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Whitening and Anti-oxidative Activities of Chemical Components Extracted from Branches of *Sorbus alnifolia*

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ABSTRACT. In this study were evaluated the whitening and anti-oxidative activities from the extracts of *Sorbus alnifolia* branches, and identified the chemical structures of the active ingredients. In the whitening tests using α-MSH stimulated B16F10 melanoma cells, the 70% ethanol extract and *n*-butanol (*n*-BuOH) fractions concentration-dependently inhibited cellular melanogenesis and intracellular tyrosinase activities without causing cell toxicity. The total polyphenol content of *n*-BuOH and ethyl acetate (EtOAc) fractions were measured to be respectively 241.1 ± 1.1 and 222.9 ± 2.4 (mg/g GAE), and the total flavonoid content of EtOAc fraction was 75.3 ± 2.0 (mg/g QE). Upon anti-oxidant studies with DPPH and $ABTS^+$ radicals, potent radical scavenging activities were observed in the EtOAc and *n*-BuOH fractions. Moreover, in the study of cell protection efficacy using HaCaT keratinocytes damaged by H_2O_2, the EtOAc and *n*-BuOH fractions showed a very positive results on prevention of oxidative stress. Phytochemical studies for this extract resulted in the isolation of four compounds; 2-oxopomolic acid (**1**), euscaphic acid (**2**), *epi*-catechin (**3**), prunasin (**4**). These results suggested that the extract of *S. alnifolia* branches containing compounds **1**-**4** as natural ingredients could be used as whitening and anti-oxidant ingredients in cosmetic formulations.

Key words: *Sorbus alnifolia*, Whitening, Anti-oxidation, Cell protective effect, Isolation

INTRODUCTION

Melanin is a pigment produced by epidermal melanocytes and is responsible for skin color and protection from UV rays.[1] However the excessive production of melanin pigments resulted in different dermatological disorders such as skin aging spots, melasma, freckles, lentigo, ephelides and melanoma.[2] Studies have shown that these physiological disturbances are primarily ascribed to the excessive accumulation of reactive oxygen species (ROS) or oxidative stress ultimately leading to hyperpigmentation.[3]

Tyrosinase, a binuclear copper containing monooxygenase, is a critical rate-limiting enzyme involved in melanin biosynthesis in the skin epidermis. Melanogenesis is initiated by the hydroxylation of L-tyrosine to L-3,4-dihydroxyphenylalanine (DOPA), which is catalyzed by tyrosinase. Tyrosinase is also participated in the subsequent enzymatic conversion of DOPA to DOPA quinone.[4] Thus, tyrosinase inhibitors are major targets in the development of anti-melanogenesis materials.[1]

ROS, which induce oxidative stress, are important molecules involved in skin aging process. ROS has been reported as key molecules that cause pigmentation because they can increase secretion of α melanocyte-stimulating hormone (MSH) in keratinocytes.[5] α-MSH binds to melanocortin 1 receptor on melanocytes and stimulates the expression of microphthalmia-associated transcription factor (MITF) through the cyclic adenosine monophosphate (cAMP)-dependent pathway, which leads to melanogenesis.[6] Therefore, anti-oxidants such as ROS scavengers may suppress melanogenesis in the epidermis.

Sorbus alnifolia (Sieb. Et Zucc.) K. Koch is a deciduous broad-leaved tree of the dicotyledonous plant Rosaceae, mainly inhabiting the shaded and rock-broken areas of dry ridges or plains from deep mountains to 100-1300 m highlands. The leaves with a length of 5-10 cm are staggered on the branches. This plant is mainly distributed in Korea, China and Japan. According to studies, *Sorbus* L. plant is recommended in ethnomedicine for respiratory and gastrointestinal diseases as well as for rheumatism, cancer and diabetes.[7] But until now, the researches on the efficacy and chemical constituents of *S. alnifolia* extract have not been reported.

Therefore, we used extracts as well as solvent fractions of *S. alnifolia* branches as research materials to evaluate their melanogenesis inhibitory properties including anti-oxidant capacities. Meanwhile, the ethyl acetate fraction was

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systematically partitioned, and the chemical structures of the purified isolates were elucidated.

EXPERIMENTAL

General Experimental Procedures

1D NMR spectra were performed on JNM-EXC 400 (FT-NMR system, 400 MHz, JEOL Co.) using pyridine-d_5, CD_3OD as solvent for measurement. The chemical shift values are reported in ppm relative to the solvent used. HR-ESI-MS analysis was performed on an ACQUITY UPLC (Waters, Milford, MA, USA) and Vion IMS QTOF (Waters MS Technologies, Manchester, UK) system using an Acquity BEH C18 column (2.1 × 100 mm, 1.7 μm, Waters, Milford, MA, USA). The ESI-MS spectra were acquired in the negative mode to produce $[M-H]^-$ ions. Merck® silica gel 60 (KGaA, Germany) and Sephadex™ LH-20 (GE healthcare Co, Sweden) were used for column chromatography, and precoated silica gel 60 F-254 plates (Merck LTD., Germany) were used for thin-layer chromatography (TLC). UV spectra were recorded on a Biochrom Libra S22 spectrophotometer. The spots on TLC were detected by spraying with 3% $KMnO_4$ aqueous solution or anisaldehyde solution with 5% H_2SO_4. 2,2-diphenyl-1-picrylhydrazyl (DPPH) and [2,2′-azino-bis (3-ethylbenzothiazoline-6-sulfonic acid)] (ABTS) were purchased from Aldrich. All solvents used were of analytical grade.

Plant Material

The *S. alnifolia* branches were collected Gwangpyeong-ri, Seogwipo city, Korea in May 2022. Voucher specimen (No. 502) was deposited at Natural Product Chemistry Laboratory, Department of Chemistry and Cosmetics, Jeju National University.

Extraction and Isolation

The *S. alnifolia* branches (820.0 g) were extracted two times with 70% aqueous ethanol (16.4 L) under stirring for 24 h at room temperature. The extracted solution was filtered, and the filtrate was concentrated under reduced pressure and freeze dried to afford a tan powder (70.7 g). A portion of the extract (56.2 g) was suspended in water (5.0 L) and fractionated into *n*-hexane (Hex, 4.8 g), ethyl acetate (EtOAc, 10.7 g), *n*-butanol (16.2 g) and water (H_2O, 24.0 g) fractions. The EtOAc soluble fraction (5.0 g) was subjected to vacuum liquid chromatography (VLC) over silica gel using step gradient solvents (*n*-Hex-EtOAc-methanol, 300 mL each) to give 32 fractions (Fr. V1-V32). A Fr. V12 (123.7 mg) was subjected by silica gel column chromatography with chloroform-methanol (30:1) eluents to yield compound **1** (18.4 mg). After, Fr. V15-16 (120.7 mg) was subjected by silica gel column chromatography with chloroform-methanol (25:1) eluents to yield compound **2** (12.6 mg). A Fr. V18 (240.7 mg) was further purified using Sephadex LH-20 column chromatography with chloroform-methanol (7:1) eluents to yield compound **3** (45.9 mg). A Fr. V25 (520.4 mg) was further purified using Sephadex LH-20 column chromatography with chloroform-methanol (5:2) eluents to afford compound **3** (23.4 mg) and **4** (15.2 mg).

Anti-melanogenesis Activities

Cell Culture

The murine melanoma cell line B16F10 was purchased from America Type Cell Culture (ATCC, Rockville, MD, USA). B16F10 cells were cultured in Dulbecco's modified Eagle's medium (DMEM) supplemented with 100 U/mL penicillin 100 μg/mL streptomycin and 10% heat-inactivated fetal bovine serum (FBS). Cells were maintained at 37 ℃ in a humidified 5% CO_2 atmosphere.

Cell Viability Assay

The cell viability after treatment with tested samples was determined using 3-(4,5-dimethylthiazol-2-yl)-2,5 diphenylt-etrazolium bromide (MTT). 2×10^4 cells were added in each well of 24-well plate. After 24 h, cells were exposed to sample at several concentrations for 72 h. MTT reagent (500 μg/mL) was added to the medium, and which was allowed to stand for 4 h. After removing the supernatant, the formazan crystals were dissolved in DMSO. Absorbance of each well was read at 570 nm using a plate reader.

Determination of Melanin Content

The B16F10 cells were seeded at density of 2×10^4 cells/well in 24-well plate and were cultured in DMEM with 10% FBS. After 24 h, the cells were treated with 100 nM α-MSH and various concentrations of the samples or with the medium only as a blank. After 72 h incubation at 37 ℃ with 5% CO_2, the cells were washed with PBS and harvested (5000 rpm × 10 min). Then, cell pellets were completely lysed with 1 N NaOH at 55 ℃. The melanin content of the cell lysates was determined by measuring the absorbance at 405 nm with a microplate reader. Melasolv (20 μM) served as a positive control. Data are expressed in terms of melanin synthesis inhibitory activity compared to the blank.

Intracellular Tyrosinase Activity Assay

Intracellular tyrosinase activity was determined based on a modification of a previously described method. 2×10^4 cells/well were treated with 100 nM α-MSH and samples. After 72 h, the incubated cells were washed twice with ice-cold PBS and harvested by centrifugation at 1,500 rpm for 5 min. The harvested cells were lysed in RIPA buffer. The total protein was collected by centrifugation at 15,000 rpm for 20 min at 4 ℃. The reaction mixture consisted of 160 μL 8 mM L-DOPA to dissolve in 67 mM sodium phosphate buffer (pH 6.8) solution and 20 μL cell-extracted protein in 96-well plate. After 1 h at 37 ℃, it was measured based on the absorbance at a wavelength of 490 nm using a microplate reader.

Anti-oxidative Activities

Determination of Total Polyphenols Content

The determination of the total polyphenol content was applied to Folin-Deinis method,[8] 900 μL distilled water and 100 μL Folin-Ciocalteu's phenol regent were added to each sample solution of 100 μL, and reacted at room temperature for 3 min. Then addition of 200 μL 7% (w/v) Na_2CO_3 solution and 700 μL distilled water, were followed by reaction at room temperature for 1 h, and measurement was conducted at the absorbance at 700 nm. After making calibration curve with different concentrations with the standard gallic acid, the total polyphenol content was calculated.

Determination of Total Flavonoid Content

The determination of the total flavonoid content was applied to Park method,[9] 100 μL of each sample solution was diluted with 300 μL 95% EtOH. Then addition of 20 μL of 10% (w/v) $Al(NO_3)_3$ solution and 20 μL of 1 M CH_3COONa solution and distilled water were made until the total volume reaches to 1 mL. After the mixtures were reacted at room temperature for 30 min, 200 μL of the reaction solution was taken in each 96-well plate, and measured the absorbance at 415 nm. After making calibration curve with different concentrations with the standard quercetin, the total flavonoid content was calculated.

DPPH Radical Scavenging Assay

2,2-diphenyl-1-picrylhydrazyl (DPPH) radical scavenging activity experiment was applied to Blois method.[10] 20 μL sample solutions with different concentrations was mixed to 180 μL 0.2 mM DPPH solution in a 96-well plate, and the mixtures were reacted at room temperature for 10 min. Absorbance was measured at 515 nm. The concentration (SC_{50}) when the scavenging activity percentage of each sample was 50% was calculated, and BHT was used as the positive control.

$ABTS^+$ Radical Scavenging Assay

2,2'-azino-bis(3-ethylbenzothiazoline-6-sulfonicacid) (ABTS) cationic free radical scavenging activity was measured by using the method of Re *et al.*[11] Mixture of 7.4 mM ABTS and 2.6 mM potassium persulfate was stored for 16 h at rt in the dark to form $ABTS^+$ free radicals. The $ABTS^+$ solution was diluted with ethanol so that its absorbance at 700 nm was 0.78 ± 0.02. 20 μL sample solutions with different concentrations was added to 180 μL $ABTS^+$ solution in a 96-well plate. The mixture was reacted at rt for 15 min, and measured the absorbance at 700 nm. The concentration (SC_{50}) was calculated. BHT was used as the positive control.

Cell Protective Effects

Cell Culture

The immortalized human keratinocyte cell line HaCaT was purchased from ATCC. HaCaT cells were cultured in DMEM supplemented with 100 U/mL penicillin 100 μg/mL streptomycin and 10% heat-inactivated FBS. Cells were maintained at 37 ℃ in a humidified 5% CO_2 atmosphere.

Cell Viability Assay

The viability of HaCaT cells was measured by the MTT assay. The cells were plated in a 96-well cell culture plate at a density of 1×10^4 cells/well and allowed to attach for 24 h then the culture medium was removed. After samples were treated separately on medium without FBS, and after 24 h of culture, MTT with a concentration of 500 μg/mL was added, reacted at 37 ℃ for 4 h, and the MTT solution was removed. Finally, DMSO was added to dissolve the formazan precipitate produced by the reaction with living cells, and the absorbance was measured at 570 nm to calculate the cell survival rate (%).

Cell Protective Effect by Hydrogen Peroxide (H_2O_2)-induced Cell Damage

The cells were plated in a 96-well cell culture plate at a density of 1×10^4 cells/well and after incubation at 37 ℃ and 5% CO_2 for 24 h, then culture medium was removed. After incubation with hydrogen peroxide at a concentration determined by cell toxicity assessment for 20 min, the H_2O_2 was removed and washed twice with Dubecco's phosphate-buffered saline (DPBS). The treated samples

were diluted according to the concentration in the medium without FBS, and after 24 h of culture, the cell viability (%) was calculated by MTT assay to verify the protective effects of cells on H_2O_2-induced cell damage.

RESULTS AND DISCUSSION

The branches of *S. alnifolia* were extracted with 70% aqueous ethanol, and the extract was suspended in water

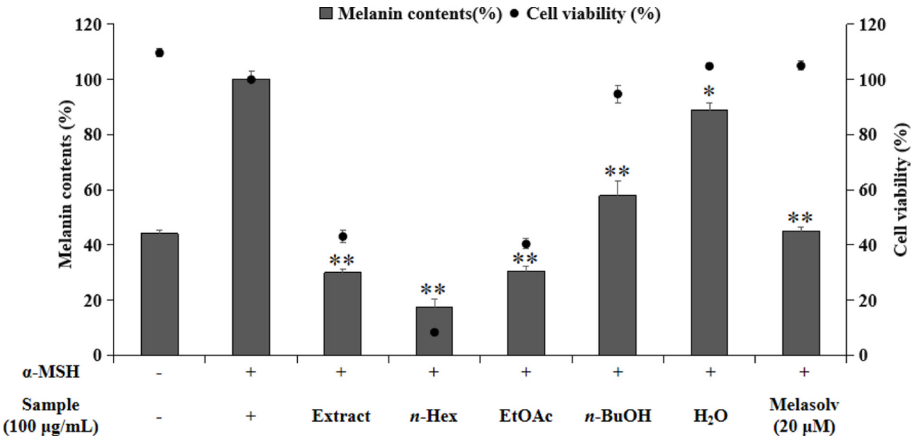

***Figure* 1.** Effects of 70% ethanol extract and solvent fractions from *S. alnifolia* branches on melanin contents and cell viability in α-MSH induced B16F10 cells. The cells were stimulated with 100 nM of α-MSH only, or with α-MSH plus fractions from *S. alnifolia* branches and melasolv (positive control, 20 μM) for 72 h. The data represent the mean ± SD of triplicate experiments ($^{*}p < 0.05$, $^{**}p < 0.01$).

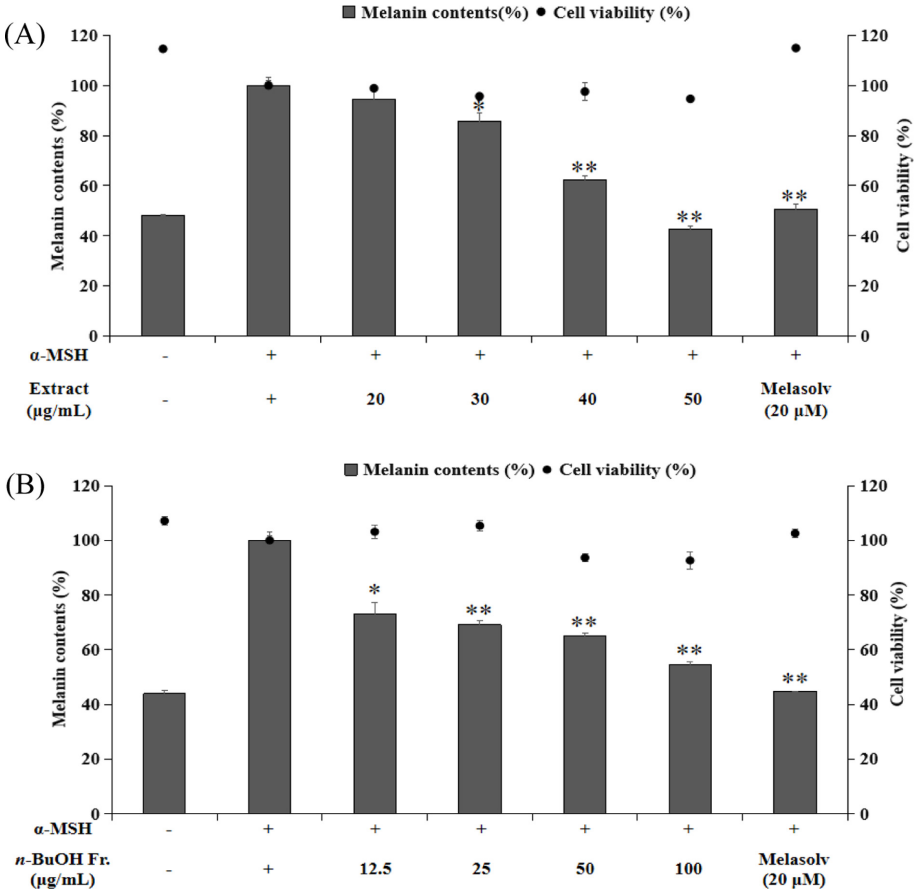

***Figure* 2.** Effects of 70% ethanol extract (A) and *n*-BuOH (B) fraction from *S. alnifolia* branches on melanin contents and cell viability in α-MSH induced B16F10 cells. The cells were stimulated with 100 nM of α-M SH only, or with α-MSH plus extract or *n*-BuOH fraction from *S. alnifolia* branches for 72 h. The data represent the mean ± SD of triplicate experiments (*$p < 0.05$, **$p < 0.01$).

and partitioned successively into *n*-hexane (*n*-Hex), ethyl acetate (EtOAc), and *n*-butanol (*n*-BuOH) to afford the respective fractions. In order to evaluate whitening effects of the 70% ethanol extract and solvent fractions from *S. alnifolia* branches, we examined melanogenesis inhibitory activity and cytotoxicity using α-MSH-stimulated B16F10 melanoma cells. As shown in *Fig.* 1, extract and each fraction showed considerable anti-melanogenesis activities. However, cell toxicities were observed at the concentration of 100 μg/mL for the extract, *n*-Hex and EtOAc fractions. On the other hand, no toxicity was appeared for the *n*-BuOH fraction at 100 μg/mL. In the case of extracts, melanin contents were decreased in a dose-dependent manner without causing cytotoxicity within a concentration range from 20 to 50 μg/mL (*Fig.* 2A). Similarly, the melanin contents and toxicity data with varying concentration (12.5-100 μg/mL) were presented for the *n*-BuOH fraction in *Fig.* 2B. Decrease of melanin contents was identified in a dose-dependent manner in this fraction.

Since cellular tyrosinase activity is also the major factor that leads to melanin synthesis, we examined its enzyme activity on α-MSH-stimulated B16F10 melanoma cells. The results showed that the 70% ethanol extract and *n*-BuOH fraction of *S. alnifolia* significantly reduced the intracellular tyrosinase activity in a concentration-dependent manner (*Fig.* 3).

Research on anti-oxidative activities was followed for the 70% ethanol extracts and fractions of *S. alnifolia* branches. First of all, the content of total phenol was determined based on calibration curve of gallic acid. The measured value is expressed by converting into the amount of gallic acid (GAE, gallic acid equivalent) contained in the sample per gram. In this experiment, higher polyphenol contents were observed in *n*-BuOH and EtOAc fractions with

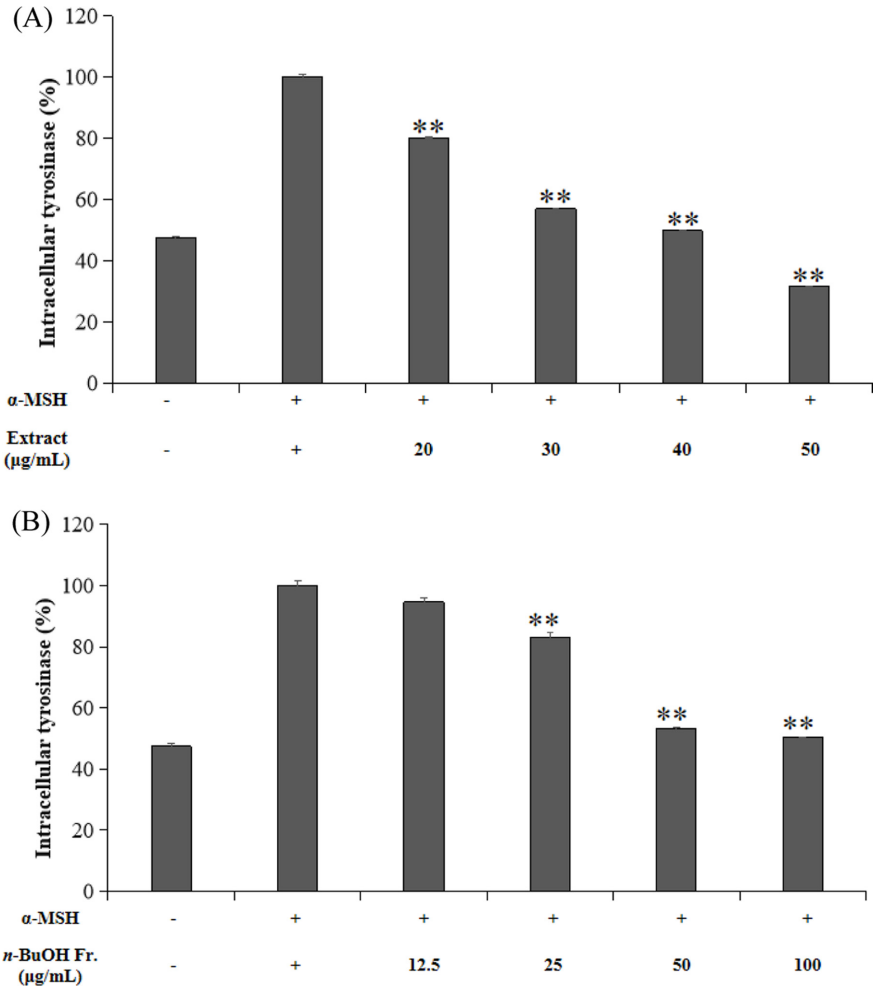

***Figure* 3.** Intracellular tyrosinase activity of 70% ethanol extract (A) and *n*-BuOH (B) fraction from *S. alnifolia* branches in α-MSH induced B16F10 cells. The cells were stimulated with 100 nM of α-MSH only, or with α-MSH plus extract or *n*-BuOH fraction from *S. alnifolia* branches for 72 h. The data represent the mean ± SD of triplicate experiments ($^{**}p < 0.01$).

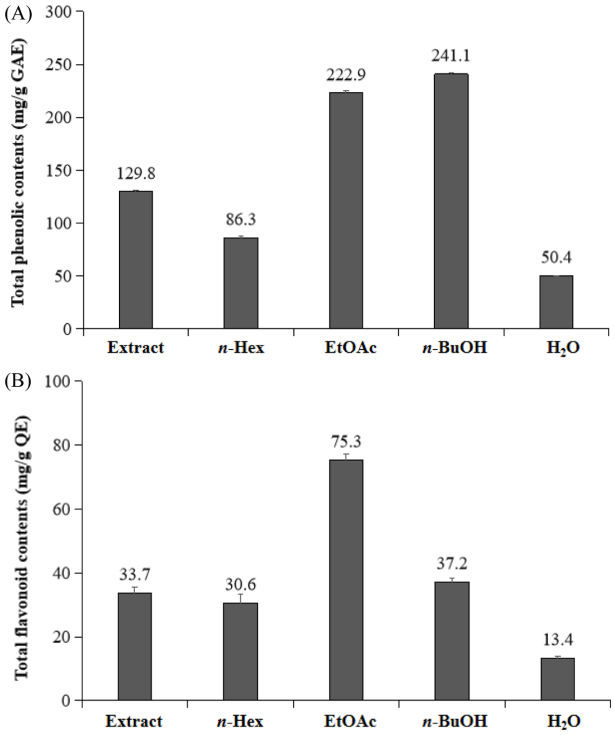

***Figure* 4.** Total polyphenol contents (A) and total flavonoid contents (B) of 70% ethanol extract and solvent fractions from *S. alnifolia* branches. The data represent the mean ± SD of triplicate experiments.

241.1 ± 1.1 and 222.9 ± 2.4 mg/g GAE values respectively (*Fig*. 4A). The content of total flavonoid was also determined by using quercetin as a standard material. The measurement is expressed by converting into the amount of quercetin in each gram of the sample (QE; quercetin equivalent). The highest content of flavonoid was observed in EtOAc fraction showing 75.3 ± 2.0 mg/g QE (*Fig*. 4B).

DPPH is a relatively stable hydrazyl free radical compound, which reacts with antioxidant substrates to be converted into neutral hydrazin substance accompanied by discoloration of its solution. As a measure of free radical scavenging activities of plant extracts, tracking the change in UV absorbance of DPPH solutions has been very efficiently utilized. The use of $ABTS^+$ is another effective measure on antioxidant capacity study, in which the color of $ABTS^+$ radicals *in situ* generated was tracked by UV spectrometer. In terms of radical scavenging capacity assay, the results of $ABTS^+$ is known to correlate significantly with those of DPPH radical.[12]

In the DPPH radical scavenging activity test for the 70% ethanol extract and fractions of *S. alnifolia* (*Table* 1), higher activities were observed in EtOAc and *n*-BuOH fractions with SC_{50} values of 26.4 and 38.1 μg/mL respectively, showing better activity than the positive control BHT (SC_{50} 44.0 μg/mL). In addition, the EtOAc and *n*-BuOH fractions showed the scavenging activity using $ABTS^+$ radicals with SC_{50} values of 34.5 and 40.9 μg/mL respectively, which are comparable free radical scavenging activities to the control group (*Table* 1).

In the following experiment, the tissue protection effect of *S. alnifolia* for the cells damaged by hydrogen peroxide. It is typical ROS, which cause oxidative damage leading to skin aging.

In order to conduct the cell protective effect of the 70% ethanol extract, first of all, its appropriate concentration without affecting cytotoxicity was calculated with HaCaT keratinocytes. When all samples were treated with 5 and 10 μg/mL, the cell survival rate was above 90% based on MTT assay. Therefore, the concentration of the samples used in this experiment was reasonably set below 10 μg/mL (*Fig.* 5).

Subsequently, H_2O_2-induced cell toxicity was investigated. HaCaT cells were treated with different concentrations (2, 4, 6, 8, 10 mM) of H_2O_2, and the cell viability was observed. Compared with the untreated control, when treated with 6 mM H_2O_2, the cell survival rate was 59.8%, which was used as a control group in this experiment (*Fig*. 6A). When EtOAc and *n*-BuOH fractions of *S. alnifolia* were treated with oxidatively damaged HaCaT cells (59.8% viability), they showed cell protective effects. For example, the EtOAc fractions of 5 and 10 μg/mL concentration were used, the cell viabilities were increased from 59.8% to 65.6% and 77.3% respectively. Similarly, *n*-BuOH fractions of 5 and 10 μg/mL concentration were treated, the cell survival rates were improved to 66.6% and 76.5% respectively (*Fig.* 6).

***Table* 1.** SC_{50} values of DPPH and $ABTS^+$ radical scavenging activities of 70% ethanol extract and solvent fractions from *S. alnifolia* branches.

SC_{50}[1)] (μg/mL)	Extract	*n*-Hex	EtOAc	*n*-BuOH	H_2O	BHT[2)]
DPPH radical	74.0	178.5	26.4	38.1	300.6	44.0
$ABTS^+$ radial	82.3	>200	34.5	40.9	>200	17.0

SC_{50}: scavenging concentration for 50% of radical
BHT: positive control

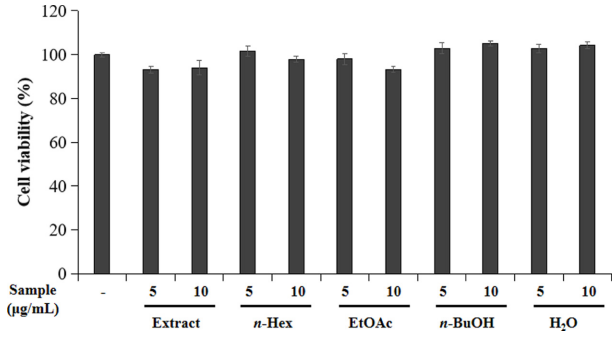

***Figure* 5.** Cell viability of 70% ethanol extract and solvent fractions from *S. alnifolia* branches in HaCaT cells. HaCaT cells were treated with different concentration of samples, and then cell toxicity was determined by MTT assay. The data represent the mean ± SD of triplicate experiments.

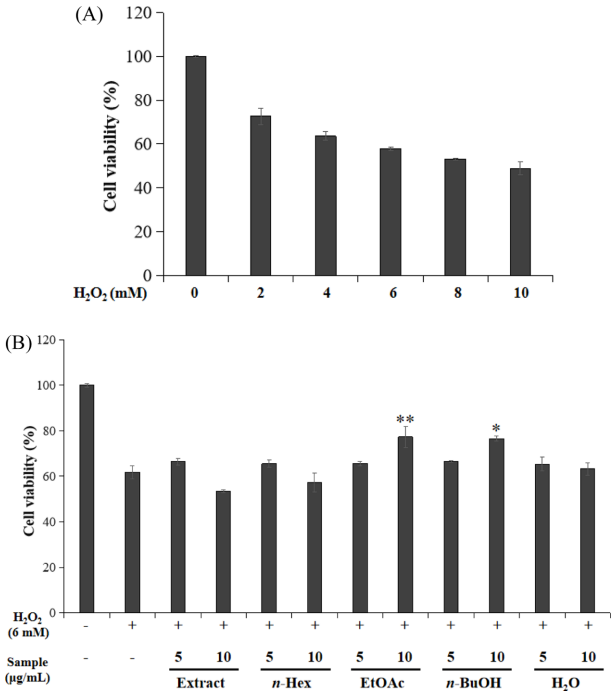

***Figure* 6.** (A) Cell viability on H_2O_2-inducted cell damage in HaCaT cells. (B) Cell protective effects of 70% ethanol extract and solvent fractions from *S. alnifolia* branches on HaCaT cells damaged by H_2O_2. HaCaT cells were treated with different concentration of sample for 24 h after being exposed to oxidative stress. The data represent the mean ± SD of triplicate experiments ($^{*}p < 0.05$, $^{**}p < 0.01$).

Phytochemical study was conducted to identify the active ingredient from the 70% ethanol extracts. Four phytochemicals were isolated from the ethyl acetate fraction of *S. alnifolia* branches by repeated column chromatography. The structures of the isolates were confirmed by ^{1}H and ^{13}C NMR and ESI-MS spectra. Compound **1** was obtained as a yellow amorphous powder. ESI-MS showed a quasi-molecular ion peak at m/z 485.3263 $[M-H]^-$ (calcd for $C_{30}H_{45}O_5$, 485.3272) corresponding to the formula $C_{30}H_{46}O_5$, agreed with its ^{1}H and ^{13}C-NMR data, and is presumed to be the triterpenoid. The NMR spectra and the comparison with the literature[13] were determined to be 2-oxopomolic acid. Compound **2** was obtained as a white amorphous powder. ESI-MS showed a quasi-molecular ion peak at m/z 487.3431 $[M-H]^-$, (calcd for $C_{30}H_{47}O_5$, m/z 487.3429). The NMR spectra signal of compound **2** was compared with that of compound **1** and found to be similar triterpene compound. Compared with the literature,[14] it was determined to be euscaphic acid. Compound **3** was obtained as a brown amorphous powder. ESI-MS showed a quasi-molecular ion peak at m/z 289.0726 $[M-H]^-$, (calcd for $C_{15}H_{13}O_6$, m/z 289.0718). Further comparison with the literature[15] determined it to be *epi*-catechin. Compound **4** was obtained as a brown amorphous powder. ESI-MS showed a quasi-molecular ion peak at m/z 294.0975 $[M-H]^-$, (calcd for $C_{14}H_{16}NO_6$, m/z 294.0983), corresponding to the formula $C_{14}H_{17}NO_6$, agreed with its ^{1}H and ^{13}C-NMR data. It was a cyanogenic glycoside compound, which was confirmed to be prunasin by further comparison with the literature.[16] All of these compounds **1**-**4** were isolated for the first time from *S. alnifolia* (*Fig*. 7).

This study showed that *S. alnifolia* branches extracts possess excellent whitening activities. Previous reports have described that triterpenoids 2-oxopomolic acid (**1**) and euscaphic acid (**2**) have potent melanogenesis inhibitory activities.[17] Therefore, it is reasonable to assume that isolates **1** and **2** are the key components for the whitening efficacy of *S. alnifolia* extracts. In addition, the potent DPPH and $ABTS^+$ radical scavenging activity as well as cell protective

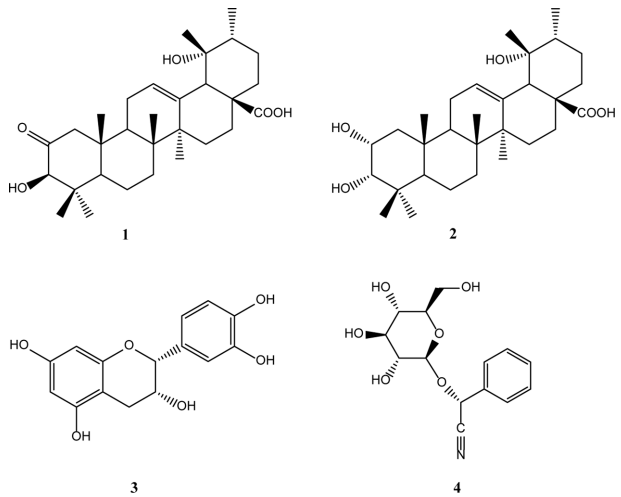

***Figure* 7.** Isolated compounds **1**-**4** from *S. alnifolia* branches.

effect of the isolated flavan-3-ol compound, *epi*-catechin (**3**), has been widely reported.[18-20] In this study, compound **3** can be considered as the main component responsible for the anti-oxidant effects of the plant extracts.

CONCLUSION

This study described the whitening and anti-oxidative activity of 70% ethanol extract and solvent fractions from *S. alnifolia* branches. In the melanogenesis inhibitory activities using α-MSH-stimulated B16F10 melanoma cells, 70% ethanol extract and *n*-BuOH fraction showed strong inhibitory activities on melanin synthesis and intracellular tyrosinase production in a dose-dependent manner. Also, anti-oxidative tests revealed that the EtOAc and *n*-BuOH fractions showed potent radical scavenging activities of DPPH and $ABTS^+$ radicals. Moreover, for the cell protective effect studies using HaCaT keratinocytes damaged by H_2O_2, the EtOAc and *n*-BuOH fractions showed a very positive results on prevention of oxidative stress. In order to identify the active ingredients, the EtOAc fraction purified by VLC followed by column chromatography to isolate four compounds; 2-oxopomolic acid (**1**), euscaphic acid (**2**), *epi*-catechin (**3**), prunasin (**4**). These compounds were isolated for the first time from *S. alnifolia*. Among them, 2-oxopomolic acid (**1**) and euscaphic acid (**2**) are considered as the active whitening constituents, and *epi*-catechin (**3**) is an extensively-studied anti-oxidative component. Therefore, the extract of *S. alnifolia* branches could be used as whitening and anti-oxidative ingredients in the fields of cosmetic industries.

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Supporting Information. Additional supporting information (NMR and HR-ESI-MS data for the compounds **1-4**) is available in the online version of this article.

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