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Original Research Article

Screening of Plants with Inhibitory Activity on Cellular Senescence

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Abstract - In this study, the effect of plant extract on the senescence action and cell survival rate in two types of cells, in which aging was derived by adriamycin, was analyzed to find the materials for suppressing cell senescence from natural resources. The results are as follows. For human umbilical vein endothelial cells (HUVECs), the fruit of *Physalis angulata* L. and the aerial part of *Synurus deltoides* (Aiton) Nakai showed excellent cell-senescence inhibition activities in a treatment concentration-dependent manner, demonstrating the high possibility for utilization as a material for prevention and treatment for vascular diseases. The water extract from the root of *Polygonatum odoratum* var. *pluriflorum for variegatum* Y. N. Lee showed potent cell-senescence inhibitory effect for human dermal fibroblasts (HDFs). Thus it is considered that the additional study on the plant needs for elucidating the possible utilization as material for skin health improvement.

Key words - Cellular senescence, HDF, HUVEC, SA-β-gal

Introduction

Recently, due to the effects of the Convention on Biological Diversity (effectuation in 1993) and the Nagoya Protocol (adopted in 2010), the trend toward the development of useful material sources and the increase in profits deriving from their utilization has increased as a result of reviews of the potentially great medical value of the plant resources possessed by countries all around the world, including Korea. Thus, efforts to improve and maintain public health through the control of dietary habits and the use of health functional foods, in addition to medical treatments, have greatly increased. The health functional food market was worth KRW1409.1 billion in 2012, representing an increase of 3% over the previous year: as such, it is necessary to develop functional materials from natural resources with safe and excellent efficacies. In this respect, since indigenous plants have been used as important food and medicinal ingredients for a long time to preserve the health of the Korean people, it is expected that they will be widely used if continuous efforts are made to exploit them as materials for improving various health-related problems that are currently on the rise.

Cellular senescence is directly or indirectly involved in the pathophysiology of aging-related diseases, and the number of senescent cells in human tissues such as the skin and liver increases with age (Dimri *et al*., 1995; Pardis *et al*., 2001). The aging of cells contributes not only to the formation and progression of cancers, but also to the aging of tissues and the organism (Patil *et al*., 2005; Campisi, 2001), and is derived from caused by a wide variety of factors including the following: telomere shortening; the activation of concogenes or tumor suppressor genes; oxidative stress; chemicals with cytotoxicity; and inflammatory cytokines (Collado *et al*., 2007). Senescent cells typically have a larger, flatter appearance, and have been observed to increase the aging-related center of the heterosome in the nucleus and the aging-related activation of β-galactosidase (SA-β-gal) and up-regulation of p53 and p161NK4 proteins. Furthermore, senescent cells secrete inflammatory cytokines such as insulin-like growth factor binding proteins (IGFBPs), interleukin-6 (IL-6), transforming growth factor-β (TGF-β), and interferons (Kuilman & Peeper, 2009). Senescent cells have been observed in inflammatory tissues in rheumatoid arthritis and tumor tissues in liver cancer, and skin diseases (Schmid *et al*., 2004; Harding *et al*., 2005; Paradis *et al*., 2001). In the case of vascular endothelial cells

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(VECs), cellular senescence plays an important role in the progression of aging-related cardiovascular diseases such as arteriosclerosis (Hayashi *et al*., 2006; Kim *et al*., 2007), and integrin β4, which is involved in cascade signal transduction such as cancer invasion, cell apoptosis and differentiation, and has increased along with VEC senescence (Guo *et al*., 2006; Lv *et al*., 2008); however, the aging of VECs is delayed if integrin β4 is knocked-down (Liu *et al*., 2007). The aging of fibroblasts and keratinocytes can be caused by UV irradiation, resulting in aging-related skin damage such as wrinkles and pigmentation, while the aging of fibroblasts in skin ulcers can influence the effects of treatments and diagnosis (Makrantonaki & Zouboulis, 2007).

Because of having many physiological efficacies such as anti-inflammatory, antioxidant and anticancer activity (Lee *et al*., 2013; Sohn *et al*., 2013; Jun *et al*., 2014), plants would be candidate resources for health improvement. Thus, this study was performed with the aim of discovering plant resources that can decrease senescence in vascular and skin cells.

Materials and Methods

Plant extract materials

The plant extracts including *Achillea millefolium* (aerial part) were provided from of Plant Extract Bank in National Institute of Horticultural and Herbal Science (NIHHS), the resources, the used parts, the extracting condition of which were shown at Table 1.

Cells and culture

Human dermal fibroblasts (HDFs) in Dulbecco's Modified Eagle medium (DMEM) with 10% fetal bovine serum (FBS),

Table 1. Plant part extracts distributed from Plant Extract Bank of NIHHS

Sample No.	Scientific name (Korean name)	Part used	Resource[z]	Extract condition[y]
1	*Achillea millefolium* (예로우)	aerial part	1	A
2	*Aeschynomene indica* L. (자귀풀)	aerial part	1	A
3	*Althaea officinalis* L. (마쉬말로우)	aerial part	1	A
4	〃	root	1	A
5	*Amaranthus paniculatus* (번수현)	aerial part	1	A
6	*Aralia cordata var. continentalis* (Kitag.) Y.C.Chu (독활)	stem	1	B
7	*Aster koraiensis* Nakai (벌개미취)	flower	1	B
8	*Boehmeria apicata* Thunb. (좀깨잎나무)	aerial part	1	A
9	*Buxux micorphylla* S.etZ. var. *koreana* Nakai (회양목)	branch	1	B
10	*Calystegia japonica* (Thunb.) Chois (메꽃)	aerial part	1	A
11	*Camellia sinensis* L. (차나무)	aerial part	3	A
12	*Celosisa cristata* L. (맨드라미)	root	1	A
13	*Dendranthema sichotense* Tzvelev (바위구절초)	whole plant	1	A
14	*Digitalis purpurea* L. (디기탈리스)	root	1	A
15	*Duchesnea chrysantha* (뱀딸기)	fruit	1	A
16	*Eleutherococcus sessiliflorus* (Rupr. & Maxim.) S.Y.Hu (오갈피나무)	fruit	4	C
17	*Eupatorium chinense* var. *simplicifolium* (등골나물)	root	1	A
18	〃	aerial part	1	A
19	*Galium verum* var. *asiaticum* (솔나물)	aerial part	1	A
20	*Heliantus annuus* L. (해바라기)	leaf	1	B
21	*Hyoscyamus niger* L. (사리풀)	aerial part	1	A
22	*Lithospermum erythrorhizon* S.etZ. (지치)	aerial part	1	A
23	*Lycium chinensis* Miller (구기자)	leaf	1	D
24	*Matricaria chamomilla* L. (카모밀라)	aerial part	1	A
25	*Melissa officinalis* (레몬밤)	aerial part	1	A
26	*Oenothera odorata* (달맞이꽃)	aerial part	1	A
27	*Phragmites communis* Trin. (갈대)	aerial part	5	A

Table 1. Continued

Sample No.	Scientific name (Korean name)	Part used	Resource[z]	Extract condition[y]
28	*Physalis angulata* L. (땅꽈리)	aerial part	1	A
29	〃	fruit	1	E
30	*Phytolacca americana* L. (미국자리공)	aerial part	1	E
31	*Plectranthus serra* Maxim. (자주방아풀)	root	1	A
32	〃	flower	1	B
33	*Polygonatum odoratum* Druce var. *pluriflorum* Ohwi (둥굴레)	aerial part	1	D
34	*Polygonatum odoratum* var. *pluriflorum* for *variegatum* Y.N.Lee (무늬둥굴레)	root	1	D
35	〃	aerial part	1	D
36	〃	aerial part	1	A
37	*Rumex obtusifolius* L. (돌소리쟁이)	aerial part	1	E
38	*Saururus chinensis* (Lour.) Baill. (삼백초)	root	1	D
39	*Saussurea lappa* Clarke (운목향)	leaf	1	A
40	*Spergularia marina* Grisebach (갯개미자리)	leaf	6	A
41	*Symphytum officinale* L. (컴프리)	aerial part	1	B
42	*Synurus deltoides* (Aiton) Nakai (수리취)	aerial part	1	A
43	*Tanacethum bungeia* (탄지)	flower	1	A
44	*Traxacum plathcarpum* H. Dahlst (민들레)	root	1	A
45	*Ulmus davidiana* var. *japonica* (Rehder) Nakai (느릅나무)	leaf	1	B
46	*Vigna sesquipedalis* L. (아스파라거스콩)	aerial part	1	A
47	*Vitex negundo* var. *incisa* (Lam.) C.B. Clarke (좀목형)	leaf	1	B

[z]1, Suwon (RDA); 2, Odaesan; 3, Mokpo; 4, Jeongseon; 5, Busan (Nakdongriver); 6, Yanggu.

[y]The plant extract samples were distributed from Plant Extract Bank of NIHHS which had been extracted by several extraction methods as below; A, methanol extraction at 50℃ by using accelerated solvent system; B, methanol extraction at 74℃ by using refluxing apparatus; C, 70% ethanol extraction; D, water extraction at room temperature; E, ethanol extraction at 85℃ by using accelerated solvent system.

1% antibiotic (penicillin-streptomycin) are plated at 1×10^5 cells per 100 ㎜ culture plate and cultured at 37°C in 5% CO_2 incubator. At 80～90% confluence of subculture, the serial passaging was conducted by adding with trypsin-ethylenediaminetetraacetic acid (EDTA) solution. Human umbilical vein endothelial cells (HUVECs) in endothelial cell growth medium (EGM)-2 were cultured using the same conditions.

Cytotoxicity analysis

MTT (3-(4,5-Dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide) assay for the verification of the cytotoxicity produced by plant extracts was performed by the method of Yang *et al*. (2010). Cells treated with adriamycin for 4 hours were isolated from culture plate by adding with trypsin-EDTA. HDFs in DMEM media with 10% FBS and 1% antibiotic were distributed at 500 cells/wells and HUVECs in EGM-2 media were distributed at 1,000 cells/well in the 96well plate, respectively. The cells were cultured at 37℃ in 5% CO_2 incubator for 24 hours. After additive treatment with 100 ㎕ of DMEM and EGM-2 media with 10% FBS and 1% antibiotic, the plant extracts being a final concentration of 10 ㎍/㎖ for HUVECs and 100 ㎍/㎖ for HDFs were treated. After dimethyl sulfoxide (DMSO) as negative control, and 5 mM N-acetylcysteine (NAC) and 500 nM Rapamycin as positive control were treated for 3 days at 37℃ in 5% incubator, the cells treated with 50 ㎕ of 0.1% MTT reagent were cultured at 37℃, 5% CO_2 humidified air for 3 hours. The MTT reagent and media were deprived from the wells. Crystals made in wells were dissolved with 100 ㎕ of DMSO. The solution were analyzed at 550 ㎚ by microplate reader.

Cell senescence inducing and senescence-associated β-galactosidase (SA-β-gal) assay

For inducing of cell senescence with adriamycin, HDFs and HUVECs were distributed at 1.5×10^5 in 100 ㎜ culture plate and cultured at 37℃ in 5% CO_2 incubator for 3 days.

The cells excluded media were washed with DMEM of 1% antibiotics two times and added with 500 nM adriamycin for 4 hours. Cells treated with adriamycin were cultures in trypsin-EDTA for 4 hours and were isolated from the plate. The isolated cells were distributed in 12 well or 24 well plates. HDFs were distributed at 5,000 cells/well in 12 wells and at 3,000 cells/well in 24 wells and HUVECs were distributed at 7,000 cells/well in 12 wells and at 5,000 cells/well in 24 wells, which were incubated at 37℃ in 5% CO_2 incubator. After the media including cells are replaced with new media, cells were treated with plant extracts, treated with DMSO as negative control, and treated 5 mM NAC and 500 nM Rapamycin as positive control. Cells were cultures at 37℃ in 5% incubator for 3 days and the staining on the SA-β -gal was conducted. Cells were washed with phosphate buffered saline (PBS), and fixed with 3.7% paraformaldehyde for 1 minute. After eliminating the fix solution from the cells, SA-β-gal staining reagent (40 mM citric acid/phosphate [pH 5.8], 5 mM potassium ferrocyanide, 5 mM potassium ferricyanide, 150 mM NaCl, 2 mM $MgCl_2$, X-gal 1 ㎎/㎖) was added in the 12 well or 24 well. The wells were incubated at 37℃ for 16 hours, washed with PBS and stained with 1% eosin for 1 minute. Cells stained blue were washed with PBS and counted under microscope. Activity of SA-β-gal was evaluated by the stained cells among the 50 cells counted. The number of stained blue cells among 50 cells in three randomized fields which were treated with DMSO were regarded 100 and the results in the stained cells among 50 cells treated with plant extracts were indicated as percentage (%) to the number of DMSO treatment.

Statistical analysis

The results were showed as average ± standard deviation and the statistical significance was determined by Student's t-test.

Results and Discussion

Effect of the plant extracts on cell proliferation

When the effects of the plant extracts on HUVECs proliferation were calculated as percentage against control, in the final concentration of 10 ㎍/㎖, the methanol extract of the aerial part of *Camellia sinensis* L., 70% ethanol extract from the fruit of *Eleutherococcus sessiliflorus* (Rupr. & Maxim.) S.Y.Hu, the methanol extract from the root of *Eupatorium chinense* var. *simplicifolium*, the methanol extract from the root of *Plectranthus serra* Maxim. showed high proliferation values over 100% (Table 2). These plants showed the high proliferation values on HUVECs compared to NAC and Rapamycin (99.6 ± 3.8% and 75.3 ± 3.0%, respectively) used as the positive control. In the final

Table 2. Effect of plant extracts on the proliferation and the senescence-associated β-galactosidase activity of HUVECs

Sample No.	Staining rate (%)[z]	Proliferation (%)[z]
Adriamycin	98.6 ± 6.6	100 ± 0
DMSO[y]	100.0 ± 0.0	88.6 ± 3.5*
NAC[x]	84.5 ± 3.5**[w]	99.6 ± 3.8
Rapamycin[x]	62.9 ± 13.2***	75.3 ± 3.0**
25	75.8 ± 12.0*	-
29	77.3 ± 9.4**	-
28	79.7 ± 12.4	85.6 ± 23.1
41	80.1 ± 16.9	-
5	80.3 ± 17.4	96.5 ± 19.6
13	81.4 ± 18.2	85.2 ± 15.6
1	83.0 ± 8.2**	69.0 ± 7.8*[z]
42	84.6 ± 13.7	-
9	85.4 ± 25.7	75.3 ± 14.9*
46	85.4 ± 11.8*	77.6 ± 10.8
16	87.0 ± 12.4	102.1 ± 34.6
21	87.3 ± 15.9	79.0 ± 21.1
18	88.3 ± 23.1	76.9 ± 9.8
30	89.0 ± 8.7	79.6 ± 9.9
27	89.5 ± 11.1	-
20	89.8 ± 13.6	83.1 ± 2.2
33	90.1 ± 21.3	-
11	90.7 ± 10.4	105.4 ± 7.9
40	90.7 ± 8.2	84.1 ± 10.7
3	91.0 ± 5.9**	-
44	91.3 ± 14.6	-
22	91.5 ± 19.5	-
7	91.6 ± 16.2	96.0 ± 32.9
31	91.6 ± 9.2	101.7 ± 7.4
43	91.7 ± 10.1*	81.0 ± 5.1
35	91.9 ± 12.0	-
10	92.0 ± 2.5	79.2 ± 13.0*
2	93.3 ± 14.3	73.1 ± 8.5
34	93.5 ± 12.3	92.2 ± 28.3
36	93.7 ± 2.0	95.8 ± 26.2
47	93.9 ± 8.5	83.9 ± 7.5
17	94.2 ± 15.2	108.1 ± 25.6
38	95.2 ± 13.0	89.2 ± 17.8
4	95.4 ± 12.8	-

Table 2. Continued

Sample No.	Staining rate (%)[z]	Proliferation (%)[z]
24	95.8 ± 9.3	79.2 ± 7.6
37	96.3 ± 14.2	88.0 ± 13.0
19	96.9 ± 9.2	81.1 ± 7.7
39	97.6 ± 8.7	78.6 ± 1.1
8	100.7 ± 3.0	89.4 ± 18.2
12	101.5 ± 6.1	-
6	103.4 ± 14.5	78.6 ± 3.9
23	104.1 ± 8.9	81.5 ± 10.2
15	105.7 ± 16.1	77.7 ± 5.3
45	108.0 ± 9.2*	82.1 ± 6.0
32	115.5 ± 2.3	88.8 ± 25.1

[z]Final concentration of the plant extract was 10 ㎍/㎖.
[y]Dimethyl sulfoxide (DMSO) was used as negative control.
[x]Final concentration of N-acetylcysteine (NAC) and Rapamycin as positive control were 5 mM and 500 nM, respectively.
[w]Symbol indicates significance in OD value; *, $p < 0.05$; **, $p < 0.01$; ***, $p < 0.001$.

concentration of 100 ㎍/㎖, the cell viabilities of the methanol extract of *Melissa officinalis* (aerial part), the methanol extract of *Symphytum officinale* L. (aerial part), the methanol extract of *Synurus deltoides* (Aiton) Nakai (aerial part) ranged from 121.0% to 170.8%.

Effects of plant extracts on the proliferation of HDFs were evaluated at the final sample concentration of 100 ㎍/㎖. Cell viabilities of the fourteen extracts such as the methanol extract of *Boehmeria apicata* Thunb. (aerial part), the methanol extract of *Camellia sinensis* L. (aerial part), the methanol extract of *Dendranthema sichotense* Tzvelev (whole plant), the methanol extract of *Digitalis purpurea* L. (root), the methanol extract of *Galium verum* var. *asiaticum* (aerial part), the methanol extract of *Heliantus annuus* L. (leaf), the methanol extract of *Melissa officinalis* (aerial part), the methanol extract of *Oenothera odorata* (aerial part), the ethanol extract of *Physalis angulata* L. (fruit), the methanol extract of *Plectranthus serra* Maxim. (root), the ethanol extract of *Rumex obtusifolius* L. (aerial part), the methanol extract of *Symphytum officinale* L. (aerial part), the methanol extract of *Synurus deltoides* (Aiton) Nakai (aerial part), the methanol extract of *Ulmus davidiana* var. *japonica* (Rehder) Nakai (leaf) were distributed from 104.9 ± 9.2% to 159.1 ± 11.1%. These proliferation values of the plants were higher than the results (91.5 ± 10.4% and 90.6 ± 4.6%) of NAC and Rapamycin (Table 3).

Table 3. Effect of plant extracts on the proliferation and the senescence-associated β-galactosidase activity of HDFs

Sample No.	Staining rate (%)[z]	Proliferation (%)[z]
Adriamycin	98.2 ± 3.4	100 ± 0
DMSO[y]	100.0 ± 0.0	91.0 ± 8.9
NAC[x]	76.8 ± 5.5***[w]	91.5 ± 10.4
Rapamycin[x]	70.9 ± 7.1***	90.6 ± 4.6
34	64.6 ± 19.3*	91.3 ± 4.7
35	71.6 ± 23.8*	92.4 ± 8.1
39	77.8 ± 16.3*	99.0 ± 7.4*
47	82.9 ± 30.2	89.6 ± 11.3
28	83.5 ± 23.3	85.9 ± 6.9*
16	84.3 ± 24.0	99.1 ± 15.6
23	85.0 ± 33.2	88.2 ± 9.0
33	85.0 ± 39.1	88.6 ± 8.9**
11	86.5 ± 10.8**	159.1 ± 11.1**
41	87.3 ± 12.5	119.6 ± 10.9**
22	87.9 ± 27.6	85.1 ± 6.7
25	89.7 ± 18.7	148.5 ± 18.4**
12	90.1 ± 3.6	89.8 ± 8.2
24	91.2 ± 15.9	93.3 ± 8.5
29	93.2 ± 13.0	115.6 ± 11.2**
40	93.4 ± 15.0	96.0 ± 16.0
38	93.5 ± 37.4	97.4 ± 13.3
17	94.1 ± 21.6	99.4 ± 10.9*
26	94.5 ± 23.1	146.8 ± 11.8**
5	95.6 ± 24.2	87.6 ± 6.8
37	97.4 ± 28.0	120.0 ± 14.1*
36	97.6 ± 19.1	88.8 ± 6.9
27	98.0 ± 19.4	90.5 ± 8.4
15	99.4 ± 18.7	88.1 ± 6.8
30	99.4 ± 22.8	84.2 ± 8.3*
10	100.1 ± 14.2	104.9 ± 9.2*
42	100.6 ± 16.8	127.2 ± 14.2**
46	101.0 ± 28.5	86.6 ± 9.9*
21	101.7 ± 5.3	85.8 ± 7.3*
8	103.1 ± 14.5	110.3 ± 16.5*
19	104.1 ± 31.6	110.6 ± 15.9*
1	106.3 ± 4.1	87.1 ± 9.7*
3	106.9 ± 25.9	85.1 ± 7.8
4	109.6 ± 23.3	90.8 ± 4.0
20	110.7 ± 7.5	109.5 ± 9.6**
44	112.2 ± 6.8	87.0 ± 8.0*
45	115.6 ± 7.0	149.0 ± 8.5**
13	116.2 ± 7.5	107.5 ± 7.4**
14	117.3 ± 17.3	113.7 ± 12.3**
31	117.5 ± 13.1	126.2 ± 9.7**

[z]Final concentration of the plant extract was 100 ㎍/㎖.
[y]Dimethyl sulfoxide (DMSO) was used as negative control.
[x]Final concentration of N-acetylcysteine (NAC) and Rapamycin as positive control were 5 mM and 500 nM, respectively.
[w]Symbol indicates significance in OD value; *, $p < 0.05$; **, $p < 0.01$; ***, $p < 0.001$.

Suppressive effect of plant extracts on cellular senescence (as SA-β-gal activity)

Dimri *et al.* (1995) reported that several human cells express a beta-galactosidase, histochemically detectable at pH 6, upon senescence in culture, and there was an age-dependent increase in this marker in dermal fibroblasts and epidermal keratinocytes. In present study, the plants were under the adriamycin-induced cellular senescence assay on HUVECs or HDFs. The SA-β-gal activity (%) of the plants in cellular senescences was evaluated compared with the number of the blue cells which were stained by only DMSO treatment as negative control. For SA-β-gal assay on HUVECs, plant extracts were tested in the final concentration of 10 ㎍/㎖. The SA-β-gal staining rate of the methanol extract from the aerial part of *Melissa officinalis* was 75.8 ± 12.0%, the lowest staining value among the plant extracts. The ethanol extract of the fruit of *Physalis angulata* L. and the methanol extract of the aerial part of *Physalis angulata* L. showed 77.3 ± 9.4% and 79.7 ± 12.4% in SA-β-gal staining rate. The SA-β-gal staining rates on HUVEC cells of other plant extracts including the aerial part of *Symphytum officinale* L., the aerial part of *Amaranthus paniculatus,* the whole plant of *Dendranthema sichotense* Tzvelev, the aerial part of *Achillea millefolium* and the aerial part of *Synurus deltoides* (Aiton) Nakai were also low or the same levels (from 84.6 ± 13.7% to 80.1 ± 16.9%) compared with the SA-β-gal activity (84.5 ± 3.5%) of NAC, a positive control. This result suggests that those plant extracts with low SA-β-gal activity have inhibitory activity on the senescence of HUVEC cells. But, eight plant extracts from the aerial part of *Boehmeria apicata* Thunb., the root of *Celosisa cristata* L., the stem of *Aralia cordata var. continentalis* (Kitag.) Y.C.Chu, the leaf of *Lycium chinensis* Miller, the fruit of *Duchesnea chrysantha,* the leaf of *Ulmus davidiana* var. *japonica* (Rehder) Nakai, and the flower of *Plectranthus serra* Maxim. showed high values (over 100%) in SA-β-gal staining rate (Table 2).

Plant extracts were also used for SA-beta-gal experiment on HDFs and the data on 40 extracts were listed (Table 3). From the experiment, the plants which have shown the low values in SA-beta-gal activity below 80% at the concentration of 100 ㎍/㎖, were the water extract from the root of *Polygonatum odoratum* var. *pluriflorum* for *variegatum* Y.N.Lee, the water extract from the aerial part of *Polygonatum odoratum* var. *pluriflorum* for *variegatum* Y.N.Lee and the methanol extract from the leaf of *Saussurea lappa* Clarke. The SA-β-gal activity of the water extract from root of *Polygonatum odoratum* var. *pluriflorum* for *variegatum* Y.N.Lee (64.6 ± 19.3%) was lower value than the values of Rapamycin (70.9 ± 7.1%) and NAC (76.8 ± 5.5%), which implies that the water extract of *Polygonatum odoratum* var. *pluriflorum* for *variegatum* Y.N.Lee (root) efficiently suppressed the senescence of HDF cells compared with two positive control materials. The water extract of *Polygonatum odoratum* var. *pluriflorum* for *variegatum* Y.N.Lee (aerial part) and the methanol extract of *Saussurea lappa* Clarke (leaf) also effectively inhibited SA-beta-gal activity to such a degree that NAC inhibited.

From the result, it is suggested that the plants used in the assay have more effective cell viability activity on HDFs than HUVECs. It implies that the samples showing comparatively low SA-beta-gal activities (below 70%) on HUVECs in the concentration of 10 ㎍/㎖ such as the methanol extract of *Melissa officinalis* (aerial part), the ethanol extract of *Physalis angulata* L. (fruit), the methanol extract of *Physalis angulata* L. (aerial part), inhibited the adriamycin-induced HUVEC senescence. And the samples having comparatively low SA-beta-gal activities (below 70%) in HDFs in the concentration of 100 ㎍/㎖ such as he water extract of *Polygonatum odoratum* var. *pluriflorum* for *variegatum* Y.N.Lee (root), the water extract of *Polygonatum odoratum* var. *pluriflorum* for *variegatum* Y.N.Lee (aerial part) and the methanol extract of *Saussurea lappa* Clarke (leaf), also indicated inhibitory efficacies on the HDF senescence.

For utilizing these plants as functional materials, the cell viabilities and SA-β-gal activities of these plant extracts on HUVEC cells were further assayed. From the assay, the extracts from *Melissa officinalis* (aerial part), *Physalis angulata* L. (fruit), *Synurus deltoides* (Aiton) Nakai (aerial part) in 100 ㎍/㎖ increased HUVEC proliferation as 170.8 ± 27.1%, 92.7 ± 4.9% and 121.0 ± 14.3%, respectively (data not shown in table or figure). The activities of SA-β-gal on HUVECs of the methanol extract of aerial part of *Melissa officinalis* were 75.8 ± 12.0% in 10 ㎍/㎖ and 80.4 ± 16.7% in 100 ㎍/㎖, respectively. The SA-β-gal activity on HUVECs

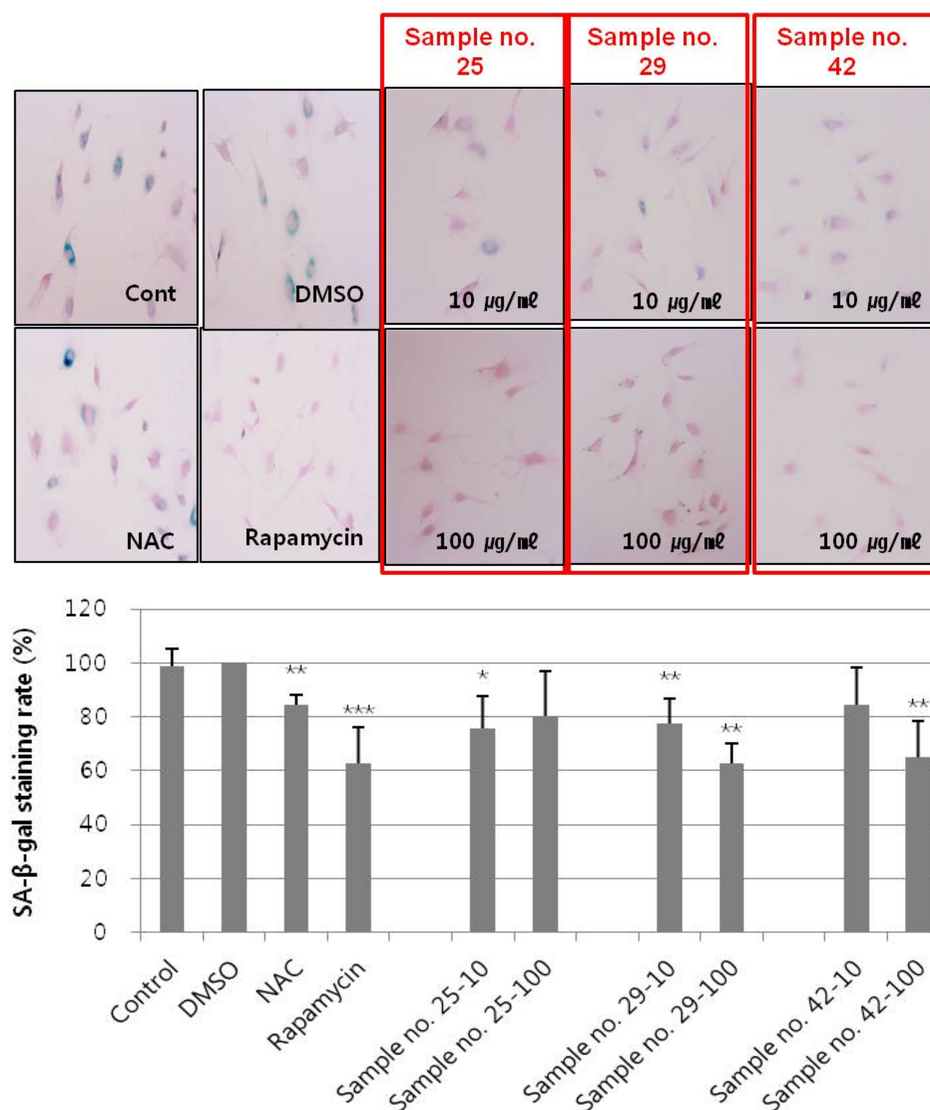

Fig. 1. Effect of the ethanol extract of *Melissa officinalsis* (aerial part, sample no. 25), *Physalis angulata* L. (fruit, sample no. 29), and the methanol extract of *Synurus deltoides* (Aiton) Nakai (aerial part, sample no. 42) on the SA-β-gal activity of senescence-induced HUVECs.

of the ethanol extract from the fruit of *Physalis angulata* L. was 62.7 ± 7.4% in 100 ㎍/㎖. The SA-β-gal activity on HUVECs of the methanol extract from the aerial part of *Synurus deltoides* (Aiton) Nakai was 65.2 ± 13.3% in 100 ㎍/㎖, respectively. The extracts from the fruit of *Physalis angulata* L. and the aerial part of *Synurus deltoides* (Aiton) Nakai showing the comparatively lower SA-β-gal activities than 65% in the concentration of 100 ㎍/㎖, indicated the similar or more effective inhibitory activities compared with the activities which NAC and Rapamycin had (84.5 ± 3.5%, 62.9 ± 13.2%) (Fig. 1).

In HDF cell viability, the effects of *Polygonatum odoratum* var. *pluriflorum* for *variegatum* Y.N.Lee (root, water extracts), *Polygonatum odoratum* var. *pluriflorum* for *variegatum* Y.N.Lee (aerial part, water extracts) and *Saussurea lappa* Clarke (leaf, methanol extract) were 91.3 ± 4.7%, 92.4 ± 8.1% and 99.0 ± 7.4% in the concentration of 100 ㎍/㎖. The activities of SA-beta-gal in HDF treated with the water extract from root of *Polygonatum odoratum* var. *pluriflorum* for *variegatum* Y.N.Lee was 72.3 ± 9.1% in the concentration of 10 ㎍/㎖. The SA-beta-gal activities of the water extract from the aerial part of *Polygonatum odoratum* var. *pluriflorum*

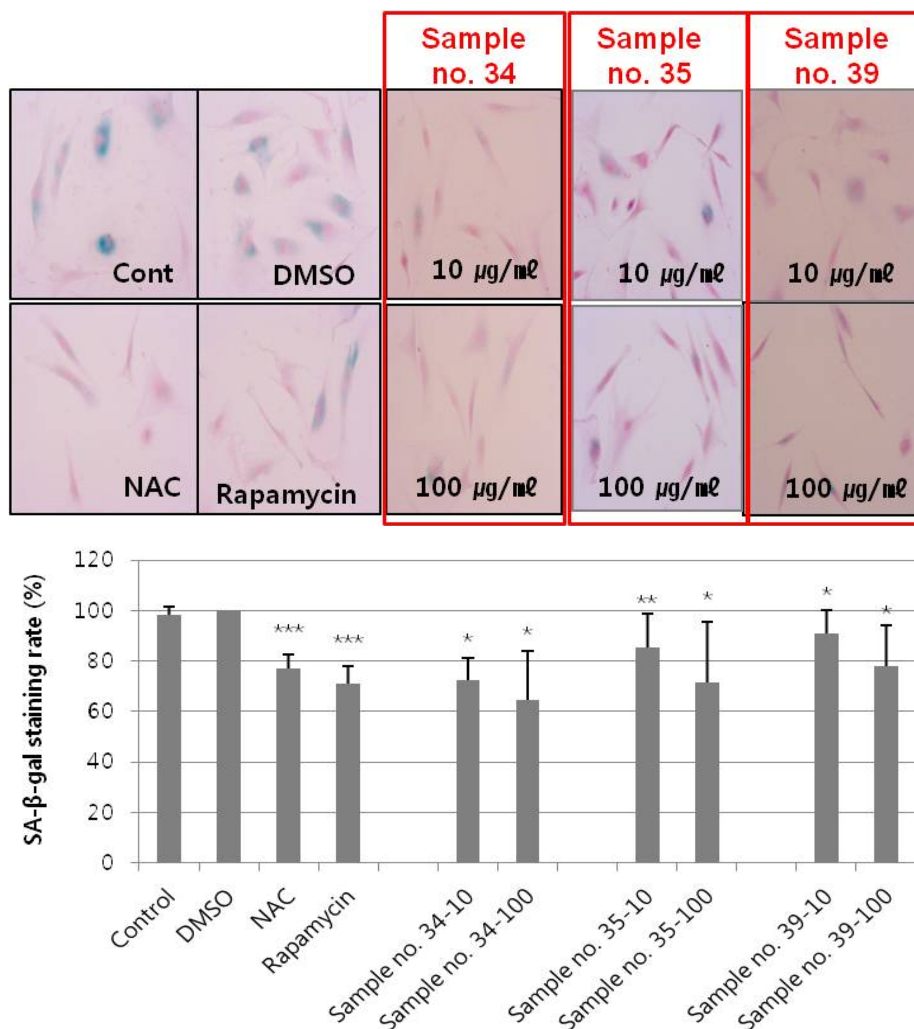

Fig. 2. Effect of the water extract of *Polygonatum odoratum* var. *pluriflorum* for *variegatum* Y.N.Lee (root, sample no. 34), the water extract of *Polygonatum odoratum* var. *pluriflorum* for *variegatum* Y.N.Lee (aeiral part, sample no. 35) and the methanol extract of *Saussurea lappa* Clarke (leaf, sample no. 39) on SA-β-gal activity of senescence-induced HDFs.

for *variegatum* Y.N.Lee in 10 ㎍/㎖ was 85.5 ± 13.3%. The SA-beta-gal activities of the methanol extract from the leaf of *Saussurea lappa* Clarke on HDF cells was 91.0 ± 9.0% (Fig. 2).

Healthy endothelials (ECs) contribute to the prevention of atherosclerosis in medium to large arteries (Cines *et al*., 1998). Cellular senescence of vascular endothelial cells (VECs) plays an important role in the progression of aging-related cardiovascular diseases such as arteriosclerosis (Hayashi *et al*., 2006; Kim *et al*., 2007). It needs to give attention to the plants indicating potent inhibitory activities on SA-β-gal production including the ethanol extract of the fruit of *Physalis angulata* L. and the methanol extract of *Synurus deltoides* (Aiton) Nakai (aerial part) as candidate materials for improving vascular health. And, the aging of fibroblasts and keratinocytes can be caused by UV irradiation, which resulted in aging-related skin damage (Makrantonaki & Zouboulis, 2007), for this reason, the water extract of the root of *Polygonatum odoratum* var. *pluriflorum* for *variegatum* Y.N.Lee having a suppressive efficacy on HDFs senescence is prospected its using as skin health increasing materials.

From the results, we conclude that the ethanol extract of *Physalis angulata* L. fruit, and the methanol extract of *Synurus deltoides* (Aiton) Nakai aerial part, which have shown the high cell viabilities and the cellular senescence inhibition activities on HUVECs in dose-dependent manner, need to further study for developing the preventive and treating

materials of vascular disorders. And we suggest that the water extract of *Polygonatum odoratum* var. *pluriflorum* for *variegatum* Y.N.Lee (root), the plant shown the high cell proliferation and the cellular senescence inhibition activities on HDFs, also needs to study for utilizing as a skin-health material.

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