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Anti-aging Potential of Extracts Prepared from Fruits and Medicinal Herbs Cultivated in the Gyeongnam Area of Korea

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ABSTRACT: Many recent studies have focused on maintaining a healthy life by preventing and/or postponing the aging process. Numerous studies have reported that continuous exposure to reactive oxygen species can stimulate skin aging and that excessive accumulation of fat can cause an impaired skin barrier and tissue structure alterations. Thus, the maintenance of antioxidant homeostasis and the suppression of adipose accumulation are important strategies for skin anti-aging. Here, we prepared three types of extracts [whole juice, acetone-perchloric acid (PCA), and ethanol] from 20 fruits and medicinal herbs native to the Gyeongnam area of Korea. The total phenolic content of each extract was analyzed, and we observed higher total phenolic contents in the medicinal herbs. Consistent with this, the results of the oxygen radical absorbance activity capacity assay indicated that the *in vitro* antioxidant activities of the medicinal herb extracts were stronger than those of the fruit extracts. The fruits and medicinal herbs had strong effects on cell-based systems, including H_2O_2-induced oxidative stress in human keratinocytes and 3T3-L1 lipid accumulation. Nishimura Wase persimmon, Taishu persimmon, wrinkled giant hyssop, sweet wormwood, Chinese cedar, red perilla, tan shen, hiyodori-jogo, and cramp bark may be natural anti-aging materials with effective antioxidant and anti-adipogenic activities. Taken together, our findings may provide scientific evidence supporting the development of functional foods and nutraceuticals from fruits and medicinal herbs.

Keywords: fruits, medicinal herbs, anti-aging, skin, gyeongnam

INTRODUCTION

Growth and aging can be affected by various factors, such as disease, injury, nutrition, exercise, stress, and numerous environmental factors. Aging is a complex process characterized by a progressive decline in physiological function, followed by dysfunction, and ultimately, death (1). Various scientific efforts have been made to slow the process of aging, and extend the maximum lifespan and average healthy lifespan. The World Health Organization reported that the percentage of the world's population of people over 60 years of age will double from 11% to 22% between 2000 and 2050 (2). Thus, many recent studies have focused on maintaining a healthy life by preventing and postponing aging.

There are extrinsic and intrinsic processes that induce skin aging. Extrinsic aging develops due to environmental factors, such as exposure to ultraviolet (UV) radiation, alcohol intake, pollution, and severe physical stress (3,4). Among these environmental factors, UV radiation contributes to up to 80% of extrinsic aging. Intrinsic skin aging occurs because of cumulative endogenous damage due to continual formation of reactive oxygen species (ROS) (5,6). ROS are broadly defined as oxygen-containing, highly reactive species. ROS are essential for biological functions and are generated constantly during normal cellular metabolism. Moderate increases in ROS such as hydrogen peroxide (H_2O_2) and superoxide anions (O_2^-) can act as an intracellular messengers in cellular events, whereas excessive production of ROS can cause oxidative stress and damage to biological molecules (7,8). ROS are ordinarily eliminated from the body through the antioxidant defense system (9). However, antioxidant defense system dysfunction and various physical and chemical factors can lead to an imbalance between ROS formation and removal, causing cells to be

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damaged by free radical reactions (10). Previous studies have pointed out that continuous ROS exposure can stimulate skin aging through antioxidant system destruction, wrinkle formation, and melanogenesis (7).

Adipose tissue is mainly found beneath the skin, around internal organs, and in breast tissue. It has various beneficial effects, including maintaining energy balance and homeostasis, insulating against heat and cold, and acting as protective padding (11). However, excessive accumulation of fat can be a cause of obesity and can lead to the development of various chronic diseases (12). Additionally, recent reports have indicated that excess adipose tissue can impair skin barrier function and connective tissue structure, causing dry skin through increased water loss and erythema (13). The wrinkle formation in skin was induced by altering the tissue structure and a collapsed between epidermis and dermis layer (14-16). Thus, maintaining antioxidant homeostasis despite free radical-induced oxidative stress and suppressing excessive adipose accumulation are strategies to prevent skin aging.

Antioxidant sources containing a variety of bioactive components from natural sources have been recommended for aging prevention (17). In addition, bioactive compounds such as isoflavones, anthocyanins, and catechins may have potent antioxidant activity against ROS (18). In this study, we prepared whole juices, juice extraction residues, and ethanol extracts of fruits and medicinal herbs found in the Gyeongnam area of Korea. The cellular antioxidant and anti-adipogenic activities of each extract were evaluated. Currently, there is little available data regarding the antioxidant and anti-adipogenic functions of natural plants inhabiting the Gyeongnam area. In addition, fruits and medicinal herbs are logically important natural materials in Gyeongnam area. Thus, in this study we investigated the antioxidant and anti-adipogenic activities of these fruits and medicinal herbs from an anti-aging point-of-view and discussed the potential of these plants as natural anti-aging materials.

MATERIALS AND METHODS

Materials

The fruits (Matsumoto Wase Fuyu, Taishu persimmon, Nishimura Wase persimmon, Jonathan apple, Tsugaru apple, pear cultivated in Hadong and Jinju, cherry tomato, green grapes, and Kyoho grapes) were purchased from a local market in Changwon, Korea. The medicinal herbs (wrinkled giant hyssop, Japanese angelica tree, sweet wormwood, Chinese cedar, cudrang, tu-chung, red perilla, tan shen, hiyodori-jogo, and cramp bark) were supplied by Gyeongsangnam-do Agricultural Research & Extension Services (Jinju, Korea) (Table 1). Oil red O (ORO), 3-isobutyl-1-methylxanthine (IBMX), dexamethasone, insulin, and 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) were purchased from Sigma-Aldrich Co. (St. Louis, MO, USA). Dulbecco's modified Eagle's medium (DMEM), penicillin-streptomycin, and phosphate-buffered saline (PBS) were obtained from WELGENE Inc. (Daegu, Korea). Fetal bovine serum (FBS) and fetal calf serum (FCS) were purchased from GE Healthcare Life Sciences Co. (Piscataway, NJ, USA). Dimethyl sulfoxide (DMSO) and isopropanol were obtained from Junsei Chemical Co., Ltd. (Tokyo, Japan), and formaldehyde was purchased from Bio Basic Inc. (Markham, ON, Canada).

Table 1. The list of fruits and medicinal herbs cultivated in the Gyeongnam area of Korea

Common name	Scientific name	Remarks
Matsumoto Wase Fuyu	*Diospyros kaki* Thunb.	MWF
Nishimura Wase persimmon	*Diospyros kaki* Thunb.	NWP
Taishu persimmon	*Diospyros kaki* Thunb.	TP
Jonathan apple	*Malus domestica* Borkh.	JA
Tsugaru apple	*Malus pumila* Mill.	TA
Pear (Hadong)	*Pyrus pyrifolia* var. *culta*	PH
Pear (Jinju)	*Pyrus pyrifolia* var. *culta*	PJ
Cherry tomato	*Solanum lycopersicum* var. *cerasiforme*	CT
Green grapes	*Vitis vinifera*	GG
Kyoho grapes	*Vitis vinifera* L. × *Vitis labrusca* L.	KG
Wrinkled giant hyssop	*Agastache rugosa*	WGH
Japanese angelica tree	*Aralia elata* (Miq.) Seem	JAT
Sweet wormwood	*Artemisia annua* L.	SW
Chinese cedar	*Cedrela sinensis* Juss	CC
Cudrang	*Cudrania tricuspidata* (Carr.) Bureau ex Lavallee	CR
Tu-chung	*Eucommia ulmoides* Oliver	TC
Red perilla	*Perilla frutescens* var. *acuta Kudo*	RP
Tan shen	*Salvia miltiorrhiza* Bunge	TS
Hiyodori-jogo	*Solanum lyratum*	HJ
Crampbark	*Viburnum sargentii* Koehne	CB

Preparation of extracts from fruit and medicinal herbs

Three types of extracts (whole juice, acetone-PCA, and ethanol) were prepared in this study. The fresh fruits and medicinal herbs (100 g of each fruit) were cleaned with deionized water, separated, and extracted with a juice extractor (HUROM HH-SBF11, HUROM, Gimhae, Korea). Then the mixture was centrifuged at 3,000 *g* for 15 min, and the supernatant was collected. The supernatant was defined as the whole juice fraction, and the remaining residue (i.e., wet pulp) was precipitated with 5% PCA (1:1 w/v) solution in a shaking incubator (200 rpm) for 10 min and extracted with 100% acetone (1:7 w/v) in a shaking incubator (200 rpm) for 30 min. Then the mixture was centrifuged at 3,000 *g* for 15 min, and the supernatant was collected. For the cellular assays, 5 g of each fresh fruit and medicinal herb were extracted with 100 mL of ethanol in a shaking incubator (200 rpm) for 72 h at room temperature and filtered through Whatman No. 1 filter paper (Tokyo, Japan). Solvents were then removed by evaporation *in vacuo*, and the dried extracts were obtained. Each dried extract was re-dissolved in DMSO to a concentration of 50 mg/mL, and then further diluted with DMSO as needed.

Determination of total phenolic content

The total phenolic content of each sample was determined according to the method of Gutfinger (19). Each sample (1.0 mL) was mixed with 1.0 mL of 10% Na_2CO_3 and allowed to stand for 3 min. Then, 1.0 mL of 50% Folin-Ciocalteu reagent was added to each mixture. After incubation at room temperature, the resulting mixtures were centrifuged at 13,400 *g* for 5 min. Absorbances were measured with a spectrophotometer (Shimadzu UV-1601, Shimadzu Corp., Tokyo, Japan) at 750 nm, and the total phenolic contents were expressed as gallic acid equivalents.

Oxygen radical absorbance capacity (ORAC) assay

The ORAC assay was carried out on a Tecan GENios fluorescence plate reader (Tecan Trading AG, Männedorf, Switzerland) with fluorescent filters (excitation wavelength 485 nm, emission wavelength 535 nm) according to Kim's method (20). In the final assay mixture, fluorescein (40 nM) was used as a target of free radical attack and 2,2'-azobis(2-methylpropionamidine) dihydrochloride (AAPH) (20 mM) was used as a peroxyl radical generator. Trolox (1 μM, prepared fresh daily) was used as a control standard. The plate reader was programmed to record the fluorescence of the final assay mixture every 2 min after AAPH was added. All fluorescence measurements were expressed relative to the initial reading. The final results were calculated based on the difference in the area under the fluorescence decay curve between the blank and each sample. $ORAC_{ROO\cdot}$ values were expressed as 1 μM of Trolox equivalents (TE). One ORAC unit is equivalent to the net protection provided by 1 μM Trolox.

MTT assay

The MTT assay was used to examine the effects of the fruit and medicinal herb extracts on human keratinocytes viability. Human keratinocytes were cultured in a 96-well plate (1×10^4 cells/well) for 24 h at 37°C with 5% CO_2. Next, the cells were treated with the fruit and medicinal herb extracts (200 μg/mL) for 24 h and then incubated with 100 μL of MTT reagent (5 mg/mL) for 1 h. Then, the reaction medium was removed and the insoluble formazan remaining in the keratinocytes was dissolved in 100 μL of DMSO at room temperature for 15 min. The absorbance of each well was measured at 540 nm using an ELISA microplate reader (BioTek Instruments, Inc., Winooski, VT, USA). The viability of the fruit and medicinal herb extract-treated cells was expressed as a percentage of the viability of untreated cells.

Intracellular ROS levels

2',7'-dichlorofluorescin diacetate (DCFH-DA), a well-established fluorescent probe, was used to evaluate the inhibition activity of fruit and medicinal herb extracts against increased H_2O_2 levels in human keratinocytes. DCFH-DA is transported across the cell membrane into the cell, where it is enzymatically deacetylated by intracellular esterases to a non-fluorescent form, dichlorofluorescin (DCFH). DCFH is further oxidized by ROS to form 2',7'-dichlorofluorescin (DCF), which is highly fluorescent. To quantify the effect of the ethanol extracts of fruits and medicinal herbs on intracellular ROS, human keratinocytes were seeded in a 96-well plate for 24 h and then incubated for 1 h with a 100 μg/mL dose of ethanol extracts prepared from fruits and medicinal herbs. Then, the medium was removed and the cells were gently washed twice with PBS. Next, the keratinocytes were treated with 600 μM H_2O_2 for 1 h, DCFH-DA was added to the culture plates, and the cells were incubated in the dark for 30 min at 37°C. A Tecan GENios fluorescence plate reader (Tecan Trading AG) was used to measure the fluorescent intensity of DCF Fluorescence was measured at an emission wavelength of 535 nm from an excitation wavelength of 485 nm.

Cell culture and adipocyte differentiation

3T3-L1 cells were purchased from the Korean Cell Line Bank (Seoul, Korea) and maintained at 37°C in a humidified, 5% CO_2 atmosphere for all procedures. The 3T3-L1 preadipocytes were seeded in a 12-well plate and cultured for 3~4 days, until confluency. Two-day post-confluent 3T3-L1 preadipocytes were maintained in DMEM supplemented with 10% FCS and 100 units/mL penicillin-streptomycin, and the medium was replaced

every 2 days. Two days after reaching confluency (Day 0~2), the preadipocytes were treated with DMEM supplemented with 10% FBS and 100 units/mL penicillin-streptomycin (FBS-medium) and containing 500 μM IBMX, 5.2 μM dexamethasone, and 167 nM insulin (differentiation medium; DM). The 3T3-L1 cells were treated with 200 μg/mL doses of the fruit and medicinal herb extracts until 2 days post-confluence. After Day 2, the medium was changed to FBS-medium with 167 nM insulin for additional 2 days (post-differentiation medium; Post-DM). Thereafter, the 3T3-L1 adipocytes were cultured in FBS-medium. The cells were harvested at Day 7 to stain for matured adipocytes (Fig. 1).

Oil red O staining

To examine the anti-adipogenic effects of fruit and medicinal herb extracts on 3T3-L1 adipogenesis, intracellular lipid accumulation in the harvested adipocyteswas measured by ORO staining. After washing with PBS, the adipocytes were fixed with 3.7% formaldehyde at room temperature for 30 min. The fixed adipocytes were then washed with tap water three times. The lipid droplets were stained with 3 mg/mL ORO in isopropanol, and the plates were gently shaken for 15 min. The stained cells were washed with tap water three times. The ORO stained lipid droplets were dissolved in DMSO and transferred to a 96-well plate (100 μL/well). The absorbance of each well at 510 nm was quantified using a microplate reader (Molecular Devices, LLC, Sunnyvale, CA, USA).

Statistical analysis

All data are presented as mean±SD. Statistical analyses were performed using SPSS software (SPSS Inc., Chicago, IL, USA). The significance of between group differences was assessed with a one-way analysis of variance (ANOVA) followed by Duncan's multiple range tests. The significance of differences between each group and the control group were determined by Student's t-test. *P*-values less than 0.05 were considered statistically significant.

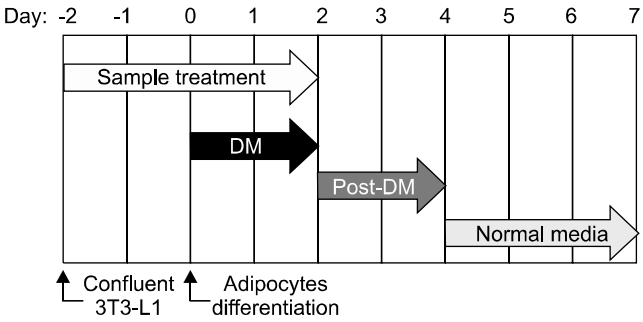

Fig. 1. Timeline of 3T3-L1 differentiation and ethanol extract treatment. Differentiation medium (DM) was composed with DMEM supplemented with 10% FBS and 100 units/mL penicillin-streptomycin (FBS-medium) and containing 500 μM IBMX, 5.2 μM dexamethasone, and 167 nM insulin. Post-diffenriation medium (Post-DM) was contained with FBS-medium and 167 nM insulin.

RESULTS

Extraction efficiency and total phenolic content

We analyzed the extraction efficiency of whole juices obtained from 10 types of fruit and 10 types of medicinal herbs (Table 2). The extraction efficiency of the fruits was higher than the extraction efficiency of the medicinal herbs. Among the fruits, the extraction efficiencies of pear (Hadong) (PH), green grape (GG), and kyoho grapes (KG) were >80%.

The total phenolic content of the ethanol extracts of 10 types of fruit and 10 types of medicinal herbs was also examined (Table 3). Overall, the total phenolic content of the medicinal herbs was higher than the total phenolic content of the fruits. The total phenolic content of Chinese cedar (CC) was the highest (1,482.4 mg/100 g)

Table 2. The extraction efficiency of whole juices prepared from fruits and medicinal herbs (unit: %)

Test sample[1)]	Extraction efficiency	Test sample[1)]	Extraction efficiency
MWF	46.06	WGH	27.16
NWP	52.63	JAT	35.41
TP	45.30	SW	1.82
JA	54.07	CC	5.32
TA	69.97	CR	21.89
PH	86.09	TC	22.75
PJ	79.36	RP	37.78
CT	66.41	TS	18.77
GG	85.63	HJ	56.40
KG	83.46	CB	8.05

[1)]Test samples listed are the same as those described in Table 1.

Table 3. The total phenolic content of ethanol extracts prepared from fruits and medicinal herbs (unit: mg/100 g)

Test sample[1)]	Total phenolic content	Test sample[1)]	Total phenolic content
MWF	20.8±0.6^{b}	WGH	742.9±2.5^{l}
NWP	14.8±0.3^{ab}	JAT	1,187.9±9.9^{p}
TP	12.4±0.5^{a}	SW	318.2±1.2^{j}
JA	67.3±1.0^{d}	CC	1,482.4±11.9^{q}
TA	77.9±2.1^{e}	CR	439.3±5.8^{k}
PH	17.4±0.3^{ab}	TC	1,047.2±2.2^{o}
PJ	28.3±0.7^{c}	RP	751.5±4.2^{m}
CT	81.5±0.3^{e}	TS	225.3±0.3^{g}
GG	253.0±1.5^{h}	HJ	264.3±1.9^{i}
KG	108.1±0.9^{f}	CB	857.6±0.3^{n}

[1)]Test samples listed are the same as those described in Table 1.
The total phenolic content of fruit and medicinal herb extracts was determinedby the Folin-Ciocalteu method.
Values followed by different superscript letters (a-q) are significantly different from one another according to ANOVA with Duncan's multiple range tests (P<0.05).

among all samples tested, followed by Japanese angelica tree (JAT) (1,187.9 mg/100 g), tu-chung (TC) (1,047.2 mg/100 g), crampbark (CB) (857.6 mg/100 g), red perilla (RP) (751.5 mg/100 g), and wrinkled giant hyssop (WGH) (742.9 mg/100 g). The total phenolic content of GG was the highest (253.0 mg/100 g) of all fruits tested.

Peroxyl radical scavenging activity

To examine the antioxidant activity of whole juice and residue extracts prepared from each of the fruits and medicinal herbs. Fig. 2A demonstrates the scavenging activity of whole fruit and medicinal herb juices on peroxyl radicals generated by AAPH. Among the extracts tested, the scavenging activity of RP against AAPH-generated peroxyl radicals was the highest (4,930.2 TE), followed by JAT (4,258.2 TE), TC (3,192.2 TE), WGH (2,584.6 TE), CB (2,141.3 TE), hiyodori-jogo (HJ) (2,121.0 TE), GG (1,901.3 TE), cudrang (CR) (1,355.5 TE), and tan shen (TS) (906.7 TE). In general, the scavenging activity of the fruit extracts against peroxyl radicals was lower than the scavenging activity of the medicinal herb extracts against peroxyl radicals. With the exception of GG (1,901.3 TE), the peroxyl radical scavenging activities of the fruit extracts were below 1,000 TE.

Similarly, the anti-peroxyl radical scavenging activity of the fruit residue extracts was lower than the anti-peroxyl radical scavenging activity of the medicinal herb residue extracts. Fig. 2B shows the peroxyl radical scavenging activities of residue extracts prepared from fruits and medicinal herbs. CB had the highest $ORAC_{ROO\cdot}$ value (5,223.6 TE), followed by WGH (4,948.5 TE), CR (3,766.0 TE), CC (3,661.8 TE), JAT (3,594.9 TE), TC (3,026.9 TE), and sweet wormwood (SW) (1,494.8 TE). Among the fruit residue extracts, GG had the highest $ORAC_{ROO\cdot}$ value (361.3 TE).

Evaluation of cytotoxic effects in human keratinocytes and 3T3-L1 cells

The MTT assay was used to test whether fruit and medicinal herb extracts could affect cell viability. Treatment of human keratinocytes with a 100 μg/mL dose of fruit and medicinal herb extracts for 24 h had only a marginal effect on keratinocyte viability. However, cell viability was significantly reduced by 400 μg/mL doses of Taishu persimmon (TP) (91.5%), JAT (83.3%), SW (72.4%), CC (78.6%), CR (86.7%), TC (81.4%), RP (75.9%), TS

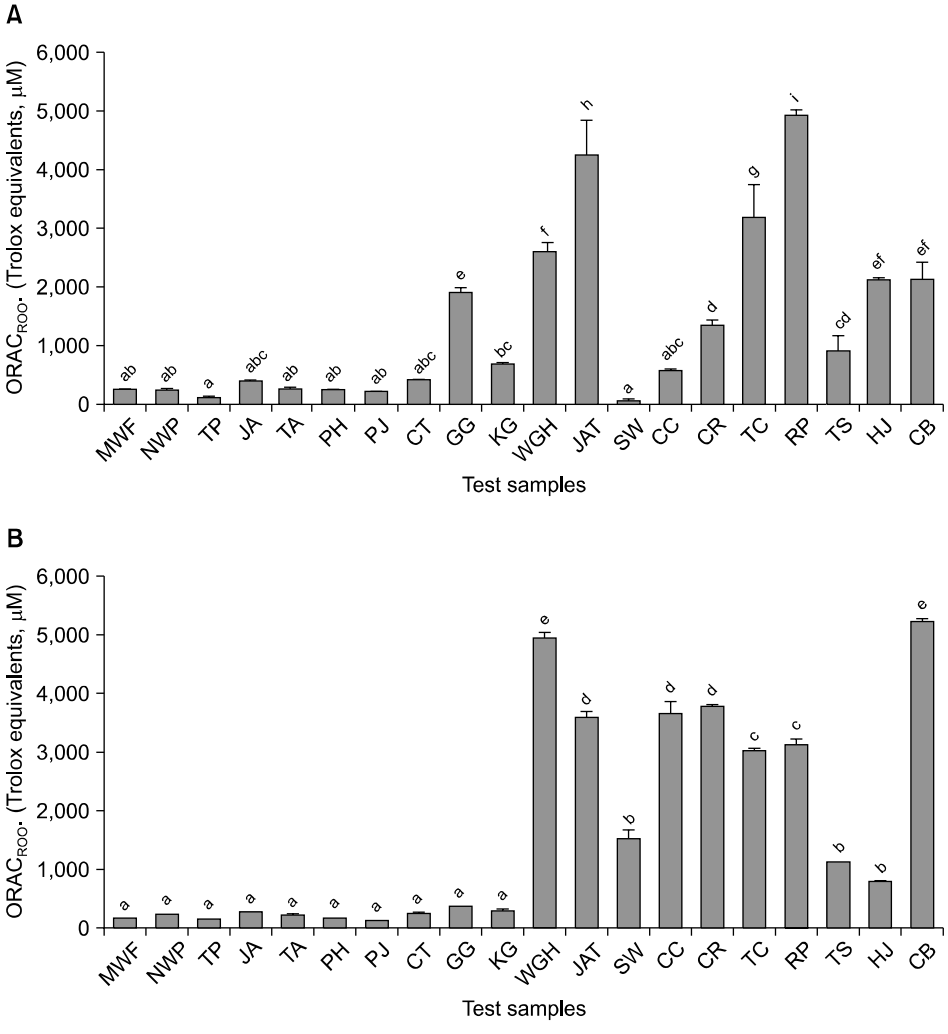

Fig. 2. Peroxyl radical scavenging activity of fruits and medicinal herbs. Whole juice (A), Acetone-PCA extracts from fruit and medicinal herb residues (i.e., wet pulp) (B). Test samples are the same as those described in Table 1. ORAC values were calculated by dividing the area under the sample curve by the area under the Trolox curve. Values with different letters (a–i) are significantly different from one another according to ANOVA with Duncan's multiple range tests (P<0.01).

(71.4%), HJ (84.2%), and CB (80.5%) extracts (Fig. 3). Treatment with up to 200 μg/mL doses of the fruit and medicinal herb extracts did not affect the viability of 3T3-L1 cells (data not shown). Thus, ethanol extract concentrations lower than 100 μg/mL were used for subsequent studies.

Inhibitory effects of fruit and medicinal herb extracts against H_2O_2-induced oxidative stress in human keratinocytes

We examined the inhibitory activity of 100 μg/mL doses of fruit and medicinal herb extracts against H_2O_2-induced oxidative stress in human keratinocytes. H_2O_2 treatment significantly increased the DCF levels of human keratinocytes. The 100 μg/mL doses of the fruit and medicinal herb extracts significantly decreased the DCF levels of H_2O_2-treated keratinocytes. The inhibitory activities of Nishimura Wase persimmon (NWP) (62.7%), TP (58.9%), WGH (60.7%), SW (60.4%), CC (57.6%), CR (54.5%), RP (67.5%), TS (60.3%), HJ (58.6%), and CB (58.2%) were greater than 50% (Fig. 4).

Inhibitory effects of fruit and medicinal herb extracts on 3T3-L1 adipogenesis

3T3-L1 cells were treated with 200 μg/mL doses of the ethanol extracts from Day-2 to Day 2 (Fig. 1). With few exceptions [i.e., Matsumoto Wase Fuyu (MWF), WGH, TS], treatment with the 200 μg/mL doses of fruit and medicinal herb extracts completely suppressed 3T3-L1 cell adipogenesis (as measured in mature adipocytes harvested at Day 7). The inhibition percentages of MWF, WGH, and TS were 37.9%, 79.1%, and 75.8%, respectively (Fig. 5).

In summary, the total phenolic content of the medicinal herbs tested was greater than that of the fruits tested. Consistent with this, the *in vitro* antioxidant activity of the medicinal herbs was stronger than the antioxidant activity of the fruits. The fruit and medicinal herb extracts had strong inhibitory activities in cell-based systems, including H_2O_2-induced oxidative stress in human keratinocytes and lipid accumulation in 3T3-L1 cells.

DISCUSSION

Recently, the aging process has been studied with fervor. Aging can be affected by various factors, including environmental, social, economic, physiological, and even spiritual factors. A number of efforts to develop a success-

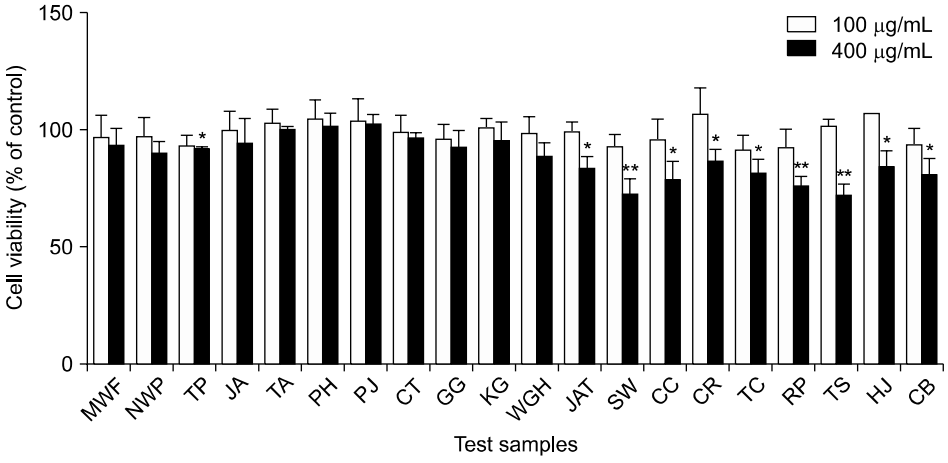

Fig. 3. Cell viability of human keratinocytes treated with ethanol extracts.Test samples are the same as those described in Table 1. Cytotoxicity was evaluated by MTT assay. Human keratinocytes were treated with 100 μg/mL and 400 μg/mL doses of fruit and medicinal herb extracts for 24 h. Control group (no treatment) vs. treatment group differences were determined by one-way ANOVA (P<0.05) followed by Student's t-tests (*P<0.05, **P<0.01).

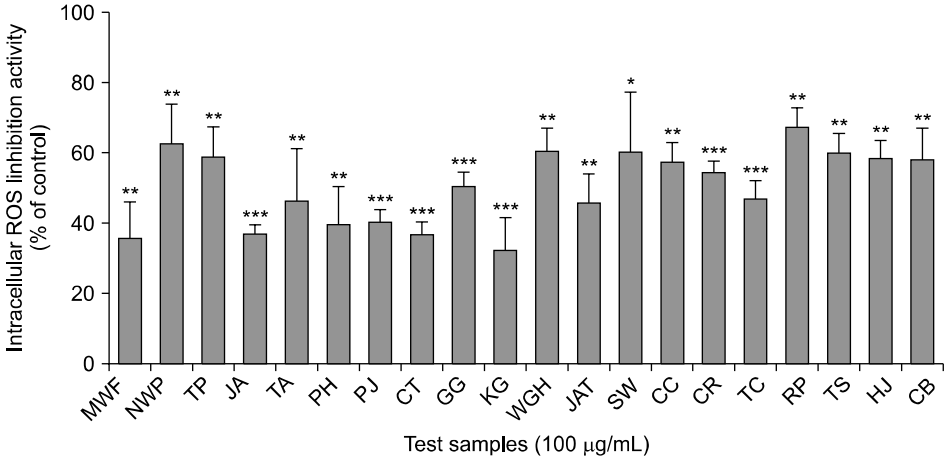

Fig. 4. Inhibitory activity of ethanol extracts against H_2O_2-induced oxidative stress in human keratinocytes. Test samples are the same as those described in Table 1. Human keratinocytes were exposed to H_2O_2 for 1 h and then treated with 100 μg/mL doses of fruit and medicinal herb extracts for 30 min. Control group (no treatment) vs. treatment group differences were determined by one-way ANOVA (P<0.05) followed by Student's t-tests (*P<0.05, **P<0.01, and ***P<0.001).

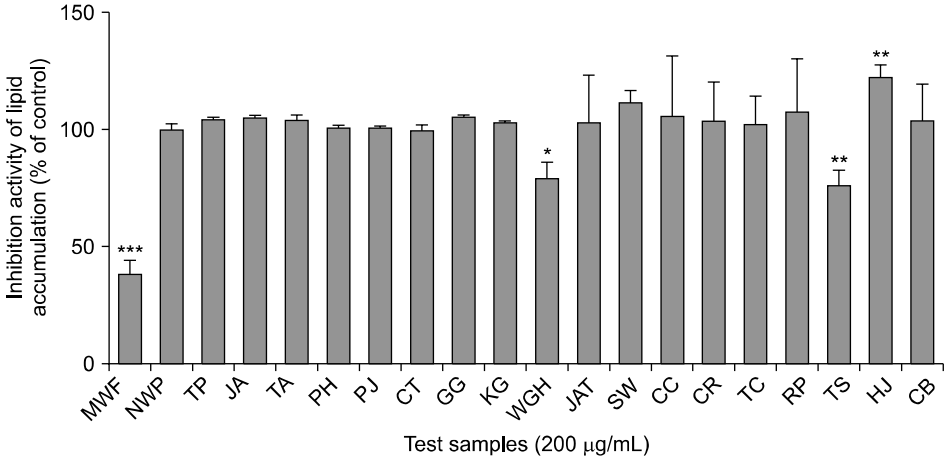

Fig. 5. Inhibitory activity of fruit and medicinal herb extracts on 3T3-L1 lipid accumulation. Test samples are the same as those described in Table 1. Inhibitory activities of 20 fruit and medicinal herb extracts on 3T3-L1 lipid accumulation as determined by ORO staining. Control group (no treatment) vs. treatment group differences were determined by one-way ANOVA ($P<0.05$) followed by Student's *t*-tests ($^{*}P<0.05$, $^{**}P<0.01$, and $^{***}P<0.001$).

ful model of skin aging have been made; however, these efforts have failed to take biological functions such as antioxidant systems and anti-adipogenic activities into account. Previous work indicates that there is a positive correlation between diminished antioxidant system capacity and stimulation of the aging process (21). Thus, we investigated whether fruit and medicinal herb extracts were able to prevent and/or postpone aging processes by scavenging radicals and inhibiting excessive lipid accumulation. Accordingly, we evaluated the antioxidant and anti-adipogenic properties of 20 types of fruits and medicinal herbs from the Gyeongnam area of Korea.

In the present study, we exhibited that the extraction efficiencies of the fruits tested were higher than the extraction efficiencies of the medicinal herbs tested, with the exception of HJ, which had an extraction efficiency of 56.4%. Previous studies suggest that the extraction efficiency of natural plant materials can be influenced by water content. While raw fruit commonly contains over 80% water by weight, medicinal herbs contain much smaller quantities of water (<30%) (22).

Conversely, we observed that the total phenolic content of ethanol extracts from medicinal herbs was greater than the total phenolic content of ethanol extracts from fruits (Table 3). The beneficial effects of plant-derived phenolics are well known. Yoon et al. (23) reported that phenolic-rich plant foods, commonly consumed in Korea, prevent and/or reduce the risk of chronic diseases because of their antioxidant, anti-inflammatory, anti-hypertensive, and anti-carcinogenic activities. Previous reports indicate that the total phenolic content of plant extracts can be affected by several factors, including water content, chemical diversity, and extraction method. They suggest that organic solvents, including ethanol, methanol, and chloroform, are more efficient for polyphenol extraction than aqueous-based methods because organic solvents degrade cell walls and seeds, which have non-polar characteristics, more effectively; this increases the release of polyphenols from plant cells (24). Likewise, the medicinal herb residue extracts obtained with acetone-PCA had higher antioxidant activities than the medicinal herb whole juices, indicating that bioactive constituents were left behind after aqueous extraction from natural plants. This was particularly true for the medicinal herbs. Further study is required to elucidate the relationship between extraction conditions and the total phenolic content of fruits and medicinal herbs.

Recent studies have focused on the intrinsic and extrinsic factors involved in skin aging. The function of the epidermis is to protect against external forces while maintaining the flexibility required to ensure tissue renewal and the ability to respond to different stimuli (25). The epidermis can be damaged by oxidative stress from radical generation, chemical hazards, and solar UV radiation (26). The epidermis is composed of several cell types, such as keratinocytes, melanocytes, Langerhans cells, and Merkel cells. Among them, keratinocytes constitute 90% of epidermal cells (27). Therefore, an important strategy in skin anti-aging is the protection of keratinocytes against oxidative stress. Several researchers have reported that the polyphenols found in natural plants can inhibit the negative cellular effects of solar UV radiation in normal human epidermal keratinocytes (28). In addition, the phenolic fractions of *Lonicera caerulea* and *Vaccinium myrtillus* fruits suppress UVB-induced keratinocyte injury, DNA breakage, and ROS generation (29). DCFH-DA is a well-known, specific probe that generates green fluorescence when reacting with intracellular ROS (30). Our results demonstrate that fruits and medicinal herbs effectively ameliorate H_2O_2-induced oxidative stress in human keratinocytes. We also observed that the antioxidant activities of fruits were stronger in cell-based assays than in *in vitro* chemical assays, such as ORAC. We used the ORAC assay, a widely accepted method for assessing the antioxidant activity of nutraceutical and pharmaceutical foods (31,32). This result may be explained by the fact that cell-based systems are affected by number of other factors, including cell membrane permeability and interactions between bioactive compo-

nents and molecular signaling pathways. Further studies are needed to determine the polyphenol profiles of fruits and medicinal herbs from the Gyeongnam area of Korea. The effect of growing district on the polyphenol composition and biological functions of plants such as fruits and medicinal herbs should also be investigated.

The relationship between moderate adipose tissue and maintaining skin health is well-known with regard to the skin barrier, sebaceous glands and sebum production, sweat glands, lymphatics, collagen structure and function, wound healing, microcirculation, and macrocirculation from previous reports (13). An increase in subcutaneous adipose tissue alters dermal structure and induces wrinkle formation (33). Ezure et al. (34) showed that excessive accumulation of subcutaneous adipose tissue impairs dermal function by decreasing dermal thickness and elasticity in obese mice. Additionally, the skin of obese mice was mechanically weaker and had a lower collagen concentration than the skin of lean mice (35). Akase et al. (23) demonstrated that the density and convolutions of collagen fibers in the dermis were reduced by UV irradiation in Tsumura-Suzuki obese diabetic (TSOD) mice. Although our 3T3-L1 cells do not fully reflect the environmental conditions of subcutaneous adipose tissue, it is clear that excessive fat accumulation can stimulate skin aging and wrinkle formation. Thus, we evaluated the anti-adipogenic activities of fruit and medicinal herb extracts in 3T3-L1 cells, which are considered an adipose tissue mimic. Intracellular lipid accumulation was quantified by ORO staining at Day 7. Treatment with the fruit and medicinal herb extracts from Day −2 to Day 2 markedly decreased 3T3-L1 adipogenesis. The results of the present study indicate that NWP, TP, WGH, SW, CC, RP, TS, HJ, and CB have as potential natural anti-aging materials with effective antioxidant and anti-adipogenic activities.

We evaluated the *in vitro* antioxidant effects, the cellular antioxidant activities, and the anti-adipogenic effects of ethanol extracts prepared from 20 fruits and medicinal herbs cultivated in the Gyeongnam area of Korea. We also examined the potential of these fruits and medicinal herbs as natural anti-aging materials. The molecular mechanism(s) underlying the antioxidant and anti-adipogenic activities of fruit and medicinal herb extracts are not fully understood. Further research should be performed to examine the effects of plant product treatment on direct biological aging markers. Our findings may provide scientific evidence for the development of functional foods and nutraceuticals.

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AUTHOR DISCLOSURE STATEMENT

The authors declare no conflict of interest.

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