



Screening of elastase and tyrosinase inhibitory activity from Jeju Island plants

Ji-Young Moon^{1,2}, Eun-Young Yim¹, Gwanpil Song¹, Nam Ho Lee^{2,3},
Chang-Gu Hyun^{1,2*}

¹Jeju Biodiversity Research Institute, Jeju High-Tech Development Institute, 699-943 Jeju Korea

²Research Group for Beauty and Cosmetics, Cheju National University, 690-756 Jeju, Korea

³Department of Chemistry, Cheju National University, 690-756 Jeju, Korea

Corresponding Author: cghyun@jejuhidi.or.kr

Abstract

To identify new active cosmetics ingredients of natural origin, we screened 299 parts of 263 plant species collected from Jeju Island, the southernmost island of the Korean Peninsula. Plant parts were investigated for their elastase and tyrosinase inhibitory activity for the purpose of identifying anti-aging and skin-whitening ingredients with the potential for use as raw materials in cosmetics. In the anti-elastase inhibition assay, 3 extracts, including *Aesculus turbinata*, *Taxillus yadoriki*, and *Cornus walteri*, showed high inhibitory activity (inhibition concentration (IC₅₀) < 50 μg/mL). The IC₅₀ of *A. turbinata* and *T. yadoriki* was 43.1 μg/mL and 36.4 μg/mL, respectively; *C. walteri* showed the highest elastase inhibition activity (IC₅₀ = 26.1 μg/mL). In the tyrosinase inhibition assay, 4 extracts, including *C. walteri* (139.2 μg/mL), *Maackia fauriei* (149.3 μg/mL), *Toxicodendron succedaneum* (142.3 μg/mL), and *Sophora flavescens* (41.6 μg/mL), showed significantly greater tyrosinase inhibition activity than the positive controls *Distylium racemosum* (145.9 μg/mL) and arbutin (180.3 μg/mL). However, they showed lower activity compared to the positive controls *Morus alba* (11.9 μg/mL) and *Morus bombycis* (22 μg/mL). These results suggest that medicinal plants possessing several biological activities may be potent inhibitors of the processes involved in pigmentation increases and aging. Further investigations will focus on in vivo assays and the chemical identification of the major active components responsible for anti-aging and whitening.

Keywords: Cosmetics, elastase, Jeju Island, medicinal plants, tyrosinase.

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INTRODUCTION

The skin is a fundamentally important organ of the body, protecting it from damage caused by direct contact with the outside environment. Of the environmental factors that injure the skin, ultraviolet (UV) irradiation is the most common and pernicious. It leads to alterations in the composition of the skin, including the accumulation of elastic fibres (Braverman and Fonferko 1982), collagen reduction and degeneration (Oikarinen and Kallionen 1989) and deposition of glycosaminoglycans (Smith et al. 1962). Such damage to the skin is believed to lead to reduced skin elasticity and the linearity of dermal elastic fibers, inducing wrinkling and sagging (Kambayashi et al. 2001). Moreover, UV irradiation sets in action an integrated

mechanism for the formation and delivery of melanin, within melanosomes, from melanocytes to keratinocytes (Costin et al. 2007, Lee et al. 2009). Elastase is the only enzyme that is capable of breaking down elastin, an insoluble elastic fibrous protein that, together with collagen, determines the mechanical properties of connective tissue (Antonicek et al. 2007). Several studies have demonstrated that both skin-aging and antiwrinkle effects are significantly correlated with decreased elastase activity. Chatterjee et al. (1990) reported an increase in the degradation of elastin after chronic UV-B

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irradiation at a suberythral dose in hairless mice. Labat-Robert et al. (2000) also reported an increase in elastase activity after chronic UV-B irradiation. Recently, a number of studies have investigated the interactions between elastase and its inhibitors (Lee et al. 1999, Kim et al. 2007c, Masuda et al. 2009, Thring et al. 2009). It has been proposed, but as yet not completely proven, that the topical application of specific inhibitors to the surface of human skin may have beneficial effects on UV-irritated and dry skin.

Tyrosinase, a copper-containing monooxygenase, is a key enzyme that catalyses melanin synthesis in melanocytes. Since the accumulation of excessive epidermal pigmentation leads to various dermatological disorders, such as melasma associated with age, freckling, age spots, and sites of actinic damage, tyrosinase inhibitors have become increasingly important in medication and in cosmetics to prevent hyperpigmentation through the inhibition of enzymatic oxidation (Briganti et al. 2003, Parvez et al. 2007).

Traditional herbal medicines have been used widely for thousands of years in many Asian countries, including Korea, Japan, and China, and they provide a largely unexplored source for the potential development of new drugs. Indeed, the potential use of traditional herbal medicines as a basis for new skin-care products recently has received increased attention. It is therefore of interest to know whether cosmetic preparations traditionally used in folk medicine have bioactivity that might be useful in modern formulations. Because of its unique ecosystem, Jeju Island - the southernmost island of the Korean Peninsula (Fig. 1) - is known for the richness and diversity of its flora, of which over 7800 species have been classified to date (Kang 2007, Yang et al. 2009). Over the past few years, we have systematically evaluated and characterised selected plant species for their putative bioactivity or potential medicinal application (Kim et al. 2007a, Kim et al. 2007b). As part of the effort to find new functional ingredients for skin-whitening and antiwrinkle preparations, we investigated 299 plant extracts indigenous to Jeju Island, evaluating their *in vitro* anti-tyrosinase and anti-elastase activity.

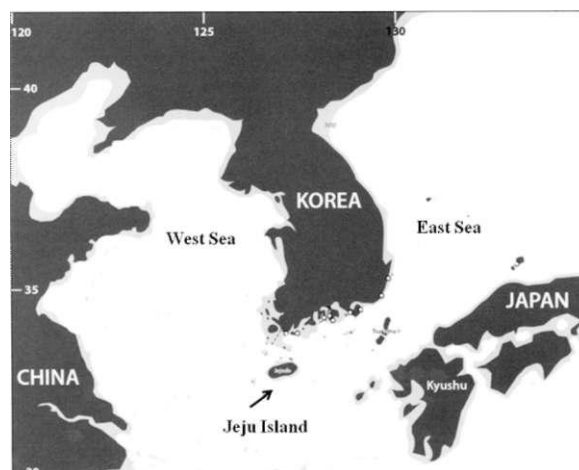


Fig. 1. Map of the Korean Peninsula.

MATERIAL AND METHODS

Plant materials and solvent extraction

Plants were collected from Jeju Island from 2006 to 2008. Voucher specimens were deposited at the herbarium of Jeju Biodiversity Research Institute (JBRI). Verification of vouchers or living plants was performed by Dr. Gwanpil Song. Excised plant parts (i.e. bark, leaf, root, fruit and whole plants) were shade dried at room temperature for 7 d and then powdered by using electric blender. The powdered samples (mesh size 1 mm) were extracted with 80% (v/v) ethanol. After the sample was filtered through two layers of cheesecloth, the filtered cakes were extracted and filtered three more times to increase the extraction yield. All the extracts were mixed together and then filtered using a sheet of Whatman No. 1 filter paper. The filtrates were concentrated under reduced pressure, freeze-dried, and stored in a closed container until testing (Yang et al. 2009).

Tyrosinase inhibition assay

Each plant extract was assayed for tyrosinase inhibition by measuring its effect on tyrosinase activity using a 96-well reader (PowerWave XS; BioTek). The reaction was carried out in a 100 mM potassium phosphate buffer (pH 6.7) containing 1.5 mM L-tyrosine and 100 U/mL mushroom tyrosinase at 37°C. The reaction mixture was pre-incubated for 15 min before adding the substrate. The change of the absorbance at 490 nm was measured. The percent inhibition of tyrosinase was calculated as follows:

$$\text{Inhibition (\%)} = [(A-B)/A] \times 100,$$

where A is absorbance at 490 nm without plant extract, and B is the change in absorbance at 490 nm with plant extract.

Elastase inhibition assay

The activity of porcine pancreatic elastase [PPE (Type IV); Sigma Chem. Co.) was examined using N-Suc-(Ala)₃-nitroanilide as the substrate, and the release of -nitroaniline at 410 nm was measured. The reaction was carried out in a 200 mM Tris-HCl buffer (pH 8.0) containing 5 mM N-Suc-(Ala)₃-nitroanilide and 10 µg/mL elastase. Plant extract was added to the reaction mixture to reach a final concentration of 500 µg/mL, and elastase inhibition was assessed at 25°C. The reaction mixture was pre-incubated for 10 min before adding the substrate. The change in absorbance was measured at 410 nm using a 96-well reader. The percent inhibition of elastase was calculated as follows:

$$\text{Inhibition (\%)} = [(A-B)/A] \times 100,$$

where A is absorbance at 410 nm without plant extract, and B is the change in absorbance at 410 nm with plant extract.

Analysis of total polyphenol

To determine the total polyphenol content in the plant extracts, the adjusted method with Folin-Ciocalteu reagent (Merck) was used. We added 550 µL of distilled water/Folin-Ciocalteu solution (10:1, v/v) to 50 µL of diluted extract (1 mg/mL of ethanol). After 3 min, 200 µL of 2 M sodium carbonate (Na₂CO₃) and 300 µL of distilled water were added. After 1 h standing at laboratory temperature, absorbance was measured at 725 nm. The total polyphenol content was calculated as a phloroglucinol equivalent from the calibration curve of phloroglucinol standard solutions (concentration range, 0–1.0 mg/mL). All measurements were conducted in triplicate.

RESULTS and DISCUSSION

In many parts of Asia, maintenance of healthy skin, skin whitening, and protection from darkening are considered desirable for cosmetic purposes. Wrinkle and melanin formation are believed to be induced mainly by UV light and other stimuli such as toxic chemical agents and daily stress. To meet the demand for cosmetic preparations with antiwrinkle and skin-whitening properties, many cosmetics companies have been

developing elastase and melanogenesis inhibitors because of their potential as active agents. In addition, the global market has seen an increased demand for natural substances, such as plant extracts, that can be used for depigmenting, antiwrinkle, and other cosmeceutical purposes (Kiken and Cohen 2002, Wang et al. 2006). Moreover, plant extracts with an inhibitory effect on melanin formation and elastase activity may be good choices for cosmetic purposes because of their relatively low incidence of side effects. At present, cosmetic preparations have been developed using plant extracts such as *Areca catechu* (Lee and Choi 1999) and *Morus alba* (Shin et al. 1998, Lee et al. 2002) as antiwrinkle and whitening agents. In the current investigation into the cosmetic properties of materials from Jeju Island indigenous plants, we examined 299 traditional Jeju Island herbal medicines (263 species of 132 different families) for their use in skin whitening and skin health. Specifically, we evaluated the effects of these medicines on elastase and tyrosinase activity.

As shown in Table 1, 192 of 273 plant extracts showed no inhibition (<30% inhibition at 500 µg/mL concentration) of PPE activity. In contrast, plant extracts from 16 species, including *Cryptomeria japonica*, *Machilus japonica*, *Machilus thunbergii*, *Melia azedarach*, *Euscaphis japonica*, *Viburnum odoratissimum*, *Cornus controversa*, *Cornus walteri*, *Actinodaphne lancifolia*, and *Taxillus yadoriki* showed more than 85% inhibition of PPE at 500 µg/mL. To determine the IC₅₀ values of the 16 plant extracts showing high biological activity, experiments to determine the dose-response relationship were performed. Table 2 presents the IC₅₀ values calculated for the 16 plant extracts. With the exception of *Machilus japonica* and *Rhus javanica*, the significant ability of the remaining 14 plant extracts to inhibit elastase is described here for the first time, as far as can be ascertained from a survey of the literature. The results clearly show the inhibitory effects on elastase activity of the 16 plant extracts in vitro. These results suggest that topical application of plant-based inhibitors of nonspecific elastase in cosmetics may provide beneficial effects for UV-irradiated and dry skin.

Previous studies on the inhibition of

Table 1. Tyrosinase and elastase inhibition of Jeju plant extracts.

Voucher specimen	Scientific name	Used part	Tyrosinase inhibition(%)		Elastase inhibition(%)
			500	pg/ml	500//g/mL
JBR002	<i>Euonymus japonicus</i> Thunb.	Le	4.9	±1.3	nd
JBR003	<i>Tetragonia tetragonoides</i> (Pall.) Kuntze	Sh	-1.9	±2.1	nd
JBR012	<i>Oenothera laciniata</i> Hill	Sh	24.4	±6.0	19.4±1.4
JBR013	<i>Artemisia capillaris</i> Thunb.	Sh	19.4	±1.4	nd
JBR016	<i>Korthalsella japonica</i> (Thunb.) Engl.	Wh	20.7	±0.6	15.3±4.7
JBR023	<i>Pinus pan/ifi lora</i> Siebold & Zuce.	Le	3.6	±4.0	19.4±1.4
JBR024	<i>Daphniphyllaceae macropodium</i> Miq.	Le	-3.6	±4.6	35.4±1.5
JBR026	<i>Farfugium japonicum</i> (L.) Kitam.	Le	19.4	±3.2	9.2±4.5
JBR028	<i>Taxus cuspidata</i> Siebold & Zuce.	Le	-85.2	±9.1	20.0±3.3
JBR029	<i>Neolitsea sericea</i> (Blume) Koidz.	Le	-12.3	±8.7	12.1 ±3.6
JBR030	<i>Ilex crenata</i> Thunb.	Le & Fr	10.2	±1.3	23.8±2.1
JBR032	<i>Melia azedarach</i> L.	Fr	7.2	±1.1	20.1 ±1.5
JBR033	<i>Hederá rhombea</i> (Miq.) Bean	Le	10.0	±4.0	nd
JBR036	<i>Euonymus japonicus</i> Thunb.	Le	4.7	±1.2	6.0±5.0
JBR039	<i>Stauntonia hexaphylla</i> (Thunb.) Decne.	Wh	9.8	±5.7	21.3±1.3
JBR040	<i>Polystichum polyblepharum</i> (Roem. Ex Kunze) C. Presi	Sh	9.4	±5.7	22.3 ±1.4
JBR041	<i>Lycopodium clavatum</i> L.	Wh	1.2	±6.9	16.3 + 1 .5
JBR042	<i>Elaeagnus glabra</i> Thunb.	Le	3.2	±2.3	18.8±2.6
JBR043	<i>Stauntonia hexaphylla</i> (Thunb.) Decne.	Sh	0.0	±1.7	nd
JBR045	<i>Pyrosia lingua</i> (Thunb.) Farw.	Wh	-0.5	±5.8	12.5±2.5
JBR046	<i>Cryptomeria japonica</i> (Thunb. ex L. f.) D. Don	Le	-8.4	±3.2	82.7 ±1.0
JBR047	<i>Dicranopteris linearis</i> (Burm. f.) Underw.	Le	4.9	±5.1	54.0±2.4
JBR048	<i>Lepisorus thunbergianus</i> (Kaulf.) Ching	Wh	10.3	±2.2	14.3±0.9
JBR049	<i>Abies koreana</i> E. H. Wilson	Le	20.2	±2.4	21.8±0.8
JBR050	<i>Cyrtomium falcatum</i> (L. f.) C. Presi	Wh	12.3	±3.2	46.3±0.9
JBR052	<i>Ligustrum japonicum</i> Thunb.	Le	9.7	±1.4	12.4±4.7
JBR053	<i>Lemmaphyllum microphyllum</i> C. Presi	Wh	14.5	±0.3	10.4±7.1
JBR055	<i>Hederá rhombea</i> (Miq.) Bean	Le	24.9	±0.1	17.1 ±2.9
JBR056	<i>Diplopterygium glaucum</i> (Thunb. ex Houtt.) Nakai	Sh	-4.5	±8.0	74.9±3.2
JBR060	<i>Dendropanax trifidus</i> (Thunb.) Makino ex H. Hara	Le	10.0	±2.7	14.9±7.1
JBR061	<i>Machilus japonica</i> Siebold & Zucc.	Le	18.8	±9.3	87.3±4.2
JBR062	<i>Fatsia japonica</i> (Thunb.) Decne. & Planch.	Le	19.6	±4.2	13.1 ±5.7
JBR063	<i>Ardisia crenata</i> Sims	Wh	17.3	±0.3	19.9±1.2
JBR064	<i>Ardisia japonica</i> (Thunb.) Blume	Wh	25.6	±1.5	51.1 ±0.2
JBR065	<i>Trachelospermum asiaticum</i> (Siebold & Zucc.) Nakai	Sh	10.2	±0.2	21.2±2.0
JBR066	<i>Rhaphiolepis indica</i> (L.) Lindl.	Le	-18.0	±0.9	65.5±0.8
JBR068	<i>Ficus oxyphylla</i> Miq. ex Zoll.	Sh	9.4	±6.8	11.7±5.7
JBR071	<i>Pinus thunbergii</i> Pari.	Le	26.8	±3.6	44.1 ±2.0
JBR072	<i>Eurya japonica</i> Thunb.	Le	1.0	±1.9	38.2±6.1
JBR073	<i>Ilex rotunda</i> Thunb.	Le	35.2	±3.7	22.1 ±2.2
JBR075	<i>Eriobotrya japonica</i> (Thunb.) Lindl.	Le	18.3	±2.8	39.4±2.9
JBR076	<i>Myrica rubra</i> Siebold & Zucc.	Le	62.8	±2.6	78.4±1.7
JBR078	<i>Nerium indicum</i> Mill.	Le	8.8	±7.6	24.7±0.4
JBR080	<i>Torreya nucifera</i> (L.) Siebold & Zucc.	Le	8.4	±2.4	17.9±0.9
JBR082	<i>Rhododendron yedoense</i> for. poukhanense (H. Lev.) M. Sugim.	Le	-20.1	±1.0	70.0 ±4.1
JBR083	<i>Elaeocarpus sylvestris</i> (Lour.) Poir.	Le	27.8	±2.4	19.2±1.6
JBR085	<i>Cycas revoluta</i> Thunb.	Le	15.3	±3.0	22.0±3.4
JBR086	<i>Illicium anisatum</i> L.	Le	27.8	±0.4	nd
JBR087	<i>Neolitsea aciculata</i> (Blume) Koidz.	Le	-3.9	±6.0	68.2±0.1
JBR088	<i>Machilus thunbergii</i> Siebold & Zucc.	Le	-0.3	±6.7	85.3±0.1
JBR090	<i>Quercus salicina</i> Blume	Le	-6.1	±3.2	70.2 ±1.6
JBR091	<i>Juniperus chinensis</i> L.	Le	20.7	±2.5	23.0±1.8
JBR093	<i>Buxus koreana</i> (Nakai ex Rehder) T. H. Chung	Le	10.9	±3.0	19.6±5.9
JBR094	<i>Ilex integra</i> Thunb.	Le	12.4	±1.3	21.2±1.7
JBR096	<i>Cleyera japonica</i> Thunb.	Le	21.5	±1.1	80.9±0.3

Abbreviations: Entire plants (Wh), Roots (Ro), Leaves (Le), Stems (St), Seeds (Se), Aerial Parts (Sh), Fruits (Fr). Nd, not determined.

Table 1. Continued.

Voucher specimen	Scientific name	Used part	Tyrosinase inhibition(%) 500 μ g/mL	Elastase inhibition(%) 500 μ g/mL
JBR098	<i>Ficus oxyphylla</i> Miq. ex Zoll.	Le	18.9 \pm 0.3	26.6 \pm 1.7
JBR099	<i>Elaeagnus macrophylla</i> Thunb.	Le	8.8 \pm 0.7	13.1 \pm 0.8
JBR100	<i>Daphniphyllaceae teijsmannii</i> Kurz ex Teijsm. & Binn.	Le	7.6 \pm 5.5	13.2 \pm 4.1
JBR102	<i>Ligustrum lucidum</i> Aiton	Le	38.1 \pm 3.0	18.9 \pm 1.3
JBR103	<i>Aucuba japonica</i> Thunb.	Le	5.7 \pm 1.1	10.8 \pm 2.4
JBR104	<i>Camellia sasanqua</i> Thunb.	Le	11.5 + 2.9	78.0 \pm 1.2
JBR106	<i>Distylium racemosum</i> Siebold & Zucc.	Le	87.6 \pm 0.3	79.8 \pm 3.7
JBR107	<i>Platanus orientalis</i> L.	Fr	4.1 \pm 5.3	19.9 \pm 2.5
JBR108	<i>Cinnamomum camphora</i> (L.) Siebold	Le	-9.2 \pm 3.6	43.7 \pm 2.1
JBR109	<i>Osmanthus fragrans</i>	Le	12.2 \pm 5.0	14.6 \pm 7.1
JBR110	<i>Chamaecyparis pisifera filifera</i> Beissn. & Hochst	Le	-18.1 \pm 0.1	47.3 \pm 4.3
JBR111	<i>Pittosporum tobira</i> (Thunb.) W. T. Aiton	Le	9.0 \pm 3.7	8.0 \pm 1.5
JBR112	<i>Abies holophylla</i> Maxim.	Le	-55.4 \pm 2.9	35.5 \pm 3.5
JBR113	<i>Eunymus japonica</i> for. <i>aureo-variegata</i>	Le	6.9 \pm 0.2	12.5 \pm 2.1
JBR114	<i>Ilex crenata</i> var. <i>microphylla</i> Maxim, ex Matsum.	Le	9.1 \pm 0.8	14.0 \pm 9.9
JBR139	<i>Vicia angustifolia</i> var. <i>segetilis</i> (Thuill.) K. Koch.	Wh	-8.5 \pm 3.6	8.0 \pm 1.9
JBR141	<i>Taraxacum officinale</i> F. H. Wigg.	Wh	7.5 \pm 4.5	nd
JBR142	<i>Brassica napus</i> L.	Wh	-1.2 \pm 2.2	8.1 \pm 2.7
JBR143	<i>Lamium amplexicaule</i> L.	Wh	19.2 \pm 4.3	nd
JBR164	<i>Spergula arvensis</i> L.	Wh	28.1 \pm 1.0	22.3 \pm 1.1
JBR165	<i>Cerastium holosteoides</i> var. <i>hallaisanense</i> (Nakai) M. Miush.	Wh	7.8 \pm 4.1	13.3 \pm 3.8
JBR209	<i>Adonis multiflora</i> Nishikawa & Koji Ito	Wh	6.4 \pm 2.2	10.8 \pm 0.5
JBR212	<i>Lathyrus japonicus</i> Willd.	Wh	2.5 \pm 0.3	21.1 \pm 1.4
JBR213	<i>Euphorbia jolkini</i> Boiss.	Sh	18.1 \pm 1.2	19.5 \pm 1.3
JBR214	<i>Peucedanum japonicum</i> Thunb.	Wh	21.8 \pm 0.3	11.5 \pm 3.2
JBR217	<i>Raphanus sativus</i> var. <i>hortensis</i> for. <i>raphanistroides</i> Makino	Wh	1.9 + 1.6	20.5 \pm 2.8
JBR219	<i>Tetragonia tetragonoides</i> (Pall.) Kuntze	Wh	3.2 \pm 0.3	45.9 \pm 4.0
JBR224	<i>Sonchus oleraceus</i> L.	Wh	5.0 \pm 0.5	nd
JBR225	<i>Rosa multiflora</i> Thunb.	Wh	18.9 \pm 2.8	82.6 \pm 1.2
JBR227	<i>Elaeagnus submacrophylla</i> Servett.	Le	8.9 \pm 0.0	nd
JBR228	<i>Elaeagnus submacrophylla</i> Servett.	Fr	4.2 \pm 2.7	nd
JBR234	<i>Rumex crispus</i> L.	Wh	1.2 \pm 0.6	21.1 \pm 1.8
JBR235	<i>Asparagus cochinchinensis</i> (Lour.) Merr.	Wh	3.6 \pm 7.5	3.7 \pm 3.2
JBR237	<i>Rumex acetosella</i> L.	Wh	6.6 \pm 2.2	25.1 \pm 3.2
JBR239	<i>Rumex acetosa</i> L.	Wh	-4.0 \pm 2.9	22.1 \pm 6.6
JBR242	<i>Allium scorodoprasum</i> var. <i>viviparum</i> Regel	Ro	-5.2 \pm 7.9	nd
JBR279	<i>Angelica japonica</i> A. Gray	Wh	3.8 \pm 3.7	63.6 \pm 0.2
JBR280	<i>Huperzia serrata</i> (Thunb.) Trevis.	Wh	-1.2 \pm 0.5	21.8 \pm 4.5
JBR281	<i>Corydalis heterocarpa</i> Siebold & Zucc.	Wh	-1.0 \pm 4.2	nd
JBR282	<i>Asparagus cochinchinensis</i> (Lour.) Merr.	Sh	2.0 \pm 5.0	nd
JBR284	<i>Suaeda maritima</i> (L.) Dumort.	Wh	-5.9 \pm 6.3	19.4 \pm 0.8
JBR293	<i>Hydrangea petiolaris</i> Siebold & Zucc.	Le	-10.2 \pm 3.7	33.2 \pm 0.7
JBR294	<i>Elaeagnus umbellata</i> Thunb.	Le	-5.8 \pm 3.2	nd
JBR296	<i>Acer pseudosieboldianum</i> (Pax) Kom. <i>Hydrangea serrata</i> for. <i>acuminata</i> (Siebold & Zucc.) E. H. Wilson	Le	-5.2 \pm 0.3	16.6 \pm 4.7
JBR297		Le	15.4 \pm 0.7	20.2 \pm 6.8
JBR298	<i>Cornus kousa</i> F. Buerger ex Miq.	Le	1.0 \pm 5.3	31.0 \pm 1.3
JBR300	<i>Quercus serrata</i> Murray	Le	-9.3 \pm 5.0	21.9 \pm 4.5
JBR301	<i>Acer pictum</i> var. <i>mono</i> (Maxim.) Maxim, ex Franch.	Le	42.5 \pm 1.9	19.1 \pm 5.3
JBR302	<i>Polygonatum odoratum</i> var. <i>pluriflorum</i> (Miq.) Ohwi	Le	-1.2 \pm 4.5	9.1 \pm 4.3
JBR303	<i>Viburnum dilatatum</i> Thunb.	Le	-3.6 \pm 6.7	48.1 \pm 1.8
JBR304	<i>Viburnum erosum</i> Thunb.	Le	-8.4 \pm 2.5	43.5 \pm 2.1
JBR305	<i>Akebia quinata</i> (Thunb.) Decne.	Le	11.2 \pm 1.4	10.6 \pm 5.8
JBR306	<i>Acer palmatum</i> Thunb.	Le	20.8 \pm 0.4	23.0 \pm 0.8
JBR307	<i>Veratrum patulum</i> Loes.	Wh	69.0 \pm 4.2	20.4 \pm 2.5
JBR309	<i>Arisaema ringens</i> (Thunb.) Schott	Wh	2.5 \pm 5.9	13.0 \pm 0.0
JBR313	<i>Viburnum furcatum</i> Blume ex Maxim.	Le	14.8 \pm 0.4	11.7 \pm 3.8
JBR314	<i>Pourthiaea villosa</i> (Thunb.) Decne	Le	-18.2 \pm 5.8	36.5 \pm 0.8

Table 1. Continued.

Voucher specimen	Scientific name	Used part	Tyrosinase	Elastase
			inhibition(%)	inhibition(%)
			ouu- μ g/mL	ouu- μ g/mL
JBR317	<i>Houttuynia cordata</i> Thunb.	Wh	6.1 \pm 2.0	17.1 \pm 3.5
JBR318	<i>Forsythia koreana</i> (Rehder) Nakai	Le	20.3 \pm 3.2	25.1 \pm 1.8
JBR319	<i>Rumex crispus</i> L.	Sh	1.2 \pm 2.8	nd
JBR319-1	<i>Rumex crispus</i> L.	Ro	67.1 \pm 0.6	nd
JBR320-1	<i>Sonchus asper</i> (L.) Hill	Ro	-2.2 \pm 0.3	9.7 \pm 0.3
JBR321	<i>Sonchus oleraceus</i> L.	Sh	2.5 \pm 2.4	nd
JBR321-1	<i>Sonchus oleraceus</i> L.	Ro	-2.2 \pm 5.2	nd
JBR323	<i>Calystegia soldanella</i> (L.) Roem. & Schult.	Wh	1.4 \pm 0.8	23.2 \pm 1.6
JBR327	<i>Smilax china</i> L.	Fr	13.0 \pm 3.0	18.7 \pm 4.4
JBR329	<i>Euonymus alatus</i> (Thunb.) Siebold	Le	-4.2 \pm 2.3	34.6 \pm 1.0
JBR331	<i>Pteridium aquilinum</i> (L.) Kuhn	Wh	1.0 \pm 0.3	23.7 \pm 1.5
JBR332	<i>Caesalpinia decapetala</i> (Roth) Alston	Le	-17.1 \pm 0.6	-3.1 \pm 8.9
JBR333	<i>Plantago lanceolata</i> L.	Wh	7.6 \pm 1.4	14.3 \pm 0.5
JBR335	<i>Oenothera stricta</i> Ledeb.	Wh	27.53.2	20.1 \pm 5.6
JBR336	<i>Ranunculus japonicus</i> Thunb.	Wh	18.3 \pm 4.4	21.8 \pm 0.4
JBR339	<i>Melia azedarach</i> L.	Le	-5.5 \pm 1.9	88.7 \pm 4.2
JBR340	<i>Pourthiaea villosa</i> var. <i>brunnea</i> (H. Lev.) Nakai	Le	22.8 \pm 2.2	21.3 \pm 2.1
JBR380	<i>Lonicera japonica</i> Thunb.	Wh	18.0 \pm 2.1	26.1 \pm 2.6
JBR381	<i>Osmunda japonica</i> Thunb.	Wh	-1.4 \pm 2.0	17.6 \pm 5.1
JBR382	<i>Robinia pseudoacacia</i> L.	Le	4.2 \pm 4.2	16.1 \pm 3.0
JBR383	<i>Lilium lancifolium</i> Thunb.	Wh	9.0 \pm 3.1	21.8 \pm 3.4
JBR384	<i>Ophiopogon jaburan</i> (Siebold) Lodd.	Wh	3.3 \pm 2.3	21.1 \pm 0.5
JBR385	<i>Asparagus schoberioides</i> Kunth	Sh	-1.1 \pm 3.2	13.7 \pm 0.6
JBR386	<i>Alnus firma</i> Siebold & Zucc.	Le	35.4 \pm 4.5	31.5 \pm 2.4
JBR387	<i>Lysimachia mauritiana</i> Lam.	Wh	6.1 \pm 1.3	16.8 \pm 0.3
JBR388	<i>Rubus parvifolius</i> L.	Le	-5.4 \pm 1.3	21.5 \pm 1.0
JBR390	<i>Morus alba</i> L.	Le	66.1 \pm 1.0	7.4 \pm 0.7
JBR391	<i>Smilax china</i> L.	Le	5.9 \pm 0.4	27.1 \pm 4.9
JBR393	<i>Mallotus japonicus</i> (L. f.) Mull. Arg.	Le	7.3 \pm 8.6	22.2 \pm 2.0
JBR394	<i>Scymplocos sawafutagi</i> Nagam.	Le	-3.5 \pm 1.7	25.1 \pm 3.4
JBR395	<i>Sedum oryzifolium</i> Makino	Wh	11.5 \pm 2.8	25.9 \pm 4.4
JBR396	<i>Desmodium podocarpum</i> subsp. <i>oxyphyllum</i> (DC.) H. Ohashi	Wh	6.0 \pm 1.3	19.9 \pm 7.6
JBR397	<i>Suaeda glauca</i> (Bunge) Bunge	Wh	-2.2 \pm 5.8	nd
JBR399	<i>Trifolium repens</i> L.	Wh	4.7 \pm 3.8	24.7 \pm 1.9
JBR401	<i>Fallopia japonica</i> (Houtt.) Ronse Deer.	Wh	11.2 \pm 6.7	26.7 \pm 2.8
JBR402	<i>Euscaphis japonica</i> (Thunb.) Kanitz	Le	-11.4 \pm 1.6	85.8 \pm 4.8
JBR403	<i>Actinidia arguta</i> (Siebold & Zucc.) Planch, ex Miq.	Le	4.7 \pm 6.1	59.2 \pm 4.0
JBR405	<i>Viburnum odoratissimum</i> Ker Gawl.	Le	3.7 \pm 2.4	85.8 \pm 3.4
JBR406	<i>Stephanandra incisa</i> (Thunb.) Zabel	Le	-14.7 \pm 11.3	42.3 \pm 1.0
JBR407	<i>Clerodendrum trichotomum</i> Thunb.	Le	3.9 \pm 5.6	nd
JBR408	<i>Cornus controversa</i> Hemsl.	Le	-15.4 \pm 2.1	90.3 \pm 3.3
JBR409	<i>Aphananthe aspera</i> (Thunb.) Planch.	Le	0.6 \pm 1.4	24.0 \pm 6.6
JBR411	<i>Actinidia polygama</i> (Siebold & Zucc.) Maxim.	Wh	-1.1 \pm 2.6	23.5 \pm 3.9
JBR412	<i>Lindera erythrocarpa</i> Makino	Le	-0.5 \pm 5.4	73.9 \pm 3.3
JBR414	<i>Boehmeria pannosa</i> Nakai & Satake	Le	-26.2 \pm 2.9	24.1 \pm 1.7
JBR415	<i>Cinnamomum japonicum</i> Siebold	Le	2.0 \pm 1.8	36.9 \pm 1.3
JBR417	<i>Ligustrum obtusifolium</i> Siebold & Zucc.	Le	4.0 \pm 6.3	18.9 \pm 6.0
JBR420	<i>Ficus erecta</i> Thunb. var. <i>sieboldii</i> (Miq.) King	Fr	5.1 \pm 0.5	19.0 \pm 1.3
JBR422	<i>Ficus erecta</i> Thunb.	Fr	1.5 \pm 2.6	23.4 \pm 1.9
JBR423	<i>Vaccinium oldhamii</i> Miq.	Le	-7.3 \pm 0.6	45.6 \pm 4.9
JBR424	<i>Eriobotrya japonica</i> (Thunb.) Lindl.	Fr	0.6 \pm 0.3	17.7 \pm 2.5
JBR425	<i>Eriobotrya japonica</i> (Thunb.) Lindl.	Se	1.7 \pm 0.8	15.2 \pm 0.7
JBR427	<i>Wisteria floribunda</i> (Willd.) DC.	Le	1.5 \pm 0.0	24.6 \pm 4.7
JBR428	<i>Magnolia denudata</i> Desr.	Le	8.1 \pm 3.1	23.6 \pm 4.0
JBR429	<i>Platycarya strobilacea</i> Siebold & Zucc.	Le	-10.7 \pm 3.0	55.6 \pm 4.1
JBR430	<i>Quercus dentata</i> Thunb.	Le	4.0 \pm 1.5	19.9 \pm 1.9
JBR431	<i>Celtis jessoensis</i> Koidz.	Le	16.5 \pm 4.5	10.8 \pm 4.4
JBR432	<i>Ostrya japonica</i> Sarg.	Le	-8.3 \pm 2.3	26.4 \pm 1.2
JBR433	<i>Carpinus turczaninowii</i> Hance	Le	13.7 \pm 1.8	9.2 \pm 0.9
JBR434	<i>Celtis aurantiaca</i> Nakai	Le	8.5 \pm 2.2	19.3 \pm 3.6
JBR435	<i>Corylus hallaisanensis</i> Nakai	Le	6.5 \pm 0.1	38.4 \pm 4.6
JBR436	<i>Carpinus cordata</i> Blume	Le	-12.5 \pm 1.1	26.5 \pm 1.9

Table 1. Continued.

Voucher specimen	Scientific name	Used part	Tyrosinase inhibition(%) 500//g/mL	Elastase inhibition(%) 500//g/mL
JBR437	<i>Carpinus tschonoskii</i> Maxim.	Le	-31.8±2.6	12.9±2.1
JBR438	<i>Carpinus laxiflora</i> (Siebold & Zucc.) Blume	Le	-22.2±9.1	17.8±5.3
JBR439	<i>Quercus acutissima</i> Carruth.	Le	7.9±0.7	19.5±1.0
JBR440	<i>Prunus X yedoensis</i> Matsum.	Le	-15.6±0.2	42.0±3.1
JBR441	<i>Rhododendron weyrichii</i> Maxim.	Le	1.1 ±1.0	55.5±0.6
JBR443	<i>Paliurus ramosissimus</i> (Lour.) Poir.	Le	2.7±2.3	18.3±1.7
JBR444	<i>Hibiscus hamabo</i> Siebold & Zucc.	Le	5.3±4.8	29.9±5.7
JBR445	<i>Salix gracilistyla</i> Miq.	Le	13.6±4.2	22.2±2.5
JBR446	<i>Callicarpa japonica</i> Thunb.	Le	29.5±5.0	nd
JBR448	<i>Sambucus sieboldiana</i> (Miq.) Blume ex Graebn.	Le	9.9±5.0	22.5 ± 1.1
JBR449	<i>Weigela florida</i> for. <i>subtricolor</i> Nakai	Le	11.2±1.3	24.7 ±0.2
JBR450	<i>Tetrapanax papyrifer</i> (Hook.) K. Koch	Le	11.8±0.9	19.6±0.3
JBR451	<i>Rhamnella franguloides</i> (Maxim.) Weberb.	Le	28.9±2.7	24.2 ±10.6
JBR452	<i>Picrasma quassioides</i> (D. Don) Benn	Le	16.7±4.6	7.0±0.0
JBR453	<i>Weigela subsessilis</i> (Nakai) L. H. Bailey	Le	16.8±0.7	31.3±6.9
JBR455	<i>Styrax japonicus</i> Siebold & Zucc.	Le	19.0±1.6	18.0±3.0
JBR456	<i>Meliosma oldhamii</i> Maxim.	Le	11.4±2.9	38.1 ±0.3
JBR457	<i>Euonymus hamiltonianus</i> Wall.	Le	6.9±3.8	3.6±0.9
JBR45S	<i>Hibiscus syriacus</i> L.	Le	7.2±1.6	22.87.5
JBR459	<i>Salix koreensis</i> Andersson	Le	15.5±0.7	9.2±8.7
JBR460	<i>Euonymus bungeana</i> Maxim.	Le	18.4±4.3	27.0±2.7
JBR461	<i>Rhus javanica</i> L.	Le	66.5±0.2	80.8 ±0.5
JBR463	<i>Magnolia kibus</i> DC.	Le	13.1 ±3.4	7.5±4.3
JBR464	<i>Clytus sieboldiana</i> Blume	Le	-2.9 ±1.0	22.7 ±1.6
JBR465	<i>Idesia polycarpa</i> Maxim.	Le	10.1 ±1.1	3.5±0.2
JBR466	<i>Cornus walteri</i> Wangerin	Le	84.1 ±2.7	89.4±4.9
JBR468	<i>Torreya nucifera</i> (L.) Siebold & Zucc.	Fr	-3.8±7.4	12.5±4.3
JBR469	<i>Ternstroemia gymnanthera</i> (Wight & Arn.) Sprague	Le	-1.1 ±0.5	34.9 ±0.9
JBR470	<i>Abelia mosanensis</i> T. H. Chung	Le	19.1 ±3.7	16.8±2.2
JBR473	<i>Ulmus parvifolia</i> Jacq.	Le	2.1 ±5.2	20.2 ±5.0
JBR474	<i>Morus bombycis</i> Koidz.	Le	73.7±1.1	12.7±6.2
JBR475	<i>Malus sieboldii</i> (Regel) Rehder in Sarg.	Le	-75.1 ±0.9	59.7±7.9
JBR476	<i>Malus baccata</i> (L.) Borkh.	Le	-146.3±0.0	56.3±0.6
JBR477	<i>Sorbus commixta</i> Hedl.	Le	14.3±2.9	28.2±5.7
JBR478	<i>Albizia julibrissin</i> Durazz.	Le	5.5±1.0	22.5±7.5
JBR479	<i>Prunus spachiana</i> (Lavallee ex Ed. Otto) Kitam.	Le	16.6±4.1	47.8 ±0.4
JBR480	<i>Sorbus alnifolia</i> for. <i>hirtella</i> (Nakai) W. T. Lee	Le	-11.4±6.8	63.6±0.0
JBR482	<i>Aria alnifolia</i> (Siebold & Zucc.) Decne.	Le	3.7±7.4	66.0±3.6
JBR483	<i>Prunus serrulata</i> var. <i>quelpaertensis</i> Uyeki	Le	-5.1 ±3.3	70.9±2.2
JBR484	<i>Cornus kousa</i> F. Buerger ex Miq.	Le	13.8±5.2	10.4±1.3
JBR485	<i>Fraxinus rynchophylla</i> Hance	Le	23.6±0.5	17.9±5.0
JBR486	<i>Ulmus davidiana</i> var. <i>japonica</i> (Rehder) Nakai	Le	-9.1 ±4.1	19.8±5.0
JBR487	<i>Styrax obassia</i> Siebold & Zucc.	Le	-18.8±7.2	22.0 ±1.5
JBR490	<i>Zanthoxylum ailanthoides</i> Siebold & Zucc.	Le	17.9±0.4	20.3±7.6
JBR491	<i>Neoshirakia japonica</i> (Siebold & Zucc.) Esser	Le	5.9±2.7	15.1 ±2.2
JBR492	<i>Maackia fauriei</i> (H. Lev.) Takeda	Le	65.0±0.4	17.4±3.4
JBR493	<i>Phellodendron amurense</i> Rupr.	Le	27.0±4.2	1.4±2.3
JBR494	<i>Actinodaphne lancifolia</i> (Siebold & Zucc.) Meisn.	Le	-0.8 ±1.1	83.6±2.2
JBR495	<i>Tilia taquetii</i> C. K. Schneid.	Le	-15.2±6.1	59.1 ±0.1
JBR496	<i>Prunus maximowiczii</i> Rupr.	Le	4.1 ±0.3	1.6±2.8
JBR497	<i>Celtis sinensis</i> Pers.	Le	27.8±2.5	4.9±5.2
JBR498	<i>Zelkova serrata</i> (Thunb.) Makino	Le	-20.5±0.9	30.3±2.2
JBR499	<i>Cudrania tricuspidata</i> (Carriere) Bureau ex Lavallee	Le	29.7±0.3	2.1 ±4.3
JBR500	<i>Broussonetia papyrifera</i> (L.) Lher. ex Vent.	Le	18.2±0.2	nd
JBR501	<i>Triadica sebifera</i> (L.) Small	Le	7.3±3.9	16.6±4.3
JBR502	<i>Securinega suffruticosa</i> (Pali.) Rehder	Le	-1.0±0.9	4.6±3.8
JBR504	<i>Fraxinus mandshurica</i> Rupr.	Le	44.0 ±0.1	0.8±7.0
JBR505	<i>Aesculus turbinata</i> Blume	Le	-11.1 ±1.7	89.6±4.2
JBR506	<i>Sophora japonica</i> L.	Le	0.2±3.8	12.9±4.2
JBR525	<i>Styrax japonicus</i> Siebold & Zucc.	Fr	10.3±0.5	20.5 ±1.0
JBR526	<i>Eleutherococcus gracilistylus</i> (W. W. Sm.) S. Y. Hu	Le	5.5±3.6	11.2 ±3.5
JBR528	<i>Euphorbia supina</i> Raf.	Wh	7.2±4.3	3.3±1.2
JBR531	<i>Suaeda japonica</i> Makino	Wh	-8.4 ±1.6	25.2±2.4

Table 1. Continued.

Voucher specimen	Scientific name	Used part	Tyrosinase	Elastase
			inhibition (%)	inhibition (%)
			$\frac{\text{OD}_{410}}{\text{DW}} \times 100$	$\frac{\text{OD}_{510}}{\text{DW}} \times 100$
JBR532	<i>Suaeda australis</i> (R. Br.) Moq.	Wh	-7.5±2.5	15.6±3.4
JBR534	<i>Salsola komarovii</i> Iljin	Wh	-4.6 ±3.0	nd
JBR543	<i>Ginkgo biloba</i> L.	Le	-2.7±6.7	53.2±1.4
JBR544	<i>Toxicodendron trichocarpum</i> (Miq.) Kuntze	Le	10.9±0.8	67.2±3.4
JBR545	<i>Elaeagnus umbellata</i> Thunb.	Le	28.5±2.1	18.7±0.8
JBR546	<i>Toxicodendron succedaneum</i> (L.) Kuntze	Le	80.5 ±1.4	24.4 ±5.8
JBR549	<i>Eucommia ulmoides</i> Oliv.	Le	-2.3±8.4	18.1 ±0.1
JBR556	<i>Staphylea bumalda</i> DC.	Le	-12.9±2.0	61.1 ±1.9
JBR563	<i>Diospyros lotus</i> L.	Le	32.9±2.7	63.5±2.4
JBR565	<i>Oenothera erythrosepala</i> Borbas	Le	8.7 ±2.0	81.0±4.0
JBR566	<i>Oenothera erythrosepala</i> Borbas	St	13.5±2.8	33.1 ±1.9
JBR570	<i>Angelica dahurica</i> (Fisch. ex Hoffm.) Benth. & Hook. f. ex Franch. & Sav.	Le	8.2±1.8	18.2 ± 1.8
JBR571	<i>Angelica dahurica</i> (Fisch. ex Hoffm.) Benth. & Hook. f. ex Franch. & Sav.	St	-3.0±0.9	18.9±3.2
JBR574	<i>Ampelopsis brevipedunculata</i> (Maxim.) Trautv.	Wh	-3.0±0.3	33.9±2.0
JBR583	<i>Suaeda malacosperma</i> H. Hara	Wh	-21.1 ±3.5	11.6 ±1.0
JBR584	<i>Pittosporum tobira</i> (Thunb.) W. T. Aiton	Fr	3.1 ±0.9	23.9±0.4
JBR585	<i>Aster spathulifolius</i> Maxim.	Wh	4.2±0.9	16.0±1.4
JBR587	<i>Limonium tetragonum</i> (Thunb.) Bullock	Wh	78.5±0.8	35.8±1.3
JBR590	<i>Physalis angulata</i> L.	Wh	2.3±2.0	15.6±0.7
JBR592	<i>Plantago asiatica</i> L.	Wh	9.0±0.9	15.3±0.7
JBR593	<i>Lycoris squamigera</i> Maxim.	Bu	-1.0±1.5	11.5 ±3.1
JBR595	<i>Akebia quinata</i> (Thunb.) Decne.	Fr	5.3±4.5	15.4±1.1
JBR596	<i>Illicium anisa turn</i> L.	Le	32.3±2.6	32.2±1.0
JBR597	<i>Illicium anisa turn</i> L.	Fr	8.1 ±1.5	18.6±3.3
JBR598	<i>Ficus erecta</i> Thunb.	Le	4.8±4.4	18.4±2.3
JBR599	<i>Ficus erecta</i> Thunb.	St	-11.4±2.5	4.4±0.4
JBR600	<i>Ficus erecta</i> Thunb. var. <i>sieboldii</i> (Miq.) King	Le	-1.3±4.8	16.8±5.0
JBR601	<i>Ficus erecta</i> Thunb. var. <i>sieboldii</i> (Miq.) King	St	69.8 + 2.6	10.2±2.3
JBR603	<i>Vernicia fordii</i> (Hensl.) Airy Shaw	Fr	51.0±3.4	31.3±7.6
JBR606	<i>Albizia julibrissin</i> Durazz.	Fr	5.6±1.5	15.6±1.8
JBR607	<i>Ficus oxyphylla</i> Miq. ex Zoll.	Fr	12.3±3.2	32.8±0.5
JBR608	<i>Salicornia europaea</i> L.	Wh	-10.8±11.0	4.7±2.1
JBR610	<i>Clerodendrum trichotomum</i> Thunb.	Le	17.9±0.9	15.7±0.7
JBR611	<i>Ficus thunbergii</i> Maxim.	Fr	-17.7±0.9	11.0±2.0
JBR613	<i>Adenophora triphylla</i> (Thunb.) A. DC.	Ro	-11.5±3.9	14.5±2.3
JBR621	<i>Stauntonia hexaphylla</i> (Thunb.) Decne.	Fr	-21.5±3.3	39.2±1.5
JBR622	<i>Actinidia arguta</i> (Siebold & Zucc.) Planch, ex Miq.	Fr	-23.2 ±1.0	14.2±2.4
JBR623	<i>Arisaema ringens</i> (Thunb.) Schott	Fr	-27.6±2.6	14.2±1.4
JBR624	<i>Asarum maculatum</i> Nakai	Wh	-7.0±6.2	2.0±1.8
JBR625	<i>Agastache rugosa</i> (Fisch. & C. A. Mey.) Kuntze	Wh	13.4±2.0	23.4±1.2
JBR629	<i>Litsea Japónica</i> (Thunb.) Juss.	Fr	4.0 ±2.1	28.0±1.8
JBR631	<i>Xylosma congesta</i> (Lour.) Merr.	Le	0.4±0.6	nd
JBR632	<i>Sageretia thea</i> (Osbeck) M. C. Johnst.	Le	-8.9 ±1.7	43.0±0.1
J07004	<i>Artemisia scoparia</i> Waldst. & Kit.	Wh	0.8±1.2	nd
J07005	<i>Artemisia japonica</i> Thunb.	Wh	-5.6 ±1.5	17.3±0.9
J07015	<i>Taxillus yadoriki</i> (Siebold ex Maxim.) Danser	Le	19.0±6.9	86.5 ±0.8
J07016	<i>Euphorbia helioscopia</i> L.	Wh	4.6±2.0	18.7±2.9
J07019	<i>Brassica juncea</i> (L.) Czern.	Wh	-21.4±4.1	15.8±0.7
J07020	<i>Podocarpus macrophyllus</i> (Thunb.) Sweet	Le	16.1 ±0.9	64.6±0.1
J07022	<i>Lotus corniculatus</i> L.	Wh	-10.9±0.8	17.1 ±1.7
J07023	<i>Corydalis platycarpa</i> (Maxim, ex Palib.) Makino	Wh	-29.9±0.5	14.8±2.3
J07025	<i>Rubus buergeri</i> Miq.	Le	-10.6±0.4	74.7±0.6
J07026	<i>Catanopsis sieboldii</i> (Makino) Hatus.	Le	21.0±0.4	17.6±1.9
J07033	<i>Cerastium glomeratum</i> Thuill.	Wh	1.5±0.9	19.3 ±1.1
J07034	<i>Lamium purpureum</i> L.	Wh	-9.1 ±1.6	21.7±2.8
J07038	<i>Sapindus mukorossi</i> Gaertn.	Le	-7.2±4.6	58.2±2.0
J07069	<i>Artemisia fukudo</i> Makino	Wh	-14.9±2.2	23.8 ±1.3
J07074	<i>Kadsura japonica</i> (L.) Dunal	Sh	-8.8±1.1	45.4±2.0
J07078	<i>Vaccinium bracteatum</i> Thunb.	Sh	4.9 ±1.4	44.3 ±0.8
J07087	<i>Camellia japonica</i> L.	Le	-9.7±5.1	55.2±2.3
J08017	<i>Litsea japonica</i> (Thunb.) Juss.	Le	6.1 ±2.1	77.4±1.8
J08022	<i>Sophora flavescens</i> Aiton	Ro	82.5±6.9	83.5±3.6

tyrosinase by a variety of compounds resulted in the use of several inhibitors as cosmetics additives or medicinal products for the reduction of hyperpigmentation (Sung et al. 2009, Zheng et al. 2009). Table 3 summarises the results of the assessment of mushroom tyrosinase inhibition by 299 plant extracts, with inhibition expressed as IC₅₀ values. Results of the current study showed that 286 of the 299 extracts had poor anti-tyrosinase activity (<60% inhibition at 500 $\mu\text{g/mL}$ concentration) compared to the reference extracts *Morus alba* and *Morus bombycis*. In the tyrosinase inhibition assay, 4 extracts, including *Cornus walteri* (139.2 $\mu\text{g/mL}$), *Maackia fauriei* (149.3 $\mu\text{g/mL}$), *Toxicodendron succedaneum* (142.3 $\mu\text{g/mL}$), and *Sophora flavescens* (41.6 $\mu\text{g/mL}$), showed significantly greater tyrosinase inhibition activity than the positive controls *Distylium racemosum* (145.9 $\mu\text{g/mL}$) and arbutin (180.3 $\mu\text{g/mL}$). However, the 4 extracts showed lower tyrosine inhibition activity than the positive controls *Morus alba* (11.9 $\mu\text{g/mL}$) and *Morus bombycis* (22 $\mu\text{g/mL}$).

To our knowledge, previous phytochemical investigations of extracts from 10 plants, including *Cornus walteri*, *Maackia fauriei*, *Toxicodendron succedaneum* and *Sophora flavescens*, did not reveal the presence of natural anti-tyrosinase compounds. However, these plants could represent potential sources of new anti-tyrosinase inhibitory agents. Further biological investigations on human melanocytes should be done to confirm the tyrosinase inhibitory activity of these plants. The isolation and structural elucidation of the active constituents of these 4 plants should provide useful leads for the development of new skin-whitening agents. Interestingly, among the tested extracts, *Taxus cuspidata*, *Malus sieboldii*, and *Malus baccata* clearly showed tyrosinase-elevating activity, indicating their potential usefulness in the development of gray-hair-prevention agents or tanning reagents (Table 4). Recently, it has been shown that the extracts from herbs such as *Polygoni multiflori* extract (Guan et al. 2008), *Capparis spinosa* (Matsuyama et al. 2009), and kava (*Piper methysticum*) rhizome and kavalactones (Matsuda et al. 2006), increase melanogenesis in B16 melanoma cells; therefore, extensive screening of natural

Table 2. Inhibitory effect of Jeju medicinal plants on elastase.

	Sample (Elastase Inhibition)	IC ₅₀ Owg/mL)
JBR494	<i>Actinodaphne lancifolia</i>	103.1
JBR505	<i>Aesculus turbinata</i>	43.1
JBR096	<i>Cleyera japonica</i>	205.9
JBR408	<i>Cornus controversa</i>	163
JBR466	<i>Cornus walteri</i>	26.1
JBR046	<i>Cryptomeria japonica</i>	108.2
JBR402	<i>Euscaphis japonica</i>	455.9
JBR061	<i>Machilus japonica</i>	189.1
JBR088	<i>Machilus thunbergii</i>	199.2
JBR339	<i>Melia azedarach</i>	293.2
JBR565	<i>Oenothera erythrosepala</i>	87.8
JBR461	<i>Rhus javanica</i>	70.5
JBR225	<i>Rosa multiflora</i>	371.9
J08022	<i>Sophora flavescens</i>	219.5
J07015	<i>Taxillus yadoriki</i>	36.4
JBR405	<i>Viburnum odoratissimum</i>	80.8
Control	<i>Areca catechu</i>	28.1

Table 3. Inhibitory effect of Jeju medicinal plants on mushroom tyrosinase.

	Sample (Tyrosinase Inhibition)	IC ₅₀ ($\mu\text{g/mL}$)
JBR466	<i>Cornus walteri</i>	139.2
JBR106	<i>Distylium racemosum</i>	145.9
JBR601	<i>Ficus erecta</i> var. <i>sieboldii</i>	219.6
JBR587	<i>Limonium tetragonum</i>	233.3
JBR492	<i>Maackia fauriei</i>	149.3
JBR076	<i>Myrica rubra</i>	437.1
JBR461	<i>Rhus javanica</i>	337.6
JBR319-1	<i>Rumex crispus</i>	306.9
J08022	<i>Sophora flavescens</i>	41.6
JBR546	<i>Toxicodendron succedaneum</i>	142.3
JBR307	<i>Veratrum patulum</i>	212.9
	Positive control	IC ₅₀ ($\mu\text{g/mL}$)
JBR390	<i>Morus alba</i>	11.9
JBR474	<i>Morus bombycis</i>	22
Control	Arbutin	180.3

resources for their potential use as tanning agents or in treatment for hair depigmentation is recommended.

Phenolics are aromatic secondary plant metabolites, widespread in the plant kingdom, that are associated with the colour, sensory qualities, and nutritional and antioxidant properties of various foods. The antioxidant activity of phenolic compounds is mainly because of their redox properties, which allow them to act as reducing agents, hydrogen donors, singlet oxygen quenchers, heavy-metal chelators, and hydroxyl-radical quenchers (Rice-Evans et al. 1995, Gupta and Prakash 2009). Therefore, the total polyphenol content of 26 extracts showing strong inhibitory activity of tyrosinase and/or elastase was assessed. As shown in Table 5,

Table 4. Activation effect of Jeju medicinal plants on mushroom tyrosinase.

Sample		Activation of Mushroom Tyrosinase (%)				
		15.6 (ng/mL)	31.3 (ng/mL)	62.5 (ng/mL)	125.0 (ng/mL)	250.0 (ng/mL)
JBR476	<i>Ma/us baccata</i>	4.9 ± 2.4	7.3 ± 0.8	16.7 ± 1.3	32.9 ± 0.1	61.5 ± 1.6
JBR475	<i>Malus sieboldii</i>	6.1 + 1	11.6 + 3.3	10.4 + 0.8	17 + 1.3	32 + 2.2
JBR028	<i>Taxus cuspidata</i>	5.9 + 1.3	12.6 + 1.9	16.5 + 2.9	24 + 2.3	43.2 + 3.4

Table 5. Polyphenol contents of Jeju medicinal plants.

Sample No.	Sample name	Polyphenol content (i/g/mg)
JBR494	<i>Actinodaphne lancifolia</i>	239.3 + 4.6
JBR505	<i>Aesculus turbinata</i>	459.5 + 0.0
JBR096	<i>Cleyera Japónica</i>	337.3 + 3.6
JBR408	<i>Cornus controversa</i>	435.0 + 4.3
JBR466	<i>Cornus walteri</i>	607.5 + 0.7
JBR046	<i>Cryptomeria Japónica</i>	205.8 + 7.1
JBR106	<i>Distylium racemosum</i>	417.5 + 2.6
JBR402	<i>Euscaphis Japónica</i>	321.7 + 0.3
JBR601	<i>Ficus erecta var. sieboldii</i>	31.3 + 0.4
JBR587	<i>Limonium tetragonum</i>	258.8 + 6.0
JBR492	<i>Maackia fauriei</i>	66.3 + 1.1
JBR061	<i>Machilus japónica</i>	264.5 + 1.3
JBR088	<i>Machilus thunbergii</i>	254.3 + 0.8
JBR476	<i>Malus baccata</i>	332.3 + 3.9
JBR475	<i>Malus sieboldii</i>	351.8 + 2.5
JBR339	<i>Melia azedarach</i>	324.5 + 4.3
JBR076	<i>Myrica rubra</i>	250.5 + 0.0
JBR461	<i>Rhus Javanica</i>	222.5 + 1.8
JBR225	<i>Rosa multiflora</i>	307.5 + 3.5
JBR319-1	<i>Rumex crispus</i>	214.8 + 1.9
J08022	<i>Sophora flavescens</i>	178.3 + 1.1
J07015	<i>Taxillus yadoriki</i>	300.0 + 0.7
JBR028	<i>Taxus cuspidata</i>	284.0 + 2.8
JBR546	<i>Toxicodendron succedaneum</i>	653.3 + 7.4
JBR307	<i>Veratrum patulum</i>	54.3 + 0.3
JBR405	<i>Viburnum odoratissimum</i>	388.2 + 7.7

5 extracts, *Toxicodendron succedaneum* (653.3 //g/mg), *Cornus walteri* (607.5 ji/g/mg), *Aesculus turbinata* (459.5 ji/g/mg), *Cornus controversa* (435.0 ji/g/mg) and *Distylium racemosum* (417.5 ji/g/mg), showed high polyphenol content (>400 ji/g/mg). Of these extracts, *T. succedaneum*, *C. walteri*, and *D. racemosum* showed the highest elastase and/or tyrosinase inhibition activity.

In conclusion, 299 Jeju Island herbal medicines were investigated in the present study for their potential effectiveness as skin-whitening and antiwrinkle agents and in the maintenance of skin health. Extracts of 10

herbal preparations were shown to be potent tyrosinase inhibitors. The results of this study indicate that, in addition to *Morus alba* extracts, which are currently used as cosmetics additives, extracts of *Cornus walteri*, *Maackia fauriei*, *Toxicodendron succedaneum*, and *Sophora flavescens* are likely candidates for the development of cosmetic applications and products. We also calculated the IC₅₀ values of 14 plant extracts that inhibit elastase, including *Aesculus turbinata*, *Taxillus yadoriki*, and *Cornus walteri*, which may be of value in the development of anti-aging cosmetics.

This may be the first systematic report on Jeju Island plants as candidates for cosmetic materials; since most regional plants have not been investigated chemically or pharmaceutically, they remain an untapped potential source of cosmetic ingredients. The results of the current study therefore provide greater knowledge of the medicinal plants of Jeju Island and compelling evidence for the rational exploration of indigenous medicinal plants as a source of cosmetic materials. Further investigations will focus on assessment of the biological activity of these plant extracts in vivo and on chemical identification of the major active components responsible for whitening and anti-aging.

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REFERENCES

- Antoniceilli F, Bellon G, Debelle L, Hornebeck W (2007) Elastin-elastases and inflamm-aging. Current Topics in Developmental Biology 79, 99-155.
- Braverman IM, Fonferko E (1982) Studies in cutaneous aging: I, the elastic fiber network. The Journal of Investigative Dermatology 78, 434-443.

- Briganti S, Camera E, Picardo M (2003) Chemical and instrumental approaches to treat hyperpigmentation. *Pigment Cell Research* 16, 101-110.
- Chatterjee R, Benzinger MJ, Ritter JL, Bissett DL (1990) Chronic ultraviolet B radiation-induced biochemical changes in the skin of hairless mice. *Photochemistry and Photobiology* 51, 91-97.
- Costin GE, Hearing VJ (2007) Human skin pigmentation: melanocytes modulate skin color in response to stress. *The FASEB Journal* 21, 976-994.
- Guan S, Su W, Wang N, Li P, Wang Y (2008) A potent tyrosinase activator from *Radix Polygoni multiflori* and its melanogenesis stimulatory effect in B16 melanoma cells. *Phytotherapy Research* 22, 660-663.
- Gupta S, Prakash J (2009) Studies on Indian green leafy vegetables for their antioxidant activity. *Plant Foods for Human Nutrition* 64, 39-45.
- Kambayashi H, Yamashita M, Odake Y, Takada K, Funasaka Y, Ichihashi M (2001) Epidermal changes caused by chronic low-dose UV irradiation induce wrinkle formation in hairless mouse. *Journal of Dermatological Science* 27, Supl. 1, 19-25.
- Kang T.H. (2007). *Data Book of Mt. Halla, 2007*. Research Institute for Mt. Halla, Jeju Special Self-Governing Province, Jeju.
- Kiken DA, Cohen DE (2002) Contact dermatitis to botanical extracts. *American Journal of Contact Dermatitis* 13, 148-152.
- Kim SS, Hyun CG, Kim JY, Lee J, Park D (2007a) Antibacterial effects of medicinal plants from Jeju Island against acne-inducing bacteria. *Journal of Applied Biological Chemistry* 50, 101-103.
- Kim SS, Hyun CG, Lee J, Lim J, Kim JY, Park D (2007b) In vitro screening of Jeju medicinal plants for cosmeceutical materials. *Journal of Applied Biological Chemistry* 50, 215-220.
- Kim YH, Kim KS, Han CS, Yang HC, Park SH, Ko KI, Lee SH, Kim KH, Lee NH, Kim JM, Son K (2007c) Inhibitory effects of natural plants of Jeju Island on elastase and MMP-1 expression. *International Journal of Cosmetic Science* 29, 487-488.
- Labat-Robert J, Fourtanier A, Boyer-Lafargue B, Robert L (2000) Age dependent increase of elastase type protease activity in mouse skin. Effect of UV-irradiation. *Journal of Photochemistry and Photobiology. B, Biology* 57, 113-118.
- Lee KK, Choi JD (1999) The effects of areca catechu L extract on anti-aging. *International Journal of Cosmetic Science* 21, 285-295.
- Lee KK, Kim JH, Cho JJ, Choi JD (1999) Inhibitory Effects of 150 Plant Extracts on Elastase Activity, and Their Anti-inflammatory Effects. *International Journal of Cosmetic Science* 21, 71-82.
- Lee SH, Choi SY, Kim H, Hwang JS, Lee BG, Gao JJ, Kim SY (2002) Mulberroside F isolated from the leaves of *Morus alba* inhibits melanin biosynthesis. *Biological and Pharmaceutical Bulletin* 25, 1045-1048.
- Lee SH, Sancheti S, Sancheti S, Seo SY (2009) Potent Antielastase and Antityrosinase Activities of *Astilbe chinensis*. *American Journal of Pharmacology and Toxicology* 4, 125-127.
- Masuda M, Murata K, Fukuhama A, Naruto S, Fujita T, Uwaya A, Isami F, Matsuda H (2009) Inhibitory effects of constituents of *Morinda citrifolia* seeds on elastase and tyrosinase. *Journal of Natural Medicines* 63, 267-273.

- Matsuda H, Hirata N, Kawaguchi Y, Naruto S, Takata T, Oyama M, Iinuma M, Kubo M (2006) Melanogenesis stimulation in murine B16 melanoma cells by Kava (*Piper methysticum*) rhizome extract and kavalactones. *Biological and Pharmaceutical Bulletin* 29, 834-837.
- Matsuyama K, Villareal MO, El Omri A, Han J, Kchouk ME, Isoda H (2009) Effect of Tunisian *Capparis spinosa* L. extract on melanogenesis in B16 murine melanoma cells. *Journal of Natural Medicines* 63, 468-472.
- Oikarinen A, Kallionen M (1989) A biochemical and immunohistochemical study of collagen in sun-exposed and protected skin. *Photo-dermatology* 6, 24-31.
- Parvez S, Kang M, Chung HS, Bae H (2007) Naturally occurring tyrosinase inhibitors: mechanism and applications in skin health, cosmetics and agriculture industries. *Phytotherapy Research* 21, 805-816.
- Rice-Evans CA, Miller NJ, Bolwell PG, Bramley PM, Pridham JB (1995) The relative antioxidant activities of plant-derived polyphenolic flavonoids. *Free Radical Research* 22, 375-383.
- Shin NH, Ryu SY, Choi EJ, Kang SH, Chang IM, Min KR, Kim Y (1998) Oxyresveratrol as the potent inhibitor on dopa oxidase activity of mushroom tyrosinase. *Biochemical and Biophysical Research Communications* 243, 801-803.
- Smith JG, Davidson EA, Tindall JP, Sams WM (1962) Alterations in human dermal connective tissue with age and chronic sun damage. *The Journal of Investigative Dermatology* 39, 347-350.
- Sung JH, Park SH, Seo DH, Lee JH, Hong SW, Hong SS (2009) Antioxidative and skin-whitening effect of an aqueous extract of *Salicornia herbacea*. *Bioscience, Biotechnology, and Biochemistry* 73, 552-556.
- Thring TS, Hill P, Naughton DP (2009) Anti-collagenase, anti-elastase and anti-oxidant activities of extracts from 21 plants. *BMC Complementary and Alternative Medicine* 9, 27.
- Wang KH, Lin RD, Hsu FL, Huang YH, Chang HC, Huang CY, Lee MH (2006) Cosmetic applications of selected traditional Chinese herbal medicines. *Journal of Ethnopharmacology* 106, 353-359.
- Yang EJ, Yim EY, Song G, Kim GO, Hyun CG (2009) Inhibition of nitric oxide production in lipopolysaccharide-activated RAW 264.7 macrophages by Jeju plant extracts. *Interdisciplinary Toxicology* 2, 245-249.
- Zheng ZP, Chen S, Wang S, Wang XC, Cheng KW, Wu JJ, Yang D, Wang M (2009) Chemical components and tyrosinase inhibitors from the twigs of *Artocarpus heterophyllus*. *Journal of Agricultural and Food Chemistry* 57, 6649-6655.

Jeju Adasi Bitkilerinde Elastaz ve Tirozinaz Inhibitor Aktivitesinin İncelenmesi

Özet

Dogal kaynakli yeni aktif kozmetik içerikler belirlemek amaciyla, Kore Yarimadasinin en güneyinde bulunan Jeju Adasindan toplanan 263 bitkinin 299 kısmi tarandi. Bitki kisimlari; kozmetik alanında ham madde olarak kullanilma potansiyeli bakımından, yaslanma önleyici ve ten beyazlatıcı içeriklerinin tanımlanması amacıyla, elastaz ve tirozinaz inhibitör aktiviteleri açısından incelendi. Anti-elastaz inhibisyon incelemesinde, *Aesculus turbinata*, *Taxillus yadoriki* ve *Cornus walteri* dahil 3 özüt yüksek inhibisyon aktivitesi sergiledi (inhibisyon konsantrasyonu $(IC)_{50} < 50$ /vg/mL). *A. turbinata* ve *T. yadoriki*'nin IC_{50} 'si sirasiyla 43,1 /vg/mL ve 36,4 /vg/mL idi. En yüksek inhibisyon aktivitesini *C. walteri* gösterdi ($IC_{50} = 26,1$ /vg/mL). Tirozinaz inhibisyon aktivitesi incelemesinde; *C. walteri* (139,2 /vg/mL), *Maackia fauriei* (149,3 /vg/mL), *Toxicodendron succedaneum* (142,3 /vg/mL) ve *Sophora flavescens* (41,6 /vg/mL) dahil 4 özüt, pozitif kontroller *Distylium racemosum* (145,9 /vg/mL) ve arbutin (180,3 /vg/mL)'den önemli ölçüde daha yüksek tirozinaz inhibisyon aktivitesi gösterdi. Ancak, pozitif kontroller *Morus alba* (11.9 v g/mL) ve *Morus bombycis* (22 v g/mL)'e kiyasla daha düşük aktivite sergilediler. Bu sonuçlar, birkaç biyolojik aktivite içeren tıbbi bitkinin, pigmentasyon artışı ve yaslanmada yer alan süreçlerin önlenmesinde etkili inhibitör olabileceğini göstermektedir. Daha sonraki araştırmalar, in vivo araştırmalarla, yaslanma karsiti ve beyazlatmadan sorumlu önemli aktif bileşiklerin tanımlanması üzerine yoğunlaşacaktır.

Anahtar Sözcükler: Elastaz, Jeju Adasi, kozmetik, tirozinaz, tıbbi bitki.

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)<