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ORIGINAL ARTICLE

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Effect of *Pinus radiata* bark extracts with different molecular weight distributions on cell growth of NIH/3T3 fibroblasts and dendrite retraction of B16 melanoma cells

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Abstract Hot water extract (HWE) of *Pinus radiata* bark was separated into monomeric polyphenol (MPP), oligomeric proanthocyanidin (OPA), and polymeric proanthocyanidin (PPA) fractions by monitored chromatography using a Sephadex LH 20 column and an UV detector at 250 nm. The effects of these fractions on NIH/3T3 fibroblasts and B16 melanoma cells were examined by evaluating cell viability, melanogenesis (melanin content), morphological changes, and tyrosinase inhibitory activity. The polyphenolic fractions had a proliferation effect on fibroblasts, with cell growth increasing significantly ($P < 0.01$) even at the high concentration of 1250 μg/ml. At 125 μg/ml, HWE, MPP, and OPA had no effect on melanogenesis, whereas PPA significantly upregulated melanogenesis ($P < 0.05$). Melanogenesis was significantly upregulated in melanoma cells treated with these fractions at the high concentration of 600 μg/ml ($P < 0.01$). B16 melanoma cells cultured with the proanthocyanidin (PA)-rich fractions (HWE, OPA, and PPA) showed marked dendrite retraction, leading to significant morphological transformation. OPA in particular showed colored adhesion on the surface of melanoma cells. All four fractions significantly inhibited mushroom tyrosinase activity when compared to arbutin and ascorbic acid 2-glucoside ($P < 0.05$). The tyrosinase inhibitory activity of OPA and PPA did not differ significantly ($P = 0.11$), indicating that the inhibitory effects had a low correlation with molecular weight distribution. The inhibition kinetics of PPA determined using a Lineweaver–Burk plot indicated that PPA is a noncompetitive inhibitor of L-3,4-dihydroxyphenylalanine oxidation by mushroom tyrosinase.

Key words *Pinus radiata* bark · Molecular weight distribution · NIH/3T3 fibroblasts · Dendrite retraction · B16 melanoma cell

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Introduction

Pine wood is commonly used in mechanical and chemical pulping, medium density fiberboard (MDF) manufacturing, and the sawmill industry. Prior to the chipping process, the bark of pine is completely removed due to the high content of lignin/polyphenol, which interferes with manufacturing processes. Pine bark is an important biomass resource, accounting for about 10%–15% of the total weight of the tree.[1] The bark removed from logs primarily causes a large surplus of waste residue. Pine bark waste has been used industrially as an energy source, fertilizer, and mulch and is also used in leather manufacturing, with the most common use being the generation of energy. Much research has been devoted to producing higher-value products from pine bark, and these efforts have expanded the bark into a source of commercial chemicals, including adhesives, biocides, and dispersants for scale-forming minerals, corrosion inhibitors, and heavy metal removers.[2] In the past few years, the awareness of the potential for effective and value-added utilization of these bark wastes has increased, in part due to their potential as a rich source of polyphenol compounds.

Until recently, many studies focused on the use of pine bark extract as dietary supplements, food ingredients, and therapy against tissue-damaging diseases. However, only a few studies have been conducted to evaluate the potential for skin protection by proanthocyanidin (PA)-rich extracts of pine bark.[3,4] Skin damage caused by exposure to ultraviolet (UV) light increases the risk of skin cancer and premature aging. Skin exposure to UV light also causes abnormal facial pigmentation such as melasma (chloasma), which is characterized by irregular light to dark brown macules and

patches on sun-exposed areas of the skin. Pycnogenol has been found to inhibit UV-induced NF-κB-dependent gene expression in a dose-dependent manner in HaCaT keratinocytes.[3] This compound has also been shown to significantly prevent UV-induced erythema in rats and humans after oral administration.[4] Additionally, Yamakoshi et al. reported that oral intake of a PA-rich extract from grape seeds improves chloasma.[5] However, the effect of pine bark extracts on melanoma cells and fibroblasts has rarely been studied, despite the significant need in the cosmetic and dermatological fields for agents that can be used to treat hyperpigmental skin disorders (melasma, freckles, and senile lentigines) and various aging effects such as whitening and wrinkling. In the present study, we separated hot water extract (HWE) from *Pinus radiata* bark into monomeric, oligomeric, and polymeric fractions since the bioactivity capacity of PA is generally recognized to be dependent on the degree of polymerization (DP). The effects of these three fractions on NIH/3T3 fibroblasts and B16 melanoma cells were then investigated to evaluate their potential value as candidate materials for cosmetic and dermatological therapies.

Materials and methods

Materials

Pinus radiata bark obtained from Sawmilling Co. Ltd., Christchurch, New Zealand, was dried at 60°C for 48 h and then ground in a Wiley mill. Sephadex LH-20 and mushroom tyrosinase (3900 unit/mg, EC 1.14.18.1) were purchased from Amersham Biosciences (Uppsala, Sweden) and Sigma (St. Louis, MO, USA), respectively. Dulbecco's modified Eagle's medium [DMEM, L-glutamine (4 mM), glucose (4500 mg/l), and sodium pyruvate] and fetal bovine serum (FBS) were purchased from Hyclone (Logan, UT, USA). Antibiotics (100×) and trypsin-ethylenediaminetetraacetic acid (EDTA) were purchased from Invitrogen (Carlsbad, CA, USA). All solvents were of HPLC grade (Duksan, Korea).

Preparation and fractionation of HWE

Preparation and fractionation of HWE were conducted as described in our previous study,[6,7] with only modification of the amounts. Briefly, bark powder (600 g oven dried, 20–80 mesh) was extracted with 6 l of deionized water for 1 h at 100°C. The bark residues were then screened immediately, squeezed in a cotton cloth bag, and then washed with 16 l of deionized water. Next, the extract was filtered through a 3-μm-filter cartridge. The filtrate was then evaporated under reduced pressure using an Eyela N-12 rotary evaporator (Kyoto, Japan) at 65°C, after which the concentrate was lyophilized for 2 days and then vacuum-dried for 2 days under P_2O_5. The HWE from *Pinus radiata* bark was fractionated using a slightly modified version of the extraction and isolation scheme suggested by Ohara et al.[8] Briefly, 25 g of HWE was dissolved in 250 ml of 70% (v/v) aqueous acetone, after which the solution was filtered and evaporated to remove the acetone. The lipids were removed from HWE by extracting five times with 200 ml of *n*-hexane. The aqueous layer was then again extracted five times with 500 ml of ethyl acetate to extract the monomeric polyphenol (MPP) and oligomeric proanthocyanidin (OPA). Next, the ethyl acetate extract (2.01 g) was eluted in a Sephadex LH-20 column with absolute ethanol. MPP (0.65 g) and OPA (1.06 g) fractions were separated by monitoring their characteristic spectra with a UV spectrophotometer.[6,7] MPP was a mixture of phenolic acids (protocatechuic acid 46 μg/g, *trans*-ferulic acid 6 μg/g, and *trans*-caffeic acid 3 μg/g) and monomeric flavonoids (taxifolin 456 μg/g, catechins 240 μg/g, and quercetin 15 μg/g). DP of OPA was determined to be within the range from 2 to 6 by matrix-assisted laser desorption/ionization time of flight (MALDI-TOF) mass spectrometry (MS) analysis.[7]

Subsequently, the aqueous layer was evaporated under reduced pressure at 65°C and lyophilized to yield 18.95 g of PA-rich powder. The lyophilized powder (5 g) was then dissolved in 50% (v/v) aqueous methanol, loaded into a Sephadex LH-20 column, and washed with 50% (v/v) aqueous methanol (5 l). Polymeric proanthocyanidin (PPA) adsorbed on the Sephadex LH-20 packing materials was recovered from the column by elution with 50% (v/v) aqueous acetone (10.5 l). The procedure to isolate PPA from the aqueous layer was duplicated. The total PPA yield was about 55% and the ^{13}C-NMR spectrum revealed that PPA comprised procyanidin (PC, 94%) and a small amount of prodelphinidin (PD, 6%), according to the spectral analysis method of Kraus et al.[9] Also, the spectral analysis showed that C2–C3 structure was mainly in the *trans* configuration. The weight- and number-average molecular mass (M_w, M_n), polydispersity (M_w/M_n), and DP of the pine bark PPA were 3800 (M_w), 1200 (M_n), 3.2, and 13, respectively.[6]

Cell viability

Samples were diluted to give 0.001%–1% solutions, and 25 μl of these diluted samples were used for cell viability testing. NIH/3T3 mouse fibroblasts (embryo fibroblasts, contact-inhibited, NIH Swiss mouse, ATTC CRL 1658) were obtained from the American Type Culture Collection. The cells were cultured in supplemented DMEM at 37°C in a humidified atmosphere containing 95% air and 5% CO_2. The cells were then subcultured three times for 3 days each. NIH-3T3 cell monolayers were treated with trypsin-EDTA, after which the cell suspensions were washed with phosphate-buffered saline (PBS) and the pellets were recovered by centrifugation at 280 × g for 5 min. The collected cells were then suspended in the culture medium, after which 200 μl were seeded into 96-well plates at 1.2×10^4 cells/well. The cells were then incubated at 37°C for 24 h. After incubation, when the cultures were at subconfluence, the medium was discarded and fresh medium (175 μl) was added to the plate, after which the cells were treated with the samples or control. After being well mixed, the cells

were incubated for 24 h and the medium and sample were discarded. Fresh DMEM (100 µl) without FBS and antibiotics was then added to the cell plate followed by the addition of 10 µl of 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT, 5 mg/ml in PBS). After 4 h of reaction, the DMEM was removed and 100 µl of dimethylsulfoxide (DMSO) was added and mixed to dissolve the purple crystals of formazan. The absorbance was then measured in a spectrophotometer at a wavelength of 540 nm. The reported values are the means of three replicates and are expressed as percentages of the control values.

Melanin content and morphology

B16 mouse melanoma cells (CRL 5323) were obtained from ATCC (Manassas, VA, USA) and cultured in 10% FBS-DMEM containing 1% antibiotics (penicillin and streptomycin) at 37°C in a humidified atmosphere of 5% CO_2 and 95% air. The harvested B16 melanoma cells were counted with a hemocytometer, after which they were seeded in 6-well plates at a density of 2.6×10^6 cells/well and cultured for 24 h. The medium was then replaced with fresh FBS-DMEM. Each polyphenolic fraction was dissolved in 50% (v/v) aqueous butylene glycol, and 375 µl of sample was added to cells in 2625 µl of the medium. After culturing for 72 h, the cells were rinsed twice with 2 ml of PBS (1×) and then collected in PBS after trypsinization (1 ml of trypsin-EDTA) for 4 min in the incubator. Four milliliters of the medium was then added to each well containing the trypsinizing solution. Next, the solution was mixed well and collected in a 15-ml centrifuge tube and centrifuged at 280 × g for 5 min. The supernatant was then carefully removed, after which 200 µl of 0.1 M NaOH was mixed with the remaining cell pellet in the centrifuge tube. The mixture containing the cells was heated in boiling water for 10 min to extract the melanin, and the reactants (200 µl) were then transferred to a 96-well plate. The absorbance of the melanin extracts was measured at 490 nm.

Tyrosinase inhibitory activity

Samples (5 mg) were diluted with 20% (v/v) aqueous DMSO to give 500 µg/ml concentrations. Arbutin and ascorbic acid-2-glucoside (AA2G), well-known tyrosinase inhibitors, were used for comparison with the polyphenolic fractions. L-3,4-Dihydroxyphenylalanine (L-DOPA) as a tyrosinase substrate was dissolved in 1/15 M phosphate buffer solution (pH 6.8) to give a concentration of 0.5 mM. Mushroom tyrosinase (3900 units/mg) was diluted with the same phosphate buffer solution to give 30 unit/ml. The reaction mixture consisted of 0.5 mM L-DOPA (1 ml), 30 unit/ml mushroom tyrosinase (1 ml), phosphate buffer (pH 6.8, 0.5 ml), and inhibitors (1 ml). The mixtures were reacted in a shaking water bath at 25°C for 10 min and the enzyme activity was then determined based on the formation of dopachrome by monitoring the absorbance at 476 nm for 10 min. The percent tyrosinase inhibition was calculated according to the following equation:

$$\% \text{ Inhibition} = \{[A_{476\text{Control}} - (A_{476\text{Sample}} - A_{476\text{Blank}})]/ A_{476\text{Control}}\} \times 100$$

where $A_{476\text{Control}}$ is the absorbance at 476 nm with L-DOPA and tyrosinase, $A_{476\text{Sample}}$ is the absorbance at 476 nm with the sample and tyrosinase with L-DOPA, and $A_{476\text{Blank}}$ is the absorbance at 476 nm with the sample and tyrosinase, but without L-DOPA.

PPA was dissolved with 20% (v/v) aqueous DMSO to give 200–1000 µg/ml. One milliliter of L-DOPA solution (0.9–3.5 mM), 500 µl of 1/15 M phosphate buffer (pH 6.8), and 1 ml of the sample solution were mixed in a test tube. After mixing, 1 ml of the aqueous solution of mushroom tyrosinase (57 units) was added to the mixtures, and the reaction for the formation of dopachrome was immediately spectrophotometrically monitored by measuring the linear increase in the absorbance at 475 nm over a 10-min period. The reaction rate was estimated from the linear portion of each curve and expressed as A_{476}/min. The inhibition type of PPA on tyrosinase was assayed using a Lineweaver–Burk plot.

Statistical analysis

The results were expressed as the means ± standard deviation, and SPSS 12.0 was used for all statistical analyses. Significant differences among the values were determined by one-way ANOVA and Duncan's multiple range tests. Significant differences were determined at $P < 0.05$ and $P < 0.01$.

Results and discussion

Cell viability

NIH/3T3 fibroblasts were employed to evaluate cell viability using the MTT reagent. As shown in Fig. 1, as the polyphenolic fractions increased, the fibroblasts still underwent a proliferation effect, with growth significantly increasing ($P < 0.01$) even at the high concentration of 1250 µg/ml. These results indicated that the polyphenols in HWE from *Pinus radiata* bark exerted no toxicity at concentrations up to 1250 µg/ml. Similarly, Shoji et al. reported that trimer-to-hexamer fractions of PC from apple polyphenol extract induced an increase in the cell growth of B16 melanoma cells to 166%–229% of that of the controls.[10] Some catecholic phenols are cytotoxic through autoxidation, which produces hydrogen peroxide and hydroxyl radicals. The majority of the proliferation effect can be ascribed to the production of low levels of these reactive oxygen species (ROS). Progression to a more pro-oxidant state, while initially enhancing the proliferation responses, subsequently increases cell death.[11] Thus, these polyphenolic fractions with a catecholic moiety may stimulate growth factors in the absence of ROS or play a key role in the reduction of ROS in fibroblasts.

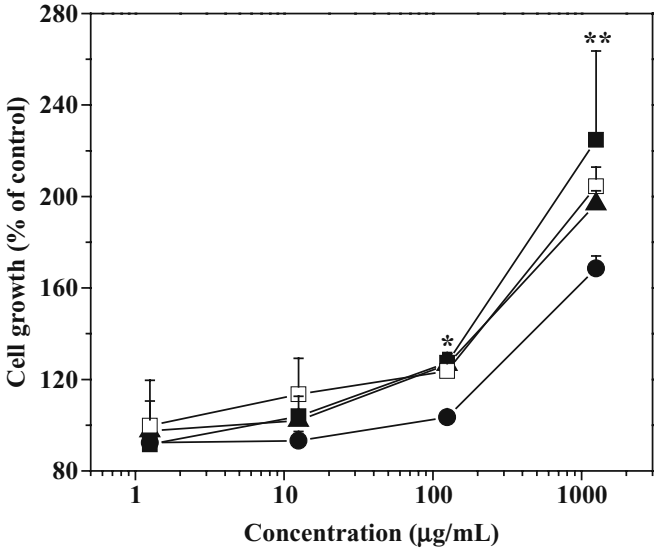

Fig. 1. Effect on NIH/3T3 fibroblast growth of polyphenolic fractions from *Pinus radiata* bark at different doses. *Solid squares*, hot water extract (HWE); *circles*, monomeric polyphenol (MPP); *triangles*, oligomeric proanthocyanidin (OPA); *open squares*, polymeric proanthocyanidin (PPA). Each value is expressed as the mean ± standard deviation ($n = 3$); * $P < 0.05$, ** $P < 0.01$, compared with the control

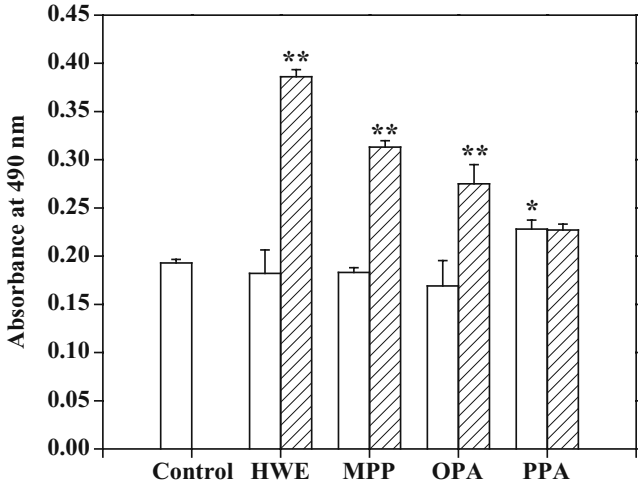

Fig. 2. Absorbance of melanin at 490 nm in B16 melanoma cells after treatment with polyphenolic fractions from *Pinus radiata* bark at concentrations of 125 (*open bars*) and 600 μg/ml (*hatched bars*). Each value is expressed as the mean ± standard deviation ($n = 3$); * $P < 0.05$, ** $P < 0.01$, compared with the control

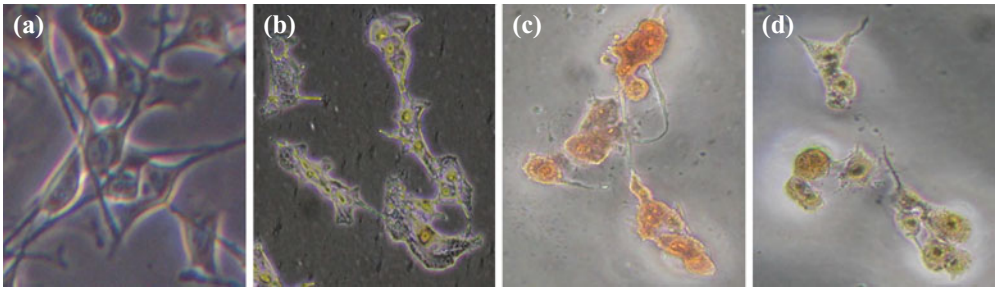

Fig. 3. B16 melanoma cells cultured with polyphenolic fractions from *Pinus radiata* bark. **a** Control cells; **b–d**, cells cultured with 125 μg/ml of HWE, OPA, and PPA, respectively. Magnification ×400

Melanogenesis inhibitory activity in melanoma cells

Melanin formed in B16 melanoma cells cultured with or without the polyphenolic fractions was estimated based on the absorbance at 490 nm. The results were compared with those of control cells to obtain statistical data regarding melanogenesis in melanoma cells treated with the fractions. As shown in Fig. 2, none of the fractions exhibited inhibitory activity against melanogenesis in cultured melanoma cells ($P > 0.05$). Specifically, HWE, MPP, and OPA had no effect on melanogenesis at 125 μg/ml, while PPA at that concentration induced a significant increase in melanogenesis ($P < 0.05$). Melanogenesis increased significantly in melanoma cells treated with a high concentration of the fractions, i.e., 600 μg/ml ($P < 0.01$). Nagata et al. reported that quercetin, one component of MPP, enhances melanogenesis by increasing tyrosinase activity and decreasing other factors such as melanogenic inhibitors.[12] These results support the increase in melanogenesis in B16 melanoma cells treated with MPP that was observed in the present study. Contrary to our results, some studies reported that PA can effectively inhibit melanogenesis in B16 melanoma cells.[5,10] Accordingly, we considered the enhancement of melanogenesis (based on the increase in absorbance at 490 nm) to be partially ascribed to the adsorption of the polyphenolic fractions at high concentration onto melanoma cells. Consequently, the morphology of melanoma cells cultured with these polyphenolic fractions was investigated.

Retraction of melanoma cell dendrites

Melanin transfer from melanocytes to keratinocytes is stimulated by a hormonal response and UV exposure and results in skin pigmentation. Figure 3 shows the morphological changes in melanocytes cultured without UV treatment. Control melanocytes showed marked dendrite elongation, whereas melanocytes treated with PA-rich fractions (HWE, OPA, and PPA) exhibited significant morphological transformation in terms of marked dendrite

retraction. Similar findings have rarely been reported and such morphological transformations have primarily been recognized as indicating inhibitory activity on melanosome transfer, although slight differences in activity were evident. Among these PA-rich fractions, OPA in particular showed colored adhesion on the surface of the melanoma cells. These findings suggest that the surface adhesion of OPA contributes to blocking melanosome transfer to keratinocytes. To date, only a few melanosome transfer inhibitors such as niacinamide (vitamin B3), centaureidin, methylophiopogonanone B, and *trans*-retinoic acid have been reported.[13–15] Hakozaki et al. reported that niacinamide suppresses melanosome transfer from melanocytes to keratinocytes in the epidermis by between 35% and 68%, thereby markedly reducing cutaneous pigmentation.[13] Ito et al. screened a large number of plant extracts for their ability to reduce melanin pigmentation in the epidermis, and identified centaureidin and methylophiopogonanone B.[14] They postulated that centaureidin inhibits dendrite outgrowth in melanocytes, suggesting that the morphological changes induced by centaureidin were associated with the inhibition of melanosome transfer. It has also been reported that methylophiopogonanone B can block melanosome transfer completely and that *trans*-retinoic acid is less effective than methylophiopogonanone B for melanocyte dendrite retraction in terms of concentration and time.[15] In the present study, our recent findings regarding the morphological transformation of melanocytes treated with PA-rich fractions suggest a new research focus for PA in terms of its effect on hyperpigmental skin disorders such as hyperpigmentation, melasma, and senile lentigines.

Tyrosinase inhibitory activity

Tyrosinase inhibitory activity was determined based on the reduction of dopachrome formation at 476 nm. All of the polyphenolic fractions from pine bark significantly inhibited mushroom tyrosinase activity when compared to arbutin and AA2G ($P < 0.05$, Fig. 4). OPA and PPA did not show a significant difference in their respective tyrosinase inhibitory activities ($P = 0.11$), demonstrating their low correlation with M_w distribution. At 143 μg/ml, MPP showed significantly higher tyrosinase inhibitory activity than the other inhibitors ($P < 0.01$), implying its competitive inhibition. These findings are similar to those of a study conducted by Nakamura et al, who reported that mushroom tyrosinase can specifically oxidize some catecholic phenols (taxifolin, catechin, quercetin, protocatechuic acid, and caffeic acid) of MPP.[16] These catecholic phenols can control the melanogenesis process because *o*-quinones induced by the catecholic moieties can conjugate with glutathione (GSH) and sulphydryl (SH) enzymes or participate in melanin formation in melanoma cells.[17]

The inhibition kinetics of PPA were analyzed using a Lineweaver–Burk plot, as shown in Fig. 5. The four lines obtained from the uninhibited enzyme and the three different concentrations of PPA intersected at the horizontal axis. These results indicated that PPA is a noncompetitive inhibitor of L-DOPA oxidation by mushroom tyrosinase. Although the inhibitory activity of mushroom tyrosinase has recently been reported as not being in good accordance with that of tyrosinase or melanin synthesis in cultured melanocytes,[16] our results support the potential use of pine bark extracts as skin whitening agents.

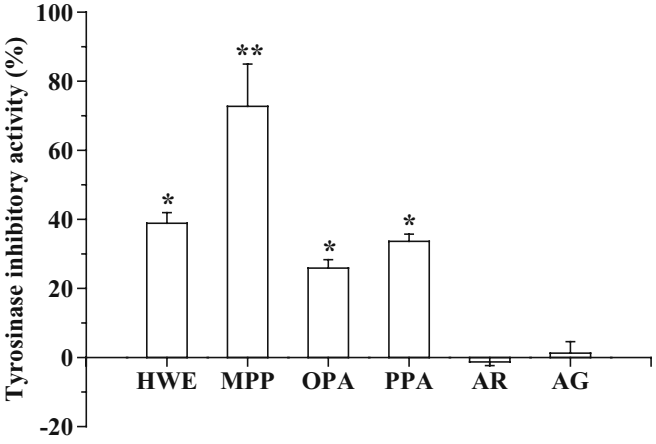

Fig. 4. Inhibitory activity of the polyphenolic fractions from *Pinus radiata* bark on mushroom tyrosinase. Sample, 143 μg/ml; tyrosinase, 30 units. Each value is expressed as the mean ± standard deviation ($n = 3$); * $P < 0.05$, ** $P < 0.01$, compared with the arbutin (*AR*) and ascorbic acid-2-glucoside (*AA2G*) references

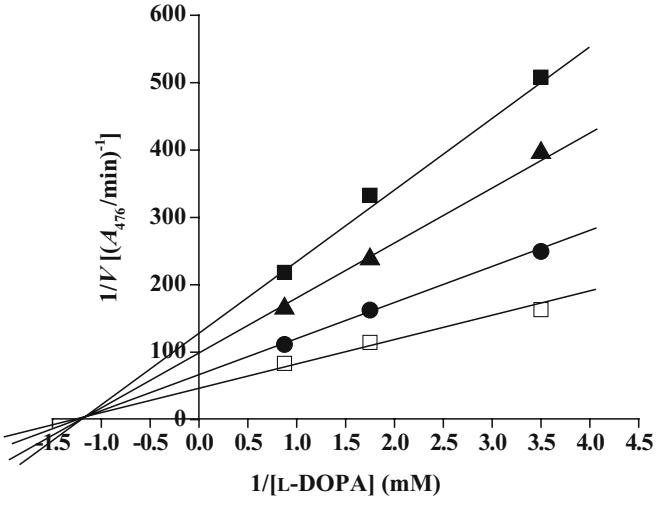

Fig. 5. Lineweaver–Burk plots of mushroom tyrosinase and L-3,4-dihydroxyphenylalanine (L-DOPA) with (*solid symbols*) and without (*open squares*) the polyphenolic fractions from *Pinus radiata* bark. Enzyme activity was measured at 476 nm in the presence of 57 μg/ml (*circles*), 143 μg/ml (*triangles*), and 286 μg/ml (*solid squares*) PPA, while L-DOPA concentrations ranged from 0.9 to 3.5 mM

Conclusions

Pinus radiata bark extracts with different molecular weight distributions exhibited cell growth effects against NIH/3T3 fibroblasts, and the PA-rich fraction noticeably retracted dendrites of B16 melanoma cells, despite the increased

melanogenesis in the cell. These effects of the polyphenolic fractions should encourage the development of a novel dendrite blocker for melanoma cells in the future. Additional studies will be directed toward further investigation of the dendrite retraction of B16 melanoma cells treated with the PA-rich fractions.

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References

1. Kofujita H, Ettyu K, Ota M (1999) Characterization of the major components in bark from five Japanese tree species for chemical utilization. Wood Sci Technol 33:223–228
2. Hon DNS, Shiraishi N (1991) Wood and cellulosic chemistry. Marcel Dekker, New York
3. Saliou C, Rimbach G, Moini H, McLaughlin L, Hosseini S, Lee J, Watson RR, Packer L (2001) Solar ultraviolet-induced erythema in human skin and nuclear factor-kappa-B-dependent gene expression in keratinocytes are modulated by a French maritima pine bark extract. Free Rad Biol Med 30:154–160
4. Svobodová A, Psotová J, Walterová D (2003) Natural phenolics in the prevention of UV-induced skin damage. Biomed Papers 147:137–145
5. Yamakoshi J, Otsuka F, Sano A, Tokutake S, Saito M, Kikuchi M, Kubota Y (2003) Lightening effect on ultraviolet-induced pigmentation of guinea pig skin by oral administration of a proanthocyanidin-rich extract from grape seeds. Pigment Cell Res 16:629–638
6. Ku CS, Mun SP (2007) Characterization of proanthocyanidin in hot water extract isolated from *Pinus radiata* bark. Wood Sci Technol 41:235–247
7. Ku CS, Mun SP (2008) Antioxidant properties of monomeric, oligomeric, and polymeric fractions in hot water extract from *Pinus radiata* bark. Wood Sci Technol 42:47–60
8. Ohara S, Suzuki K, Ohira T (1994) Condensed tannins from *Acacia mearnsii* and their biological activities. Mokuzai Gakkaishi 40:1363–1374
9. Kraus TEC, Yu Z, Preston R, Dahlgren RA, Zasoski RJ (2003) Linking chemical reactivity and protein precipitation to structural characteristics of foliar tannins. J Chem Ecol 29:703–730
10. Shoji T, Masumoto S, Moriichi N, Kobori M, Kanda T, Shinmoto H, Tsushida T (2005) Procyanidin trimers to pentamers fractionated from apple inhibit melanogenesis in B16 mouse melanoma cells. J Agric Food Chem 53:6105–6111
11. Perluigi M, De Marco F, Foppoli C, Coccia R, Blarzino C, Marcante ML, Cini C (2003) Tyrosinase protects human melanocytes from ROS-generating compounds. Biochem Biophys Res Commun 305:250–256
12. Nagata H, Takekoshi S, Takeyama R, Homma T, Osamura RY (2004) Quercetin enhances melanogenesis by increasing the activity and synthesis of tyrosinase in human melanoma cells and in normal human melanocytes. Pigment Cell Res 17:66–73
13. Hakozaki T, Minwalla L, Zhuang J, Chhoa M, Matsubara A, Miyamoto K (2002) The effect of niacinamide on reducing cutaneous pigmentation and suppression of melanosome transfer. Br J Dermatol 147:20–31
14. Ito Y, Kanamaru A, Tada A (2006) Centaureidin promotes dendrite retraction of melanocytes by activating Rho. Biochim Biophys Acta 1760:487–494
15. Ito Y, Kanamaru A, Tada A (2006) Effects of methylophiopogonanone B on melanosome transfer and dendrite retraction. J Dermatol Sci 42:68–70
16. Nakamura Y, Torikai K, Ohigashi H (2001) A catechol antioxidant protocatechuic acid potentiates inflammatory leukocyte-derived oxidative stress in mouse skin via a tyrosinase bioactivation pathway. Free Rad Biol Med 30:967–978
17. Ito S (2003) A chemist's view of melanogenesis. Pigment Cell Res 16:230–236