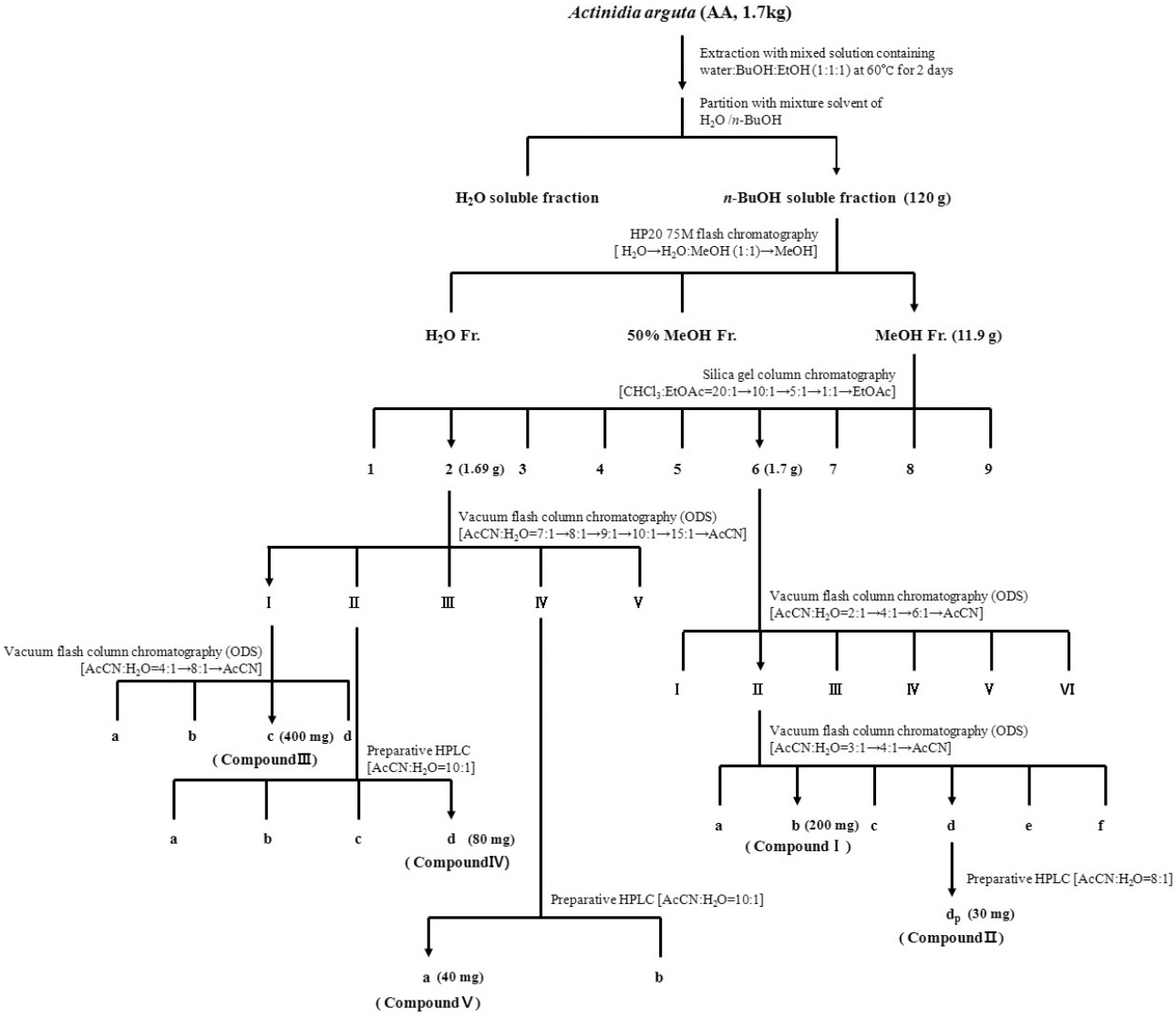

Fig. 1. Isolation scheme of five active compounds from *Actinidia arguta.*

set at 4 ml/min. The purity of the compound IV (AAM-2-II-d, 80 mg) was checked by TLC, NMR and EI-MS.

Isolation of compound V

The separation of compound V from AAM-2-IV fraction was performed by the same method used in the isolation of active compound from AAM-2-II. The mobile phase was an isocratic solvent, AcCN in water (AcCN:H_2O=10:1) and flow rate was set at 4 ml/min. The purity of the compound V (AAM-2-IV-a, 40 mg) was checked by TLC, NMR and EI-MS.

Cell line and culture procedures

RBL-2H3 cells, which are from the mast cell line, were used for bioassay. RBL-2H3 cells were purchased from American Type Culture Collection (ATCC, Rockville, MD, USA). RBL-2H3 cells were maintained in monolayer culture in Minimum Essential Medium (MEM, Gibco-BRL, Grand Island, NY, USA) supplemented with 15% (v/v) fetal bovine serum (FBS, Gibco-BRL, Grand Island, NY, U.S.A.) and penicillin-streptomycin (100 μg/ml) at 37°C in a 5% CO_2 humidified air atmosphere.

Down-regulatory activities on IL-4 production and cell viability in cultured RBL-2H3 cells

The down-regulatory activities on IL-4 production *in vitro* test system were evaluated by the method reported previously (Choi *et al.*, 2007; Kim *et al.*, 2009). To measure the effect on IL-4 , RBL-2H3 cells were plated at 2×10^5 cells/well in the 24-well plates and cultured in 0.5 ml of MEM (Sigma, MO, USA) containing 15% FBS for 6 hours. The cells were incubated in the absence or the presence of test samples for 30 mins before stimulation with 1 μM of A23187 (Sigma, MO, USA). After 12 hours, the cultured media were taken to determine the level of IL-4 by ELISA (R&D systems, Minneapolis, MN, USA). The cell viability was evaluated via analysis by scanning electron microscopy. Based on the concentrations without cytotoxicity, all samples were treated in RBL-2H3 cells under optimal con-

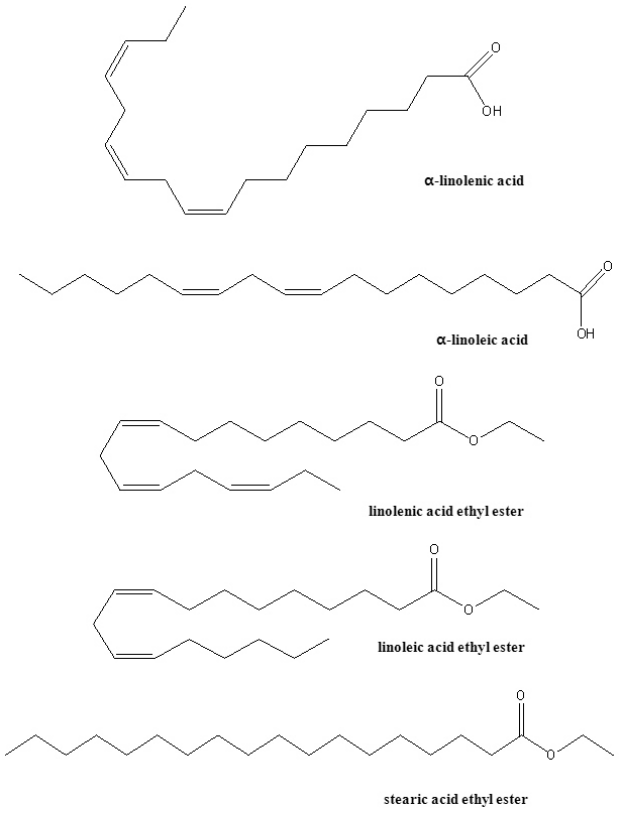

Fig. 2. Chemical structures of compounds isolated from *Actinidia arguta.*

ditions.

Statistics

Values are expressed as means ± SEM. Statistical analysis was performed using one-way analysis of variance (ANOVA) followed by Student's *t*-test. Statistical significance was set at $p<0.05$.

RESULTS

Structure elucidation of compound I

Compound I was a colorless oil. The molecular formula of I was determined to be $C_{18}H_{30}O_2$ by EI-MS (obsd $[M]^+$ at m/z 278) and ^{13}C NMR (18 carbon signals) data. ^1H NMR spectral data including correlations from the ^1H-^1H COSY experiment illustrated signals attributable to a tri-unsaturated fatty acid, which were six olefinic methine protons of a multiplet resonance at δ 5.28, allylic methylene protons at δ 2.80 (4H, t, *J*= 6.0 Hz), δ 2.34 (2H, t, *J*=7.5 Hz) and δ 2.06 (4H, m), numerous methylene protons at δ 1.63-1.32 (10H, m), and terminal methyl proton at δ 0.90 (3H, t, *J*=7.5 Hz). The ^{13}C NMR and gHSQC spectra of I allowed all proton signals to be assigned to their respective carbon signals. The olefinic protons correlated with carbons at δ 132.1, 130.4, 128.5, 128.4, 128.0, and 127.3, while the methylene protons correlated with 10 methylene carbons at δ 34.3-20.8, and the aliphatic methyl protons correlated with a carbon signal at δ 14.5. The ^{13}C NMR spectrum of I also showed an additional signal attributed to a carboxylic carbon at δ 180.4. By comparing all spectroscopic data with literature values, compound I was identified as 9, 12, 15-octadecatrienoic acid, also called α-linolenic acid (Falkenstein *et al.*, 1991).

Structure elucidation of compound II

Compound II was colorless oil. The EI-MS of compound II showed a molecular ion peak at m/z 280 for a molecular formula of $C_{18}H_{32}O_2$. The analysis of the ^1H and ^{13}C NMR spectra together with gCOSY data indicated that II is a sort of di-unsaturated fatty acid. The ^1H NMR spectral data of II showed a similarity to that of compound I except for the number of olefinic methine protons and aliphatic methylene protons. In the ^1H NMR spectrum of II, characteristic signals representing four olefinic methine protons at δ 5.34, and allylic methylene protons at δ 2.78 (2H, t, *J*=7.0 Hz), δ 2.36 (2H, t, *J*=7.5 Hz), and δ 2.06 (4H, m) were observed. The ^1H NMR spectrum of II also showed methylene protons (δ 1.64-1.33, 16H) and terminal methyl proton (δ 0.90, 3H, t, *J*=7.0 Hz). The analysis of the ^1H NMR data was inferred that compound II was (C18:2) fatty acid. All carbon resonances were fully assigned by ^{13}C NMR, ^1H-^1H gCOSY and gHSQC spectra. The ^{13}C -NMR spectrum of II showed carboxylic carbon (δ 180.2), four olefinic carbons (δ 130.5, 130.4, 128.3 and 128.1), terminal methyl carbon (δ 14.3), and 12 aliphatic methylenes signals (δ 34.3-22.8). On the basis of the spectral data of II and previously reported literature values of (C18:2) fatty acids (Marcel *et al.*, 1997; Kim *et al.*, 2004) the structure of compound II was determined to be 9, 12-octadecadienoic acid, also called α-linoleic acid.

Structure elucidation of compound III

Compound III was isolated as a colorless oil that analyzed for the molecular formula $C_{20}H_{34}O_2$ by EI-MS (m/z 306 $[M]^+$) and NMR methods. The ^1H and ^{13}C NMR spectra for III were almost identical to those of the aliphatic chain in I. The major difference between the spectra for I and III was the presence of an ethoxy group. ^1H NMR spectral data illustrated signals attributable to the ethoxy group at δ 4.09 (2 H, t, *J*=7.5 Hz) and δ 1.23 (3 H, t, *J*=7.5 Hz). The gHSQC experiment showed that these proton signals correlated with ^{13}C NMR signals at δ 60.3 ($-OCH_2-$) and δ 14.5 (methyl unit). As expected, the ester carbon signal was observed at δ 174.0 in the ^{13}C NMR spectrum of III. The structure of compound III was thus determined to be linolenic acid ethyl ester (9, 12, 15-octadecatrienoic acid ethyl ester) (Falkenstein *et al.*, 1991).

Structure elucidation of compound IV

Compound IV was a colorless oil. EI-MS data of IV showed a molecular ion peak at m/z 308. The ^1H NMR and ^{13}C NMR spectra of IV were similar to those of compound II except for the additional signals attributable to an ethoxy group at δ_H 4.13 (2H, t, *J*=7.0 Hz), δ_H 1.26 (3H, t, *J*=7.0 Hz), δ_C 60.4, and δ_C 14.5. An ester carbon signal was also observed at δ 174.1 in ^{13}C NMR spectrum of IV. The structure of compound IV was thus identified as linoleic acid ethyl ester ($C_{20}H_{36}O_2$), 9, 12-octadecadienoic acid ethyl ester (Pouchert and Behnke, 1993; Kim *et al.*, 2004).

Structure elucidation of compound V

Compound V was isolated as a colorless oil. The EI-MS showed the molecular ion peak at m/z 312. In comparison with

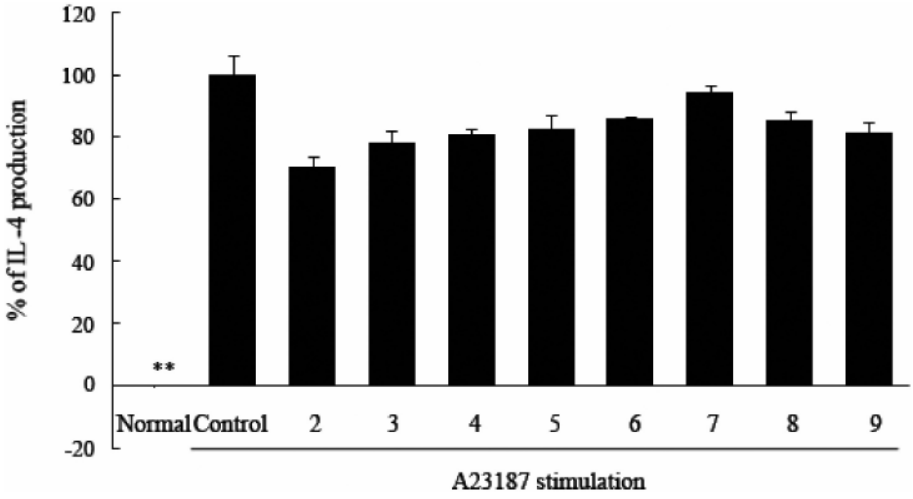

Fig. 3. Inhibitory effects of ten subfractions obtained from the AAM fraction on IL-4 production in RBL-2H3 cells. RBL-2H3 cells were incubated in the absence or the presence of test samples for 30 mins before stimulation with 1 μM of A23187. Test samples were treated at the concentration of 1 μg/ml without cytotoxicity. After 12 hours, the cultured media were taken to determine the level of IL-4 by ELISA. The AAM-2 fraction shows effectively decrease of IL-4 production at 1 μg/ml concentration. Values are the means ± SEM. * indicates the significance at $p<0.05$ compared to control cells (**$p< 0.01$).

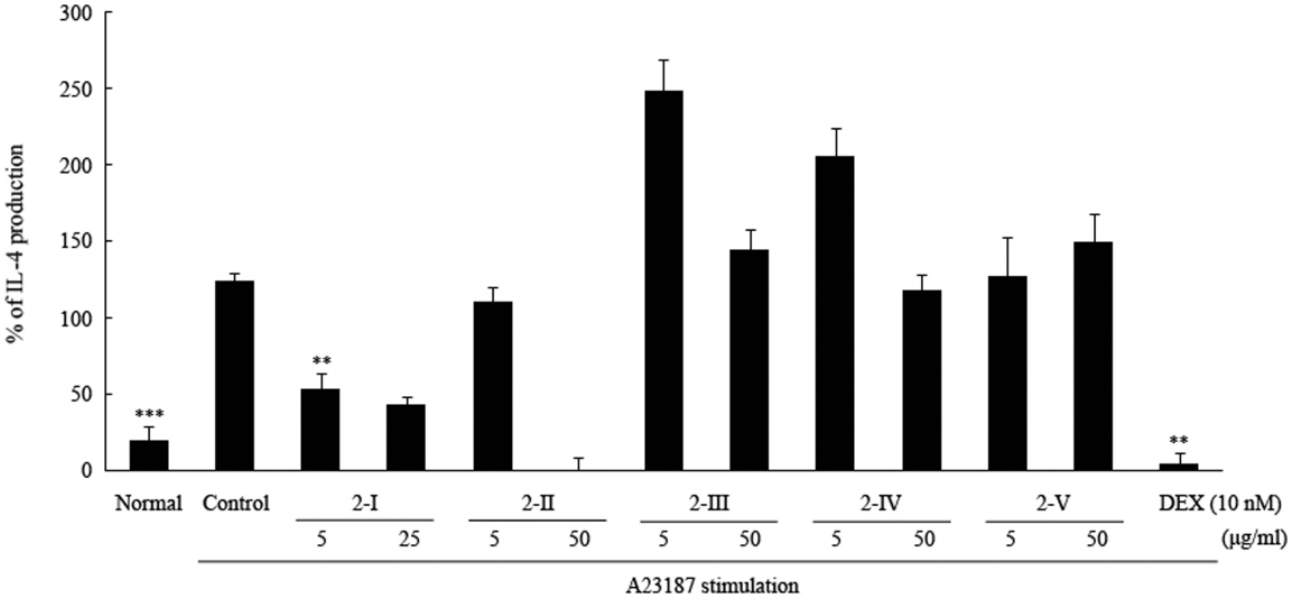

Fig. 4. Inhibitory effects of five subfractions obtained from the AAM-2 fraction on IL-4 production in RBL-2H3 cells. RBL-2H3 cells were incubated in the absence or the presence of test samples for 30 mins before stimulation with 1 μM of A23187. After 12 hours, the cultured media were taken to determine the level of IL-4 by ELISA. The AAM-2-I fraction shows effectively decrease of IL-4 expression at the concentration of 25 μg/ml without cell toxicity. Values are the means ± SEM. * indicates the significance at $p<0.05$ compared to control cells (**$p<0.01$ and ***$p< 0.001$).

^{1}H-NMR data of other compounds isolated from AAM-2 fraction, it was similar to a C18 saturated fatty acid ethyl ester. Compound V was identified as octadecanoic acid ethyl ester, also known as stearic acid ethyl ester ($C_{20}H_{40}O_2$) through the comparison of the spectral data with literature values (Christie, 2003).

Inhibitory effects of *Actinidia arguta* on IL-4 expression in RBL-2H3 cells

Based on the activity-guided isolation technique, each fractions isolated from AA was examined for down-regulatory activities on IL-4 production in RBL-2H3 cells. RBL-2H3 cells were pre-treated with test samples at concentration that did not affect cell viability and then stimulated with A23187 for 12 hrs. Test samples were evaluated for suppression of the production of IL-4 in the concentration range without cell toxicity in A23187-simulated RBL-2H3 cells.

The MeOH fraction obtained from the first flash column chromatography showed significant IL-4 production inhibitory effect with no cell toxicity. The final concentration of test samples diluted with culture media was 1 μg/ml (dissolved in 0.1% DMSO). The MeOH fraction was subjected to silica gel column chromatography with an *n*-hexane-EtOAc mixture and yielded nine subfractions. As a result of down-regulatory activity of subfractions on IL-4 production in cultured RBL-2H3 cells, The subfraction 2 showed a higher inhibitory effect (about 20%) than others (Fig. 3). AAM-1 had a quantity too small to be subjected to the column chromatography.

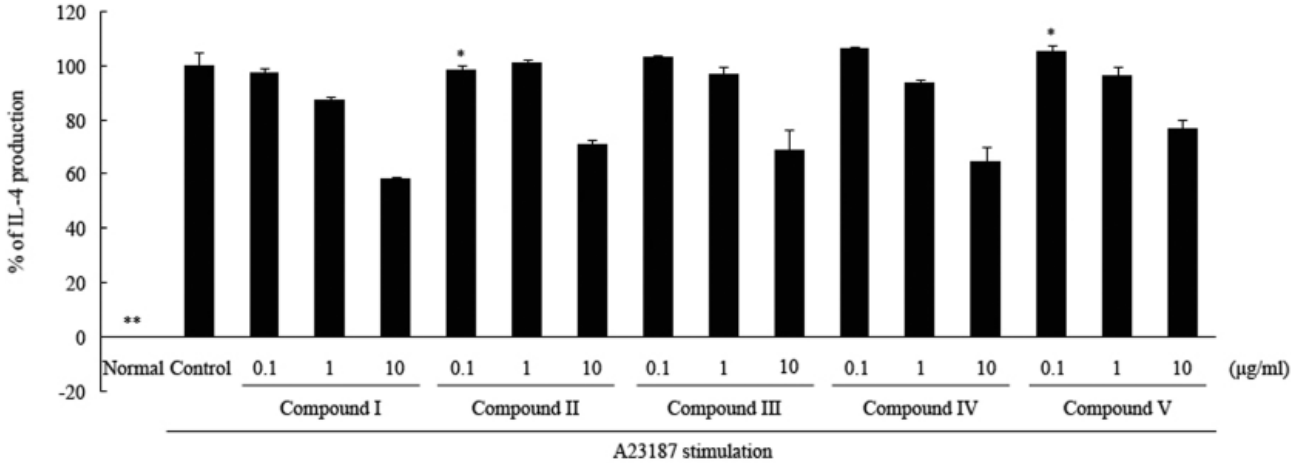

Fig. 5. Inhibitory effects of subfractions containing five compounds isolated from AA on IL-4 production in RBL-2H3 cells. RBL-2H3 cells were incubated in the absence or the presence of test samples for 30 mins before stimulation with 1 μM of A23187. After 12 hours, the cultured media were taken to determine the level of IL-4 by ELISA. All subfractions that had measurable level of secreted IL-4 showed significant down-regulatory effects compared with the control at the concentration of 10 μg/ml without cytotoxicity. Values are the means ± SEM. * indicates the significance at $p<0.05$ compared to control cells (*$p<0.05$ and **$p<0.01$).

The combined fraction AAM-2 was chromatographed on C-18 using a mixture solvent (AcCN-H_2O) and yielded five subfractions. These fractions were examined for inhibitory activity on IL-4. Compared with A23187-treatment, AAM-2-I showed significant decreases of IL-4 production (about 80%) and no cell viability at the concentration of 25 μg/ml (Fig. 4).

AAM-6 included major compounds which showed a similar TLC pattern in comparison with AAM-2. So, the fractionation of AAM-6 was performed by a C18 vacuum flash column chromatography and then yielded fractions were purified by reverse phase prep-HPLC. The down-regulatory effects on A23187-induced IL-4 production of six subfractions prepared by chromatographic fractionation of AAM-6-II were measured by ELISA for the level of IL-4. All subfractions that had measurable level of secreted IL-4 showed significant down-regulatory effects compared with the control at the concentration of 10 μg/ml. Compound I (AAM-6-II-b) showed the highest down-regulatory effect (about 43%) (Fig. 5).

DISCUSSION

Atopic dermatitis, also known as atopic eczema, is a chronic inflammatory skin disorder which is most prevalent in childhood (Peroni *et al.*, 2007). A substantial increase in atopic diseases has been observed during the last decades (Byberg *et al.*, 2007). Among the various treatments, herbal treatments are often used for dermatological conditions such as atopic dermatitis. AA has been demonstrated to possess both *in vivo* and *in vitro* anti-inflammatory effects (Park *et al.*, 2005). Although AA has high inhibitory effects on allergic diseases, the active compounds causing these effects have not been identified. The aim of the present study was to isolate components with anti-allergic activities from AA and elucidate their structures. Moreover, the deduction of these active compounds will be meaningful as candidates for regulatory reagents of atopic diseases.

The five compounds (compounds I-V) from the dried fruits of AA were isolated using activity-guided isolation technique. Activity tests were performed by ELISA assay to examine the down-regulatory effects on IL-4 production in the RBL-2H3 mast cell line. Structures of the compounds isolated were elucidated using EI-MS, ^{1}H-NMR and ^{13}C-NMR and confirmed by comparison of all spectroscopic data with literature values. All isolated compounds were determined to the series of fatty acids through spectral analyses. Compound I and II were α-linolenic acid (C18:3) and linoleic acid (C18:2), respectively. Compound III, IV and V were α-linolenic acid ethyl ester (ethyl linolenate, C18:3), linoleic acid ethyl ester (ethyl linoleate, C18:2) and stearic acid ethyl ester (ethyl stearate, C18:0), respectively. It has been reported that various fatty acids such as α-linolenic acid ethyl ester, linoleic acid ethyl ester and α-linolenic acid have been isolated from *Actinidia* species (Matich *et al.*, 2003; Ren *et al.*, 2007).

Fatty acids including these compounds isolated from AA have been reported to have anti-allergic effects. Fatty acids are an essential component of the plasma cell membrane, and they also play important roles in the control of inflammation (Kawamoto *et al.*, 2001). There are two principal families of polyunsaturated fatty acids (PUFAs), the *n*-6 (or ω-6) and the *n*-3 (or ω-3) families. The simplest members of each family are linoleic acid (18:2n-6) and α-linolenic acid (18:3*n*-3) (Calder, 2006). Regarding the immunosuppressive effects of *n*-3 PUFAs, various studies have reported that *n*-3 PUFAs decrease the production of inflammatory mediators and the expression of adhesion molecules and *n*-3 PUFAs are potential anti-inflammatory agents (Calder, 2003). A recently published study showed that α-linolenic acid isolated from *Actinidia Polygama* fruits was found to show *in vivo* and *in vitro* anti-inflammatory activity (Ren *et al.*, 2007). Also, another study showed a significant improvement of atopic dermatitis after fatty acid supplementation in the form of sunflower oil (63% linoleic acid). As an essential component of the epidermal barrier system, the effect of linoleic acid was positively correlated with an increased level of 13-hydroxyoctadecadienoic acid in skin (Giménez-Arnau *et al.*, 1997).

Compound III, IV and V are esterification products of ethanol. It is possible that fatty acids released from esters after hydrolysis could mediate adjuvant activity of stable water-in-oil (w/o) emulsions (Bomford, 1981). The studies about anti-allergic effects of FAEEs compared with essential fatty acids such as linoleic acid and α-linolenic acid are hardly reported. In this study, according to the results of the down-regulatory effects on IL-4 production in RBL-2H3 cell, five compounds isolated had significant inhibitory activities. The results of the present study suggest that linolenic acid ethyl ester, linoleic acid ethyl ester, stearic acid ethyl ester, linolenic acid and linoleic acid, the five active compounds isolated from AA, are potent inhibitors of A23187-induced IL-4 production in RBL-2H3 cells. These findings increase the importance of AA as a natural product and the five fatty acids as components for the suppression of IL-4 production correlated with an attack of allergic diseases.

Considering the multiplicity and complex interactions of T_H2 cytokine in allergic diseases, Measurements of the effects on other pathologic cytokines such as IL-13 or IL-5 are needed.

In conclusion, the present results demonstrate that various compounds, such as linoleic acid, α-linolenic acid, ethyl linoleate, ethyl linolenate and ethyl stearate isolated from AA fruits, have potential as IL-4 production inhibitor.

ACKNOWLEDGMENTS

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SUPPORTING INFORMATION

EI-MS, ^{1}H NMR, ^{13}C NMR, ^{1}H-^{1}H gCOSY and ^{1}H-^{13}C gHSQC spectra of the five compounds isolated from *Actinidia arguta* are described herein.

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