



Effects of magnolialide isolated from the leaves of *Laurus nobilis* L. (Lauraceae) on immunoglobulin E-mediated type I hypersensitivity *in vitro*



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ABSTRACT

Ethnopharmacological relevance: *Laurus nobilis* L. (Lauraceae) has been used for folk medicines in the Mediterranean area and Europe to treat various disorders including skin inflammation (dermatitis) and asthma.

Aim of the study: Our aim was to investigate the scientific evaluation of the compounds from *Laurus nobilis* L. on immunoglobulin E (IgE)-mediated type I hypersensitivity responses *in vitro* such as atopic dermatitis and asthma.

Methods and materials: Seven compounds were isolated and examined for the mast cell stabilizing effect on IgE-sensitized RBL-2H3 mast cells by measuring the β -hexosaminidase activity. In addition, the effects on interleukin (IL)-4 production and IL-5-dependent Y16 early B cell proliferation were investigated as well as their cytotoxic effects on RBL-2H3 cells.

Results: Among the seven isolated compounds, magnolialide attenuated the release of β -hexosaminidase from RBL-2H3 cells with an IC₅₀ value of 20.2 μ M, while the other compounds revealed no significant effects at concentrations tested. Furthermore, magnolialide significantly inhibited the IL-4 release with an IC₅₀ value of 18.1 μ M and IL-4 mRNA expression with an IC₅₀ value of 15.7 μ M in IgE-sensitized RBL-2H3 cells. In addition, the inhibition of IL-5-dependent proliferation of early B cells (Y16 cells) by magnolialide was demonstrated with an IC₅₀ value of 18.4 μ M.

Conclusion: These results suggest that the magnolialide might be a candidate for the treatment of IgE-mediated hypersensitivity responses such as atopic dermatitis and asthma by inhibiting mast cell degranulation, the IL-4 production, and IL-5-dependent early B cell proliferation, key factors in the development and amplification of type I hypersensitivity reactions.

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1. Introduction

Type I hypersensitivity responses have been implicated in the cause of several diseases including allergic asthma and atopic dermatitis (Fuller et al., 1986). These responses are characteristically associated with immunoglobulin E (IgE) and mast cells play a crucial role in the development of IgE-mediated hypersensitivity reactions (Hu et al., 2007). Mast cells possess a high-affinity IgE receptor (Fc ϵ RI) that binds to IgE (Onose et al., 2008). In type I hypersensitivity reactions, allergen interacts with mast cell-bound IgE and activates cells by cross-linking IgE–Fc ϵ RI complexes

(Gounni, 2006). The activation of mast cells following the engagement of IgE–Fc ϵ RI complex by antigens results in a process called degranulation (Matsubara et al., 2004), and the activated mast cells release preformed molecules from its cytoplasmic granules, such as histamines, proteoglycans, serine proteases, and β -hexosaminidase, which are capable of inducing bronchoconstriction, mucus secretion, and mucosal edema, all features of asthma (Bradding et al., 2006). Degranulation of mast cells with the resulting release of histamine and β -hexosaminidase elicits the production of cytokines including interleukin (IL)-4, which activates pre-T helper (pre-Th) cells and transforms into T helper 2 (Th2) cells (Biedermann et al., 2004), and the overexpression of the IL-4 affects Th2 differentiation and IgE class switch (Guo et al., 2004). IL-4 levels are elevated in patients with severe asthma and atopic dermatitis, and this increase in the IL-4 levels also correlates with the severity of these diseases (Cho et al., 2005;

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Dhar et al., 2005). IL-5, one of the main pro-inflammatory mediators produced by Th2 cells, stimulates eosinophilic inflammation and mucus production (Sampson, 2001) and induces early B cell proliferation and immunoglobulin secretion (Togawa et al., 2001). IL-5 acted as a stimulator of immunoglobulin productions from activated B cells and the proliferation of eosinophils, and its synthesis is up-regulated by mast cells activated in type I hypersensitivity allergic reactions (Kouro and Takatsu, 2009; Sampson, 2001).

The rat basophilic leukemia mast cell line (RBL-2H3) has been commonly used as an *in vitro* model for IgE-mediated responses in mast cells (Suzuki et al., 2001). RBL-2H3 cells produce high-affinity FcεRI receptors for IgE on the cell-surface membrane and aggregation of the IgE–FcεRI complex caused by antigens activates various cascades of intracellular events that result in mast cell degranulation and the consequent release of chemical mediators of IgE-mediated responses (Qu et al., 2005). Y16 cell is characterized as an early B cell and has been used for an IL-5-dependent early B cell proliferation assay (Sato et al., 1994).

Laurus nobilis L. (Lauraceae), commonly known as Bay, Sweet Bay, True Laurel, or Roman Laurel, is widely spread in the Mediterranean area and Europe (Dall'Acqua et al., 2006), and leaves of this plant are used as a folk medicine in the treatment of arthritis (Lev and Amar, 2000), rheumatic pains (Bruni et al., 1997), skin inflammation (Pieroni et al., 2004), and asthma (Loi et al., 2004). Previously, phytochemical studies have led to isolation of several classes of secondary metabolites of *Laurus nobilis* L. including glycosylated flavonoids (Fiorini et al., 1998), megastigmane and phenolic components (De Marino et al., 2004), monoterpenes, and sesquiterpenes (Dall'Acqua et al., 2006; Julianti et al., 2012). Sesquiterpene lactones, most diverse metabolites of bay leaf, have been investigated for various pharmacological effects such as neuroprotective (Ham et al., 2011), cytotoxic (Barla et al., 2007), and anti-inflammatory activities (Hall et al., 1979), but little has been investigated to validate the scientific evaluation of *Laurus nobilis* L. for type I hypersensitivity disorders such as atopic dermatitis and asthma. In this study, the effects of sesquiterpenes isolated from the leaves of *Laurus nobilis* L. especially focusing on mast cell degranulation, IL-4 production in RBL-2H3 cells and IL-5-dependent B cell proliferation were investigated.

2. Materials and methods

2.1. Chemicals and reagents

Dulbecco's modified Eagle's medium (DMEM), RPMI-1640 medium, monoclonal anti-dinitrophenyl (DNP) IgE mouse antibody, 4-nitrophenyl N-acetyl-β-glucosaminide, DNP-conjugated human serum albumin (HSA), and 2-mercaptoethanol, doxorubicin hydrochloride (purity ≥ 98%), cromoglycate (purity ≥ 95%), ketotifen fumarate salt (purity ≥ 99%), and tyrphostin AG 490 (purity ≥ 98%) were purchased from Sigma-Aldrich (St. Louis, MO, USA). Fetal bovine serum (FBS) was obtained from Invitrogen (Carlsbad, CA, USA). Prime RT-PCR premix™ was purchased from Genet Bio (Seoul, Korea), and easy-BLUE™ total RNA extraction kits were purchased from iNTRON Biotechnology (Kyunggi, Korea).

2.2. Plant material

The leaves of *Laurus nobilis* L. were purchased from Orege Forest Agricultural and Food Products Foreign Trade, Turkey, and identified by Dr. Yeong-han Kwon of Korea National Arboretum. A voucher specimen (NPRI-Q003) has been deposited in Natural Products Research Institute, Seoul National University.

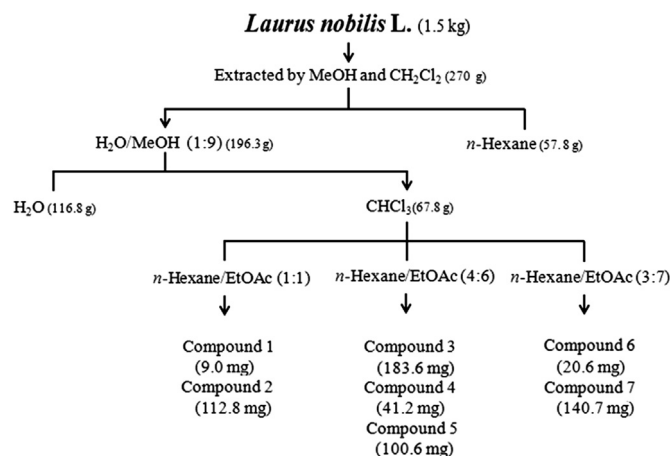


Fig. 1. A schematic overview of the extraction steps.

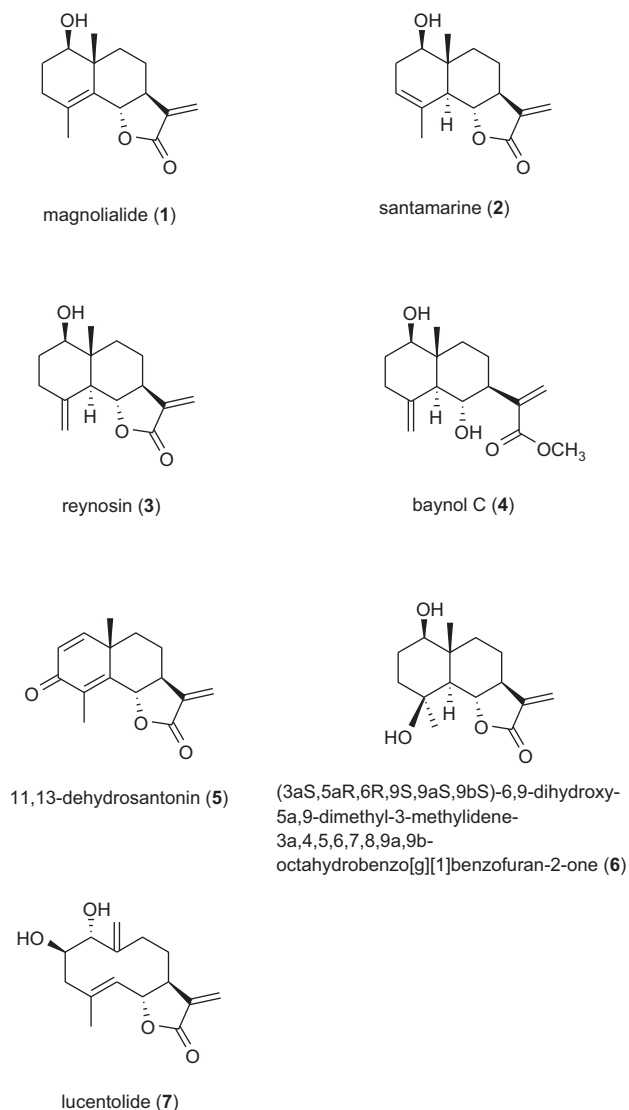


Fig. 2. Chemical structures of isolated compounds from *Laurus nobilis*. Compound 1: magnolialide, compound 2: santamarine, compound 3: reynosin, compound 4: baynol C, compound 5: 11,13-dehydrosantonin, compound 6: (3aS,5aR,6R,9S,9aS,9bS)-6,9-dihydroxy-5a,9-dimethyl-3-methylidene-3a,4,5,6,7,8,9a,9b-octahydrobenzo[g][1]benzofuran-2-one, and compound 7: lucentolide.

2.3. Extraction and isolation

To prepare a sample, 1.5 kg of the leaves of *Laurus nobilis* L. was air-dried and were extracted repeatedly with CH_2Cl_2 ($2\text{L} \times 2$) and MeOH ($2\text{L} \times 2$) (Fig. 1). The combined filtrates were evaporated under reduced pressure using a rotary vacuum evaporator to yield a dark green gum (dry wt, 270 g) which was stored in the dark at -20°C until use. The extract was partitioned between *n*-hexane (dry wt, 57.8 g) and MeOH/ H_2O (9:1) (dry wt, 196.3 g), then the latter was repartitioned between H_2O (dry wt, 116.8 g) and CHCl_3 (dry wt, 67.8 g). The inhibition of mast cell degranulation was performed and bioactivity-guided fractionation procedure was performed to obtain seven isolates (Fig. 2) as follows, the CHCl_3 fraction was subjected to silica normal-phase vacuum flash chromatography with *n*-hexane and ethyl acetate gradients (elution order: *n*-hexane, *n*-hexane/EtOAc (9:1 $\rightarrow$ 3:7), EtOAc, acetone) and finally MeOH. The sub-fraction (970.0 mg) eluted with *n*-hexane/EtOAc (1:1) mixture was subjected to semi-preparative high performance liquid chromatography (HPLC; YMC ODS-A column, $5\ \mu\text{m}$, $1 \times 25\ \text{cm}$, MeOH/ H_2O (6:4), 2 mL/min, RI detector). The final purification of compound was accomplished by HPLC (YMC ODS-A column, $5\ \mu\text{m}$, $1 \times 25\ \text{cm}$, MeOH/ H_2O (1:1), 2 mL/min, RI detector) to yield 9.0 mg and 112.8 mg of magnolialide (**1**: retention time, 37.2 min) (Elferaly et al., 1979), santamarine (**2**: 24.3 min) (Devivar and Jimenez, 1965). The sub-fraction (1220.0 mg) eluted with *n*-hexane/EtOAc (4:6) mixture was subjected to semi-preparative HPLC (YMC ODS-A column, $5\ \mu\text{m}$, $1 \times 25\ \text{cm}$, MeOH/ H_2O (55:45), 2 mL/min, RI detector), then further purified by HPLC (YMC ODS-A column, $5\ \mu\text{m}$, $1 \times 25\ \text{cm}$, MeOH/ H_2O (1:1), 2 mL/min, RI detector) to yield 183.6 mg, 41.2 mg, and 100.6 mg of reynosin (**3**: 15.0 min) (Fraga, 2002), baynol C (**4**: 24.2 min) (Rodrigues et al., 1978), and 11,13-dehydrosantonin (**5**: 30.0 min) (Iida et al., 1993), respectively. Finally, the sub-fraction (381.1 mg) eluted with *n*-hexane/EtOAc (3:7) mixture was subjected to semi-preparative HPLC (YMC ODS-A column, $5\ \mu\text{m}$, $1 \times 25\ \text{cm}$, MeOH/ H_2O (1:1), 2 mL/min, RI detector), then further purified by HPLC (YMC ODS-A column, $5\ \mu\text{m}$, $1 \times 25\ \text{cm}$, ACN/ H_2O (15:85), 2 mL/min, RI detector) to yield 20.6 mg of (3a,5,5aR,6R,9S,9aS,9bS)-6,9-dihydroxy-5a,9-dimethyl-3-methylidene-3a,4,5,6,7,8,9a,9b-octahydrobenzo[g][1]benzofuran-2-one (**6**: 22.3 min) (Julianti et al., 2012) and 140.7 mg of lucentolide (**7**: 27.0 min) (Marco et al., 1997). Based on electrospray ionization mass spectrometry and ^1H NMR and ^{13}C NMR spectral data, the purified compounds exhibited a comparable spectroscopy to previously published values.

2.4. Cell culture

Rat basophilic leukemia (RBL-2H3) cells (ATCC no. CRL-2256) were cultured at 37°C , 5% CO_2 in DMEM supplemented with 10% (*v/v*) fetal bovine serum, 100 U/mL penicillin, and 100 $\mu\text{g}/\text{mL}$ streptomycin.

2.5. Cell viability test

MTT (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide) assay was conducted to examine cell viability (Twentyman and Luscombe, 1987). RBL-2H3 cells (5×10^5 cells/mL, 100 $\mu\text{L}/\text{well}$) were plated into a 96-well plate. After 24 h of incubation, cells were incubated with various concentrations of 7 isolated compounds for 3 h and medium was replaced with MTT dissolved in phenol-red free medium (250 $\mu\text{g}/\text{mL}$) and incubated at 37°C for 4 h. The medium was carefully discarded and formazan was resuspended in 200 μL of dimethyl sulfoxide (DMSO). The absorbance was measured at 595 nm using a microplate reader. Values measured from untreated cells were considered to represent 100% viability.

2.6. Measurement of β -hexosaminidase release from RBL-2H3 cells

Release of β -hexosaminidase was measured based on the previously described method (Mastuda et al., 2002). Briefly, RBL-2H3 cells (5×10^5 cells/mL, 100 $\mu\text{L}/\text{well}$) seeded in 96-well plates were incubated with monoclonal mouse anti-dinitrophenyl IgE antibody (anti-DNP IgE) (1 $\mu\text{g}/\text{mL}$) for 24 h at 37°C . The IgE-sensitized RBL-2H3 cells were washed twice with PBS buffer to remove excess IgE in medium. After washing, the cells were pre-incubated with each isolated compound in 100 μL of fresh medium at 37°C for 20 min followed by 400 ng/mL of multivalent antigen, DNP-HSA for 1 hr. Supernatants (40 μL) were transferred to a 96-well plate and incubated for 1.5 h at 37°C with 50 μL of substrate solution (5 mM of 4-nitrophenyl N-acetyl- β -D-glucosaminide in 50 mM of citric acid buffer, pH 4.5). The reactions were quenched by the addition of 50 μL of stop solution (0.5 M $\text{Na}_2\text{CO}_3/\text{NaHCO}_3$, pH 10.0) and the absorbance at 405 nm was measured. Activity values measured from cells treated with anti-DNP IgE and DNP-HSA were considered to represent 100% of degranulation.

2.7. Measurement of IL-4 cytokine release from RBL-2H3 cells

RBL-2H3 cells (5×10^5 cells/mL, 300 $\mu\text{L}/\text{well}$) were sensitized for 24 h with 1 $\mu\text{g}/\text{mL}$ of anti-DNP IgE in 48-well plates. Later, the IgE-sensitized RBL-2H3 cells were treated with each isolated compound for 3 h followed by DNP-HSA for an additional 2 h. The amount of IL-4 released in culture medium was measured using Rat IL-4 Platinum ELISA™ kit (eBioscience, USA), according to the manufacturer's instruction.

2.8. Measurement of IL-4 mRNA expression in RBL-2H3 cells by reverse transcription-polymerase chain reaction (RT-PCR)

RBL-2H3 cells (5×10^5 cells/mL, 500 $\mu\text{L}/\text{well}$) were cultured for 24 h in 24-well plates with anti-DNP IgE (1 $\mu\text{g}/\text{mL}$). The RBL-2H3 cells were washed two times with PBS buffer and replaced with fresh media, then incubated with each isolated compound for 3 h at 37°C followed by DNP-HSA at 400 ng/mL for an additional 1 h. Total RNA was isolated with easy-BLUE™ (iNtRON Biotechnology, Korea), according to the manufacturer's instruction. cDNA synthesis was performed from 500 ng of total RNA by using ONE-STEP RT-PCR PreMix kit (iNtRON Biotechnology, Korea). The following primer pairs were synthesized. (Bioneer, Korea). IL-4, 5'-GGGTGCTTCGCAATTTTACTT-3' (sense) and 5'-ACCGAGAACCCC AGACTTGTT-3' (antisense); and β -actin, 5'-TCTGTGTGGATTGGTGGCTCTA-3' (sense) and 5'-CTGCTTGCTGATCCACATCTG-3' (antisense). The denaturation, annealing, extension, and cycle conditions were as follows, IL-4: 94°C for 45 s, 58°C for 45 s, 72°C for 45 s and 34 cycles; β -actin: 94°C for 45 s, 55°C for 45 s, 72°C for 45 s and 30 cycles. The PCR reaction was performed with a 2720 Thermal Cycler (Applied Biosystems, USA). Aliquots of the PCR reactions were electrophoresed in 1.5% (*w/v*) agarose gels stained with SYBR Safe DNA gel stain (Invitrogen, USA). DNA bands were visualized with LAS 1000plus (FUJIFILM, Japan).

2.9. Y16 early B cell proliferation assay

Y16 cells (Takaki et al., 1990) (5×10^5 cells/mL, 100 $\mu\text{L}/\text{well}$) were plated to a 96-well microplate with RPMI-1640 supplemented with 24 mM NaHCO_3 , 100 U/mL penicillin, 100 $\mu\text{g}/\text{mL}$ streptomycin, 50 μM 2-mercaptoethanol, and 10 U/mL of IL-5 (Prospec, Israel). Cells were treated with isolated compounds or a positive control compound, tyrphostin AG490 (Sigma-Aldrich, USA), for 48 h. MTT assay was performed to measure cell proliferation by measuring the absorbance at 540 nm using a microplate reader.

2.10. Statistical analysis

All data were presented as the mean $\pm$ S.D. of at least three independent experiments performed in triplicate. Statistical significance was determined using GraphPad Prism (GraphPad Software, USA). The differences among groups were evaluated by one-way analysis of variance (ANOVA) with Bonferroni's multiple comparison method. p -value less than 0.05 was considered to be statistically significant.

3. Results

The combined MeOH and CH_2Cl_2 extract of leaves of *Laurus nobilis* L. was subfractionated (Fig. 1) and seven isolates (Fig. 2) were obtained from CHCl_3 sub-fraction. Cytotoxic effects of 7 compounds were determined after 24 h of treatment in RBL-2H3 cells using the MTT assay at various concentrations up to 100 μM (Fig. 3). In this experiment, bayanol C and lucentolide revealed relatively strong cytotoxic effects at a concentration of 100 μM (70.4% survival and 79.2% survival, respectively), whereas magnolialide revealed a relatively weak cytotoxic effect (91.1% survival at 100 μM), and the other compounds showed a little or non-cytotoxic ($> 95.2\%$ survival at 100 μM). A positive control compound, doxorubicin, revealed a cytotoxic effect with an IC_{50} value of 29.2 μM . The degranulation of RBL-2H3 mast cells, after sensitization with anti-DNP IgE and reaction with DNP-HSA, was evaluated by measuring the activity of β -hexosaminidase released. Treatment with magnolialide inhibited the release of β -hexosaminidase with an IC_{50} value of 20.2 μM , and mast cell stabilizers, cromoglycate and ketotifen, used as positive control compounds, revealed IC_{50} values of 74.3 and 58.4 μM , respectively (Fig. 4). The effect of magnolialide on the release of IL-4 was determined by measuring the IL-4 concentration

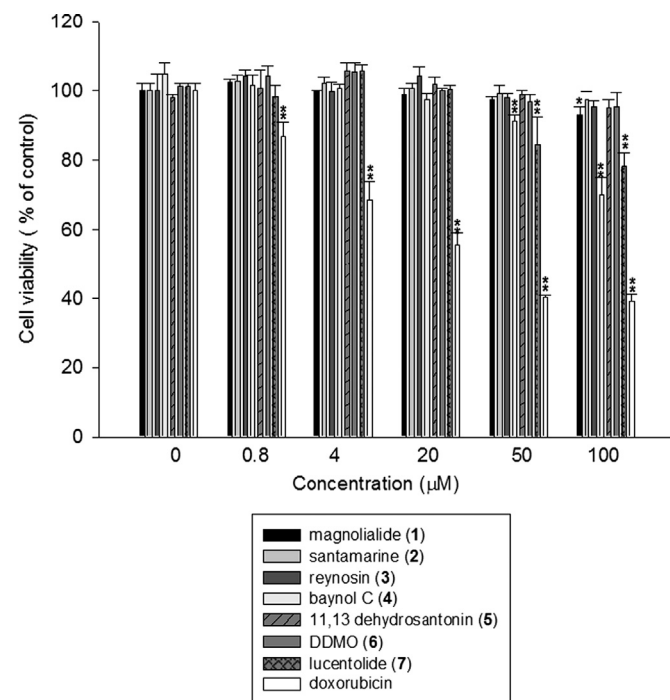


Fig. 3. Cytotoxic effects of compounds isolated from *Laurus nobilis* on RBL-2H3 cells. RBL-2H3 cells were treated with each isolated compound or doxorubicin (a positive control compound) for 24 h and cell viability was determined by MTT assay. All data were obtained from three independent experiments performed in triplicate. Data represent the mean $\pm$ S.D. of three independent experiments, * $p < 0.05$, ** $p < 0.01$, compared with vehicle treatment group. DDMO: (3aS,5aR,6R,9S,9aS,9bS)-6,9-dihydroxy-5a,9-dimethyl-3-methylidene-3a,4,4,5,6,7,8,9a,9b-octahydrobenzo[g][1]benzofuran-2-one.

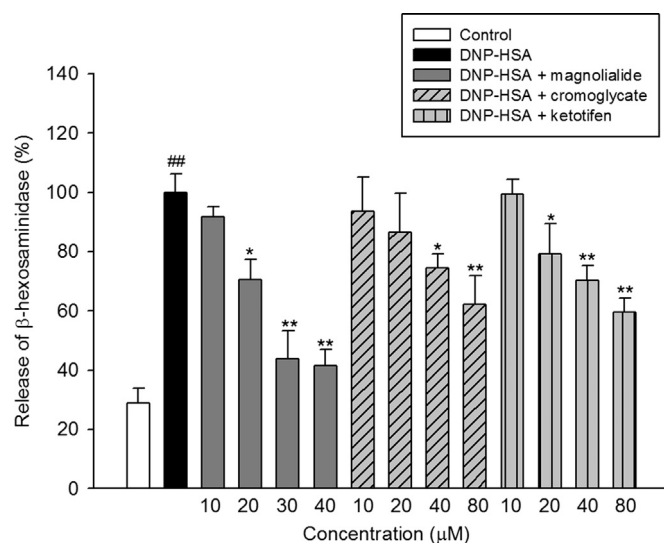


Fig. 4. Inhibitory effects of magnolialide on β -hexosaminidase release from RBL-2H3 cells. Values measured from cells treated with anti-DNP IgE and DNP-HSA were considered to represent 100% of degranulation. Cells were treated with magnolialide or positive control compounds (cromoglycate and ketotifen) for 20 min followed by DNP-HSA for an hour. Data represent the mean $\pm$ S.D. of three independent experiments; ## $p < 0.01$, compared with control group (anti-DNP IgE treatment alone); * $p < 0.05$ and ** $p < 0.01$, compared with anti-DNP IgE and DNP-HSA treatment, respectively.

in the cultured medium by using the ELISA kit. Magnolialide inhibited the IL-4 release with an IC_{50} value of 18.1 μM ; the positive control compounds, cromoglycate and ketotifen, also inhibited IL-4 release with IC_{50} values of 7.4 and 15.2 μM , respectively (Fig. 5). In addition, the effect on the expression of IL-4 mRNA was investigated. Magnolialide reduced the IL-4 mRNA expression with an IC_{50} value of 15.7 μM , cromoglycate with an IC_{50} value of 8.1 μM , and ketotifen with an IC_{50} value of 19.4 μM (Fig. 6). Other six isolated compounds santamarine, reynosin, bayanol C, 11,13-dehydrosantonin, (3aS,5aR,6R,9S,9aS,9bS)-6,9-dihydroxy-5a,9-dimethyl-3-methylidene-3a,4,4,5,6,7,8,9a,9b-octahydrobenzo[g][1]benzofuran-2-one, and lucentolide did not show any significant inhibitory effects at concentrations tested. In addition, the inhibitory effect of magnolialide on IL-5-stimulated Y16 cell proliferation was examined and inhibition was also observed in a dose dependent manner (Fig. 7). The IC_{50} values of magnolialide and of the positive control compound tyrphostin AG490 were 18.4 μM and 24.9 μM , respectively.

4. Discussion

The mast cell has been reported to associate with skin inflammation (Harvima and Nilsson, 2011) and asthma (Carroll et al., 2002), and mast cell degranulation along with several cytokines produced from activated mast cells is considered to play pivotal roles in these responses (Bradding et al., 1994). In this study, we investigated several isolated compounds from the leaves of *Laurus nobilis* L. which was traditionally used for skin disease and asthma therapy, and especially focused on their activity against IgE-mediated hypersensitivity responses from mast cells. First, we isolated seven sesquiterpene lactones from the leaves of *Laurus nobilis* L., and found that magnolialide significantly inhibited IgE-mediated degranulation from RBL-2H3 mast cells. No significant toxicity was observed at the range of concentrations tested in this experiment. The inhibition of mast cell degranulation was compared with cromoglycate (Theoharides et al., 1980) and ketotifen (Juanola et al., 1998), mast cell stabilizers. The inhibitory effects of

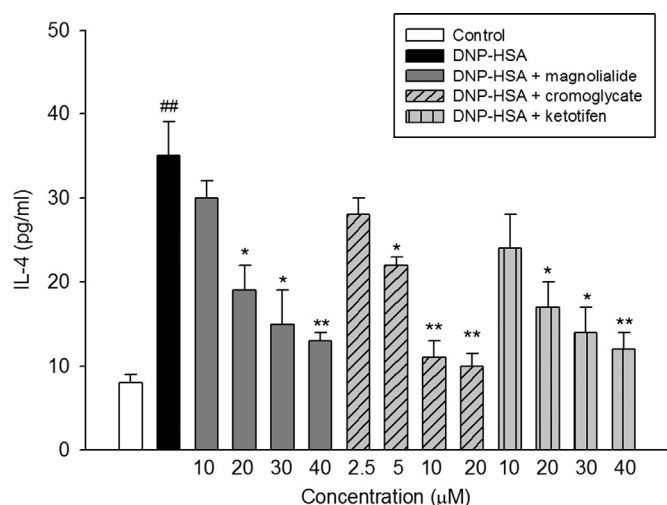


Fig. 5. Inhibitory effect of magnolialide on IL-4 release from RBL-2H3 cells. Cells were treated with magnolialide or positive control compounds (cromoglycate and ketotifen) for 3 h followed by DNP-HSA for 2 h. The release of IL-4 was measured by ELISA as described in materials and methods. Data represent the mean $\pm$ S.D. of three independent experiments; ^{##} $p < 0.05$, compared with control group (anti-DNP IgE treatment alone); ^{*} $p < 0.05$ and ^{**} $p < 0.01$, compared with anti-DNP IgE and DNP-HSA treatment, respectively.

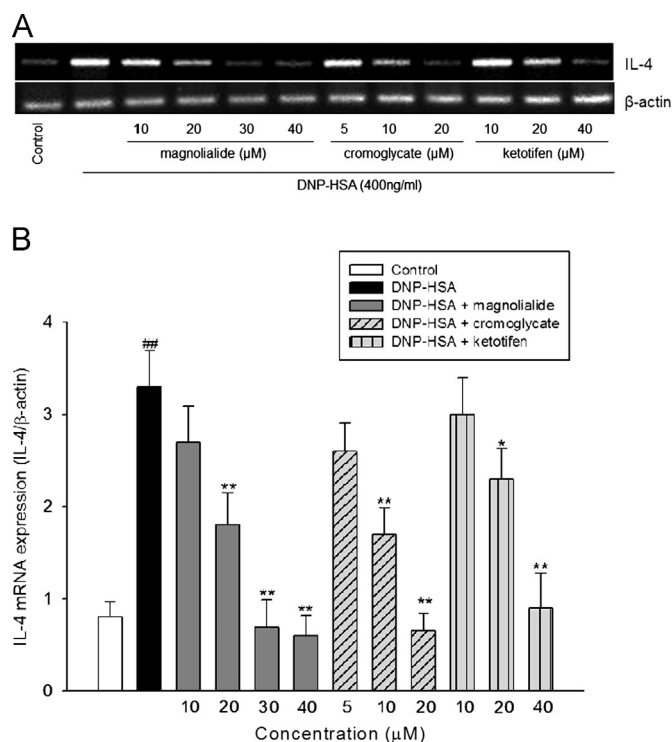


Fig. 6. Inhibitory effect of magnolialide on the expression of IL-4 mRNA in RBL-2H3 cells. Cells were treated with magnolialide or positive control compounds (cromoglycate and ketotifen) for 3 h followed by DNP-HSA for an hour. (A) Inhibitory effects of magnolialide, cromoglycate, and ketotifen on the expression of IL-4 mRNA in RBL-2H3 cells. (B) Quantitative representation of expression of IL-4 and β -actin mRNA using Image J software. Data represent the mean $\pm$ S.D. of three independent experiments; ^{##} $p < 0.01$, compared with control group (anti-DNP IgE treatment alone); ^{*} $p < 0.05$ and ^{**} $p < 0.01$, compared with anti-DNP IgE and DNP-HSA treatment, respectively.

these two agents were relatively weak compared to magnolialide (Table 1).

Besides degranulation during IgE-mediated reactions, mast cells also concomitantly synthesize and release a variety of cytokines,

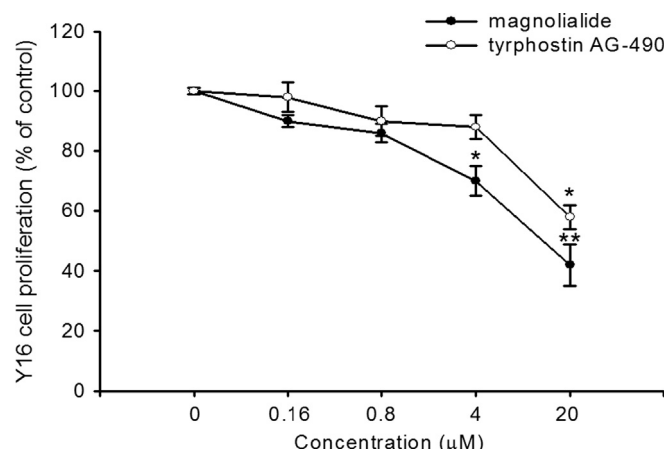


Fig. 7. Inhibitory effects of magnolialide on IL-5-dependent proliferation of Y16 cells. Y16 cells were cultured with IL-5 with or without magnolialide or tyrphostin AG 490 for 48 h. Cell growth was measured by MTT assay. IL-5 treated cells with vehicle treatment were used as representing 100% cell proliferation. Data represent the means $\pm$ S.D. of three independent experiments; ^{*} $p < 0.05$ and ^{**} $p < 0.01$, compared with vehicle treatment group.

including IL-4, IL-5, IL-13, and tumor necrosis factor- α , during the late-phase reaction (Metcalf et al., 1997). Among those cytokines, IL-4 derived from mast cells is known to be important in the mucosal inflammatory responses and in the development and amplification of IgE-dependent allergic responses (Bradding et al., 1994). The reduction of IL-4 production by cromoglycate and ketotifen in IgE-mediated RBL-2H3 cells has been demonstrated (Matsubara et al., 2004). In the present study, IL-4 release and IL-4 mRNA expression levels in RBL-2H3 cells were significantly inhibited by magnolialide. In comparison with cromoglycate and ketotifen, magnolialide was less potent than cromoglycate but it has a similar inhibitory effect to ketotifen (Table 1). Thus, magnolialide might contribute to alleviate the inflammatory and late allergic symptoms by suppressing the expression and production of IL-4 in mast cells.

Besides IL-4, it was reported that IL-5, another pro-inflammatory cytokine, is produced by Th2 cells and activates mast cells (Bradding et al., 1994). The mast cell-derived IL-5 induces early B cell proliferation and subsequently increases in immunoglobulin secretion (Yamaguchi et al., 1988). In this study, treatment with magnolialide significantly inhibited the proliferation of IL-5-dependent Y16 early B cells, and the inhibitory effect was 1.3-times more potent than that of tyrphostin AG490, a control compound with a specific inhibitory effect against Janus kinase 2 (JAK2) which is critical for the anti-apoptotic effect induced by IL-5 (Pazdrak et al., 1998). These results demonstrated that magnolialide might be able to control IgE-mediated hypersensitivity reactions by not only suppressing the early phases of mast cell activation, but also suppressing subsequent IgE amplification by the IL-5-dependent proliferation of B cells.

The present study demonstrated for the first time that magnolialide isolated from the leaves of *Laurus nobilis* L. effectively inhibited the mast cell degranulation and IL-4 production in IgE-involved responses in RBL-2H3 mast cells, and suppressed the proliferation of IL-5-dependent Y16 early B cells. Some sesquiterpene lactones such as parthenin, frullanolide, and alantolactone are reported to cause allergic contact dermatitis, which is a type-IV T-cell-mediated reaction (Mitchell et al., 1970). However, several studies have reported the possible therapeutic effects of sesquiterpene lactones for a type-I IgE-mediated-hypersensitivity. An extract of the plant feverfew (*Tanacetum parthenium*), a rich source of sesquiterpene lactones, inhibited anti-IgE-induced histamine release from rat peritoneal mast cells (Hayes and Foreman, 1987). Also, sesquiterpene lactones such as isobutyrolyplenolin and seneciopylenin from *Centipeda minima* exhibited significant

Table 1

Inhibitory effects of compounds from *Laurus nobilis* L. on the β -hexosaminidase release and IL-4 production from antigen-induced RBL-2H3 cells, and IL-5-dependent proliferation of Y16 cells.

Compound	β -hexosaminidase release (IC ₅₀ $\pm$ SD, μ M)	IL-4 production (IC ₅₀ $\pm$ SD, μ M)		IL-5-dependent proliferation (IC ₅₀ $\pm$ SD, μ M)
		Release	mRNA expression	
Magnolialide (1)	20.2 $\pm$ 4.9	18.1 $\pm$ 4.1	15.7 $\pm$ 3.1	18.4 $\pm$ 4.2
Santamarine (2)	> 100	> 100	> 100	> 100
Reynosin (3)	> 100	> 100	> 100	> 100
Baynol C ^a (4)	> 20	> 20	> 20	> 20
11,13 Dehydrosantonin (5)	> 100	> 100	> 100	> 100
DDMO (6)	> 100	> 100	> 100	> 100
Lucentolide ^a (7)	> 20	> 20	> 20	> 20
Cromoglycate (control)	74.3 $\pm$ 5.5	7.4 $\pm$ 3.7	8.1 $\pm$ 4.0	n.d.
Ketotifen (control)	58.4 $\pm$ 3.3	15.2 $\pm$ 4.2	19.4 $\pm$ 4.9	n.d.
Tyrphostin AG490 (control)	n.d.	n.d.	n.d.	24.9 $\pm$ 3.9

The IC₅₀ values were determined in a semilogarithmic graph depicting the relationship between at least 4 different concentrations of compounds and the each percentage of inhibition.

n.d.: Not determined.

DDMO: (3aS,5aR,6R,9S,9aS,9bS)-6,9-dihydroxy-5a,9-dimethyl-3-methylidene-3a,4,5,6,7,8,9a,9b-octahydrobenzo[g][1]benzofuran-2-one.

^a Cytotoxic at a concentration higher than 20 μ M.

anti-allergic activity in passive cutaneous anaphylaxis (PCA) test with p.o. administration (Wu et al., 1991). It was also reported that parthenolide, a sesquiterpene lactone, suppressed IL-4 production in peripheral blood T cells by blocking NF- κ B binding to IL-4 promoter regulatory regions, suggesting a treatment of IL-4-mediated allergic inflammation (Li-Weber et al., 2002). In conclusion, our results suggest that magnolialide might have therapeutic potential for a type-I IgE-mediated allergic inflammatory disorder such as asthma and atopic dermatitis through a mast cell stabilizing effect and inhibition of IL-4 production and proliferation of IL-5-dependent early B cells, although further investigation of magnolialide involved in IL-4-mediated and IL-5-mediated signal transduction processes is warranted.

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Appendix A. Supporting information

Supplementary data associated with this article can be found in the online version at <http://dx.doi.org/10.1016/j.jep.2013.07.015>.

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Effects of Magnolialide Isolated from the Leaves of *Laurus nobilis* L. (Lauraceae) on Immunoglobulin E-Mediated Type I Hypersensitivity *in Vitro*

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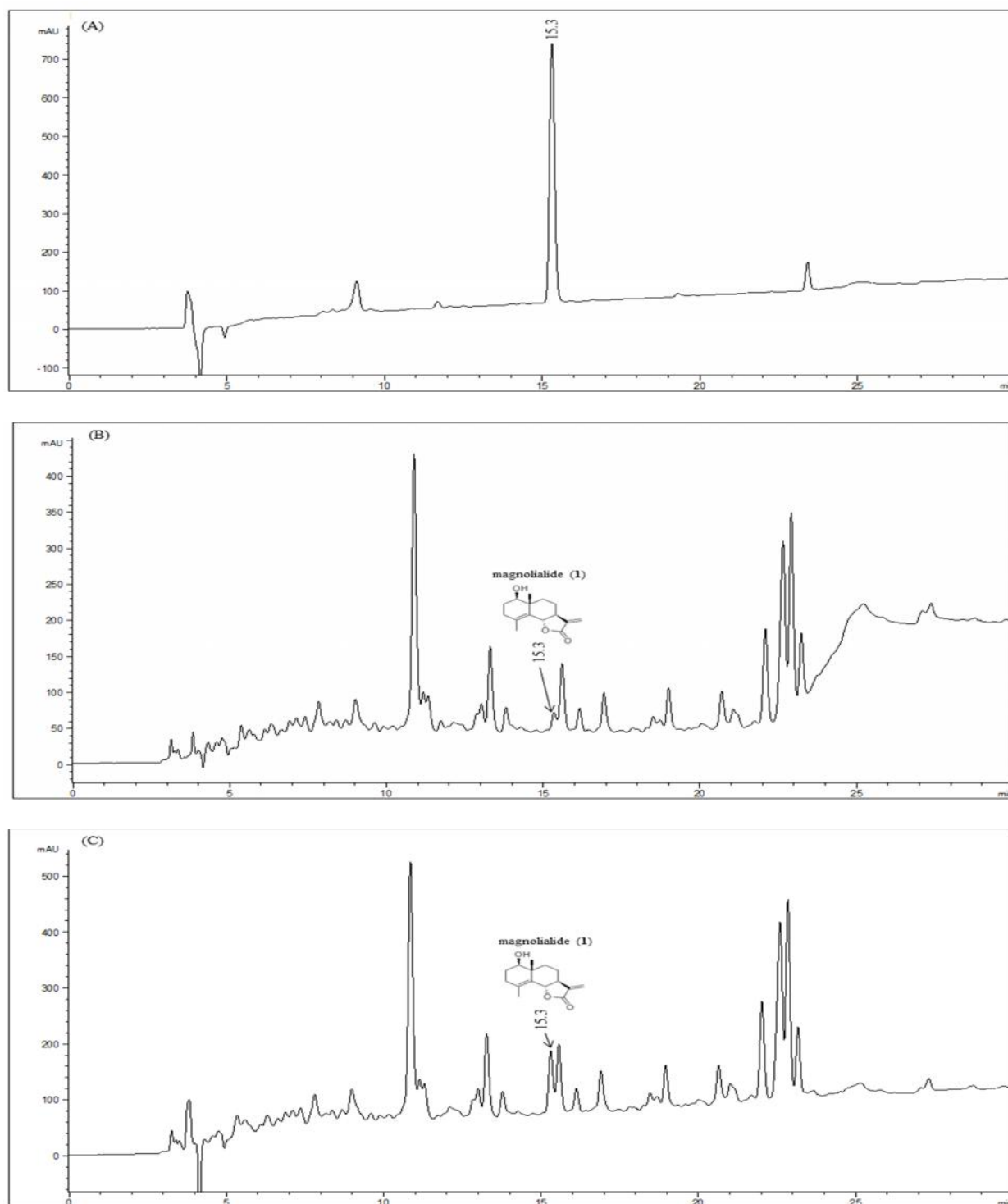
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Figure.S1. The HPLC chromatograms of magnolialide (**1**) (A), the total extract of the leaves of *Laurus nobilis* (B), and co-injection of magnolialide (**1**) with the total extract of the leaves of *Laurus nobilis* (C).



HPLC Condition:

The HPLC system consisted of an Agilent 1200 series HPLC equipped with an autosampler, column oven, binary pump, diode array detector (Agilent Technologies, Waldbronn, Germany) and degasser (Agilent Technologies, Tokyo, Japan). Chemstation software (Agilent Technologies, Avondale, CA, USA) was used to operate this HPLC-DAD system. Separation was performed on a Shiseido Capcell Pak C18 (5 μ m, 4.6 mm $\times$ 250 mm) analytical column. A gradient elution of A (water with 0.1% formic acid) and B (MeOH with 0.1% formic acid) was used (0 min, 50% B; 30 min, 100% B in A v/v). Ten-microliter of magnolialide (**1**) (1.8 mg/mL), total extract (10.6 mg/mL), or co-injection (total extract : magnolialide (**1**), 6:1, v/v) were directly injected to the HPLC system. The mobile phase flow rate was 0.7 mL/min, and the column temperature was set at 25 °C.