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Inhibitory effects of natural plants of Jeju Island on elastase and MMP-1 expression

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Synopsis

In order to search for new active cosmetic ingredients of natural origin, we screened about 60 plants collected from Jeju Island, which is located in the southernmost part of the Republic of Korea. We investigated their free radical scavenging activity, elastase inhibition activity, and reduction of MMP-1 mRNA expression for the development of anti-aging ingredients as raw materials for use in cosmetics. In the free radical scavenging capacity assay, 12 extracts, including *Typha orientalis* (seed) and *Torreya nucifera* (leaf), showed significant free radical scavenging activity (up to SC_{50} < 30 μg/ml). Among these extracts, *Nymphaea tetragona* (rhizome) extract showed the highest free radical scavenging activity (SC_{50} = 4.7 μg/ml). In the anti-elastase inhibition assay, seven extracts, including *Typha orientalis* (seed) and *Persicaria hydropiper* (whole plant), showed high inhibitory activity (>50% at 100 μg/ml). Among these extracts, *Persicaria hydropiper* (whole plant) extract showed the highest elastase inhibition activity (IC_{50} = 46.7 μg/ml). In the MMP-1 expression assay using RT-PCR, *Typha orientalis* (seed), *Pyrrosia hastata* (root), and *Capsicum annum* (whole plant) showed slightly lower inhibition activity than EGCG, which was used as a control. Furthermore, four extracts, including *Persicaria hydropiper* (whole plant), *Filipendula glaberrima* (root), *Nymphaea tetragona* (root), and *Camellia japonica* (leaf), completely inhibited the expression of MMP-1 in human fibroblast cells. The results showed that four of the 60 plant extracts may hold potential for use as natural active ingredients for anti-aging cosmetics.

INTRODUCTION

The skin consists of two distinct layers, epidermis and dermis. The dermis is the thicker, deeper layer of the skin underlying the epidermis, and is mainly composed of connective

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tissues, such as collagen and elastic fibers; it also contains proteins, nerves, blood vessels, lymph, and muscles. Among these, collagen fiber is the main component of the extracellular matrix (ECM) as the representative connective tissue and comprises about 90% of the dermis; collagen has a direct influence on skin tension. The maintenance of the collagen structure relates to the intrinsic aging and photo-aging of the skin (1,2). Therefore, a variety of investigations has been focused on protection against skin aging by the inhibition of collagenase activity, which disintegrates the ECM proteins (3).

It in generally known that, among the matrix metalloproteinases (MMPs), the enzymes related to collagenase mRNA include MMP-1, MMP-8, MMP-13, and MMP-18 (4). MMPs also play crucial roles in the degradation of basal membranes and ECM for tumor metastasis and permeation in situations such as tumor invasion, migration, and host immune escape, as well as in the destruction of connective tissue due to inflammatory diseases like rheumatoid arthritis, periodontal disease, osteoarthritis, gastric ulcer, and arteriosclerosis in pathological conditions (5,6). MMPs can be divided into four categories according to the preferred substrate: collagenase (MMP-1,8,13,18), gelatinase (MMP-2,9), stromelysin (MMP-3,10), and membrane-type MMPs (MMP-14,15,16,17) (7,8). Since the enzymatic activity of interstitial collagenase in degrading the collagen triple helix was reported by Gross and Lapiere in 1962 (9), a considerable amount of research has been focused on the MMP-1 of these matrix metalloproteinases. Therefore, it is suggested that the evaluation of the inhibition efficacy of materials on MMP-1 gene expression could be used as a screening method to find promising candidates that inhibit the degradation of collagens (10–12).

Elastin is another fibrous protein of the skin. Even though the distribution of elastin is much lower than that of collagen, elastin has an influence on skin elasticity (13).Because this elastic fiber is easily decomposed by elastase secretion and activation due to UV or ROS (reactive oxygen species), an approach that inhibits elastase activity could also be used as a useful method in protecting against skin aging (14).

Moreover, free radical species and ROS have been of interest to pharmacologists, biochemists, and other health professionals because they cause oxidative damage, and because substances generated from oxidative stress are believed to cause melanin and wrinkle formation (15). These species are formed by the body, sometimes resulting from exposure to sunlight, chemicals, and microbes (16). Evidence shows that polymeric substrates that play roles in retaining the elasticity of the skin, such as collagen, hyaluronic acid, and elastin, break down when exposed to active oxygen species (17,18). In this study, 60 plants, collected from Jeju Island, were extracted, and their efficacies for activities, such as scavenging of free radicals that cause photo-aging, inhibition of elastin degradation by elastase, and inhibition of the expression of MMP-1 to induce wrinkles on the skin, were evaluated. Because of the regional and climatic diversity, we chose natural plants living on Jeju Island for this screening.

Jeju Island is located south of the Korean Peninsula, and is the largest volcanic island in the country. The nature of the volcanic island remains well-preserved in its prehistoric state. Because Mt. Halla rises 1950 meters above sea level in the center of Jeju Island, the island has the diversity of a plant community at a variety of altitudes and latitudes. It is generally known that about 1800 plant species exist on this island (19).

MATERIALS AND METHODS

MATERIALS AND EQUIPMENT

Porcine pancreatic elastase (Type IV), DPPH (2,2-diphenyl-1-picrylhydrazyl), EGCG ((-)epigallocatechin-3-gallate), and N-Succinyl-Ala-Ala-Ala-*p*-nitroanilide (Mw, 454.4) were purchased from Sigma (Sigma-Aldrich, St. Louis, MO). All of the community available chemicals used in this study were reagent-grade, and were used in the form that they were received.

A UV-spectrophotometer (Hewlett-Packard, HP-8453) was used in the free radical scavenging activity test and the elastase inhibition activity assay. An ELISA reader (TECAN, A-5082 Sunrise) and PCR (Bio-Rad, Mycycler™ thermal cycler) were used in the cytotoxicity assay and the inhibition assay on MMP-1 expression.

PREPARATION OF PLANT EXTRACTS

Native plants were collected from all parts of Jeju Island and authenticated by Dr. C. S. Kim, director of the research institute for Mt. Halla in Mt. Halla National Park. The collected plants were dried in the shade at room temperature and stored in a dark, cold room until use. Dried plants were extracted twice with 70% (v/v) ethanol (ten times as much as the weight of the dried plants) for 24 h at room temperature. The extracts of the plants were filtered through Whatman paper (No. 5) and then evaporated at 60°C. After evaporation, the viscous residue was lyophilized in order to yield the product.

FREE RADICAL SCAVENGING ACTIVITY TEST

The free radical scavenging capacity assay was carried out according to the method previously reported by Blois (20). The DPPH (1,2-diphenyl-2-picrylhydrazyl) radical has a deep violet color due to its unpaired electron, and radical scavenging capacity can be followed spectrophotometrically by a loss of absorbance at 525 nm. DPPH (0.2 mM) 95% ethanolic solution (1 ml) was added to the sample stock (2 ml). Each sample stock solution was diluted with a 70% ethanolic solution to final concentrations of 100, 50, and 10 μg/ml, and the samples were then agitated. After 10 min, the optical density at 525 nm was measured by UV/Vis spectrophotometer. The free radical scavenging activity of the samples was calculated according to the formula:

$$\text{DPPH radical scavenging activity (\%)} = [1 - (OD_s - OD_b))/OD_c] \times 100$$

where OD_s is the absorbance of the experimental sample, OD_b is the absorbance of the blank, and OD_c is the absorbance of the control at 525 nm.

The results were reported in terms of SC_{50} (SC_{50}: concentration needed to reduce 50% of DPPH). BHT (di-*t*-butyl hydroxy toluene), a representative antioxidant, was used as a control.

ELASTASE ACTIVITY INHIBITION ASSAY

The evaluation of elastase activity was performed according to the method previously reported by Kraunsoe *et al.* (21). In order to evaluate the inhibition of elastase activity, the released amount of *p*-nitroaniline, hydrolyzed from the substance N-Succinyl-Ala-Ala-Ala-p-nitroanilide by elastase, was read with a maximum absorbance at 410 nm.

N-Succinyl-Ala-Ala-Ala-*p*-nitroanilide (1.015 mM) was prepared in 0.1232 M Tris-Cl buffer (pH 8.0) This solution (1300 µl) was added to the sample stock (100 µl). Each sample stock solution was diluted to final concentrations of 100, 50, and 10 µg/ml. The solutions were then vortexed and preincubated for 10 min at 25°C, and elastase (0.0375 unit/ml) stock solution (100 µl) was added. After vortexing, the solutions were placed in a water bath for 10 min at 25°C. The absorbance was measured at 410 nm.

CYTOTOXICITY ASSAY ON HUMAN FIBROBLASTS

Human fibroblast cells (American Type Culture Collection, ATCC, CRL-2076) were seeded in 24-well plates with DMEM +10% FBS at a density of 1×10^5 cells per well and cultured at 37°C in 5% CO_2. After one day, the medium was exchanged with fresh media containing 2% serum, and the cells were allowed to incubate in a CO_2 incubator at 37°C in the presence of samples (100 µl/ml) for 24 h. The cells were then treated with 100 µl of 2.5 mg/ml MTT and incubated at 37°C for an additional 4 h. The medium containing the MTT was discarded, the MTT formazan produced was extracted with 1 ml of DMSO, and the absorbance was read at a wavelength of 570 nm with a reference wavelength of 650 nm. The level of cell viability was calculated as:

$$\text{Cell viability } (\%) = (OD_{570(\text{sample})}/OD_{570(\text{control})}) \times 100$$

where $OD_{570(\text{sample})}$ is the absorbance of the treated cells at 570 nm and $OD_{570(\text{control})}$ is the absorbance of the negative control (non-treated cells) at 570 nm.

INHIBITION ASSAY ON MMP-1 EXPRESSION BY RT-PCR

Human fibroblasts were cultured with DMEM + 10% FBS, 50 U/mol penicillin, and 50 µg/ml of streptomycin; the medium was changed every two or three days. Cells were cultured at 37°C in 5% CO_2. When the cells reached confluence, they were separated by treatment with 0.25% trypsin-0.03% EDTA (ethylenediamine tetraacetic acid) solution. Cells were seeded into a 100-mm dish at a density of 2×10^6 cells and cultured at 37°C in 5% CO_2. After one day, fresh medium containing 2% serum was added to the cells, which were then treated with samples for 24 hours. Total RNA was isolated from the cells with TRIzol (Invitrogen) according to the instructions of the manufacturer. First-strand cDNA synthesis was performed using random hexamers. The sequences of primers are as follows: 5′-TGGGAGCAAACACATCTGA-3′ (sense) and 5′-ATCACTTCTCCCCGAATCGT-3′ (anti-sense) for MMP-1; 5′-GAGACCTTCA-ACACCCCAGCC-3′ (sense) and 5′-GGCCATCTCTTGCTCGAAGTC-3′ (anti-sense) for β-actin. For MMP-1 RT-PCR reactions, reverse transcription was performed at 50°C for 30 min, and denaturing was performed at 96°C for 3 min, followed by 22 cycles at 94°C for 1 min, 48°C for 1 min, and 72°C for 1 min, followed by an extension step cycle at 72°C for 10 min. For β-Actin RT-PCR reactions, reverse transcription was performed at 50°C for 30 min, and denaturing was performed at 96°C for 3 min, followed by 29 cycles at 94°C for 1 min, 70°C for 1 min, and 72°C for 1 min, followed by an extension step cycle at 72°C for 10 min. The final products were detected with 1.5% agarose gel. The gels were photographed, and the intensity of the stained PCR fragments was quantified from photographs by densitometric analysis using Gel Doc 2000 (Bio-Rad Laboratories, Segrate, Milan, Italy). EGCG ((−)epigallocatechin-3-gallate) was used as a positive control.

RESULTS AND DISCUSSION

PREPARATION OF PLANT EXTRACTS

About 60 plants obtained from Jeju Island were extracted with 70%(v/v) aqueous ethanol at room temperature. The yields were calculated by comparison of the amount of raw material used with that of the extracts obtained. The results of these calculations are shown in Table I. The highest yield was 32.70% for *Hizikia fusiformis* (whole plant) extract, and the yield was 26.15% for *Cimicifuga acerina* (root), 22.14% for *Daucus carota* L. (leaf), and 21.08% for *Cassia nomame* (whole plant).

FREE RADICAL SCAVENGING ACTIVITY

It was reported that free radicals induced by ultraviolet radiation or oxidative stress accelerate skin aging (16). Free radical scavenging capacity assays were carried out using the DPPH method. DPPH is one of a limited number of stable, commercially available organic nitrogen radicals, and has a UV-Vis absorption maximum at 515 nm. Upon reduction, the color of the solution fades; the progress of the reaction is conveniently monitored by a spectrophotometer. The free radical scavenging capacity of native plant extracts was measured at each concentration (1 ~ 400 μg/ml), and the results are shown in Table II. The free radical scavenging capacity was expressed as SC_{50}, the concentration needed to reduce 50% of DPPH.

Twelve extracts, *Erigeron annuus* (leaves and twigs, root), *Typha orientalis* (seed), *Torreya nucifera* (leaf), *Cassia nomame* (whole plant), *Myriophyllum spicatum* L. (whole plant), *Miscanthus sinensis* (flower), *Persicaria hydropiper* (whole plant), *Euphorbia jolkini* (whole plant), *Elsholtzia splendens* (whole plant), *Nymphaea tetragona* (rhizome), *Cimicifuga acerina* (root), and *Pyrrosia hastata* (root) had efficient free radical scavenging capacity (SC_{50} < 30 μg/ml). Among these, *Nymphaea tetragona* (rhizome) extract had the highest free radical scavenging activity (SC_{50} = 4.7 μg/ml). The SC_{50} values were 6.1 μm/ml for *Euphorbia jolkini* (whole plant), 9.4 μg/ml for *Euphorbia jolkini* (seed), and 9.5 μg/ml for *Persicaria hydropiper* (whole plant). These extracts showed extremely high free radical scavenging activity compared to BHT (di-*t*-butyl hydroxytoluene) (SC_{50} = 25.4 μg/ml), which was used as a positive control (Figure 1). Most radical scavengers that exist in a natural substance are phenolic or poly-phenolic compounds, such as flavonoids or anthocyanins. Their antioxidative activity is based on the inhibition of free radical transfer mechanisms. Therefore, it is suggested that 12 extracts with superior free radical scavenging capacity have certain levels of compounds, such as flavonoids or anthocyanins, and it is also believed that radical scavenging activity results from the presence of those compounds.

ELASTASE ACTIVITY INHIBITION

The 60 plant extracts, obtained from Jeju Island and prepared at concentrations of 10, 50, and 100 μg/ml, were tested for their inhibitory effect on porcine pancreatic elastase. The results are summarized in Table III. Seven extracts, *Typha orientalis* (seed), *Persicaria hydropiper* (whole plant), *Capsicum annum* (whole plant), *Nymphaea tetragona* (rhizome),

Table I
Plants and Their Ethanolic Extract Yields

Plant	Part used[a]	Gathering season (month)	Yield (%)
Acanthopanax koreanum	LF	8	18.51
Acanthopanax koreanum	ST	8	10.04
Acanthopanax koreanum	RT	8	15.87
Aruncus aethusifolius	RT	2	18.26
Aster spathulifolius	LT	1	10.07
Aster spathulifolius	RT	1	14.49
Brassica campestris	ST	4	2.30*
Brassica campestris	FL	4	2.50*
Brassica campestris	RT	4	3.60*
Camellia japonica	LF	3	9.5
Camellia sienensis	SD	1	9.86*
Capsicum annum	WP	12	8.61
Cassia nomame	WP	8	21.08
Chrysanthemum indicum	WP	12	16.48
Chrysanthemum zawadskii	WP	11	9.91
Cimicifuga acerina	RT	2	26.15
Citrus grandis	LF	11	13.23
Citrus grandis	ST	11	8.00
Citrus unshiu	LF	7	12.75
Citrus unshiu	ST	7	8.19
Clematis terniflora	LT	9	14.45
Clerodendron trichotomum	LF	8	17.56
Clerodendron trichotomum	ST	8	7.49
Commelina communis L.	WP	9	5.64
Crinum asiaticum	SD	11	8.75*
Crinum asiaticum	RT	5	6.80*
Crinum asiaticum	LT	5	5.60*
Daphniphyllum macrophodum	ST	8	9.20
Daphniphyllum macropodum	LF	8	11.53
Daucus carota L.	LF	12	22.14
Elsholtzia splendens	WP	12	6.31
Erigeron annuus	RT	6	8.58
Erigeron annuus	LT	6	11.23
Euphorbia esula L.	WP	11	10.38
Euphorbia helioscopia	WP	5	9.27
Euphorbia jolkini	WP	11	9.00
Euphorbia jolkini	SD	12	7.26
Farfugium japonicum	WP	1	19.61
Filipendula glaberrima	RT	2	18.54
Hizikia fusiformis	WP	6	32.70
Kummerowiastriata	WP	8	12.54
Lycoris aurea	RT	11	8.61*
Maackia fauriei	ST	12	6.38
Miscanthus sinensis	FL	10	3.55
Miscanthus sinensis	RT	10	1.94
Miscanthus sinensis	ST	10	5.42
Myriophyllum spicatum L.	WP	8	13.53
Narcissus tazetta	LT	1	10.95
Nymphaea tetragona	RH	1	5.72
Persicaria hydropiper	WP	9	5.94
Potamogeton crispus L.	WP	8	11.38
Pyrrosia hastata	LT	2	12.16

(continued)

Table I (continued)

Plant	Part used[a]	Gathering season (month)	Yield (%)
Pyrrosia hastata	RT	2	14.03
Sasa quelpaertensis	LT	5	7.65*
Scirpus tabernaemontani	LT	8	5.72
Solanum nigrum L.	WP	9	8.88
Solanum tuberosum L.	RH	5	1.10*
Thymus quinquecostatus	WP	12	11.52
Torreya nucifera	ST	10	6.23
Torreya nucifera	LF	10	14.11
Typha orientalis	SD	8	4.98
Zingiber mioga	ST	8	13.11

[a] Part used: LF (leaf), ST (stem), LT (leaves and twigs), RT (root), WP (whole plant), SD (seed), FL (flower), RH (rhizome).
* Yields were calculated from the fresh plant, and the others were calculated from the dried plant.

Filipendula glaberrima (root), *Pyrrosia hastata* (root), and *Camellia japonica* (leaf), showed high inhibition efficacy (>50% at 100 μg/ml). Among these, *Persicaria hydropiper* (whole plant) extract was found to have the highest elastase inhibition activity (IC_{50} = 46.7 μg/ml). The IC_{50} values for the elastase inhibition activity of *Typha orientalis* (seed), *Nymphaea tetragona* (rhizome), and *Capsicum annum* (whole plant) were 60.1 μg/ml, 70.4 μg/ml, and 71.1 μg/ml, respectively (Figure 2). Furthermore, five of the extracts listed above, *Typha oreintalis* (seed), *Persicaria hydropiper* (whole plant), *Nymphaea tetragona* (rhizome), *Filipendula glaberrima* (root), and *Pyrrosia hastata* (root), also showed significantly high DPPH radical scavenging activity, which suggests that the origins of the two activities were similar.

Elastase is a highly cationic enzyme, and its active site holds its hydrophilic property. It has been reported that the binding of elastase to hydrophobic substrates or ligands is so weak that the degradation of substrates is inhibited (23). Tixier *et al.* (24) reported that condensed polyphenols, such as proanthocyanidin, inhibited catalytic activity by binding to elastin. Therefore, it is likely that the seven extracts with the highest elastase inhibition activity possess relatively hydrophobic compounds or phenolic compounds, such as polyphenols and flavonoids.

CYTOTOXICITY ASSAY ON HUMAN FIBROBLASTS

Out of 60 native plants of Jeju Island, seven plant extracts with superior effects on free radical scavenging activity and elastase inhibition were selected; those plants were *Typha orientalis* (seed), *Persicaria hydropiper* (whole plnat), *Capsicum annum* (whole plant), *Nymphaea tetragona* (rhizome), *Filipendula glaberrima* (root), *Pyrrosia hastata* (root), and *Camellia japonica* (leaf). The cytotoxicities of these seven extracts on human fibroblasts (ATCC, CRL-2076) are shown in Figure 3. They did not show any cytotoxicity (cell viability >99%) compared to EGCG, which was used as a positive control at a concentration of 100 μg/ml. These findings suggest that effective active ingredients could be produced with no cytotoxicity. Conversely, extracts such as *Filipendula glaberrima* (root), *Capsicum annum* (whole plant), and *Nymphaea tetragona* (rhizome) had cell proliferation activities measured at 108.8%, 111.5%, and 111.6%, respectively.

Table II
Free Radical Scavenging Activity of Plant Extracts

Plant: Botanical name (part used)[a]	DPPH radical scavenging activity (%) 400 µg/ml	200 µg/ml	100 µg/ml	50 µg/ml	10 µg/ml	1 µg/ml	SC_{50}* (µg/ml)
Acanthopanax koreanum (LF)	—	83.6	80.8	78.5	17.3	—	31.2
Acanthopanax koreanum (RT)	—	72.1	35.6	6.3	—	—	137.6
Acanthopanax koreanum (ST)	—	77.6	67.2	29.7	1.7	—	77.3
Aruncus aethusifolius (RT)	—	81.2	84.4	84.6	27.1	—	26.1
Aster spathulifolius (LT)	53.2	28.3	14.2	—	—	—	372.3
Aster spathulifolius (RT)	42.6	22.9	11.8	—	—	—	>400
Brassica campestris (ST)	32.7	—	—	—	30	—	>400
Brassica campestris (FL)	88	45.3	—	—	27.4	—	250
Brassica campestris (RT)	40	—	—	—	8.9	—	>400
Camellia japonica (LF)	—	64.3	48.7	32.2	10	—	120
Camellia sinensis (SD)	38.2	21.7	11.1	—	—	—	>400
Capsicum annum (WP)	57	32.1	21.3	14.3	9.4	—	345.3
Cassia nomame (WP)	—	—	91.2	89.6	35.6	1.9	20.7
Chrysanthemum indicum (WP)	—	—	62.5	35.4	—	—	72.7
Chrysanthemum zawadskii (WP)	32	18.6	—	—	—	—	>400
Cimicifuga acerina (RT)	—	70.6	37.2	19.1	5.4	—	138.5
Citrus grandis (LF)	85.8	66.8	37.1	19.9	—	—	143.3
Citrus grandis (ST)	64.4	35.8	20.4	10.2	—	—	299.5
Citrus unshiu (LF)	—	53.1	39.8	22.3	6.5	—	175
Citrus unshiu (ST)	76.5	44.3	24.9	11.1	3.4	—	280
Clematis terniflora (LT)	71.1	39.2	—	—	—	—	267.9
Clerodendron trichotomum (LF)	—	—	88.1	78.1	15.3	1.3	32
Clerodendron trichotomum (ST)	—	—	86.3	70.6	15.6	1.5	35
Commelina communis L. (WP)	82.9	76	47.4	—	—	—	108.3
Crinum asiaticum (SD)	8.5	4.4	—	—	—	—	>400
Crinum asiaticum (LT)	—	—	—	0.5	2.9	—	>400
Crinum asiaticum (RT)	—	—	0.8	4.1	8	—	>400
Daphniphyllum macropodum (LF)	—	90	81.4	48.9	—	—	62.1
Daphniphyllum macropodum (ST)	—	88.9	61.7	33.8	—	—	76.1
Daucus carota L. (LF)	—	68.8	38.2	24.1	9.2	—	138.7
Elsholtzia splendens (WP)	—	90	88.2	82.3	19	—	29.8
Erigeron annuus (LT)	—	—	90.3	74.3	32.4	7.8	22.2
Erigeron annuus (RT)	—	—	74.2	72.1	16.1	3.1	34.1
Euphorbia esula L. (WP)	—	77.8	43.7	25.5	7.6	—	136.3
Euphorbia helioscopia (WP)	—	—	79.7	73.4	25.6	—	31
Euphorbia jolkini (SD)	—	91.8	91.4	90.3	58.7	14.5	9.4
Euphorbia jolkini (WP)	—	—	—	87.7	69.9	23.1	6.1
Farfugium japonicum (WP)	—	75.3	50.3	27.6	9.2	—	99.9
Filipendula glaberrima (RT)	—	87.7	88.7	89.2	35.6	—	20.5
Hizikia fusiformis (WP)	14.9	—	—	—	2.4	—	>400
Kummerowiastriata (WP)	—	—	82.4	57.9	7.7	—	43.6
Lycoris aurea (RT)	19.7	10.5	—	—	—	—	>400
Maackia fauriei (ST)	78.6	49.8	29.8	20.5	—	—	201.8
Miscanthus sinensis (FL)	—	—	91.1	82.4	17.9	2.5	29.9
Miscanthus sinensis (RT)	89.7	72.2	39.2	—	—	—	132.4
Miscanthus sinensis (ST)	—	—	84.8	53.1	—	—	46.8
Myriophyllum spicatum L. (WP)	—	—	88.2	86.7	39.2	—	14.4
Narcissus tazetta (LT)	59.8	33.3	16.8	—	—	—	324.3
Nymphaea tetragona (RH)	—	—	93	93	91.8	20.1	4.7
Persicaria hydropiper (WP)	—	—	—	86.5	52.7	5.7	9.5

(continued)

Table II (continued)

Plant: Botanical name (part used)[a]	DPPH radical scavenging activity (%) 400 μg/ml	200 μg/ml	100 μg/ml	50 μg/ml	10 μg/ml	1 μg/ml	SC_{50}* (μg/ml)
Potamogeton crispus L. (WP)	—	87.9	74	39.4	—	—	65
Pyrrosia hastasts (LT)	—	77.2	75.3	42.3	9.3	—	61.6
Pyrrosia hastata (RT)	—	81.5	83.9	79.6	19.9	—	30.1
Sasa quelpaertensis (LT)	50	—	—	—	14.9	—	>400
Scirpus tabernaemontani (LT)	—	—	85.2	78.5	16.7	2.2	31.5
Solanum nigrum L. (WP)	—	—	—	—	—	—	>400
Solanum tuberosum L. (RH)	30.1	—	—	—	3.7	—	>400
Thymus quinquecostatus (WP)	—	79.8	52.8	26.7	—	—	94.6
Torreya nucifera (LF)	—	—	90.4	89.2	31.9	6.5	22.7
Torreya nucifera (ST)	—	72.6	46.4	28.9	—	—	112.8
Typha orientalis (SD)	—	—	89.6	88.9	34.1	6.7	21.6
Zingiber mioga (ST)	30.2	—	—	—	—	—	>400
BHT	—	—	82.5	64.1	33.1	6.2	25.4

[a] Part used: LF (leaf), ST (stem), LT (leaves and twigs), RT (root), WP (whole plant), SD (seed), FL (flower), RH (rhizome).

* SC_{50}; Concentration (μg/ml) at which the inhibition % of DPPH radical scavenging activity is 50%. BHT: di-*t*-butyl hydroxyl toluene.

ASSAY ON MMP-1 EXPRESSION BY RT-PCR

It was mentioned above that the major component of ECM in the dermis is collagen fiber, and ECM degradation is associated with the generation of wrinkles. The enzymes related to collagenase mRNA, and which degrade collagen fiber, are known as MMPs (matrix metalloproteinases) (4). They can be divided into four main classes: collagenase (MMP-1,8,13,18), gelatinase (MMP-2, 9), stomelysin (MMP-3,10), and membrane-type MMPs (MMP-14,15,16,17), (7,8). Since Gross and Lapiere reported the ability of MMP-1 to degrade the collagen triple helix 1962 (9), it has been used in the evaluation of collagen degradation activity by estimating the amount of MMP-1 mRNA. In this study, in order to screen anti-aging candidates that would inhibit the degradation of collagen fiber, we investigated the reduction of MMP-1 expression for seven extracts with superior elastase inhibition activity using the RT-PCR method. EGCG was used as a positive control because its activities were well-known for the inhibitory effect on collagenase and stromelysin mRNA expression induced by L-1β (25) and for the protective effect against skin damage due to UVB (26).

The MMP-1 expression assay in human fibroblasts was carried out with a gel image analyzer (Bio-Rad, Gel DOC 2000). The relative amounts of MMP-1 expression extracts were compared at a concentration of 100 μg/ml, and the results from seven plants are shown in Figure 4. The results showed that MMP-1 expression rates of *Typha orientalis* (seed), *Pyrrosia hastata* (root), and *Capsicum annum* (whole plant) were a little higher or similar to that of EGCG, while four extracts, *Persicaria hydropiper* (whole plant), *Filipendula glaberrima* (root), *Nymphaea tetragona* (rhizome), and *Camellia japonica* (leaf), showed up to 100% inhibition of MMP-1 expression.

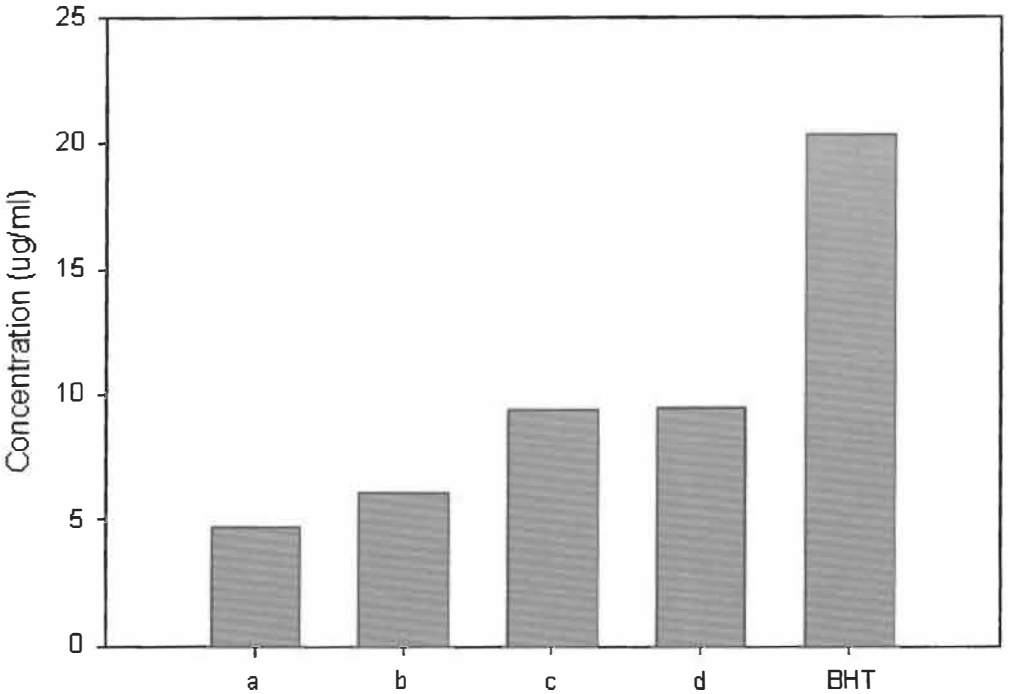

Figure 1. SC_{50} of several extracts on DPPH reduction. a: *Nymphaea tetragona* (rhizome). b: *Euphorbia jolkini* (whole plant). c: *Euphorbia jolkini* (seed). d: *Persicaria hydropiper* (whole plant). BHT (di-*t*-butyl hydroxy toluene) as a positive control.

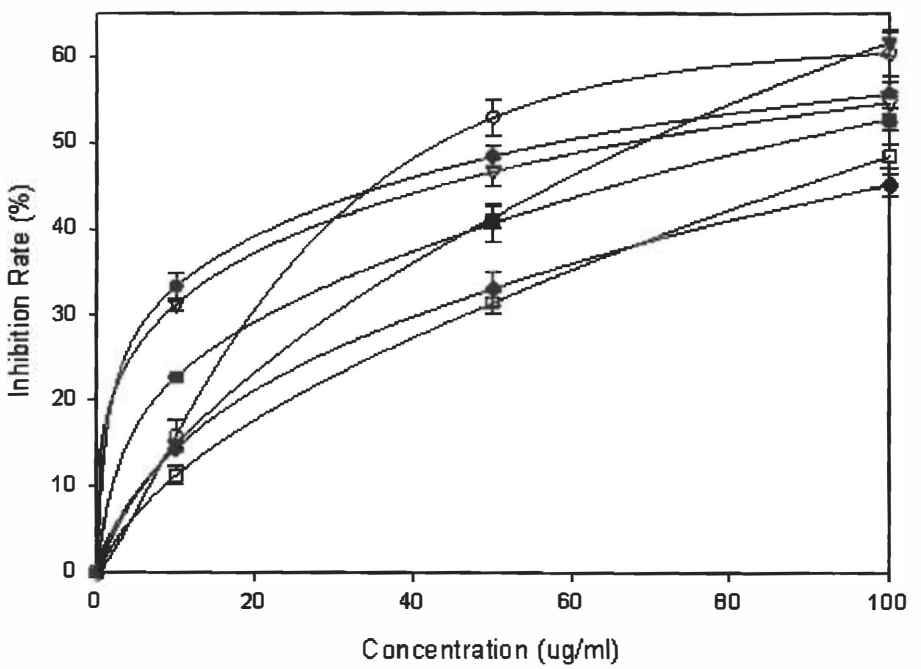

Figure 2. Anti-elastase activity of seven plant extracts. ◆: *Camellia japonica* (leaf). ○: *Persicaria hydropiper* (whole plant). ●: *Typha orientalis* (seed). ▼: *Capsicum annum* (whole plant). ■: *Filipendula glaberrima* (root). □: *Pyrrosia hastata* (root). ▽: *Nymphaea tetragona* (rhizome).

Table III
Elastase Inhibition Activity of Plant Extracts

Plant: Botanical name (part used)[a]	Inhibition (%)			IC_{50} (μg/ml)[b]
	100 μg/ml	50 μg/ml	10 μg/ml	
Acanthopanax koreanum (LF)	—	—	—	—
Acanthopanax koreanum (RT)	—	9.2	5.2	—
Acanthopanax koreanum (ST)	—	1.3	3.5	—
Aruncus aethusifolius (RT)	6.7	4.1	8.7	—
Aster spathulifolius (LT)	17.8	20.1	19.4	—
Aster spathulifolius (RT)	25.5	26.2	26	—
Brassica campestris (ST)	13	12.6	17.4	—
Brassica campestris (FL)	15.6	23.5	21.2	—
Brassica campestris (RT)	15.5	13	15.4	—
Camellia japonica (LF)	45.2	33.1	14.3	124.6
Camellia sinensis (SD)	14.9	17.1	17	—
Capsicum annum (WP)	61.7	41.4	14.7	71.1
Cassia nomame (WP)	29.9	22.8	10.7	—
Chrysanthemum indicum (WP)	—	2.3	3.5	—
Chrysanthemum zawadskii (WP)	11.4	10.4	10.1	—
Cimicifuga acerina (RT)	6.9	1.1	4	—
Citrus grandis (LF)	13.9	12.5	12	—
Citrus grandis (ST)	5.7	8.6	7.3	—
Citrus unshiu (LF)	3.7	22.9	—	—
Citrus unshiu (ST)	—	—	7.1	—
Clematis terniflora (LT)	8.8	11	10.5	—
Clerodendron trichotomum (LF)	2.5	—	—	—
Clerodendron trichotomum (ST)	1.5	—	1.1	—
Commelina communis L. (WP)	8.2	0.6	3	—
Crinum asiaticum (SD)	0.4	—	—	—
Crinum asiaticum (LT)	10	15.7	18.8	—
Crinum asiaticum (RT)	14.2	16.3	22.1	—
Daphniphyllum macropodum (LF)	—	5.9	11.8	—
Daphniphyllum macropodum (ST)	—	3.7	13.2	—
Daucus carota L. (LF)	3.9	7.9	7.3	—
Elsholtzia splendens (WP)	1.6	6.5	4.5	—
Erigeron annuus (LT)	—	—	0.4	—
Erigeron annuus (RT)	—	—	—	—
Euphorbia esula L. (WP)	14.5	15	15.1	—
Euphorbia helioscopia (WP)	40.4	36.7	22.3	—
Euphorbia jolkini (SD)	14.7	12.6	3.5	—
Euphorbia jolkini (WP)	7.3	15.1	39.4	—
Farfugium japonicum (WP)	—	—	1.8	—
Filipendula glaberrima (RT)	52.8	40.8	22.6	88.2
Hizikia fusiformis (WP)	30.2	30.1	31.9	—
Kummerowiastriata (WP)	5.4	—	—	—
Lycoris aurea (RT)	6.4	17.3	18.9	—
Maackia fauriei (ST)	2.5	10	4.9	—
Miscanthus sinensis (FL)	20	17	12.8	—
Miscanthus sinensis (RT)	10.8	7.6	8.8	—
Miscanthus sinensis (ST)	12.9	15.8	11.9	—
Myriophyllum spicatum L. (WP)	19.7	19	12.7	—
Narcissus tazetta (LT)	15.4	15.2	14.8	—
Nymphaea tetragona (RH)	54.7	46.7	31.2	70.4
Persicaria hydropiper (WP)	60.4	52.9	15.8	46.7

(continued)

Table III (continued)

Plant: Botanical name (part used)[a]	DPPH radical scavenging activity (%)			IC_{50} (μg/ml)
	100 μg/ml	50 μg/ml	10 μg/ml	
Potamogeton crispus L. (WP)	6.5	5.1	6.4	—
Pyrrosia hastata (LT)	8.4	1.8	6	—
Pyrrosia hastata (RT)	48.5	31.5	11.23	105.1
Sasa quelpaertensis (LT)	24.9	25.2	25	—
Scirpus tabernaemontani (LT)	28.1	22.2	11.3	—
Solanum nigrum L. (WP)	6.9	8.5	6.3	—
Solanum tuberosum L. (RH)	40.4	38	34.9	—
Thymus quinquecostatus (WP)	9.5	15.1	17.7	—
Torreya nucifera (LF)	8.6	7.9	6.5	—
Torreya nucifera (ST)	1.8	4	3.7	—
Typha orientalis (SD)	55.7	48.5	33.3	60.1
Zingiber mioga (ST)	13.3	14.9	10.3	—

[a] Part used: LF (leaf), ST (stem), LT (leaves and twigs), RT (root), WP (whole plant), SD (seed), FL (flower), RH (rhizome).
[b] IC_{50}: Concentration (μg/ml) at which the inhibition % of elastase activity is 50%.

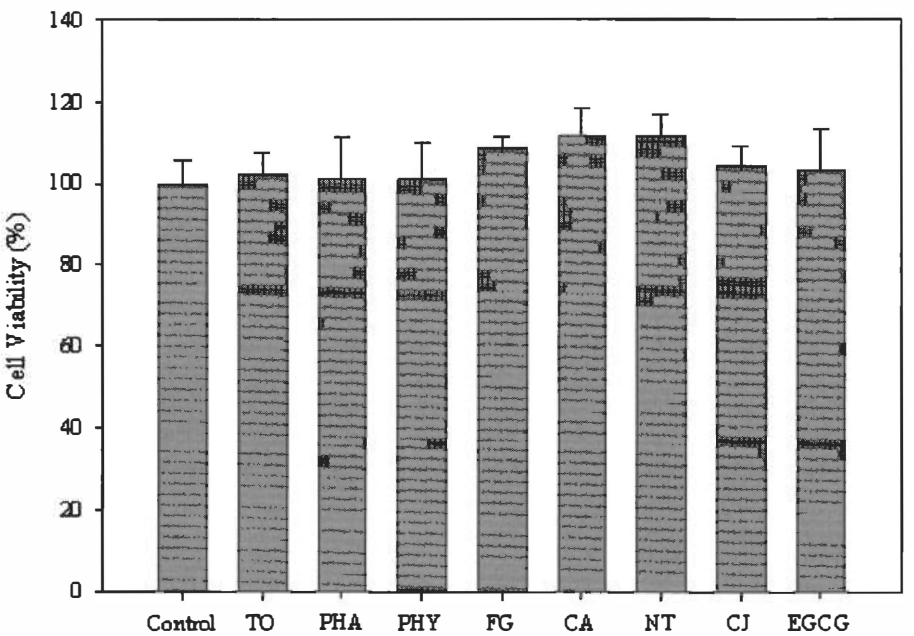

Figure 3. Cytotoxicity in human fibroblasts (ATCC, CRL-2076). All plant extracts were used at 100 μg/ml, except EGCG (25 μM). TO: *Typha orientalis* (seed). PHA: *Pyrrosia hastata* (root). PHY: *Persicaria hydropiper* (whole plant). FG: *Filipendula glaberrima* (root). CA: *Capsicum annum* (whole plant). NT: *Nymphaea tetragona* (rhizome). CJ: *Camellia japonica* (leaf). EGCG: (-)-*epigallocatechin-3-gallate*.

CONCLUSIONS

Jeju Island is the largest island located in the southernmost part of the Republic of Korea. Mt. Halla rises 1950 meters above sea level in the center of the island. Therefore, due to its altitude and latitude, a variety of plant species are present on the island in spite

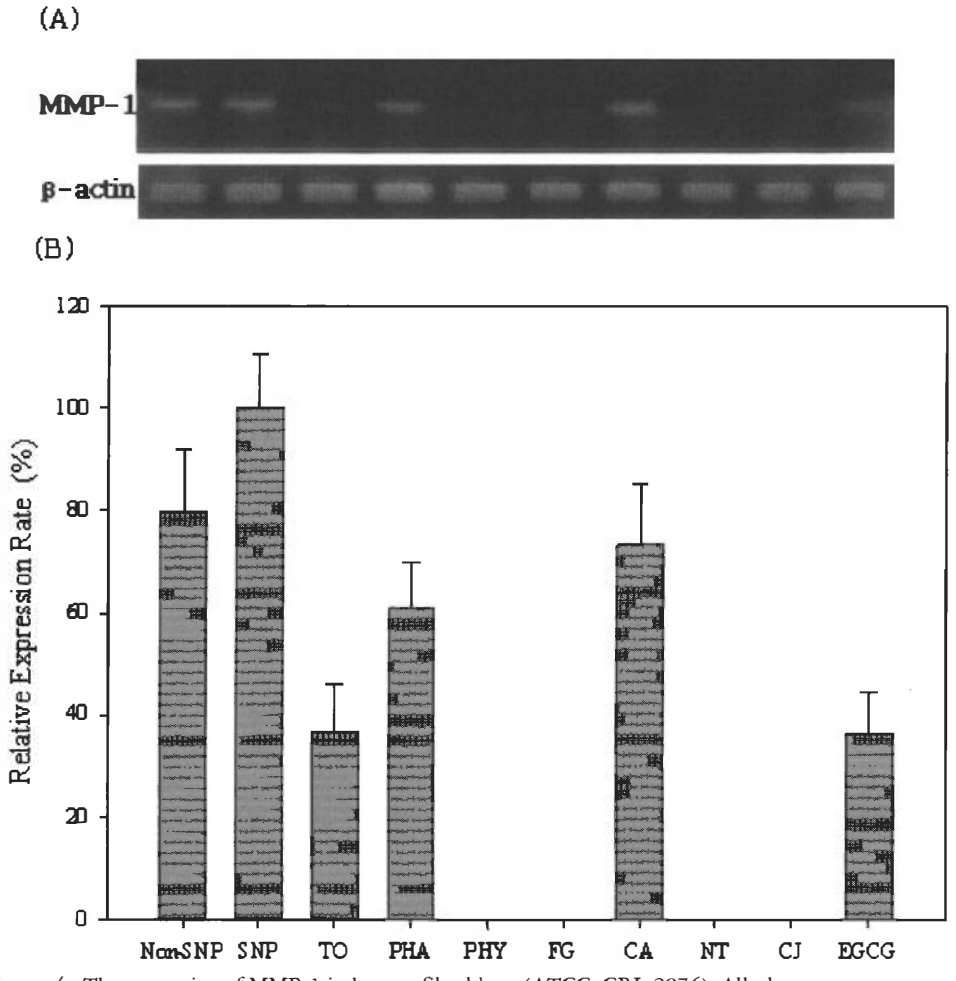

Figure 4. The expression of MMP-1 in human fibroblasts (ATCC, CRL-2076). All plant extracts were used at 100 μg/ml, except EGCG (25 μM). Non-SNP: in the absence of sodium nitroprusside. SNP: in the presence of sodium nitroprusside. TO: *Typha orientalis* (seed). PHA: *Pyrrosia hastata* (root). PHY: *Persicaria hydropiper* (whole plant). FG: *Filipendula glaberrima* (root). CA: *Capsicum annum* (whole plant). NT: *Nymphaea tetragona* (rhizome). CJ: *Camellia japonica* (leaf). EGCG: (-)epigallocatechin-3-gallate.

of its small area. We searched for plants with effective anti-aging compounds for the skin among the natural plants of Jeju Island.

Sixty plants from Jeju Island were extracted, and their activities were investigated for free radical scavenging activity, elastase inhibition activity, and reduction of the expression of MMP-1 mRNA related to collagenase, which is known to degrade collagenase and induce wrinkling of the skin. As a result, we found twelve plants with highly effective DPPH radical scavenging activity and seven plants with good anti-elastase activity. Five of the seven species with superior effects on elastase activity inhibition also had high DPPH radical scavenging activity, which suggests that there is a certain correlation between the causes of the two activities. When comparing the inhibition activities of the seven extracts on MMP-1 expression in human fibroblasts with EGCG as a positive control, four extracts, *Persicaria hydropiper* (whole plant), *Filipendula glaberrima* (root),

Nymphaea tetragona (rhizome), and *Camellia japonica* (leaf) completely inhibited MMP-1 expression in human fibroblasts and could be developed as active ingredients for anti-aging cosmetics. These results encourage us to find the active compounds of those extracts. Further study regarding the active compounds is necessary, and we hope to report the results of these investigations in the near future.

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