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Inhibitory effects of norlignans isolated from *Anemarrhena asphodeloides* on degranulation of rat basophilic leukemia-2H3 cells



Jong Phil Bak^{a,1}, Young Mi Cho^{b,1}, Inhye Kim^b, Dae Won Park^b, Jung Eun Kwon^b,
 Yong Joon Jeong^b, Jong Hwan Kwak^{c,*}, Se Chan Kang^{b,*}

^a The Clinical Center for Bio-industry, Semyung University, Jecheon 390-711, South Korea

^b Department of Oriental Medicine Biotechnology, College of Life Sciences, Kyung Hee University, Yongin 17104, South Korea

^c School of Pharmacy, Sungkyunkwan University, Suwon 440-746, South Korea

ARTICLE INFO

Article history:

Received 29 August 2016

Received in revised form 5 October 2016

Accepted 13 October 2016

Keywords:

Anemarrhena asphodeloides

Norlignans

Rat basophilic leukemia cell (RBL-2H3)

Isolated rat primary mast cell (PMC)

Anti-allergy

ABSTRACT

Anemarrhena asphodeloides is known to suppress inflammation and lower various fevers. To determine the active component of *A. asphodeloides*, ethanol (EtOH) extract of *A. asphodeloides* rhizomes was fractionized. The compounds isolated from the dichloromethane (CH₂Cl₂) soluble fraction were identified as 4'-O-methylnyasol (1), nyasol (2), 3''-methoxynyasol (3), 3''-hydroxy-4''-methoxy-4''-dehydroynyasol (4), 4-hydroxybenzaldehyde (5), and 4-hydroxyacetophenone (6). The four norlignans (1–4) potently inhibited the release of β-hexosaminidase from immunoglobulin E (IgE)/dinitrophenol-conjugated bovine serum albumin (DNP-BSA)-treated rat basophilic leukemia (RBL)-2H3 and A23187 plus phorbol 12-myristate 13-acetate co-treated isolated rat primary mast cells, as markers of degranulation and histamine release. The intraperitoneal treatment with the EtOH extract significantly suppressed the fetal reaction, and serum histamine release induced by compound 48/80 in mice. These results suggest that the four active norlignan compounds and the EtOH extract of *A. asphodeloides* may have potential to be developed as medicines for the treatment of allergies by inhibiting the activation of mast cells.

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1. Introduction

An allergy is a hypersensitivity immune reaction, which results in inflammation and tissue damage. The mast cell is a central player in allergic diseases [1]. Allergies can be classified as humoral or cellular immune responses depending on the immune cells involved in the reaction. The humoral immune response is further categorized into three types including an immediate reaction (type 1), a cytotoxic reaction (type 2), and an immune complex-mediated reaction (type 3). In general, a type 1 allergic reaction results in anaphylaxis, which is induced by degranulation of mast cells, as is the case in atopic dermatitis, rhinitis, and asthma. In particular, immunoglobulin E (IgE) plays a key role in type 1 allergic reactions. The IgE molecule activates mast cells, which degranulate allergic mediators including histamine and serotonin [2–4]. This action induces a variety of biological processes including inflammation of surrounding tissues, vasodilation, mucous secretion, and bronchoconstriction [2–4]. The

β-hexosaminidase enzyme, which is stored in the secretory granules of mast cells, is released concomitantly with histamine when mast cells are immunologically activated [5]. Therefore, the release of β-hexosaminidase in culture media has been used as a critical marker of mast cell degranulation [6,7].

Natural herbs are used as traditional medicines for the treatment of various disorders. In particular, they have received considerable attention as potential medicinal agents for human allergies [8–13]. The dried rhizomes of *Anemarrhena asphodeloides* (Liliaceae) are well-known traditional Chinese medicine used as an antipyretic, diuretic, sedative, and antitussive [14]. Previous studies of *A. asphodeloides* have investigated various biological activities including antiinflammatory, antipyretic, hypoglycemic, antidiabetic, estrogenic, antiangiogenesis, antiviral, and antifungal [14–18]. In addition, the antiallergic activity of major steroidal saponins isolated from the rhizomes of *A. asphodeloides* was reported without any bioassay-guided fractionation [19]. Recently, we reported that the dichloromethane (CH₂Cl₂) fraction obtained from the ethanol (EtOH) extract of *A. asphodeloides* displayed *in vitro* antiallergic activity and we verified nyasol as an active ingredient [20]. In this study, we performed further activity-guided fractionation and purification of the CH₂Cl₂ fraction from the EtOH

* Corresponding authors.

E-mail addresses: jhkwak@skku.edu (J.H. Kwak), sckang@khu.ac.kr (S.C. Kang).

¹ These authors contributed equally to this work.

extract of *A. asphodeloides*, which yielded four norlignans as well as two phenolics, 4-hydroxybenzaldehyde and 4-hydroxyacetophenone phenols. Here, we report the isolation of three norlignans (**1**, **3**, and **4**), nyasol (**2**), and two phenolic compounds (**5**, **6**) from *A. asphodeloides*. In addition, we investigated their inhibitory effects on β -hexosaminidase release from rat basophilic leukemia (RBL)-2H3 cells treated with dinitrophenol-conjugated bovine serum albumin (DNP-BSA) or A23187 plus phorbol 12-myristate 13-acetate (PMA), as a marker of the degranulation activity of the isolated compounds. Finally, we determined the *in vivo* anti-allergic activities of the EtOH extract of *A. asphodeloides*.

2. Materials and methods

2.1. Plant material

The rhizomes of *A. asphodeloides* were purchased in October 2006 from a traditional medicine market at Kyung-dong yak-ryong-si in Seoul, Korea, and a voucher specimen was deposited at Kyung Hee University (No. NMR-CA03-062).

2.2. Instruments and reagents

Nuclear magnetic resonance (NMR) experiments were performed using a Varian Unity Inova 500 spectrometer with the usual pulse sequences. The electron ionization mass spectrometry (EIMS) data were obtained using a HP 6890 series gas chromatograph (GC) system equipped with a 5973 mass selective detector. Column chromatography was conducted on Si gel 60 (Merck, 230–400 mesh) and Sephadex LH-20 (lipophilic Sephadex, Sigma-Aldrich, 25–100 μ). High-performance liquid chromatography (HPLC) and recycling HPLC were performed using a Phenomenex Luna 10 μ C18 (2) column (250 $\times$ 10 mm) and Jaigel-GS310 column, respectively with an ultraviolet (UV) detector (254 nm).

2.3. Extraction and isolation

The dried rhizomes of *A. asphodeloides* were extracted thrice with EtOH at room temperature. The EtOH extract was evaporated to dryness under reduced pressure, and the residue was suspended in distilled water, and then consecutively partitioned with *n*-hexane, CH_2Cl_2 , ethyl acetate (EtOAc), and *n*-butanol (BuOH). The extract was separated using bioassay-guided chromatographic fractionation. The CH_2Cl_2 extract, which showed inhibitory activity against antigen-induced degranulation in mast cells, was subjected to column chromatography on silica gel with sequential elution using *n*-hexane/ CH_2Cl_2 /MeOH (10:10:1, 10:10:2, and 10:10:3) and CH_2Cl_2 /MeOH (5:1 and 1:1) to obtain sub-fractions (D1–D7). The first sub-fraction (D1) was chromatographed on silica gel eluting sequentially with *n*-hexane/EtOAc (7:1, 5:1, 3:1, and 1:1) and the resulting fourth fraction was further separated using Sephadex LH-20 column chromatography (CH_2Cl_2 /MeOH, 2:3) to yield compound **1**. Fractions D2 and D3 were combined and then re-chromatographed on silica gel and Sephadex LH-20, eluting with *n*-hexane/EtOAc (5:1, 3:1, and 1:1) and CH_2Cl_2 /MeOH (2:3), respectively to obtain compound **2** and a mixture of compounds **3–6**. Compounds **3** and **4** were separated by C18 reversed-phase HPLC (75% MeOH) and recycling HPLC (Jaigel, GS310) with MeOH as the eluent, and compounds **5** and **6** were obtained by C18 reversed-phase HPLC (75% MeOH) and silica gel column chromatography (*n*-hexane/EtOAc, 3:1).

The 4'-O-methylnyasol (**1**) spectral data. EIMS: m/z 266 [M^+], 251, 235, 207, 158, 145, 121, 107. Proton (^1H)-NMR (CDCl_3 , 500 MHz) δ : 3.83 (3H, s, OCH_3), 4.53 (1H, dd, J = 10.0, 6.0 Hz, H-3), 5.15–5.22 (2H, m, H-5), 5.70 (1H, dd, J = 11.5, 10.0 Hz, H-2), 6.04 (1H, ddd, J = 17.5, 10.0, 6.0 Hz, H-4), 6.56 (1H, d, J = 11.0 Hz, H-1), 6.80 (2H, d,

J = 8.5 Hz, H-3'', 5''), 6.89 (2H, d, J = 8.5 Hz, H-3', 5'), 7.13 (2H, d, J = 8.5 Hz, H-2'', 6''), 7.25 (2H, d, J = 8.5 Hz, H-2', 6'). ^{13}C NMR (CDCl_3 , 125 MHz) δ : 55.5 (OCH_3), 113.9 (C-3'), 115.3 (C-5), 115.6 (C-3'', 5''), 128.9 (C-1), 129.1 (C-2'', 6''), 129.9 (C-1'), 130.1 (C-2', 6'), 131.9 (C-2), 135.9 (C-1''), 141.0 (C-4), 154.3 (C-4''), 158.8 (C-4').

The nyasol (**2**) spectral data. EIMS: m/z 252 [M^+], 237, 207, 158, 145, 131, 107. ^1H NMR (CDCl_3 , 500 MHz) δ : 4.51 (1H, dd, J = 10.0, 5.8 Hz, H-1), 5.15–5.21 (2H, m, H-5), 5.70 (1H, dd, J = 11.5, 10.0 Hz, H-2), 6.03 (1H, ddd, J = 17.0, 10.8, 6.0 Hz, H-4), 6.54 (1H, d, J = 12.0 Hz, H-1), 6.79 (2H, d, J = 8.5 Hz, H-3'', 5''), 6.81 (2H, d, J = 8.0 Hz, H-3', 5'), 7.12 (2H, d, J = 8.5 Hz, H-2'', 6''), 7.19 (2H, d, J = 8.0 Hz, H-2', 6'). ^{13}C NMR (CDCl_3 , 125 MHz) δ : 115.3 (C-5), 115.4 (C-3', 5'), 115.6 (C-3'', 5''), 128.8 (C-1), 129.1 (C-2'', 6''), 130.1 (C-1'), 130.3 (C-2', 6'), 132.0 (C-2), 135.9 (C-1''), 141.0 (C-4), 154.3 (C-4''), 154.8 (C-4').

The 3''-methoxynyasol (**3**) spectral data. EIMS: m/z 282 [M^+], ^1H NMR (CDCl_3 , 500 MHz) δ : 3.85 (3H, s, OCH_3), 4.48 (1H, dd, J = 9.7, 6.2 Hz, H-3), 5.16 (1H, dt, J = 10.2, 1.5 Hz, H-5a), 5.19 (1H, dt, J = 17.2, 1.5 Hz, H-5b), 5.68 (1H, dd, J = 11.4, 10.0 Hz, H-2), 6.02 (1H, ddd, J = 17.2, 10.2, 6.0 Hz, H-4), 6.53 (1H, d, J = 11.4 Hz, H-1), 6.72 (1H, d, J = 1.8 Hz, H-2''), 6.75 (1H, dd, J = 8.1, 1.8 Hz, H-6''), 6.79 (2H, d, J = 8.5 Hz, H-3', 5'), 6.86 (1H, d, J = 8.1 Hz, H-5''), 7.18 (2H, d, J = 8.5 Hz, H-2', 6'). ^{13}C NMR (CDCl_3 , 125 MHz) δ : 47.5 (C-3), 56.1 (OCH_3), 110.4 (C-2''), 114.6 (C-5''), 115.3 (C-3', 5'), 115.4 (C-5), 120.5 (C-6''), 128.9 (C-1), 130.0 (C-1'), 130.2 (C-2', 6'), 131.8 (C-2), 135.6 (C-1''), 140.9 (C-4), 144.3 (C-4''), 146.7 (C-3''), 154.8 (C-4').

The 3''-hydroxy-4''-methoxy-4''-dehydroxynyasol (**4**) spectral data. EIMS: m/z 282 [M^+], ^1H NMR (CDCl_3 , 500 MHz) δ : 3.89 (3H, s, OCH_3), 4.54 (1H, dd, J = 9.3, 6.3 Hz, H-3), 5.14 (1H, m, H-5a), 5.19 (1H, m, H-5b), 5.67 (1H, dd, J = 11.2, 10.1 Hz, H-2), 6.01 (1H, ddd, J = 16.8, 10.0, 6.1 Hz, H-4), 6.49 (1H, d, J = 11.4 Hz, H-1), 6.68 (1H, dd, J = 8.1, 2.0 Hz, H-6''), 6.77 (1H, d, J = 8.1 Hz, H-5''), 6.77 (2H, d, J = 8.4 Hz, H-3', 5'), 6.81 (1H, d, J = 2.0 Hz, H-2''), 7.10 (2H, d, J = 8.4 Hz, H-2', 6'). ^{13}C NMR (CDCl_3 , 125 MHz) δ : 47.0 (C-3), 56.2 (OCH_3), 110.6 (C-5''), 115.0 (C-2''), 115.3 (C-5), 115.6 (C-3', 5'), 120.9 (C-6''), 128.9 (C-1), 129.1 (C-2', 6'), 130.8 (C-1'), 132.3 (C-2), 135.8 (C-1''), 140.8 (C-4), 145.4 (C-3''), 145.8 (C-4''), 154.3 (C-4').

The 4-hydroxybenzaldehyde (**5**) spectral data. EIMS: m/z 122 [M^+], 121, 93, 75, 65, 53. ^1H NMR (CD_3OD , 500 MHz) δ : 6.91 (2H, d, J = 8.5 Hz, H-3, 5), 7.76 (2H, d, J = 8.5 Hz, H-2, 6), 9.75 (1H, s, H-8). ^{13}C NMR (CD_3OD , 125 MHz) δ : 117.0 (C-3, 5), 130.5 (C-1), 133.5 (C-2, 6), 165.3 (C-4), 193.0 (C-8).

The 4-hydroxy acetophenone (**6**) spectral data. EIMS: m/z 136 [M^+], 121, 107, 93, 77, 65, 53. ^1H NMR (CDCl_3 , 500 MHz) δ : 2.58 (3H, s, H-8), 6.95 (2H, d, J = 8.5 Hz, H-3, 5), 7.91 (2H, d, J = 8.5 Hz, H-2, 6). ^{13}C NMR (CDCl_3 , 125 MHz) δ : 26.5 (C-8), 115.8 (C-3, 5), 130.0 (C-1), 131.4 (C-2, 6), 161.5 (C-4), 198.6 (C-7).

2.4. Cell culture

The RBL-2H3 rat mast cell line (American Type Culture Collection, ATCC, Rockville, MD) was maintained in minimum essential medium (MEM) with 15% fetal bovine serum (FBS), 2 mM l-glutamine, 100 U/mL penicillin, and 100 $\mu\text{g}/\text{mL}$ streptomycin at 37 $^\circ\text{C}$ in a humidified incubator with 5% CO_2 .

2.5. Animals

Specific-pathogen-free 8-week-old male C57BL/6 mice were purchased from SLC Inc. (Shizuoka, Japan) and housed in an air-conditioned animal room with a 12-h light/12-h dark cycle at a temperature of 22 ± 1 $^\circ\text{C}$ and humidity of $50 \pm 10\%$. The mice were fed a standard laboratory diet with tap water *ad libitum*. All experimental protocols involving the use of animals were conducted in accordance with National Institutes of Health (NIH) guidelines and approved by the Committee on Animal Care, Semyung University.

2.6. β -Hexosaminidase release assay

The IgE-antigen-stimulated degranulation of mast cells was evaluated by performing a β -hexosaminidase release assay. The RBL-2H3 cell line was cultured in MEM supplemented with 15% FBS and L-glutamate. Prior to the assay, the cells were seeded in 24-well plates at a concentration of 5×10^5 cells/well in phenol red-free media and cultured overnight. The cells were sensitized by incubation in media containing 0.1 μ g/mL mouse monoclonal IgE for 4 h at 37 °C in 5% CO₂. The cells were then washed in Siraganian buffer (pH 7.2, 119 mM NaCl, 5 mM KCl, 0.4 mM MgCl₂, 25 mM PIPES, and 40 mM NaOH) and preincubated with 180 μ L of Siraganian buffer containing 5.6 mM glucose, 1 mM CaCl₂, and 0.1% BSA for an additional 10 min at 37 °C. The cells were exposed to 20 μ L of the test material for 20 min, followed by treatment with 20 μ L of the antigen (DNP-BSA, 1 μ g/mL) for 30 min at 37 °C to activate the cells and evoke the allergic reaction (degranulation). The reaction was stopped by cooling in an ice bath for 10 min. The reaction mixture was then centrifuged at 1000 rpm for 10 min and 25 mL of the supernatant was transferred to 96-well plates and incubated with 25 mL of the substrate (1 mM *p*-nitrophenyl-N-acetyl- β -D-glucosaminide) for 1 h at 37 °C. The stop solution was added, and the absorbance was measured at 405 nm using an enzyme-linked immunosorbent assay (ELISA) reader (Spectra190, Molecular Devices, USA).

2.7. Mast cell purification and histamine release assay

The peritoneal mast cells of rats were sensitized by administering an intraperitoneal injection of 2 mL of a 1/200 dilution of the

Table 1

Inhibitory activities of isolated compounds (**1–6**) on β -hexosaminidase release from rat basophilic leukemia (RBL)-2H3 cells stimulated by dinitrophenol-conjugated bovine serum albumin (DNP-BSA).

Compounds	IC ₅₀ (μ M)
1	52.67
2	36.25
3	18.48
4	2.85
5	183.79
6	322.52
ketotifen	10.12

1, 4'-O-methylnyasol; **2**, nyasol; **3**, 3''-methoxynyasol; **4**, 3''-hydroxy-4''-methoxy-4''-dehydroynyasol; **5**, 4-hydroxybenzaldehyde; **6**, 4-hydroxyacetophenone; IC₅₀, half-maximal inhibitory concentration.

monoclonal IgE preparation. Two days later, the rats were euthanized under ether anesthesia, and peritoneal cells were recovered by washing the cavity with 20 mL of Tyrode solution containing 5 units/mL of heparin. Peritoneal washings containing peritoneal exudate cells (PEC) were pooled and spun down at $45 \times g$ for 5 min. The cells were washed twice with Tyrode solution by centrifugation at $45 \times g$ for 5 min and resuspended in a small volume of the same solution. The mast cells were purified by density gradient centrifugation with Percoll [21–23]. Isosmotic Percoll (90%) was prepared by mixing 9 volumes of Percoll with 1 volume of a 10-fold concentration of phosphate free Tyrode solution. This was further diluted with Tyrode solution to make a 60% Percoll solution, with a density of 1.09 and 12 mL was placed in a plastic tube, and then 1 mL of the PEC suspension was layered over the Percoll. After centrifugation at $45 \times g$ for 15 min, the

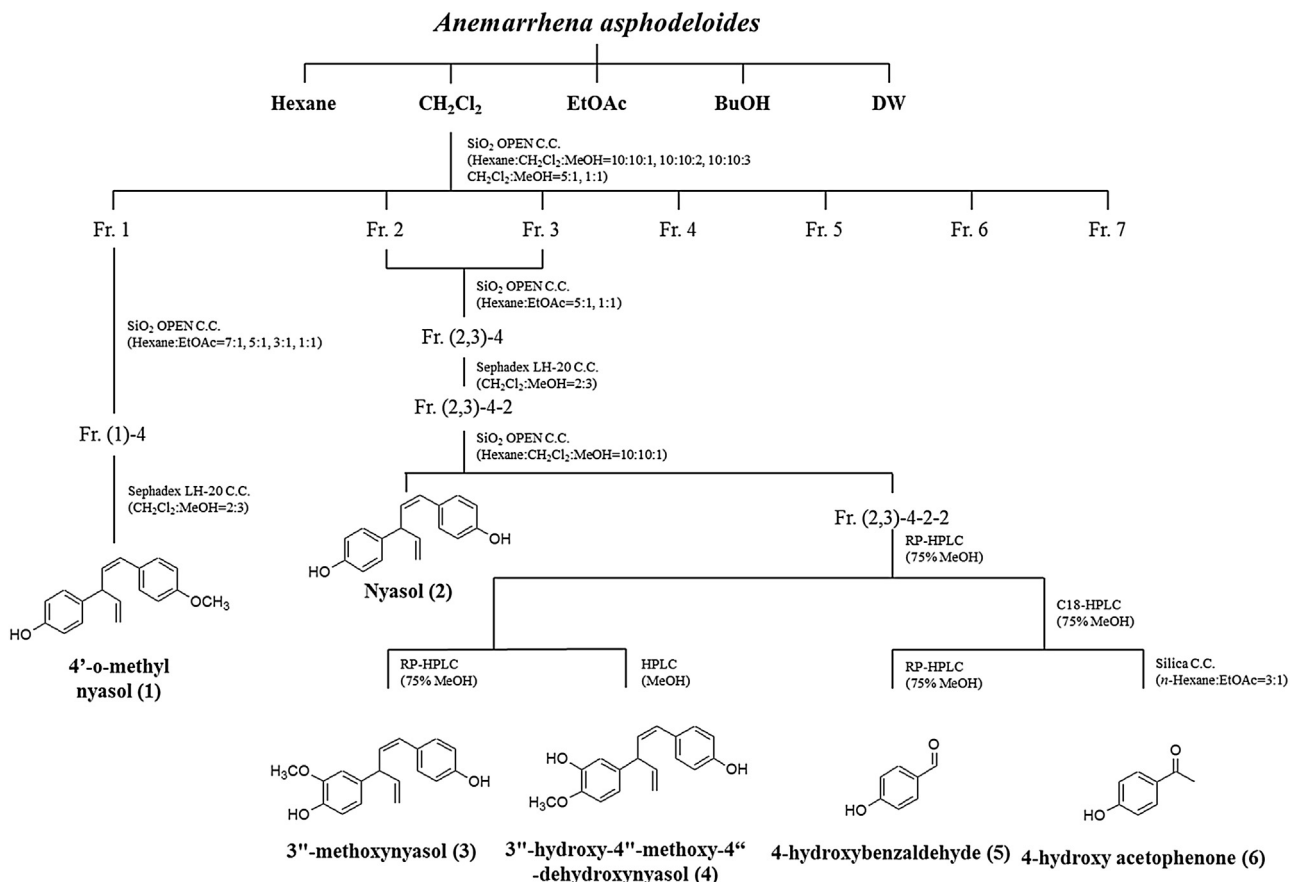


Fig. 1. Schematic diagram of the extraction, isolation, and structures of compounds **1–6** isolated from *Anemarrhena asphodeloides*.

purified mast cells (PMCs) at the bottom of the tube were collected and suspended in Tyrode solution. The inhibition of histamine release from the PMCs co-treated with A23187 plus PMA was evaluated using a procedure similarly to that used to determine antigen-induced degranulation. In this procedure, the PMCs were preincubated with the four norlignans at the indicated concentration for 10 min prior to incubation with either A23187 or PMA instead of anti-DNP IgE and DNP-BSA. The serum histamine content was measured using a histamine ELISA assay kit (IBL, Hamburg, Germany).

2.8. The 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) assay

The cytotoxicity of the test extracts was determined using the 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) assay [24]. Briefly, NIH-3T3 cells were placed in 96-well flat-bottomed micro-culture plates in the presence or absence of various concentrations of the isolated compounds (in triplicate) and incubated at 37 °C in a 5% humidified CO₂ incubator for 12 h. Then, 10 µL of MTT (5 mg/mL) was added to each well and the incubation was continued for another 2 h at 37 °C. The medium was discarded, and then 100 µL of dimethyl sulfoxide (DMSO) was added to each well. Following the complete solubilization of the dye, the plates were read at 570 nm using an ELISA plate reader. The reference wavelength was 690 nm.

2.9. Compound 48/80 induced systemic anaphylaxis

The mice (n = 10/group) were administered an intraperitoneal injection of 8 mg/kg body weight of the mast cell degranulation compound 48/80. The *A. asphodeloides* EtOH extract (5, 25, 125, or 625 mg/kg body weight) was dissolved in saline and orally administered 1 h prior to injecting compound 48/80. The mortality was monitored for 1 h following the induction of anaphylactic shock [25]. After the mortality test, blood was collected from each mouse heart, centrifuged at 400g for 10 min to obtain the serum, which was analyzed for the histamine content using an ELISA assay (IBL, Hamburg, Germany).

2.10. Statistical analysis

The results were expressed as means ± standard error of mean (SEM) for all experiments. The Student's *t*-test was used to statistical compare the difference between groups. Results with *p* < 0.05 were considered statistically significant.

3. Results and discussion

The *in vitro* antiallergic activity-guided fractionation and purification of the EtOH extract of the rhizomes of *A. asphodeloides* led to the isolation of four norlignans and two simple phenolic compounds as active components. The norlignan compounds

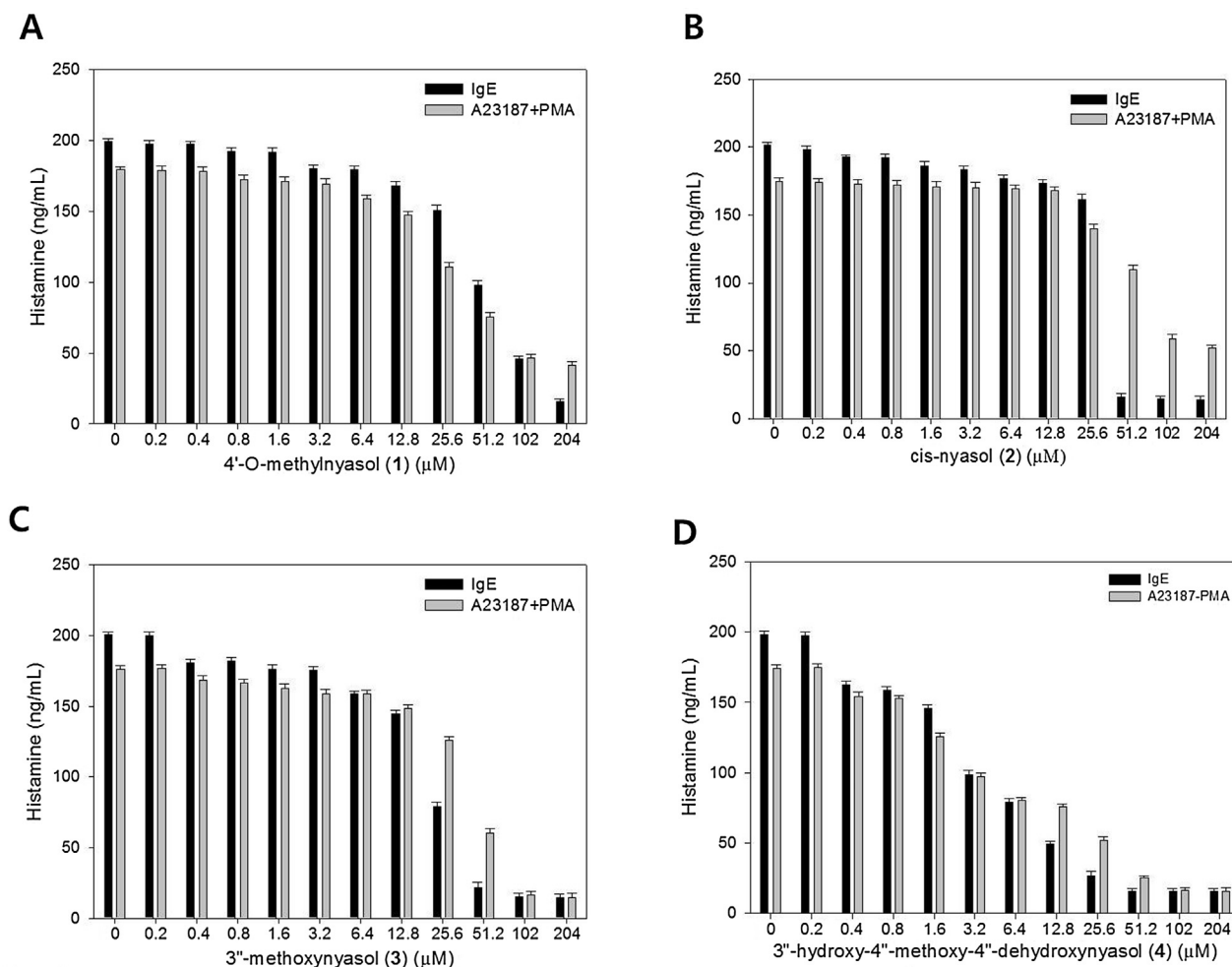


Fig. 2. Inhibition of histamine release by compounds 1–4 treatment in primary mast cells (PMCs). Cells were treated with compounds 1–4 for 10 min prior to incubation with either anti-DNP IgE and DNP-BSA or A23187 and PMA. (A) 4'-O-methylnyasol, compound 1, (B) Nyasol, compound 2, (C) 3"-methoxyynyasol, compound 3, (D) 3"-hydroxy-4"-methoxy-4"-dehydroxyynyasol, compound 4. IC₅₀, half-maximal inhibitory concentration; IgE, immunoglobulin E; PMA, phorbol 12-myristate 13-acetate.

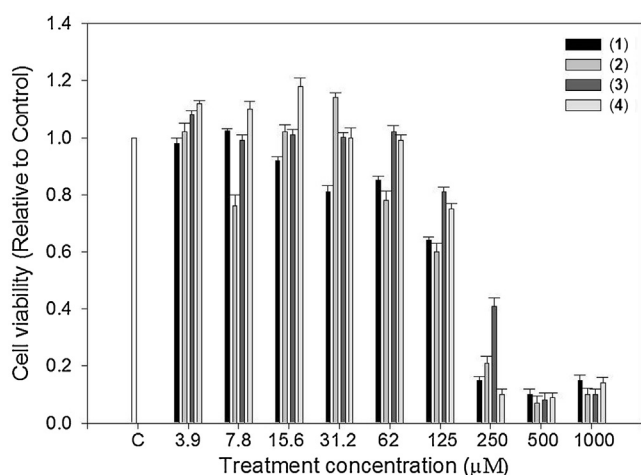


Fig. 3. Viability of NIH-3T3 cells treated with compounds **1–4** isolated from *Anemarrhena asphodeloides*. (1) 4'-O-methylnyasol, compound **1**, (2) nyasol, compound **2**, (3) 3''-methoxynyasol, compound **3**, (4) 3''-hydroxy-4''-methoxy-4''-dehydroxynyasol, compound **4**.

isolated from the CH_2Cl_2 soluble fraction were identified as 4'-O-methylnyasol (**1**) [26], nyasol (**2**) [27,28], 3''-methoxynyasol (**3**) [29], and 3''-hydroxy-4''-methoxy-4''-dehydroxynyasol (**4**) [30] by comparing their spectral data with standard published values in the literature. In addition, the 4-hydroxybenzaldehyde (**5**) and 4-hydroxyacetophenone (**6**) were identified by direct comparison of their spectral data with authentic samples (Fig. 1).

The inhibition of antigen-induced degranulation by the isolated compounds was tested using an *in vitro* assay involving the inhibition of β -hexosaminidase release in RBL-2H3 cells stimulated by DNP-BSA, according to previously described protocols [31]. The results of the determination of their inhibitory activities are

summarized in Table 1. The compound **2** inhibited the β -hexosaminidase release by 50% at a concentration of 36.25 μM . Compound **1** is structurally derived by the replacement of a hydroxyl group with a methoxy group on the A-ring of the compound **2**, and was relatively less effective in inhibiting β -hexosaminidase release than compound **2**. Interestingly, compound **4**, which has *p*-methoxy and *m*-hydroxy groups on the B-ring of the compound **2**, displayed the most potent activity among the norlignans tested with a half-maximal inhibitory concentration (IC_{50}) of 2.85 μM . Compound **3**, which has a *m*-methoxy group on the B-ring of the compound **2**, was less active (IC_{50} , 18.48 μM) than compound **4** was. The activity of **4** was much stronger than that of the second-generation H_1 -antihistamine ketotifen (IC_{50} , 10.12 μM), which is used to treat allergic conjunctivitis [32]. Therefore, these results indicate that the structural alteration of the A- or B-ring of compound **2** is crucial to significantly increasing the inhibition of β -hexosaminidase release. The two simple phenolic compounds **5** and **6**, weakly inhibited β -hexosaminidase release.

We isolated four norlignans from *A. asphodeloides* and evaluated their activity against histamine release from PMCs stimulated with IgE or A23187 plus PMA co-treatment (Fig. 2). The level of released histamine was barely detectable in the unstimulated cells; however, the PMCs released a high level of histamine when stimulated with either A23187 plus PMA or anti-DNP IgE sensitization followed by a DNP-BSA challenge. The norlignans isolated from *A. asphodeloides* dose-dependently inhibited histamine release mediated by both A23187 plus PMA and IgE treatment. It is noteworthy that the *in vitro* anti-histamine release activity of **4** (IC_{50} , 3.18 and 2.32 μM against IgE and A23187 plus PMA, respectively) was higher than that of the other compounds (**1**, IC_{50} , 50.41 and 40.64 μM ; **2**, IC_{50} , 36.25 and 74.03 μM ; **3**, IC_{50} , 21.31 and 40.15 μM against IgE and A23187 plus PMA, respectively). These results indicate that mast cell-mediated immediate-type allergic reactions are inhibited by norlignans. It is known that stimulation of mast cells following co-treatment with A23187 plus

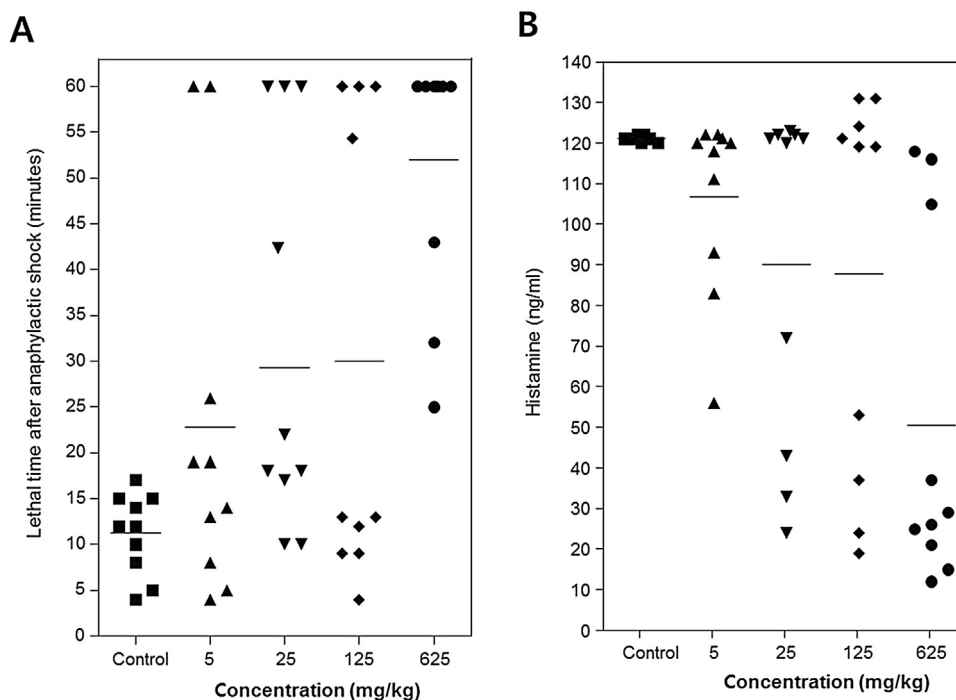


Fig. 4. *In vivo* inhibitory effects of the *Anemarrhena asphodeloides* ethanol (EtOH) extract against systemic allergic reactions. (A) Lethal time after the anaphylactic shock, (B) Serum histamine release. Mice were injected with 8 mg/kg body weight of the mast cell degranulation compound 48/80. A bar indicates the average of group.

PMA or anti-DNP IgE activates a signal transduction pathway, which leads to histamine release.

The MTT conversion assay was used to determine the viability of NIH-3T3 cells exposed to the four norlignans. As shown in Fig. 3, the cell viability was almost 100% after exposure to 15.6 μ M of the four norlignans for 24 h. However, doses of the norlignans from 62 μ M–1 mM revealed dose-dependent cytotoxicity in the NIH-3T3 cells. IC₅₀ of the compound 3 displayed 220.5 which was the lowest cytotoxicity compared to compounds 1, 2, and 4 (IC₅₀ 159.52, 160.0, and 172.5, respectively).

The EtOH extract of *A. asphodeloides* showed a higher inhibition of degranulation than the other fractions, in the *in vitro* assay [20]. We also determined whether the EtOH extract has *in vivo* anti-allergy effects using a mouse allergy model. The model involved injecting mice intraperitoneally with 8 mg/kg of compound 48/80 to induce a systemic fetal allergic reaction. The control group mice, which were subsequently treated with intraperitoneal injections of PBS died within 1 h. However, mice treated intraperitoneally with the EtOH extract survived and this survival was dose-dependent (Fig. 4A). The mice treated with 625 mg/kg of the EtOH extract exhibited a significantly decreased death rate after 1 h. In addition, mice treated with the fraction showed a concentration-dependent decrease in histamine release (Fig. 4B).

In conclusion, *A. asphodeloides* is used in traditional medicine to treat diabetes mellitus and cold-induced febrile diseases. Recently, the *A. asphodeloides*-derived compound 2 was reported to possess estrogen modulatory activity. This present study is the first report of the antiallergic activity of *A. asphodeloides* and its active compound designated as 4. The results have shown that these compounds inhibited the degranulation of allergic mediators and activation of mast cells *in vitro* and improved systemic anaphylaxis *in vivo*. The data suggest that *A. asphodeloides*-derived compounds and extracts have the potential for further development as anti-allergy agents for use in humans.

Conflict of interest

The authors state that they have no conflict of interest to declare.

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