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Article

Screening and Compound Isolation from Natural Plants for Anti-allergic Activity

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Drug discovery for the treatment of allergic disease is an important field in human health. Natural plants and medicinal herbs used commercially in Korea were screened for degranulation inhibitory activities of 164 plant species in the RBL-2H3 cell line, and their activities were investigated using the β-hexosaminidase release assay; 34 species were active. *Lactuca indica* var. laciniata had the highest degranulation inhibitory activity, and *Plantago asiatica*, *Cimicifuga heracleifolia*, *Poncirus trifoliata*, *Lycopus ramosissimus*, *Quercus mongolica*, *Anemarrhena asphodeloides*, *Eugenia caryophyllata*, and *Curcuma longa* exhibited degranulation inhibitory capacity higher than 50% at 125 μg/mL. One compound was isolated by activity-guided fractionation and isolation from methanol (MeOH) extract of *A. asphodeloides*, which has not yet been identified as constituent for anti-allergic and atopic effects. The structure of isolated compound was elucidated on the basis of NMR and Mass spectrometric data as nyasol, which was highly effective at very low dose (70.98±1.57% at 50 μg/mL). These results demonstrate that natural products and contained compounds may be useful in the therapy of allergic diseases, such as atopic dermatitis and asthma.

Key words: allergy, degranulation, medicinal herbs, RBL-2H3

Plants have been used as traditional natural medicines for healing many diseases. In particular, various oriental medicinal herbs are reported to have anti-allergic activity both *in vitro* and *in vivo* [Choo *et al*., 2003; Kim *et al*., 2004; Kim *et al*., 2006; Kim and Shin, 2006]. However, the active constituents and mechanisms of action of most herbs are largely unknown.

β-Hexosaminidase is located in the secretory granules of mast cells where histamines are stored, and is released along with histamine when mast cells are immunologically activated [Marquardt and Wasserman, 1983]. For this reason, β-hexosaminidase is considered as a degranulation marker and has been widely used in biochemical studies of allergies as a screening method for anti-allergic agents [Fischer *et al*., 1995; Choi *et al*., 1996].

Several therapeutic agents that inhibit the release of pharmacologically active chemical mediators from mast cells or basophils are now in clinical use [Marone *et al*., 2002]. Moreover, many kinds of natural products have been reported to show anti-allergic effects [Choo *et al*., 2003]. To investigate the anti-allergic activity of the selected plants, the rat basophil leukemia cell line, RBL-2H3 was used. In addition, the cytotoxicity of these plants on the human fibroblast cell line, NIH-3T3 was investigated.

Materials and Methods

Plant materials and preparation of crude extracts. A total of 190 plant extracts [95% ethanol (EtOH) extracts] were purchased from the Plant Extract Bank of Korea (DaeJeon, Korea). The plant extracts were dissolved in dimethylsulfoxide (DMSO) and used as samples for screening tests. The rhizomes of *Anemarrhena*

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asphodeloides were purchased on October, 2008 from a traditional medicine market, Kyung-dong yak-ryong-si, Seoul, Korea.

Extraction and isolation. The dried rhizomes of *A. asphodeloides* were extracted with methanol (MeOH) at room temperature three times. The MeOH extract was evaporated to dryness under reduced pressure. The residue was suspended in distilled water and then partitioned with *n*-hexane, dichloromethane (CH_2Cl_2), ethyl acetate (EtOAc), and butanol (*n*-BuOH), consecutively. The extract was separated by bioassay-guided chromatographic fractionation. The CH_2Cl_2 extract, which showed inhibitory activity of antigen-induced degranulation in mast cells, was subjected to column chromatography over 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 give seven sub-fractions (Fr.1~Fr.7). Fractions Fr.2 and Fr.3 were combined, and then re-chromatographed on silica gel sephadex LH-20, and silica gel with *n*-hexane:EtOAc (5:1, 3:1 and 1:1), CH_2Cl_2:MeOH (2:3), and *n*-hexane: CH_2Cl_2:MeOH (10:10:1), to give nyasol (scheme 1).

Nyasol – EI-MS *m/z*: 252[M^+], 237, 207, 158, 145, 131, 107; ^{1}H-NMR (500 MHz, $CDCl_3$, δ ppm): 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 (125 MHz, $CDCl_3$, δ ppm): 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') (Fig. 1).

Cell lines and reagents. RBL-2H3 and NIH-3T3 cells were purchased from the Korean Cell Line Bank (Seoul, Korea). Dulbecco's Modified Eagle Medium (DMEM) and other cell-culture reagents were purchased from HyClone (Logan, UT). *p*-Nitrophenyl-*N*-acetyl-β-D-glucosaminide was purchased from Sigma (St. Louis, MO), anti-dinitrophenol (DNP)-IgE was purchased from Zymed (San Francisco, CA), and DNP-bovine serum albumin (BSA) was purchased from Molecular Probes (Eugene, OR). 4-amino-5-(4-chlorophenyl)-7-(t-butyl) pyrazolo[3,4-d]pyrimidine (PP2) was purchased from Calbiochem (Darmstadt, Germany).

Assay of anti-allergic activity. The inhibitory activities of plant extracts against the release of β-hexosaminidase from RBL-2H3 cells were evaluated according to the techniques outlined by Choi *et al*. [1996]. RBL-2H3 cells were grown in DMEM supplemented with 15% fetal bovine serum and L-glutamine. Before the experiment, cells were dispensed into 24-well plates at 5×10^5 cells per well. The cells were then sensitized by incubation in medium containing 0.5 μg/mL of mouse monoclonal IgE overnight at 37°C in 5% CO_2. They were subsequently washed with 500 μL of siraganian buffer (pH 7.2, 119 mM NaCl, 5 mM KCl, 0.4 mM $MgCl_2$, 25 mM PIPES (piperazine-N,N'-bis(2-ethanesulfonic acid)), and 40 mM NaOH) and incubated in 180 μL siraganian buffer containing 5.6 mM glucose, 1 mM $CaCl_2$, and 0.1% BSA for an additional 10 min at 37°C. The cells were then exposed to 20 μL of test material for 20 min, followed by treatment with 20 μL of antigen (DNP-BSA, 1 μg/mL) for 30 min at 37°C to activate the cells and evoke allergic reactions (degranulation). The reaction was stopped by cooling in an ice bath for 10 min. The reaction mixture was centrifuged at 1,000 rpm for 10 min, and 25-μL aliquots of the supernatant were transferred to a 96-well plate and incubated with 25 μL of substrate (1 mM *p*-nitrophenyl-*N*-acetyl-β-D-glucosaminide) for 1 h at 37°C. The reaction was stopped by adding 200 μL of 0.1 M $Na_2CO_3/NaHCO_3$. Absorbance was measured using an ELISA plate reader at 405 nm.

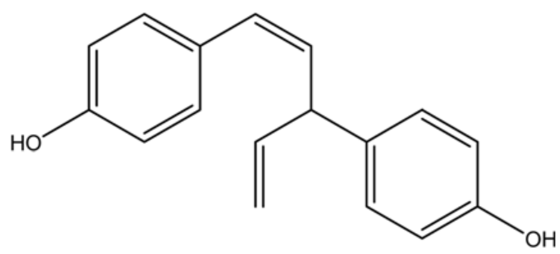


Fig. 1. The structure of nyasol from *A.asphodeloides*.

Results and Discussion

RBL-2H3 cells were used for *in vitro* assay to assess the anti-allergic activity of various plant extracts. RBL-2H3 cells were stimulated by DNP-BSA. The inhibitory activities on degranulation were measured with the ethanol extracts of 106 medicinal plants (Table 1) and 84 native Korean plants (Table 2). Thirty-four extracts strongly inhibited degranulation in cells (Table 3). *Plantago asiatica*, *Cimicifuga heracleifolia*, *Poncirus trifoliata, Lycopus ramosissimus*, *Quercus mongolica*, *Anemarrhena asphodeloides*, *Eugenia caryophyllata*, *Curcuma longa*, and *Lactuca indica* extracts showed potent inhibitory activity. These extracts exhibited concentration-dependent degranulation inhibitory activities of more than 50% at 125 μg/mL.

C. longa and *P. asiatica* were reported to have anti-inflammatory activities in arachidonic acid-induced mouse ear edema [Murai *et al*., 1995; Chainani-Wu, 2003]. Although the mechanism of these effects is unknown, *P.*

Table 1. Anti-allergic activities of medicinal plants

Family	Scientific Name	Common Name	Activity
Acoraceae	*Acorus gramineus* Soland.	Acori graminei rhizoma	++
Alismataceae	*Alisma plantago-aquatica* L.	Alismatis rhizoma	+
Amaranthaceae	*Achyranthes japonica* Nakai	Achuranthis radix	++
Anacardiaceae	Rhus javanica L.	Galla rhois	+
Anthericaceae	*Anemarrhena asphodeloides* Bunge	Anemarrhenae rhizoma	+++
Araceae	*Arisaema amurense* Maxim.	Arisaematis rhizoma	-
	Pinellia ternata (Thunb.) Breit.	Pinelliae tuber	+
Araliaceae	*Acanthopanax senticosus* Harms	Acanthopanacis cortex	-
	Aralia cordata Thunb.	Araliae cordatae radix	-
	Panax notoginseng (Burkill) Chen ex Yunnan Inst.Bot.	Notoginseng radix	++
	Acanthopanax sessiliflorum Seem.	Acanthopanacis cortex	++
	Panax ginseng C.A.Mey.	Ginseng radix	++
Asteraceae	*Carthamus tinctorius* L.	Cartami flos	+
Campanulaceae	*Adenophora stricta* Miq.	Adenophorae radix	-
Caprifoliaceae	*Lonicera japonica* Thunberg	Lonicerae flos	+
Compositae	*Atractylodes japonica* Koidz.	Atractylodis rhizoma	++
	Chrysanthemum indicum L.	Chrysanthemi flos	-
	Chrysanthemum zawadskii Herbich	Chrisanthemi sibirici Herba	+
	Atractylodes japonica Koidz.	Atractylodis Rhizoma alba	-
	Saussurea lappa C.B.Clarke	Saururi herba	+
	Taraxacum platycarpum Dahlst.	Taraxaci herba	+
Convallariaceae	*Liriope platyphylla* F.T.Wang & T.Tang	Liriopis tuber	-
Convolvulaceae	*Cuscuta japonica* Chois.	Cuscutae semen	+
Cornaceae	*Cornus officinalis* Siebold & Zucc.	Corni fructus	-
Cruciferae	*Raphanus sativus* L.	Raphani semen	-
	Draba nemorosa L.	Drabae semen	++
Cupressaceae	*Thuja orientalis* L.	Biotae orientalis folium	++
Dioscoreaceae	*Dioscorea batatas* Decne.	Dioscoreae rhizoma	-
Ephedraceae	*Ephedra sinica* Stapf	Ephedrae herba	+
Ericaceae	*Vaccinium koreanum* Nakai	Pruni nakaii semen	+
Eucommiaceae	*Eucommia ulmoides* Oliver	Eucomiae cortex	-
Fagaceae	*Castanea crenata* Siebold & Zucc.	Castaneae semen	-
Flacourtiaceae	*Hydnocarpus anthelmintica* Pierre	Hydnocarpi semen	-
Gramineae	*Coix lacryma-jobi* L. var. *ma-yuen* Stapf	Coicis semen	++
	Sasa japonica Makino	Bambusae folium	++
Labiatae	*Scutellaria salvia* H.Lév.	Salviae radix	-
Labiatae	*Mentha arvensis L.*	Menthae herba	-
	Perilla frutescens Britton.	Perillae folium	++
	Leonurus japonicus Miq.	Leonuri herba	+
	Lycopus ramosissimus (Makino) Makino	Lycopi herba	+++
	Nepeta japonica Maxim.	Nepetae spica	+
	Scutellaria baicalensis Georgi	Scutellariae radix	+
Lardizabalaceae	*Akebia quinata* Decne.	Akebiae caulis	-
Lauraceae	*Cinnamomum cassia* D.Don	Cinnamomi ramulus	-
	Cinnamomum cassia D.Don	Cinnamomi cortex	-

trifoliata and *L. lucidus* were reported to exhibit passive cutaneous anaphylaxis inhibitory activity and mast cell-mediated anti-allergic effects [Park *et al*., 2003; Shin *et al*., 2005]. However, *L. indica*, *E. caryophyllata*, *A. asphodeloides*, *C. heracleifolia,* and *Q. mongolica* have not been reported to have any anti-inflammatory activity

Table 1. Continued

Family	Scientific Name	Common Name	Activity
Leguminosae	*Pueraria lobata* (Willd.) Ohwi	Puerariae radix	-
	Pueraria lobata (Willd.) Ohwi	Puerariae flos	-
	Glycyrrhiza uralensis Fisch. ex DC.	Glycyrrhizae radix	-
	Cassia obtusifolia L.	Cassiae semen	+
	Sophora flavescens Ait.	Sophorae radix	+
	Albizia julibrissin Durazz.	Albizziae cortex	+
	Astragalus membranaceus Bunge	Astragali radix	++
Liliaceae	*Polygonatum officinale* All.	Polygonati officinalis rhizoma	++
Magnoliaceae	*Schizandra chinensis* K.Koch	Schizandrae fructus	++
	Magnolia officinalis Rehder & E.H.Wilson	Magnoliae cortex	+
Malvaceae	*Malva verticillata* L.	Malvae semen	+
Meliaceae	*Melia azedarach* L.	Meliae cortex	+
Moraceae	*Morus alba* L.	Mori radicis cortex	+
	Morus alba L.	Mori fructus	++
	Morus alba L.	Mori folium	+
Myrtaceae	*Eugenia caryophyllata* Thunb.	Caryophylli flos	+++
Oleaceae	*Forsythia viridissima Lindl.*	Forsythiae fructus	-
Orchidaceae	*Gastrodia elata* Blume	Gastrodiae rhizoma	++
Paeoniaceae	Paeonia japonica	Paeoniae Radix alba	-
	Paeonia obovata Maxim.	Paeoniae radix	+
	Paeonia suffruticosa Andrews	Moutan cortex radicis	-
Piperaceae	*Saururus chinensis* Hort. ex Loud.	Saussureae radix	+
Plantaginaceae	*Plantago asiatica* L.	Plantaginis semen	+++
Polygalaceae	*Polygala tenuifolia* Poir.	Polygalae radix	++
	Rheum undulatum Pall.	Rhei rhizoma	+
	Polygonum multiflorum Thunb.	Polygoni multiflori radix	++
	Poria cocos Wolf	Hoelen rubra	+++
Ranunculaceae	*Pulsatilla koreana* (Y.Yabe ex Nakai) T.Mori	Pulsatillae radix	-
	Cimicifuga heracleifolia Kom.	Cimicifugae rhizoma	+++
Rhamnaceae	*Zizyphon jujubum* St.Lag.	Zizyphi fructus	-
Rosaceae	*Prunus persica* Stokes	Persicae semen	-
	Rubus coreanus Miq.	Rubi fructus	-
	Eriobotrya japonica (Thunb.) Lindl.	Eriobotriae folium	-
	Crataegus pinnatifida Bunge	Crataegi fructus	-
	Prunus humillis Bunge	Pruni nakaii semen	+
Rubiaceae	*Oldenlandia diffusa* (Willd.) Roxburgh	Hedyotidis diffusae herba	-
	Uncaria rhynchophylla (Miq.) Jacks.	Uncariae Ramulus et Uncus	++
	Uncaria rhynchophylla Miq.	Vitidis viniferae caulis	+
	Citrus tangerina Hort. ex Tanaka	Citri tangerinae Semen	-
	Citrus nobilis Lour.	Citri unshiu Pericarpium	-
	Poncirus trifoliatus Raf.	Ponciri fructus	+++
	Citrus unshiu Marcow.	Aurantii nobilis pericarpium	++
	Phellodendron amurense Rupr.	Phellodendri cortex	++
Scitamineae	*Zingiber officinale* Rosc.	Zingiberis rhizoma	+
Scrophulariaceae	*Rehmannia glutinosa* Steud.	Rhemaniae radix	-
	Scrophularia kakudensis Franch.	Scrophulariae radix	+

or anti-allergic effects. β-Hexosaminidase is located in the secretory granules of mast cells where histamine is stored, and is released along with histamine when mast cells are immunologically activated [Schwartz *et al.*,

Table 1. Continued

Family	Scientific Name	Common Name	Activity
Smilacaceae	*Poria cocos* Wolf	Hoelen	-
Ulmaceae	*Ulmus davidiana* Planch.	Salicis radicis cortex	+
Umbelliferae	*Ostericum koreanum* (Maxim.) Kitag.	Angelicae koreanae Radix	-
	Angelica sinensis (Oliv.) Diels	Angelicae sinensis radix	-
	Ledebouriella seseloides H.Wolff	Ledebouriellae radix	-
	Angelica dahurica Maxim.	Angelicae dahuricae radix	+
	Bupleurum falcatum Turcz.	Bupleuri radix	+
	Cnidium officinale Makino	Ligustici rhizoma	++
Viscaceae	*Viscum album* L.	Loranthi ramulus	+
Vitaceae	*Vitis vinifera* L.	Vitidis vinferae radix	+
Zingiberaceae	*Curcuma aromatica* Salisb.	Curcumae longae Rhizoma	++
	Amomum xanthioides Wall.	Amomi semen	-
	Curcuma longa L.	Curcumae longae Rhizoma	+++
	Alpinia globosa Horan.	Alpiniae katsumadaii semen	++
-	-	Massa medicata fermentata	-

Activity: β-hexosaminidase inhibition activity, +++: 50~60%, ++: 40~50%, +: 20~40 %, -: no effect

Table 2. Anti-allergic activity of native Korean plants

Family	Scientific Name	Part	Activity
Acoraceae	*Acorus gramineus* [Soland.]	Leaf	+
Alangiaceae	*Alangium platanifolium* var. *macrophylum*	Whole plant	-
Anacardiaceae	*Rhus chinensis* Mill.	Stem	-
Apiaceae	*Ostericum koreanum* (Maxim.) Kitag	Whole plant	-
Araceae	*Arisaema takesimense* Nakai	Aerial part	+
Asparagaceae	*Asparagus cochinchinensis* Merr.	Aerial part	+
Asteraceae	*Aster scaber* Thunb.	Whole plant	-
	Arctium lappa L.	Whole plant	+
	Artemisia princeps var. *orientalis*	Leaf	-
	Aster koraiensis Nakai	Whole plant	-
	Lactuca indica var. *laciniata*	Whole plant	+++
	Ligularia fischeri Turcz.	Whole plant	-
		Leaf	-
Betulaceae	*Betula platyphylla* var. *japonica*	Bark	+
Brassicaceae	*Brassica juncea* var. *integrifolia*	Whole plant	-
	Brassicaceae Diplotaxis muralis (L.) DC.	Stem	-
	Capsella bursa-pastoris (L.) Medik.	Leaf	-
	Cardamine flexuosa With.	Aerial part	-
	Cardamine leucantha (Tausch) O.E.Schulz	Aerial part	-
	Erysimum aurantiacum Leyb.	Leaf	-
	Rorippa indica (L.) Stehle	Whole plant	-
	Wasabia koreana Nakai	Whole plant	-
Cactaceae	*Opuntia ficus-indica* (L.) Mill.	Aerial part	+
Caprifoliaceae	*Weigela subsessilis* L.H.Bailey	Flower	-
Convallariaceae	*Convallaria keiskei* Miq.	Whole plant	-
Cucurbitaceae	*Schizopepon bryoniaefolius* Maxim.	Fruit	-
Ebenaceae	*Diospyros kaki* L.f.	Leaf	-
Ericaceae	*Vaccinium koreanum* Nakai	Stem and Leaf	-
Eucommiaceae	*Eucommia ulmoides* Oliver	Stem and Leaf	-

Table 2. Continued

Family	Scientific Name	Part	Activity
Fagaceae	*Quercus mongolica* Fisch. ex Turcz.	Bark	+++
	Fagus crenata var. *multinervis*	Whole plant	-
		Leaf	-
	Quercus acuta Thunb. ex A.Murray	Stem and Leaf	-
		Leaf	-
Fagaceae	*Quercus acutissima* Carruth.	Leaf	+
		Bark	+
		Duramen	+
	Quercus aliena J.G.Jack	Stem and Leaf	-
	Quercus gilva Blume	Bark	-
		Duramen	-
		Leaf	-
	Quercus glauca Thunb.	Leaf	-
		Duramen	-
	Quercus salicina Blume	Leaf	+
		Bark	+
		Duramen	+
	Quercus serrata Roxb.	Bark	-
		Duramen	-
		Leaf	+
	Quercus variabilis Blume	Leaf	-
	Quercus dentata Wats.	Bark	-
		Leaf	-
Ginkgoaceae	*Ginkgo biloba* L.	Leaf	+
Hostaceae	*Hosta longipes* Nakai	Leaf	-
Lamiaceae	*Mentha arvensis* var. *piperascens*	Leaf	-
	Perilla frutescens var. *japonica*	Leaf	+
Leguminosae	*Desmodium caudatum* DC.	Leaf	-
	Albizzia julibrissin	Stem	+
	Sophora flavescens Ait.	Whole plant	-
Liliaceae	*Crinum asiaticum* var. *japonicum*	Whole plant	-
Moraceae	*Cudrania tricuspidata* Bureau ex Lavallée	Seed	-
		Fruit	-
		Stem	-
	Broussonetia kazinoki var. *humilis*	Stem	+
		Leaf	+
	Ficus nipponica Franch. & Sav.	Stem	-
	Morus bombycis Koidzumi	Leaf	+
		Flower	-
		Root	-
Moraceae	*Morus bombycis* Koidzumi	Bark	+
		Duramen	+
Nymphaeaceae	*Nuphar japonicum* DC.	Hole part	-
Pinaceae	*Abies koreana* E.H.Wilson	Hole part	-
	Pinus densiflora Siebold & Zucc.	Leaf	-
		Bark	-

1979]. Therefore, β-hexosaminidase is designated as a ‘degranulation marker,’ and the release of β-hexosaminidase has been used to determine the extent of degranulation and to evaluate anti-allergic activities

Table 2. Continued

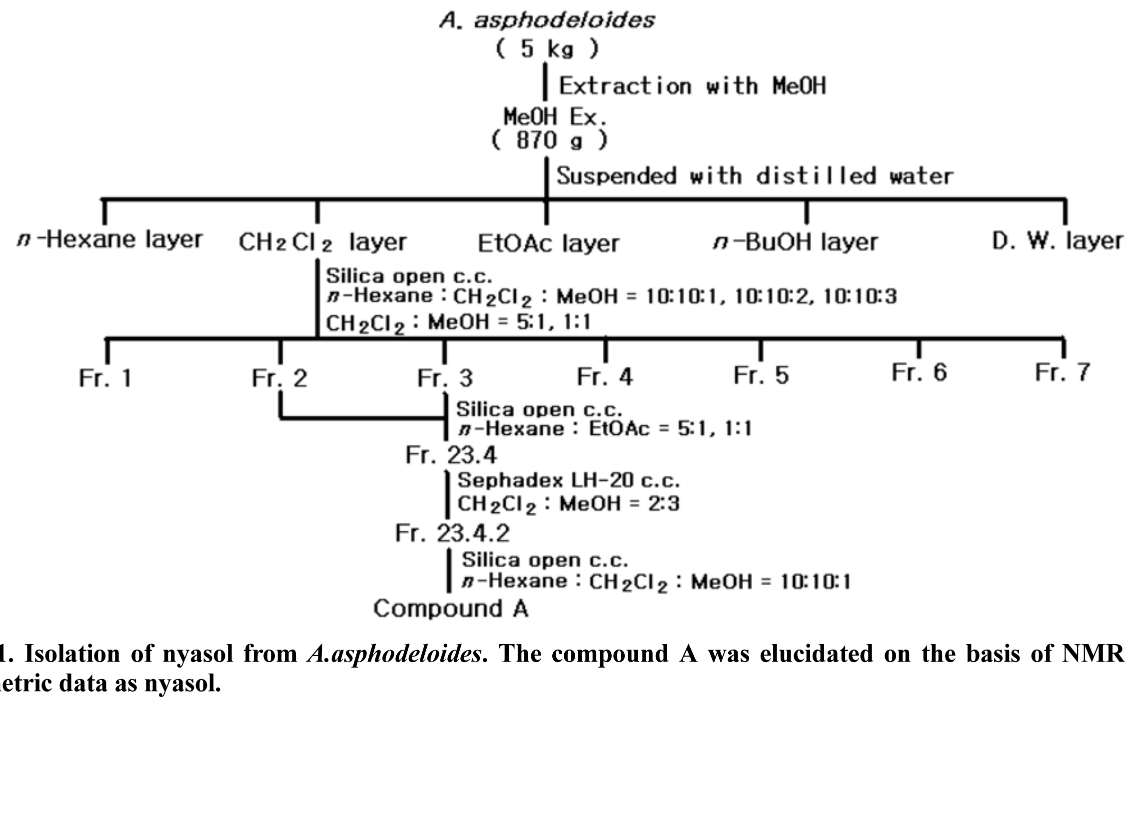
Family	Scientific Name	Part	Activity
Plantaginaceae	*Plantago asiatica* L.	Hole part	+
Rosaceae	*Crataegus pinnatifida* Bunge	Leaf	-
	Prunus persica (L.) Batsch	Stem and Leaf	-
Rutaceae	*Poncirus trifoliata* (L.) Raf.	Fruit	+
Saururaceae	*Houttuynia cordata* Thunb.	Hole part	-
Theaceae	*Thea sinensis* L.	Stem and Leaf	-
Ulmaceae	*Ulmus davidiana* var. *japonica*	Bark	-
Verbenaceae	*Verbena officinalis* L.	Hole part	-
Vitaceae	*Parthenocissus tricuspidata* Planch.	Stem	-

Activity: β-hexosaminidase inhibition activity, +++: 50~60%, ++: 40~50%, +: 20~40 %, -: no effect

[Fischer *et al*., 1995]. One compound was isolated by activity-guided fractionation and isolation from MeOH extract of *A. asphodeloides*, whose constituents for anti-allergic and atopic effects have not yet been identified (Scheme 1 and Table 4). The CH_2Cl_2 extract, which showed the highest inhibitory activity among the solvent fractions from MeOH extract of *A. asphodeloides*, was subjected to column chromatography over 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 give sub-fractions (Fr.1~Fr.7). Because fractions Fr.2 and Fr.3 showed similar activity, Fr.2 and Fr.3 were combined, and then re-chromatographed on silica gel sephadex LH-20, and silica gel with *n*-hexane: EtOAc (5:1, 3:1 and 1:1), CH_2Cl_2:MeOH (2:3), and *n*-hexane:CH_2Cl_2:MeOH (10:10:1), respectively, to give nyasol (Scheme 1). The structure of isolated compound was elucidated on the basis of NMR and Mass spectrometric data as nyasol (Fig. 1) [Bae *et al*., 2007].

There are two stereochemical isomers of hinokiresinol, *cis*- and *trans*-hinokiresinol and *cis*-form hinokiresinol referred to as nyasol. Some biological activities of nyasol have been described previously, including inhibitions of angiogenesis, anti-viral, anti-oxidant, and inhibiting activity for passive cutaneous anaphylaxis [Jeong *et al*., 1999; Lee and Ryu, 1999; Bae *et al*., 2007]. Moreover, anti-allergic and anti-inflammatory activities were previously reported [Lee and Ryu, 1999; Lim *et al*, 2009]. However, this is the first report to demonstrate anti-allergic activity of nyasol from *A. asphodeloides.*

Although the results discussed above are limited to *in vitro* anti-allergic activities, the results of this study suggest that many natural plants and constituents could be good sources for natural anti-allergic agents.



Scheme 1. Isolation of nyasol from *A.asphodeloides*. The compound A was elucidated on the basis of NMR and Mass spectrometric data as nyasol.

Table 3. Effect of natural products on antigen-induced degranulation in mast cells

				Percent inhibition of concentrations (%)		
Family	Species	Plant Part	PEB No.	5 μg/mL	25 μg/mL	125 μg/mL
Acoraceae	*Acorus gramineus* [Soland.]	Root	CA02-055	0.22±0.38	13.90±0.52	44.64±0.64
Amaranthaceae	*Achyranthes japonica* Nakai	Root bark	CA03-028	9.69±1.25	20.19±1.25	48.13±1.25
Anthericaceae	*Anemarrhena asphodeloides* Bunge	Root	CA03-062	11.62±0.73	21.01±0.73	55.33±0.73
Apiaceae	*Cnidium officinale* Makino	Root	CA04-047	4.79±1.46	17.33±1.46	45.51±1.46
Araliaceae	*Acanthopanax sessiliflorum* Seem.	Stem bark	CA02-079	2.65±1.29	11.25±1.29	44.25±1.29
	Panax ginseng C.A.Mey.	Root bark	CA03-041	9.63±1.47	19.39±1.47	48.56±1.47
	Panax notoginseng (Burkill) Chen ex Yunnan Inst.Bot.	Root bark	CA02-048	12.06±1.76	20.75±1.76	44.59±1.76
	Atractylodes japonica Koidz.	Root	CA03-069	6.66±0.75	16.92±0.75	44.05±0.75
Asteraceae	*Lactuca indica* var. laciniata	Aerial part	023-076	11.73±0.72	25.94±0.72	64.40±0.72
	Draba nemorosa L.	Seed	CA03-050	5.81±1.51	16.11±1.51	47.04±1.51
Brassicaceae	*Polygonatum officinale* All.	Root	CA01-042	2.86±1.09	4.753±1.09	41.81±1.09
Convallariaceae	*Thuja orientalis* L.	Leaf	CA03-076	5.18±1.41	17.29±1.41	45.50±1.41
Cupressaceae	*Quercus mongolica* Fisch. ex Turcz.	Leaf	016-074	2.11±0.64	16.44±0.64	55.30±0.64
Fagaceae	*Coix lacryma-jobi* L.	Seed	CA04-035	10.55±1.71	29.02±1.71	47.66±1.71
Gramineae	*Perilla frutescens* Britton.	Leaf	CA02-062	1.15±1.69	12.83±1.69	43.48±1.69
Labiatae	*Lycopus lucidus* Turcz. ex Benth.	Aerial part	CA04-062	4.08±1.54	17.21±1.54	53.91±1.54
Lamiaceae	*Leonurus japonicus* Houtt.	Aerial part	CA02-089	7.81±1.54	20.13±1.44	49.91±1.54
	Scutellaria baicalensis Georgi	Root bark	CA04-087	5.06±1.3	14.76±1.30	46.08±1.30
	Astragalus membranaceus Bunge	Root bark	CA04-090	3.77±0.62	7.445±0.62	49.41±0.62
Leguminosae	*Schizandra chinensis* K.Koch	Fruit	CA02-081	2.53±1.87	12.14±1.87	43.02±1.87
Magnoliaceae	*Morus alba* L.	Fruit	CA04-027	11.87±0.97	21.76±0.97	43.38±0.97
Moraceae	*Eugenia aromatica* Sond. ex O.Berg	Flower	CA02-093	5.35±1.93	21.29±1.93	55.61±1.93
Myrtaceae	*Gastrodia elata* Blume	Root	CA04-048	5.12±1.70	16.38±1.70	45.21±1.70
Orchidaceae	*Plantago asiatica* L.	Seed	CA04-044	8.09±1.11	20.98±1.11	50.91±1.11
Plantaginaceae	*Sasa japonica* Makino	Leaf	CA03-056	7.38±1.90	8.161±1.90	49.98±1.90
Poaceae	*Polygala tenuifolia* Poir.	Root bark	CA02-086	5.25±1.92	18.04±1.92	45.53±1.92
Polygalaceae	*Polygonum multiflorum* Thunb.	Root bark	CA03-047	5.53±1.39	14.92±1.39	45.96±1.39
Polygonaceae	*Cimicifuga heracleifolia* Kom.	Root	CA02-065	3.12±1.36	17.30±1.36	51.04±1.36
Ranunculaceae	*Uncaria rhynchophylla* Miq.	Stem bark	CA03-053	4.19±0.12	16.89±0.38	46.50±1.00
Rubiaceae	*Poncirus trifoliata* (L.) Raf.	Fruit	CA03-065	11.02±1.57	21.11±1.57	52.01±1.57
Rutaceae	*Citrus unshiu* Marcow.	Fruit bark	CA03-068	2.52±1.42	16.14±1.42	46.19±1.42
	Alpinia katsumadai Hayata	Seed	CA03-075	8.31±1.29	17.33±1.29	41.89±1.29
Zingiberaceae	*Curcuma aromatica* Salisb.	Root	CA02-007	2.39±1.28	5.127±1.28	45.84±1.28
	Curcuma longa L.	Root	CA03-030	4.22±1.07	20.16±1.07	56.82±1.07
	0.01%DMSO				1.2±1.30	
	10 μM PP2				85.5±0.35	

Percent inhibition of degranulation was determined by measuring the release of the granule marker β-hexosaminidase in the media as described in Materials and Methods. Negative control: 0.01% DMSO; positive control: 10 μM PP2.

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Table 4. Effects of active compound, sub-fractions, and solvent-fractions of *A. asphodeloides* on antigen-induced degranulation in mast cells

				Percent inhibition of concentrations (%)		
Crude extract	Solvent-fractions	Sub-franctions	compound	5 μg/mL	25 μg/mL	125 μg/mL
MeOH extract				13.18±0.42	22.08±1.34	59.48±1.95
	n-hexane layer			3.75±0.26	3.72±0.19	3.08±0.71
	CH_2Cl_2 layer	Fr. 1		8.56±0.68	7.39±0.72	8.35±0.69
		Fr. 2		36.84±2.01	60.21±2.22	58.44±1.84
		Fr. 3		40.39±2.62	68.33±3.62	60.66±2.98
			nyasol	55.21±2.04	70.98±1.57	65.87±2.03
		Fr. 4		20.22±2.89	34.52±1.61	43.01±2.56
		Fr. 5		8.60±1.69	12.92±2.13	11.95±1.98
		Fr. 6		3.21±0.54	6.66±0.63	6.99±0.39
		Fr. 7		4.01±0.58	5.21±0.28	4.63±0.53
	EtOAc layer			11.45±0.84	15.29±0.77	15.03±0.76
	n-BuOH layer			5.96±0.33	5.32±0.51	4.84±0.69
	D.W layer			3.33±0.27	6.21±0.58	6.33±0.88
0.01% DMSO					1.1±0.89	
10 μM PP2					83.3±1.02	

Negative control: 0.01% DMSO; positive control: 10 μM PP2.

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