DOI:10.1021/jf102650d

JOURNAL OF AGRICULTURAL AND FOOD CHEMISTRY
ARTICLE

Extract of Passion Fruit (*Passiflora edulis*) Seed Containing High Amounts of Piceatannol Inhibits Melanogenesis and Promotes Collagen Synthesis

Yuko Matsui,*,† Kenkichi Sugiyama,† Masanori Kamei,† Toshio Takahashi,† Tamio Suzuki,‡ Yohtaro Katagata,§ and Tatsuhiko Ito||

†Research Institute, Morinaga and Company, Limited, 2-1-1 Shimosueyoshi, Tsurumi-ku, Yokohama 230-8504, Japan, ‡Department of Dermatology, Yamagata University School of Medicine, Yamagata University, 2-2-2 Iidanishi, Yamagata 990-9585, Japan, §Faculty of Agriculture and Life Science, Hirosaki University, 3 Bunkyo-cho, Hirosaki 036-8561, Japan, and ||Health Care Division, Morinaga and Company, Limited, 2-1-1 Shimosueyoshi, Tsurumi-ku, Yokohama 230-8504, Japan

The effect of passion fruit, the fruit of *Passiflora edulis*, on melanin inhibition and collagen synthesis was studied using cultured human melanoma and fibroblast cells. Passion fruit was divided into three parts, rind (PF-R), pulp (PF-P), and seed (PF-S), and each part was extracted using 80% ethanol. The concentration of polyphenols was higher in PF-S than in PF-R or PF-P. Treatment of melanoma cells with PF-S led to inhibition of melanogenesis. In addition, the production of total soluble collagen was elevated in dermal fibroblast cells cultured in the presence of PF-S. PF-R and PF-P did not yield these effects. Furthermore, the removal of polyphenols from PF-S led to the abolishment of the effects described above. We discovered that piceatannol (3,4,3′,5′-tetrahydroxy-*trans*-stilbene) is present in passion fruit seeds in large amounts and that this compound is the major component responsible for the PF-S effects observed on melanogenesis and collagen synthesis.

KEYWORDS: *Passiflora edulis*; piceatannol; passion fruit seed; melanin; collagen

INTRODUCTION

Tropical fruits and vegetables are beneficial for human health. Passifloraceae is a well-known tropical plant from the South American tropical forests, and its leaves, vines, and flowers are used as medicinal herbs. There are many reports on the actions of Passifloraceae herbs, which include its anti-anxiety effect in humans (*1*) and its anti-inflammatory and cough-suppressant effects in mice (*2*, *3*). However, studies on the fruit of *Passiflora edulis* from the Passifloraceae family, which is known as passion fruit, are limited.

Passion fruit is usually eaten in its natural state, with the seeds, or processed as tropical juice. Passion fruit contains many phytochemicals, such as polyphenolic compounds (*4*) and carotenoid families (*5*), and vitamin C (*6*), which are known as being beneficial for the skin. The health effects of the polyphenols contained in many natural plants, including tropical fruits, have been increasingly and energetically studied. The content of polyphenols in various tropical fruits (*7*), as well as the bioactive effects of tropical fruits, such as acai and mangosteen (*8*, *9*), have been reported.

Skin abnormalities, such as aging, are caused by genetic and environmental factors. In addition, the damage to skin by UV exposure, mental and environmental stress, eating habits, etc., leads to skin pigmentation, wrinkles, and even skin cancer. Melanin is the pigment that colors the skin. Although melanin is important for protection from UV rays, production to an excessive degree leads to skin erythema, freckles, and other skin disorders. Many bioactive substances from naturally occurring plants are studied for the prevention of melanogenesis. In those reports, the effect of polyphenols from safflower seed, grape seed (*10*, *11*), and many other edible plants are described. Collagen, which is another molecule that is essential for skin health, plays many important roles in the body, including cell−cell adhesion, cell proliferation, and cell differentiation. The functional properties of the skin considerably depend upon the quantity and condition of the collagen present in the dermis. Some foods and food components contribute to the maintenance of the collagen condition in the skin or inhibit collagen-degrading factors, e.g., royal jelly (*12*) and polyphenols, such as catechin and flavonoids (*13*, *14*). To prevent skin damage and maintain its protective potency against environmental agents, there is an increased research focus on compounds that target the skin. As described above, polyphenols play important roles in dermal cells; thus, we predicted the effectiveness of passion fruit in the promotion and maintenance of skin health.

The aim of this paper was to investigate the effect of passion fruit on melanogenesis and collagen synthesis in dermal cells and identify the component responsible for these effects.

MATERIALS AND METHODS

Materials. Piceatannol (3,4,3′,5′-tetrahydroxy-*trans*-stilbene), resveratrol (3,5,4′-trihydroxy-*trans*-stilbene), and polyvinylpolypyrrolidone

*To whom correspondence should be addressed: Research Institute, Morinaga and Co., Ltd., 2-1-1 Shimosueyoshi, Tsurumi-ku, Yokohama 230-8504, Japan. Telephone: +81-45-571-2504. Fax: +81-45-571-6109. E-mail: y-matsui-jd@morinaga.co.jp.

 Published on Web 09/07/2010

(PVPP) were purchased from Sigma-Aldrich Japan (Tokyo, Japan). Culture reagents, such as Dulbecco's modified Eagle's medium (DMEM), Medium 106S, phosphate-buffered saline (PBS), low-serum growth supplement (LSGS), glutamine, penicillin, streptomycin, gentamicin, amphotericin B, and trypsin−ethylenediaminetetraacetic acid (EDTA), were purchased from Invitrogen (Carlsbad, CA), and fetal bovine serum (FBS) was purchased from Biowest (Maine-et-Loire, France). Synthetic melanin was purchased from Nacalai Tesque (Kyoto, Japan), and the Sircol soluble collagen assay kit was purchased from Biocolor Life Science Assays (Newtownabbey, U.K.). Other reagents were purchased from Wako Pure Chemicals (Tokyo, Japan).

Sample Preparation. Commercial whole passion fruit (product of Kagoshima, Japan) was divided into three parts: rind, pulp, and seed. Each part was freeze-dried, milled, and extracted twice using 10-fold amounts of 80% (v/v) ethanol, by shaking at room temperature. The extract solution was centrifuged at 3000*g* for 10 min to obtain the supernatant, which was filtered through a paper filter to remove the sediment. Aliquots containing the extracts were evaporated, freeze-dried, and dissolved in distilled water at the concentration of 100 mg/mL (for rind and pulp) or 20 mg/mL (for seeds). The extract solutions were filtered using a hydrophilic membrane filter (Kanto Kagaku, Japan), with a pore size of 0.45 μm. The rind, pulp, and seed of passion fruit extracts were termed PF-R, PF-P, and PF-S, respectively.

Polyphenol Quantification. The polyphenol concentration was measured according to a modified Folin−Ciocalteu method, as described previously (*15*), using (−)-epicatechin to obtain the standard curve for polyphenol. To examine whether polyphenols contribute to the inhibition of melanogenesis and collagen synthesis, polyphenols were removed from PF-S via absorption with PVPP because the amide bonds of PVPP form hydrogen bonds with the hydroxyl groups of polyphenols. PVPP was swollen in distilled water, added to 20 mg/mL PF-S, and stirred for 30 min. The solution was centrifuged at 12000*g* for 15 min to obtain PVPP-treated PF-S. The polyphenol concentration was measured again using the Folin−Ciocalteu method, to confirm that the polyphenols were removed. The experiment was carried out in triplicate.

Fractionation of PF-S. High-performance liquid chromatography (HPLC) analysis was carried out using a Shimadzu system controller SCL-10A, a UV detector RF-10A, and a fraction collector FRC-10A (Shimadzu, Kyoto, Japan). Samples were fractionated via reverse-phase HPLC using an octa decyl silyl (ODS) column (Mightysil RP-18 GP250-10, 250 × 10 mm inner diameter, 5 μm, Kanto Kagaku, Tokyo, Japan). A total of 4 mL of 20 mg/mL PF-S was injected into the HPLC apparatus using a linear gradient of 0−30% at 0−10 min from solvent B to solvent A, where solvent A consisted of a water/acetonitrile/acetic acid (400:10:1, v/v/v) mix and solvent B consisted of a solvent A/methanol (2:1, v/v) mix. The temperature of the column was maintained at 40 °C. Measurements were carried out at a flow rate of 3 mL/min, and the wavelength used for UV detection was 280 nm. The absorbance of each fraction was measured with a spectrophotometer (V-630, JASCO Corporation, Tokyo, Japan). The HPLC chromatogram was divided into three fractions. Fraction 1 (tubes 1−17; 0−30 min), fraction 2 (tubes 18−23; 31−40 min), and fraction 3 (tubes 23−39; 41−70 min). The solvents used for HPLC were eliminated from each fraction by evaporation and freeze-drying, and the remaining extracts were dissolved in distilled water to maintain a concentration of 100 μg/mL. Each fraction and 100 μg/mL of PF-S served as samples for the measurement of melanin and soluble collagen, as described below.

Qualitative Analysis and Determination of Piceatannol. Ground passion fruit seeds were extracted with 70% (v/v) acetone 3 times, with shaking at room temperature. Samples were evaporated and freeze-dried to obtain crude extracts. A total of 100 mg of crude extract was suspended in 50 mL of 50% methanol solution and centrifuged at 1500*g* for 5 min. This supernatant was separated via reverse-phase liquid chromatography using a linear-gradient mode, as follows. Chromatographic measurements were carried out using an Agilent 1100 series liquid chromatography/mass spectrometry (LC/MS) system (Agilent Technologies, Tokyo, Japan) that included a photodiode array (PDA) detector. The HPLC column used in this study was Inertsil ODS-3 (150 × 2.1 mm inner diameter, 5 μm, GL Science, Tokyo, Japan). The mobile phase consisted of (A) water and (B) acetonitrile (v/v) using an initial gradient elution of 10% B and a gradient of 10−45% B at 0−25 min. The column temperature was maintained at 45 °C. All measurements were carried out at a flow rate of 0.25 mL/min using a detector wavelength of 280 nm. The mass spectrometric data were collected in full-scan mode, from m/z 200 to 1000.

Cells and Cell Culture. Melanin-producing MNT-1 human melanoma cells (a gift from Dr. V. J. Hearing, Laboratory of Cell Biology, National Cancer Institute, National Institutes of Health, Bethesda, MD) were cultured in DMEM containing 10% FBS, 4 mM glutamine, 100 units/mL penicillin, and 100 μg/mL streptomycin.

SF-TY human dermal fibroblast cells (Health Science Research Resources Bank, Tokyo, Japan) were cultured in Medium 106S supplemented with 5% LSGS, 10 μg/mL gentamicin, and 0.25 μg/mL amphotericin B. Melanoma and fibroblast cells were incubated at 37 °C under 5% CO_2 and 95% air.

Measurement of Melanin Content. MNT-1 cells were seeded at a density of 7.0×10^4 cells/well in 12-well culture plates and were cultured for 24 h. Subsequently, the medium was replaced with fresh DMEM containing various concentrations of PF-R, PF-P, PF-S, and kojic acid [5-hydroxy-2-(hydroxymethyl)-4-pyrone], which was used as a positive control. After 72 h of culture, cells were washed with PBS and trypsinized with 0.25% trypsin containing 0.02% EDTA. The number of cells harvested was counted. To measure the melanin produced in these cells, harvested cells were washed twice with PBS and centrifuged at 300*g* for 5 min at 4 °C, to obtain cell pellets. The cell pellets were dissolved in 500 μL of 1 N NaOH and quantified for melanin content using spectrophotometry at a wavelength of 415 nm. The concentration of the melanin produced was calculated from the standard curves using synthetic melanin dissolved in 1 N NaOH. The melanin produced was expressed as a concentration ratio relative to control MNT-1 cells that were not treated with any of the extracts or kojic acid.

Measurement of Soluble Collagen Content. SF-TY cells were seeded at a density of 1.7×10^4 cells/well in 48-well culture plates and were cultured for 24 h. Subsequently, the medium was replaced with fresh medium containing various concentrations of PF-R, PF-P, PF-S, and ascorbate, which was used as a positive control. After 72 h of culture, the medium was collected and assayed for soluble collagen content using the Sircol soluble collagen assay kit, according to the instructions of the manufacturer. Briefly, supernatants of SF-TY cells were centrifuged at 12000*g* for 4 min, and 100 μL of each supernatant was mixed with 1 mL of Sircol dye and shaken for 30 min. The aliquot was then centrifuged at 12000*g* for 10 min to pellet the collagen−dye complex. After the suspension was decanted, droplets were dissolved in 0.75 mL of Sircol alkali reagent. The concentration of collagen was measured using spectrophotometry at a wavelength of 540 nm, using soluble collagen for the standard curve. The value of the blank (i.e., the medium alone) was subtracted from each sample, to remove the contribution of the collagen contained in FBS. Total soluble collagen was expressed as a concentration ratio relative to control SF-TY cells that were not treated with any of the extracts or ascorbate. Adherent cells were washed twice with PBS and harvested using trypsin, for cell counting.

Statistical Analyses. Data represent the mean ± standard deviation (SD) for the indicated number of experiments. Statistical significance of the difference between the corresponding control was carried out using the paired t test, where ∗, ∗∗, and ∗∗∗ represented $p < 0.1$, $p < 0.05$, and $p < 0.01$, respectively.

RESULTS

Total Polyphenol Content. Total polyphenol content in freeze-dried rind, pulp, and seed was measured using the Folin−Ciocalteu method. The results showed that PF-S contained a much larger amount of polyphenols compared to PF-R and PF-P, because polyphenols represented 33% of the freeze-dried seeds. The rind and pulp contained only 4 and 0.2% polyphenols, respectively. The part ratio occupying raw passion fruit was as follows: rind, 40%; pulp, 48%; and seed, 12%, and the freeze-drying process reduced the weight of each part by 14, 18, and 45%, respectively. This means that the calculated polyphenol content in the raw fruit was 0.22, 0.02, and 1.8%, respectively. Although the seed represents only 12% of the whole fruit, in weight, 88% of the total polyphenol content was found in the seed.

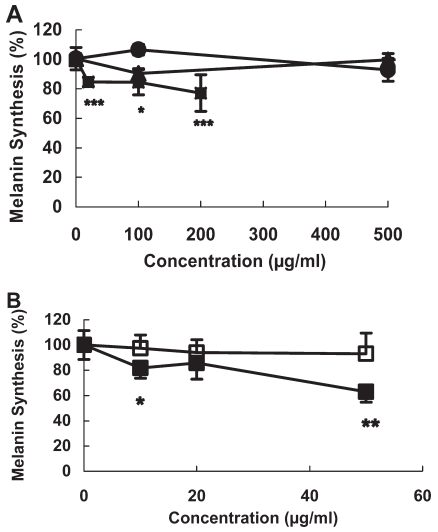

Figure 1. Comparison of melanin synthesis associated with (A) 80% ethanol extracts of passion fruit rind (PF-R; ●), pulp (PF-P; ▲), and seed (PF-S; ■). (B) PF-S (■) and PVPP-treated PF-S (□). Data are expressed as means ± SD (n = 4). Statistical analyses were performed using the paired t test: ∗, $p < 0.1$; ∗∗, $p < 0.05$; ∗∗∗, $p < 0.01$.

Inhibition of Melanogenesis by PF-R, PF-P, and PF-S in Melanoma Cells. Melanin synthesis was measured in melanin-producing human melanoma cells. Cells were cultured in the presence or absence of passion fruit extracts, and the melanin produced was compared to that of control cells, which were cultured in the absence of extracts. None of the samples inhibit cell growth at the concentrations examined (data not shown). As shown in **Figure 1A**, a significant decrease in melanin synthesis was observed when PF-S was applied to the melanoma cell culture at 20 μg/mL. In contrast, PF-R and PF-P did not inhibit melanin synthesis, although the concentration of these extracts was higher than that of PF-S. Kojic acid, which was applied for positive control, showed statistical significance ($p < 0.05$) at 10 μg/mL. To examine whether the polyphenols contained in PF-S are effective in the prevention of melanogenesis, polyphenols were removed from PF-S via PVPP treatment. More than 95% of the polyphenols were removed. PF-S inhibited melanin synthesis significantly, while PVPP-treated PF-S did not lead to inhibition of melanin synthesis (**Figure 1B**).

Soluble Collagen Production in Dermal Fibroblast Cells after PF-R, PF-P, and PF-S Treatment. Total soluble collagen was quantified in the culture medium of human dermal fibroblast cells (**Figure 2A**). Cells were cultured in the presence or absence of passion fruit extracts, and the collagen produced was compared to that of control cells, which were cultured in the absence of extracts. None of the samples inhibit cell growth at the concentrations examined (data not shown). Soluble collagen synthesis increased in a dose-dependent manner after the addition of PF-S to the cell culture. Ascorbate, which was applied for positive control, showed statistical significance ($p < 0.1$) at 25 μg/mL. However, similar to what was observed in melanoma cells, PF-R and PF-P did not increase collagen synthesis. Furthermore, PF-S led to a significant increase in the soluble collagen concentration in the medium (at 100 μg/mL) (**Figure 2B**), whereas the removal of polyphenols from PF-S via PVPP treatment did not lead to an increase of soluble collagen content in the culture medium.

Fractionation of PF-S and Measurement of Melanin and Soluble Collagen Synthesis. To determine the component of PF-S that was responsible for the inhibition of melanogenesis and soluble collagen synthesis, PF-S was fractionated using HPLC.

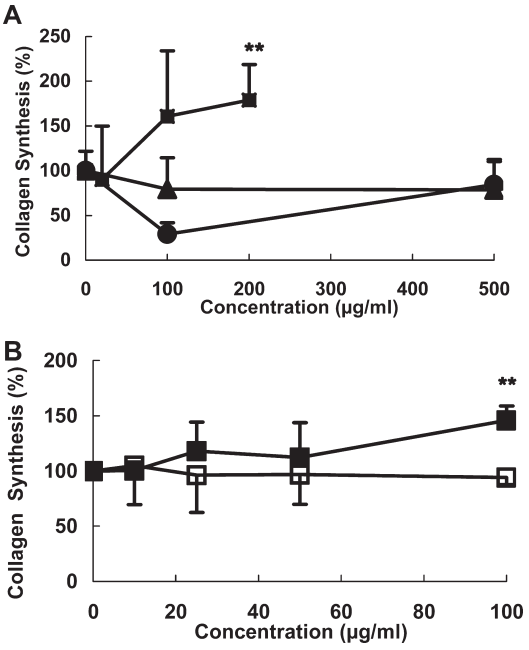

Figure 2. Soluble collagen synthesis associated with (A) 80% ethanol extracts of passion fruit rind (PF-R; ●), pulp (PF-P; ▲), and seed (PF-S; ■). (B) PF-S (■) and PVPP-treated PF-S (□). Data are expressed as means ± SD (n = 3). Statistical analyses were performed using the paired t test: ∗, $p < 0.1$; ∗∗, $p < 0.05$.

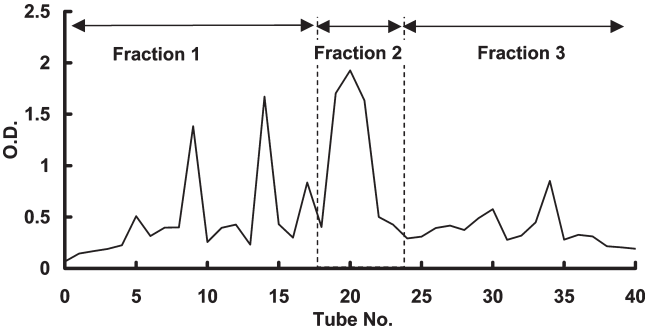

Figure 3. Absorbance of the fractioned 80% ethanol extracts of passion fruit seed (PF-S), obtained using reverse-phase chromatography with an ODS column. The three fractions were divided as fraction 1 (tubes 1−17; 0−18 min), fraction 2 (tubes 18−23; 19−22 min), and fraction 3 (tubes 23−39; 23−70 min).

Figure 3 shows the absorbance of each fraction of PF-S obtained by HPLC. One significant peak was observed at a retention time of ∼20 min. PF-S was divided into three fractions, fractions 1−3, where fraction 2 contained the significant peak and fractions 1 and 3 were fractions with a retention time that was earlier and later, respectively, compared to fraction 2. The three fractions, with a concentration equivalent to 100 μg/mL, were added to MNT-1 and SF-TY cells to examine inhibition of melanogenesis and soluble collagen synthesis, as described previously. As shown in **Figure 4A**, fraction 2 inhibited melanin synthesis to a similar extent, as did PF-S ($p = 0.103$). Soluble collagen synthesis was also elevated in fraction-2-treated cells, which demonstrates that the components responsible for these effects are contained in fraction 2 (**Figure 4B**). However, only PF-S showed a significant increase in collagen synthesis.

Determination of Piceatannol. The polyphenol contained in passion fruit seed was determined by chromatographic measurements. The specific peak 1, as described in **Figure 5**, corresponded

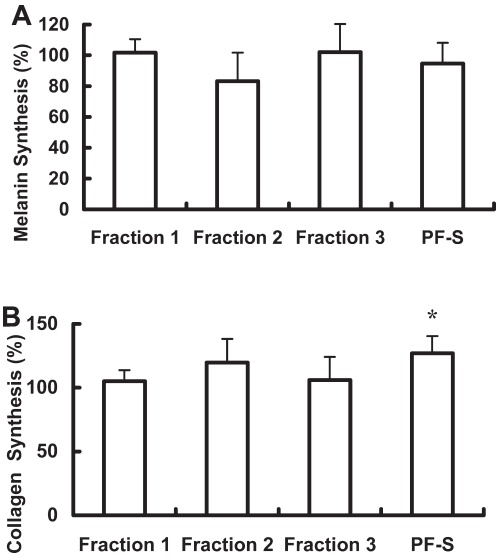

Figure 4. (A) Melanin synthesis associated with 100 μg/mL PF-S and 100 μg/mL PF-S fractionated using an ODS column. (B) Collagen synthesis associated with 100 μg/mL PF-S and 100 μg/mL PF-S fractionated using an ODS column. Data are expressed as means $\pm$ SD (n = 3). Statistical analyses were performed using the paired t test: $*$, $p < 0.1$.

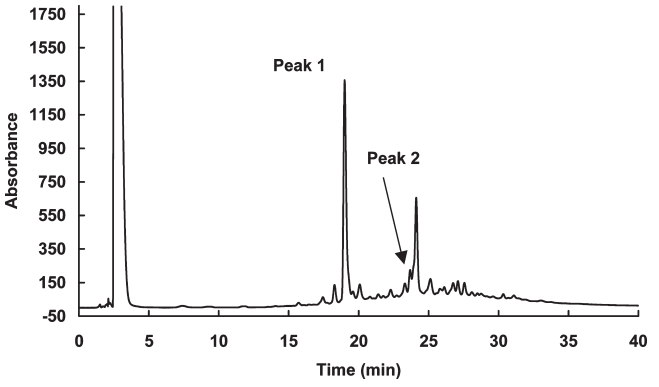

Figure 5. Determination of the concentration of piceatannol and resveratrol in passion fruit seed using chromatographic measurements. Peak 1 represents piceatannol, and peak 2 represents resveratrol. Standard piceatannol and peak 1 showed retention times of 19.01 and 19.13 min and UV (λ_{max}) of 236, 303, and 324 nm and 238, 303, and 325 nm, respectively. Standard resveratrol and peak 2 showed retention times of 23.43 and 23.61 min and UV (λ_{max}) of 237, 306, and 319 nm and 236, 306, and 319 nm, respectively.

to piceatannol, which was present in passion fruit seed in large amounts (**Figure 6A**). The retention time and the UV spectrograph coincided with those of standard piceatannol, with piceatannol and peak 1 showing retention times of 19.01 and 19.13 min and UV (λ_{max}) of 236, 303, and 324 nm and 238, 303, and 325 nm, respectively. The LC/MS data of PF-S also coincided with piceatannol, with the $[M - H]^-$ peak at m/z 243 (piceatannol molecular weight of 244). Piceatannol was not detected in the rind or pulp. Resveratrol (**Figure 6B**) was also determined and is represented in peak 2. The retention time and the UV spectrograph coincided with those of standard resveratrol, with resveratrol and peak 2 showing retention times of 23.43 and 23.61 min and UV (λ_{max}) of 237, 306, and 319 nm and 236, 306, and 319 nm, respectively. The LC/MS data of PF-S also coincided with resveratrol, with the $[M - H]^-$ peak at m/z 227 (resveratrol molecular weight of 228). The amount of piceatannol and

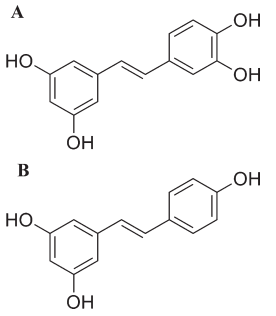

Figure 6. Chemical formulas of (A) piceatannol (3,4,3′,5′-tetrahydroxy-*trans*-stilbene) and (B) resveratrol (3,5,4′-trihydroxy-*trans*-stilbene).

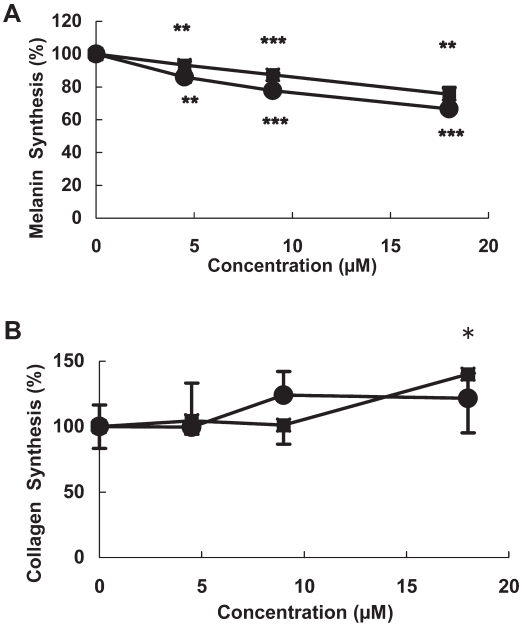

Figure 7. Comparison of the effect of PF-S (●) and piceatannol reagent (■) on (A) melanin synthesis and (B) collagen synthesis. Data are expressed as means $\pm$ SD (n = 3). Statistical analyses were performed using the paired t test: $*$, $p < 0.1$; $**$, $p < 0.05$; $***$, $p < 0.01$.

resveratrol contained in freeze-dried passion fruit seed was 4.8 and 0.22 mg/g, respectively, which translates to 2.2 and 0.1 mg/g, respectively, in raw passion fruit seed.

Comparison of the Effects of Piceatannol from PF-S and Piceatannol Reagent in Dermal Cells. To evaluate the contribution of piceatannol to the effects of PF-S on melanin inhibition and collagen synthesis, piceatannol extracted from PF-S and a commercially available piceatannol reagent were prepared to the same concentration, to compare their activity. As shown in **Figure 7A**, piceatannol from PF-S showed a stronger effect in the inhibition of melanin synthesis, although piceatannol reagent also yielded a strong inhibition of melanin synthesis. Both piceatannol samples decreased melanin synthesis significantly, at 4.5 μM. Soluble collagen synthesis was increased after treatment with both piceatannol samples; however, piceatannol reagent showed a significant difference at 18.5 μM (**Figure 7B**).

Comparison of the Effects of Piceatannol and Resveratrol Reagents in Dermal Cells. Piceatannol and resveratrol were examined to compare their effects on dermal cells. As shown in **Figure 8A**, both compounds inhibited melanin synthesis in MNT-1 cells, although piceatannol yielded a higher melanin inhibitory effect compared to resveratrol, because piceatannol led to a significant decrease in melanin synthesis at 5 μM, whereas

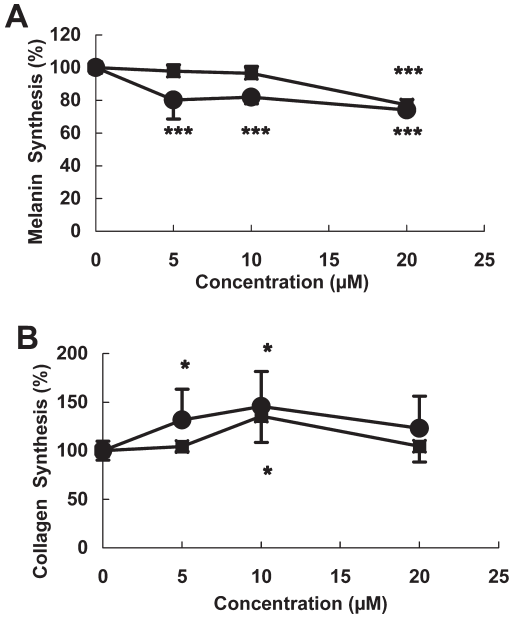

Figure 8. Comparison of the effect of piceatannol (●) and resveratrol (■) reagent on (A) melanin synthesis and (B) collagen synthesis. Data are expressed as means ± SD ($n = 3$). Statistical analyses were performed using the paired t test: ∗, $p < 0.1$; ∗∗, $p < 0.05$; ∗∗∗, $p < 0.01$.

resveratrol led to a significant decrease in melanin synthesis at 20 μM. Moreover, both piceatannol and resveratrol increased soluble collagen in SF-TY cells; piceatannol increased soluble collagen significantly at 5 μM, whereas resveratrol increased soluble collagen significantly at 10 μM (**Figure 8B**).

DISCUSSION

Passion fruit is a tropical fruit that is widely grown in South America and is eaten with the seeds, in its natural state, or converted to juice. The phytochemical compounds of passion fruit juice have been studied (*4*−*6*), although there are not many on seeds. However, it is reported that passiflin, a novel protein found in passion fruit seed, acts as an antifungal protein (*16*) and that the insoluble fiber from passion fruit seed acts as a potential hypocholesterolemic ingredient (*17*). The oil content in the seed is approximately 23%, of which 72% is linoleic acid (*18*).

Polyphenols are scavengers of reactive oxygen species (ROS). Although ROS are a biogenic substance, exposure to UV rays, stress, medication, and many other environmental conditions lead to ROS production to an excessive degree. The total polyphenol content and the antioxidant activity, as assessed using superoxide dismutase (SOD) activity, which is a well-known antioxidant index, were especially high in passion fruit seeds (data not shown). SOD activity is often used as an index of the strength of antioxidant activity. Polyphenols are thought to be natural antioxidants, and polyphenols, such as tea catechins (*19*) and proanthocyanidin (*11*), have been reported as having antioxidant activity in dermal cells. The high antioxidant activity found in PF-S may derive from its high polyphenol content.

Piceatannol is a tetrahydric polyphenol and an analogue form of resveratrol. The content of piceatannol in freeze-dried passion fruit seed was 4.8 mg/g when extracted using 70% acetone, which translates into 2.2 mg/g of raw material. There are many studies on the functions of piceatannol, which include anti-allergic effects (*20*) and inhibition of melanin synthesis (*21*). Piceatannol is found in a limited number of plants, in limited amounts: 138−422 ng/g of dry sample of highbush blueberry (*Vaccinium corymbosum*) and deerberry (*Vaccinium stamineum*) (*22*) and 0.052 μg/g of fresh weight from grapes (*Vitis vinifera* L., cv. Cabernet Sauvignon) (*23*). Because of these reasons, Ku et al. (*24*) reported that piceatannol can be produced in a large amount from the callus of peanut by UV irradiation. Our results showed that passion fruit contains a very high content of piceatannol in a natural state, which is nearly 50 times larger than that found in grapes (described above). Here, we report for the first time that piceatannol is present in passion fruit seed and that this content of piceatannol is much higher than that known for any other plants. Piceatannol was not detected in the rind or pulp of passion fruit. Resveratrol was also detected in passion fruit seeds, at a concentration of 0.22 mg/g of freeze-dried seeds.

Piceatannol is often compared to resveratrol because of their similar chemical structure. Resveratrol is one of the well-known polyphenols contained in grapes and peanuts (*23*, *25*) and reportedly contributes to skin photoprotection and antioxidation (*26*, *27*), among other functions. Moreover, piceatannol is a derivative of resveratrol and is metabolized from resveratrol via the cytochrome P450 enzyme CYP1B1, which is involved in cancer prevention (*28*). We compared the melanin-inhibitory and collagen-synthesis effects of piceatannol and resveratrol. Both compounds led to the inhibition of melanin synthesis and the production of soluble collagen; however, piceatannol yielded stronger effects in both dermal cell types. The larger number of hydroxyl groups in piceatannol may contribute to its stronger effect compared to resveratrol; in addition, the catechol structure may yield a high antioxidant effect.

To evaluate the contribution of piceatannol to the effects observed for PF-S, we prepared a reagent at the same concentration of the piceatannol contained in PF-S and compared their activity. The data show that piceatannol contributes to the inhibition of melanin synthesis and that the effect of PF-S depended strongly upon the effect of piceatannol. The slightly stronger effect of PF-S may be due to other bioactive compounds (e.g., polyphenols, such as resveratrol or other antioxidant pigments). These *in vitro* results suggest that piceatannol contributes greatly to the inhibitory effect of PF-S on melanin synthesis and to the PF-S-mediated promotion of collagen synthesis.

We found that piceatannol was the major polyphenol contained in passion fruit seed and that its melanin-synthesis inhibitory activity was higher compared to the other parts of the passion fruit, although the rind and pulp also contain polyphenols (*4*, *29*) and other pigments, such as carotenoids (*5*). Studies on the mechanism of the biogenesis of melanin and the inhibition of melanogenesis have progressed actively. Cytokines, such as endothelin-1 (ET-1) and the α-melanocyte stimulating hormone (α-MSH), induce melanogenesis; conversely, suppression of these cytokines inhibits melanogenesis (*30*,*31*). Moreover, tyrosinase is the main enzyme that contributes to melanogenesis. Tyrosinase catalyzes several steps of the melanin pigment biosynthetic pathway, via oxidation. There are reports on the inhibitory action of polyphenols from plant seeds on tyrosinase, e.g., those of safflower seeds (*10*), and also of grape-seed-mediated inhibition of melanogenesis in guinea pig (*11*). In addition, gnetol (2,3′,5′,6-tetrahydroxy-*trans*-stilbene), which is another tetrahydroxyl stilbene compound isolated from gnetum, with a structure similar to that of piceatannol, has a strong inhibitory effect on tyrosinase and suppresses melanin biosynthesis in melanoma cells (*32*). Luteolin, the polyphenol contained in the rind of passion fruit (*29*), inhibits melanin synthesis in B16 melanoma cells (*33*); however, our results did not show inhibitory effects for rind extracts. Yokozawa et al. (*21*) compared the melanin inhibitory activities of piceatannol and resveratrol in melanoma cells and concluded that the melanin inhibitory activity of piceatannol was higher than that of resveratrol because of the higher

antioxidative properties of piceatannol. Similar results were obtained in the present study. Piceatannol contains an additional hydroxyl residue, which provides a stronger reduction effect. The high content of polyphenols in PF-S may inhibit tyrosinase activity, and its high antioxidative activity may scavenge ROS; however, further studies are needed to identify the precise mechanism underlying these effects.

Collagen represents $^1/_3$ of the total protein content in the body and plays many important roles, including cell−cell adhesion, cell proliferation, and cell differentiation. Collagen is produced in fibroblast cells, and approximately 70% of the dermis consists of collagen. The functional properties of the skin depend upon the quality and condition of the collagen present in the dermis. Some food components promote collagen synthesis effectively in the skin. Other substances act as co-enzymes of prolyl hydroxylase and lysyl hydroxylase, which are the key enzymes in collagen synthesis, and some induce transforming growth factor β1 (TGF-β1), which stimulates the accumulation of type-I procollagen mRNA in human fibroblast cells (*12*). Inhibition of the degradation of collagen via inhibition of matrix metalloproteinases (MMPs) is another effective approach to the maintenance of collagen in the dermis. Piceatannol is an inhibitor of the JAK1/STAT-1 pathway, which induces the expression of the *MMP-1* gene in cultured human dermal fibroblasts (*34*). Moreover, catechins, well-known polyphenols, also inhibits UV-induced expression of MMPs in mouse skin (*13*). These reports led us to speculate that the PF-S-induced increase in the levels of collagen may be the result of the inhibition of MMPs or of their activity by the polyphenols present in PF-S; however, additional experiments are required to elucidate these issues.

In conclusion, we found that piceatannol was contained in passion fruit seeds at very high levels and that it exerted positive effects on cultured dermal cells: inhibition of melanogenesis and synthesis of collagen. The synthesis of melanin and the degradation of collagen are accompanied by the production of ROS, and ROS scavengers are effective for these damages. We postulate the possibility from our *in vitro* data that oral and topical application of PF-S may contribute to a decrease in the skin damage, which leads to skin melanogenesis, wrinkles, and other skin abnormalities. However, additional *in vitro* and *in vivo* studies are needed for human application. Although passion fruit is usually eaten with the seeds in its natural state, the seeds are discarded when it is processed to tropical juice. Extraction of piceatannol from seeds could be an efficient use of the seeds wasted during the production of passion fruit juice.

ABBREVIATIONS USED

PF-R, passion fruit rind extract; PF-P, passion fruit pulp extract; PF-S, passion fruit seed extract; PVPP, polyvinylpolypyrrolidone; DMEM, Dulbecco's modified Eagle's medium; PBS, phosphate-buffered saline; SOD, superoxide dismutase; HPLC, high-performance liquid chromatography; ODS, octa decyl silyl; LC/MS, liquid chromatography/mass spectrometry; PDA, photodiode array; ROS, reactive oxygen species; ET-1, endothelin-1; α-MSH, α-melanocyte stimulating hormone; TGF-β1, transforming growth factor β1; MMP, matrix metalloproteinase.

LITERATURE CITED

(1) Movafegh, A.; Alizadeh, R.; Hajimohamadi, F.; Esfehani, F.; Nejatfar, M. Preoperative oral *Passiflora incarnata* reduces anxiety in ambulatory surgery patients: A double-blind, placebo-controlled study. *Anesth. Analg.* **2008**, *106*, 1728−1732.

(2) Montanher, A. B.; Zucolotto, S. M.; Schenkel, E. P.; Fröde, T. S. Evidence of anti-inflammatory effects of *Passiflora edulis* in an inflammation model. *J. Ethnopharmacol.* **2007**, *10*, 281−288.

(3) Dhawan, K.; Sharma, A. Antitussive activity of the methanol extract of *Passiflora incarnata* leaves. *Fitoterapia* **2002**, *73*, 397−399.

(4) Talcott, S. T.; Percival, S. S.; Pittet-Moore, J.; Celoria, C. Phytochemical composition and antioxidant stability of fortified yellow passion fruit (*Passiflora edulis*). *J. Agric. Food Chem.* **2003**, *51*, 935−941.

(5) Mercadante, A. Z.; Britton, G.; Rodriguez, D. B. Carotenoids from yellow passion fruit (*Passiflora edulis*). *J. Agric. Food Chem.* **1998**, *46*, 4102−4106.

(6) Suntornsuk, L.; Gritsanapun, W.; Nilkamhank, S.; Paochom, A. Quantitation of vitamin C content in herbal juice using direct titration. *J. Pharm. Biomed. Anal.* **2002**, *28*, 849−855.

(7) Gorinstein, S.; Zemser, M.; Haruenkit, R.; Chuthakorn, R.; Grauer, F.; Martin-Belloso, O.; Trakhtenberg, S. Comparative content of total polyphenols and dietary fiber in tropical fruits and persimmon. *J. Nutr. Biochem.* **1999**, *10*, 367−371.

(8) Mertens-Talcott, S. U.; Rios, J.; Jilma-Stohlawetz, P.; Pacheco-Palencia, L. A.; Meibohm, B.; Talcott, S. T.; Derendorf, H. Pharmacokinetics of anthocyanins and antioxidant effects after the consumption of anthocyanin-rich acai juice and pulp (*Euterpe oleracea* Mart.) in human healthy volunteers. *J. Agric. Food Chem.* **2008**, *56*, 7796−7802.

(9) Kondo, M.; Zhang, L.; Ji, H.; Kou, Y.; Ou, B. Bioavailability and antioxidant effects of a xanthone-rich Mangosteen (*Garcinia mangostana*) product in humans. *J. Agric. Food Chem.* **2009**, *57*, 8788−8792.

(10) Roh, J. S.; Han, J. Y.; Kim, J. H.; Hwang, J. K. Inhibitory effects of active compounds isolated from safflower (*Carthamus tinctorius* L.) seeds for melanogenesis. *Biol. Pharm. Bull.* **2004**, *27*, 1976−1978.

(11) Yamakoshi, J.; Otsuka, F.; Sano, A.; Tokutake, S.; Saito, M.; Kikuchi, M.; Kubota, Y. Lightening effect on ultraviolet-induced pigmentation of guinea pig skin by oral administration of a proanthocyanidin-rich extract from grape seeds. *Pigm. Cell Res.* **2003**, *16*, 629−638.

(12) Koya-Miyata, S.; Okamoto, I.; Ushio, S.; Iwaki, K.; Ikeda, M.; Kurimoto, M. Identification of a collagen production-promoting factor from an extract of royal jelly and its possible mechanism. *Biosci., Biotechnol., Biochem.* **2004**, *68*, 767−773.

(13) Vayalil, P. K.; Mittal, A.; Hara, Y.; Elmets, C. A.; Katiyar, S. K. Green tea polyphenols prevent ultraviolet light-induced oxidative damage and matrix metalloproteinases expression in mouse skin. *J. Invest. Dermatol.* **2004**, *122*, 1480−1487.

(14) Lim, H.; Kim, H. P. Inhibition of mammalian collagenase, matrix metalloproteinase-1, by naturally occurring flavonoids. *Planta Med.* **2007**, *73*, 1267−1274.

(15) Sanoner, P.; Guyot, S.; Marnet, N.; Molle, D.; Drilleau, J. P. Polyphenol profiles of French cider apple varieties (*Malus domestica* sp.). *J. Agric. Food Chem.* **1999**, *47*, 4847−4853.

(16) Lam, S. K.; Ng, T. B. Passiflin, a novel dimeric antifungal protein from seeds of the passion fruit. *Phytomedicine* **2009**, *16*, 172−180.

(17) Chau, C. F.; Huang, Y. L. Effects of the insoluble fiber derived from *Passiflora edulis* seed on plasma and hepatic lipids and fecal output. *Mol. Nutr. Food Res.* **2005**, *49*, 786−790.

(18) Liu, S.; Yang, F.; Li, J.; Zhang, C.; Ji, H..; Hong, P. Physical and chemical analysis of *Passiflora* seeds and seed oil from China. *Int. J. Food Sci. Nutr.* **2008**, *59*, 706−715.

(19) Jeon, S. E.; Choi-Kwon, S.; Park, K. A.; Lee, H. J.; Park, M. S.; Lee, J. H.; Kwon, S. B.; Park, K. C. Dietary supplementation of (+)-catechin protects against UVB-induced skin damage by modulating antioxidant enzyme activities. *Photodermatol., Photoimmunol. Photomed.* **2003**, *19*, 235−241.

(20) Matsuda, H.; Tomohiro, N.; Hiraba, K.; Harima, S.; Ko, S.; Matsuo, K.; Yoshikawa, M.; Kubo, M. Study on anti-Oketsu activity of rhubarb II. Anti-allergic effects of stilbene components from *Rhei undulati* rhizoma (dried rhizome of *Rheum undulatum* cultivated in Korea). *Biol. Pharm. Bull.* **2001**, *24*, 264−267.

(21) Yokozawa, T.; Kim, Y. J. Piceatannol inhibits melanogenesis by its antioxidative actions. *Biol. Pharm. Bull.* **2007**, *30*, 2007−2011.

(22) Rimando, A. M.; Kalt, W.; Magee, J. B.; Dewey, J.; Ballington, J. R. Resveratrol, pterostilbene, and piceatannol in vaccinium berries. *J. Agric. Food Chem.* **2004**, *52*, 4713−4719.

(23) Bavaresco, L.; Fregoni, M.; Trecisan, M.; Mattivif, F.; Vrhovsk, U.; Falchetti, R. The occurrence of the stilbene piceatannol in grapes. *Vitis* **2002**, *41*, 133−136.
(24) Ku, K. L.; Chang, P. S.; Cheng, Y. C.; Lien, C. Y. Production of stilbenoids from the callus of *Arachis hypogaea*: A novel source of the anticancer compound piceatannol. *J. Agric. Food Chem.* **2005**, *53*, 3877−3881.
(25) Sanders, T. H.; McMichael, R. W., Jr.; Hendrix, K. W. Occurrence of resveratrol in edible peanuts. *J. Agric. Food Chem.* **2000**, *48*, 1243−1246.
(26) Nichols, J. A.; Katiyar, S. K. Skin photoprotection by natural polyphenols: Anti-inflammatory, antioxidant and DNA repair mechanisms. *Arch. Dermatol. Res.* **2010**, *302*, 71−83.
(27) Hung, L. M.; Su, M. J.; Chu, W. K.; Chiao, C. W.; Chan, W. F.; Chen, J. K. The protective effect of resveratrols on ischaemia-reperfusion injuries of rat hearts is correlated with antioxidant efficacy. *Br. J. Pharmacol.* **2002**, *135*, 1627−1633.
(28) Potter, G. A.; Patterson, L. H.; Wanogho, E.; Perry, P. J.; Butler, P. C.; Ijaz, T.; Ruparelia, K. C.; Lamb, J. H.; Farmer, P. B.; Stanley, L. A.; Burke, M. D. The cancer preventative agent resveratrol is converted to the anticancer agent piceatannol by the cytochrome P450 enzyme CYP1B1. *Br. J. Cancer.* **2002**, *86*, 774−778.
(29) Ichimura, T.; Yamanaka, A.; Ichiba, T.; Toyokawa, T.; Kamada, Y.; Tamamura, T.; Maruyama, S. Antihypertensive effect of an extract of *Passiflora edulis* rind in spontaneously hypertensive rats. *Biosci., Biotechnol., Biochem.* **2006**, *70*, 718−721.
(30) Imokawa, G.; Yada, Y.; Miyagishi, M. Endothelins secreted from human keratinocytes are intrinsic mitogens for human melanocytes. *J. Biol. Chem.* **1992**, *267*, 24675−24680.
(31) Thody, A. J.; Graham, A. Does α-MSH have a role in regulating skin pigmentation in humans? *Pigm. Cell Res.* **1998**, *11*, 265−274.
(32) Ohguchi, K.; Tanaka, T.; Iliya, I.; Ito, T.; Iinuma, M.; Matsumoto, K.; Akao, Y.; Nozawa, Y. Gnetol as a potent tyrosinase inhibitor from genus *Gnetum*. *Biosci., Biotechnol., Biochem.* **2003**, *67*, 663−665.
(33) Choi, M. Y.; Song, H. S.; Hur, H. S.; Sim, S. S. Whitening activity of luteolin related to the inhibition of cAMP pathway in α-MSH-stimulated B16 melanoma cells. *Arch. Pharm. Res.* **2008**, *31*, 1166−1171.
(34) Kim, S.; Kim, Y.; Lee, Y.; Chung, J. H. Ceramide accelerates ultraviolet-induced MMP-1 expression through JAK1/STAT-1 pathway in cultured human dermal fibroblasts. *J. Lipid Res.* **2008**, *49*, 2571−2581.

Received for review July 8, 2010. Revised manuscript received August 18, 2010. Accepted August 25, 2010.