



# LOWBUSH BLUEBERRY EXTRACT PROTECTS NORMAL HUMAN (*HOMO SAPIENS* L.) EPIDERMAL KERATINOCYTES FROM DAMAGE BY UV-B IRRADIATION

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**Keywords:** flavonoids, normal human epidermal keratinocytes (NHEKs), polyphenols, proanthocyanidins, ultraviolet-B (UV-B).

To search for natural materials that protect human skin from damage caused by ultraviolet-B (UV-B) irradiation, we prepared water extracts of the following four dry berries: lowbush blueberry (LBB) (*Vaccinium angustifolium* L.), highbush blueberry (HBB) (*Vaccinium corymbosum* L.), cranberry (CB) (*Vaccinium macrocarpon* Ait.) and grape (*Vitis vinifera* L.). Normal human epidermal keratinocytes (NHEKs) were pretreated with each of the extracts and then subjected to transient UV-B irradiation (15.0 W m<sup>-2</sup>) for 80 s. The results showed that pretreatment of NHEKs with LBB extract significantly attenuated UV-B-induced damage. The concentrations of proanthocyanidins, total flavonoids and total polyphenols in LBB extract were higher than those in the other three berry extracts. Therefore, these compounds might mediate the protection of NHEKs from UV-B-induced damage.

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## INTRODUCTION

Chlorofluorocarbons cause continual depletion of the stratospheric ozone layer, which increases the amount of solar ultraviolet-B (UV-B; 280–320 nm) irradiation [UV-B (+)] passing through to the earth's surface.<sup>1</sup> UV-B (+) is harmful to living organisms. UV-B is the chief cause of skin reddening and sunburn, which damages the skin's more superficial epidermal layers. Direct absorption of UV-B by DNA induces mutations and leads to the development of carcinomas in cutaneous spinous cells.<sup>2,3</sup> Protection from UV-B (+) is crucial and is primarily achieved by the application of topical sunscreen treatments. Sunscreen contains UV-light absorbers and UV-light scattering agents. UV-light absorbers are usually phenolic compounds found in various oils whereas UV-light scattering agents are minerals such as titanium oxide or zinc oxide. However, these components sometimes cause allergies. Thus, it is essential to identify natural and safe compounds that do not cause allergies and that still protect skin from UV-B-mediated damage.

Red and purple berries, such as lowbush blueberry (LBB) (*Vaccinium angustifolium* L.), highbush blueberry (HBB) (*Vaccinium corymbosum* L.), cranberry (CB) (*Vaccinium macrocarpon* Ait.) and grape (*Vitis vinifera* L.) provide a readily available source of dietary phenolic compounds. Berries also contain many flavonoids.<sup>4-6</sup> A flavonoid fraction from CB extract inhibits the proliferation of human tumor cell lines.<sup>7</sup> Flavonoids from LBB and CB inhibit matrix metalloproteinases (MMPs), which degrade the extracellular cell matrix (ECM) during cancer metastasis<sup>8,9</sup> and in human prostate cancer cells.<sup>10,11</sup> Monomeric flavonoids that form oligomers and polymers are called

proanthocyanidins or condensed tannins. Feeding proanthocyanidins to hairless mice reduced UV-B-induced skin carcinogenesis.<sup>12</sup> These combined results suggest that flavonoids and proanthocyanidins have beneficial dietary activities that promote health. However, the full effects of these berry compounds have not been elucidated. Plant flavonoids absorb UV irradiation;<sup>13-15</sup> therefore, berry extracts may absorb UV irradiation when added to a topical treatment. Our aim is to determine whether the addition of berry extracts to topical treatments could protect human skin from UV-B (+).

Although water extracts of living tissues can result in low recovery rates, they are simple to prepare, easy to use and provide a safe and biologically compatible matrix for bioactive compounds. We previously prepared water extracts from the following four dry berries: LBB, HBB, CB and grape (designated as the 'berry extract' in the present study). We showed that these four berry extracts efficiently absorb UV radiation, primarily UV-B.<sup>16</sup> Pretreatment of cucumber (*Cucumis sativus* L.) cotyledons with LBB, HBB and CB extracts attenuates UV-B-induced damage without causing any side effects.<sup>16</sup> We used high-performance liquid chromatography (HPLC) to analyze 51 polyphenols in the four berry extracts. The LBB extract contained primarily chlorogenic acid, caffeic acid and protocatechuic acid; the HBB extract contained primarily chlorogenic acid, caffeic acid and syringic acid; and the CB extract contained primarily protocatechuic acid, myricetin and *p*-coumaric acid.<sup>16</sup> We proposed that these compounds contributed to the protection of cucumber plants against UV-B-induced damage.<sup>16</sup>

Similar to the UV-B protective effects of LBB, HBB and CB extracts in cucumber cotyledons, pretreatment of human skin with these extracts might also offer protection against UV-B-induced damage without any side effects. With the aim of developing a safe sunscreen for use in humans in the present study, we investigated whether the four berry extracts could protect human cells from UV-B-induced damage. First, we pretreated normal human (*Homo sapiens* L.) epidermal keratinocytes (NHEKs) with the four berry extracts, and then tested the effects of transient UV-B (+) on

NHEK survival. Second, we performed a spectrophotometric analysis to determine the concentrations of proanthocyanidins, total flavonoids and total polyphenols in the four berry extracts. Finally, we discuss the potential defense mechanisms elicited by the berry extracts in NHEKs in response to UV-B (+).

## EXPERIMENTAL

### Preparation of berry extracts

Commercially produced dry fruits of lowbush blueberry (LBB) (*Vaccinium angustifolium* L.), highbush blueberry (HBB) (*Vaccinium corymbosum* L.), cranberry (CB) (*Vaccinium macrocarpon* Ait.) and grape (*Vitis vinifera* L.) were purchased from the local supermarket Aeon (Aeon Co., Ltd., Chiba, Japan) in Fukuoka prefecture on September 27, 2012 and July 11, 2013, and were used before their expiry dates. For each of the four species, 5 g dried berries were ground using a mortar and pestle at room temperature, and then 15 ml of distilled water (pH 7.5) was added. Samples were placed at 4 °C, and the ground tissue was extracted into distilled water for 14 h. Then, samples were centrifuged at 6,000 rpm for 15 min at 4 °C. The crude supernatant was transferred to a new tube and centrifuged at 12,000 rpm for 10 min at 4 °C, and the clear supernatant was collected. This clear supernatant is designated as the berry extract. The Brix value of the four berry extracts was measured with a pocket sugar content meter (APAL-J; Atago Co., Ltd., Tokyo, Japan). The Brix values of LBB, HBB, CB and grape extracts were 19.4 %, 18.7 %, 22.4 % and 21.6 %, respectively. The extraction efficiencies [(collected extract weight / dried fruit weight) × Brix] of LBB, HBB, CB, and grape extracts were 45.7 %, 42.4 %, 43.25 and 56.1 %, respectively. The four berry extracts were stored at 4 °C during the experiment.

### Analysis of berry extract dose-response effects on normal human epidermal keratinocytes (NHEKs) under normal light radiation [UV-B (-)]

The maximum amount of each berry extract that could be added to NHEKs (KK-4109; Kurabo Industries, Ltd., Osaka, Japan) without affecting cell viability under UV-B (-) was investigated by ACEL, Inc. (Kanagawa, Japan). NHEKs ( $2 \times 10^4$ ) were transferred to 100  $\mu$ l keratinocyte basal medium (KBM, HuMedia-KG2; Kurabo Industries, Ltd., Osaka, Japan) in 32 wells of a 96-well culture plate (MS-8096F; Sumitomo Bakelite Co., Ltd., Tokyo, Japan), and cells were cultured in a dark humidified (5 % CO<sub>2</sub> atmosphere) incubator for 24 h at 37 °C. Cell growth and proliferation were confirmed. Then, 100  $\mu$ l KBM per culture well was replaced with 100  $\mu$ l KBM supplemented with 0 % (defined as a control in this study), 0.00064 %, 0.0032 %, 0.016 %, 0.08 %, 0.4 %, 2 % or 10 % (v/v) of each berry extract ( $n=4$ ). One culture plate was used for each of the four berry extracts (a total of four culture plates were used for the experiment). The cells were cultured for 24 h at 37 °C before assessing cell viability using the Cell Count Reagent SF colorimetric assay (Nacalai Tesque, Inc., Kyoto, Japan). Briefly, 10  $\mu$ l Cell Count Reagent SF was added to each well and incubated for 2 h at 37 °C. Then, cell viability was assessed colorimetrically by measuring the optical density at

450 nm (OD<sub>450</sub>) using a microplate reader (Precision Microplate Reader; Molecular Devices Co., Tokyo, Japan). In this assay, the relative number of viable cells was expressed as the change in OD<sub>450</sub> over a period of 1 h (OD<sub>450</sub> h<sup>-1</sup>).

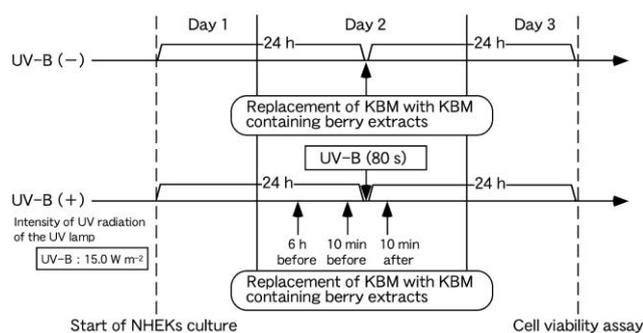
### Investigation of UV-B-induced lethality in NHEKs

The effect of transient UV-B irradiation [UV-B (+)] on NHEK survival was tested by ACEL, Inc. (Kanagawa, Japan). First, the time required for UV-B (+) to cause cell death in approximately 50 % of the NHEKs (LD<sub>50</sub>) was determined as follows. The NHEKs ( $2 \times 10^4$ ) were transferred to 100  $\mu$ l KBM per well in 6 wells in the middle of a 96-well culture plate, and cells were cultured in a dark humidified (5 % CO<sub>2</sub> atmosphere) incubator for 24 h at 37 °C. Cell growth and proliferation were confirmed. Then, the culture plates were placed 8 cm below a UV lamp (UVLMS-38; Funakoshi, Co., Ltd., Tokyo, Japan) with the following intensities of UV-A, UV-B and UV-C: 2.4, 15.0 and 1.7 W m<sup>-2</sup>, respectively. UV-A, UV-B and UV-C intensities were measured with a digital UV intensity meter (UVX radiometer; Funakoshi, Co., Ltd.) equipped with a sensor for UV-A (Radiometer Sensor UVX-36, 365 nm; Funakoshi, Co., Ltd.), UV-B (Radiometer Sensor UVX-31, 310 nm; Funakoshi, Co., Ltd.) or UV-C (Radiometer Sensor UVX-25, 254 nm; Funakoshi, Co., Ltd.). Thus, the UV lamp primarily radiates UV-B. This UV lamp was adopted as a transient emitter of UV-B (+) for the present study. The culture plates were irradiated with the UV lamp for 0, 20, 100, 200 or 1,000 s, and then cultured again in a dark humidified (5 % CO<sub>2</sub> atmosphere) incubator for 24 h at 37 °C. Therefore, a total of five culture plates were needed for the study. Cell viability was evaluated after 24 h using the Cell Count Reagent SF colorimetric assay described in the previous section.

### Effect of transient UV-B (+) on NHEKs that were pretreated with berry extracts

The effect of transient UV-B (+) on the survival of NHEKs that had been pretreated with berry extracts was investigated by ACEL, Inc. (Kanagawa, Japan). The experimental strategy is illustrated in Figure 1. For UV-B (-) of NHEKs,  $2 \times 10^4$  cells were cultured in 100  $\mu$ l KBM per well in 8 wells in the middle of a 96-well culture plate in a dark humidified (5 % CO<sub>2</sub> atmosphere) incubator for 24 h at 37 °C, and cell growth and proliferation were confirmed. Then, the culture medium in 4 wells was replaced with 100  $\mu$ l KBM supplemented with berry extract at the maximum concentration that did not affect viability (as determined above), and cells were again cultured under the same conditions for 24 h (Figure 1). The remaining four wells were used as controls. Because there were four berry extracts, a total of four culture plates were needed. Cell viability was evaluated after 24 h using the Cell Count Reagent SF colorimetric assay as described above. For transient UV-B (+) of NHEKs, the same protocol as for UV-B (-) was used with the following modification: the time for replacement of KBM with KBM containing berry extracts was different. KBM medium was replaced at 6 h before, 10 min before and 10 min after transient UV-B (+) (Figure 1). Transient UV-B (+) of NHEKs was conducted 24 h after the start of the experiment (Figure 1). Briefly,  $2 \times 10^4$  cells were

cultured in 100  $\mu\text{l}$  KBM per well in 16 wells in the middle of a 96-well culture plate in a dark humidified (5 %  $\text{CO}_2$  atmosphere) incubator at 37  $^\circ\text{C}$  (Figure 1). Then, the culture medium in 4 wells was replaced with 100  $\mu\text{l}$  KBM supplemented with berry extract at the maximum concentration that did not affect viability (as determined above) at each of three different times [6 h before, 10 min before and 10 min after transient UV-B (+)], and cells were again cultured under the same conditions (Figure 1). Therefore, a total of 12 wells were needed. The remaining four wells were used as controls. Because there were four berry extracts, a total of four culture plates were required. Cell viability was evaluated using the Cell Count Reagent SF colorimetric assay as described above.



**Figure 1.** Illustration of the experimental protocol for assessing the effects of UV-B (-) and transient UV-B (+) on the viability of NHEKs that were treated with berry extracts. Standard KBM medium was removed and replaced with KBM containing berry extracts 6 h before, 10 min before and 10 min after transient UV-B (+) for 80 s.

#### Quantitative spectrophotometric analysis of proanthocyanidin concentrations in berry extracts

Proanthocyanidin concentrations in the berry extracts were analyzed according to a modified method which was reported previously.<sup>17</sup> Briefly, 1 ml berry extract was added to 4 ml of 0.07 % (w/v) iron (II) sulfate solution, and the reactions were incubated at 95  $^\circ\text{C}$  for 1 h. Then, 4 ml of 0.6 N hydrochloric acid:*n*-butanol (1:1, v/v) was added, and the sample absorbance was measured at 550 nm with ultraviolet and visible spectrophotometry (UVIDEC-4; Jasco Co., Tokyo, Japan). Delphinidin solution was used to prepare a standard calibration curve, and proanthocyanidin concentration was calculated with respect to the delphinidin calibration curve ( $\mu\text{g } \mu\text{l}^{-1}$ ). The proanthocyanidin concentration in each berry extract was expressed as the average of three replicates.

#### Quantitative spectrophotometric analysis of the total flavonoid concentration in berry extracts

The total flavonoid concentration was analyzed according to the method which was reported previously.<sup>18</sup> Briefly, 1 ml berry extract was added to 4 ml distilled water and 0.3 ml of 5 % (w/v) sodium nitrite solution, and the reaction was allowed to stand for 5 min at room temperature. Then, 0.3 ml of 10 % (w/v) aluminum chloride solution was added and the tube was vortexed. After 1 min, 2 ml of 1.0 M sodium hydroxide solution was added, and the tube was vortexed

again. Then, 2.4 ml of distilled water was added, and the sample absorbance was measured at 510 nm with ultraviolet and visible spectrophotometry (UVIDEC-4; Jasco Co., Tokyo, Japan). A calibration curve was prepared using (+)-catechin, and the total flavonoid concentration was calculated with respect to the (+)-catechin calibration curve ( $\mu\text{g } \mu\text{l}^{-1}$ ). Total flavonoid concentration in each berry extract was expressed as the average of three replicates.

#### Quantitative spectrophotometric analysis of the total polyphenol concentration in berry extracts

The total polyphenol concentration was analyzed according to the method of Folin-Denis.<sup>19</sup> Briefly, 50  $\mu\text{l}$  berry extract was added to 4 ml distilled water and 1 ml phenol reagent (a 5-fold dilution of Folin-Ciocalteu reagent with distilled water). After vortexing, 1 ml of 10 % (w/v) sodium carbonate solution was added and the tube was vortexed again. Then, the samples were allowed to stand in the dark for 1 h at room temperature. Sample absorbance was measured at 760 nm with ultraviolet and visible spectrophotometry (UVIDEC-4; Jasco Co., Tokyo, Japan). A calibration curve was prepared using (+)-catechin solution, and the total polyphenol concentration was calculated with respect to the (+)-catechin calibration curve ( $\mu\text{g } \mu\text{l}^{-1}$ ). Total polyphenol concentration in each berry extract was expressed as the average of three replicates.

#### Statistical analysis

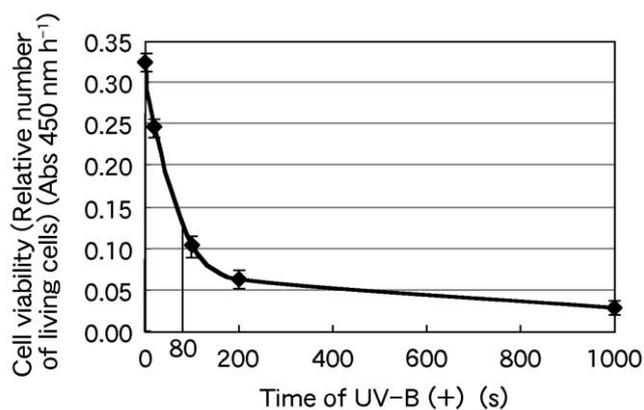
NHEK cell viability and the concentrations of proanthocyanidins, total flavonoids and total polyphenols in each berry extract were expressed as the mean  $\pm$  standard error. Statistically significant differences were assessed by Dunnett's test (<http://www.gen-info.osaka-u.ac.jp/MEPHAS/dunnett.html>, March 6, 2017).

## RESULTS

#### Pretreatment of NHEKs with berry extract protects against transient UV-B-induced cell death.

First, we determined the maximum concentrations of berry extracts (expressed as % of pure extract) that did not reduce NHEK cell viability more than 70 % of the value in control NHEKs under UV-B (-). The maximum concentrations of LBB, HBB, CB and grape extracts that did not affect NHEK cell viability were 2 %, 10 %, 10 % and 10 % (v/v), respectively. Because excessive dilution of the KBM media inhibits cell growth, the maximum percentage of extract that could be added to the cells was limited to 10 %. Second, the time for UV-B (+) that caused cell death in approximately 50 % of the NHEKs ( $\text{LD}_{50}$ ) was determined; this time was 80 s (Figure 2).

We have confirmed that the cell viability of NHEKs pretreated with berry extracts (Figures 3Ab–Db) was more than 70 % of that of control NHEKs under UV-B (-) (Figures 3Aa–Da). The decrease in cell viability of NHEKs pretreated with berry extracts (Figures 3Ab–Db) suggests that these four berry extracts are toxic to cells.



**Figure 2.** Effect of transient UV-B (+) at  $15.0 \text{ W m}^{-2}$  on NHEK viability. Values represent the average of six replicates (six wells in the culture plate).

The decline in cell viability of NHEKs treated with transient UV-B (+) (Figures 3Ac–Dc) was less than 50 % of that of NHEKs treated with UV-B (–) (Figures 3Aa–Da). Cell viability of NHEKs pretreated with 2 % LBB extract 6 h before transient UV-B (+) (Figure 3Ad) was higher than that of NHEKs treated with transient UV-B (+) ( $P < 0.01$ ) (Figure 3Ac).

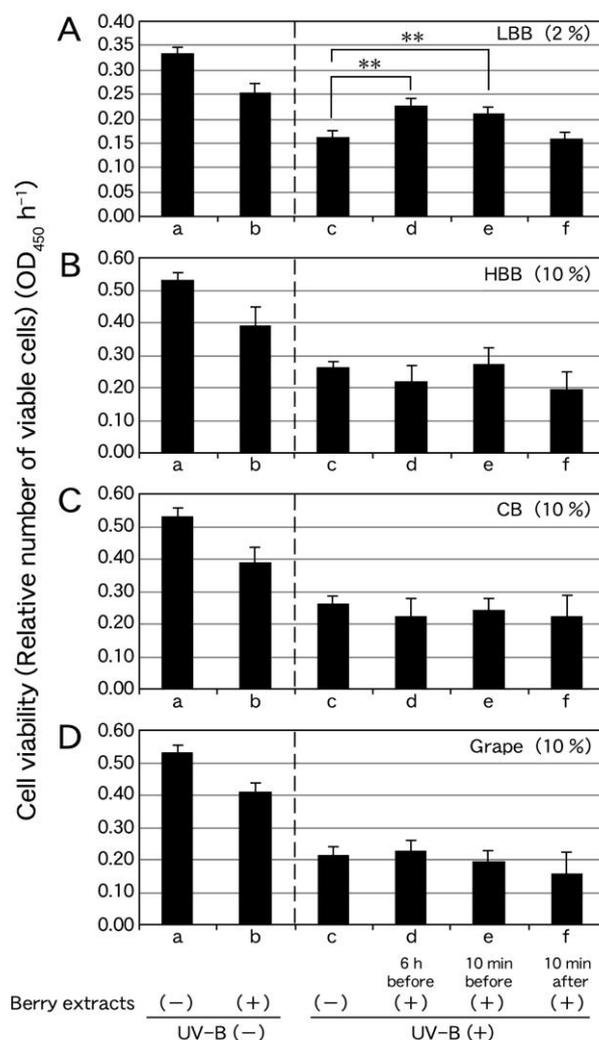
Cell viability of NHEKs pretreated with 2 % LBB extract 10 min before transient UV-B (+) (Figure 3Ae) was higher than that of NHEKs treated with transient UV-B (+) ( $P < 0.01$ ) (Figure 3Ac). By contrast, cell viability of NHEKs treated with 2 % LBB extract 10 min after transient UV-B (+) (Figure 3Af) was not significantly different from that of NHEKs treated with transient UV-B (+) (Figure 3Ac).

Cell viability of NHEKs treated with 10 % HBB extract at 6 h and 10 min before (Figure 3Bd, Be) and 10 min after (Figure 3Bf) transient UV-B (+) was not higher than that of NHEKs treated only with transient UV-B (+) (Figure 3Bc). Similarly, cell viability of NHEKs treated with 10 % CB extract 6 h and 10 min before (Figure 3Cd, Ce) and 10 min after (Figure 3Cf) transient UV-B (+) was not more significant than that of NHEKs treated only with transient UV-B (+) (Figure 3Cc). Cell viability of NHEKs treated with 10 % grape extract 6 h and 10 min before (Figure 3Dd, De) and 10 min after (Figure 3Df) transient UV-B (+) was not higher than that of NHEKs treated only with transient UV-B (+) (Figure 3Dc). These results indicate that pretreatment of NHEKs with LBB extract protected the cells from UV-B-induced damage.

#### Spectrophotometric analysis of compounds in the berry extracts

To evaluate the presence of compounds that absorb UV radiation in the berry extracts, the concentrations of proanthocyanidins, total flavonoids and total polyphenols were analyzed. The proanthocyanidin concentrations in LBB, HBB, CB and grape extracts were  $27.7 \pm 0.44$ ,  $14.5 \pm 0.63$ ,  $18.0 \pm 1.1$  and  $6.1 \pm 0.2 \mu\text{g ml}^{-1}$ , respectively (Figure 4A). Therefore, the proanthocyanidin concentration in LBB extract was 1.91-, 1.54- and 4.57-fold greater than those in HBB ( $P < 0.01$ ), CB ( $P < 0.01$ ) and grape ( $P < 0.01$ ) extracts, respectively. The total flavonoid concentrations in LBB, HBB, CB and grape extracts were  $150.8 \pm 7.8$ ,  $83.2 \pm$

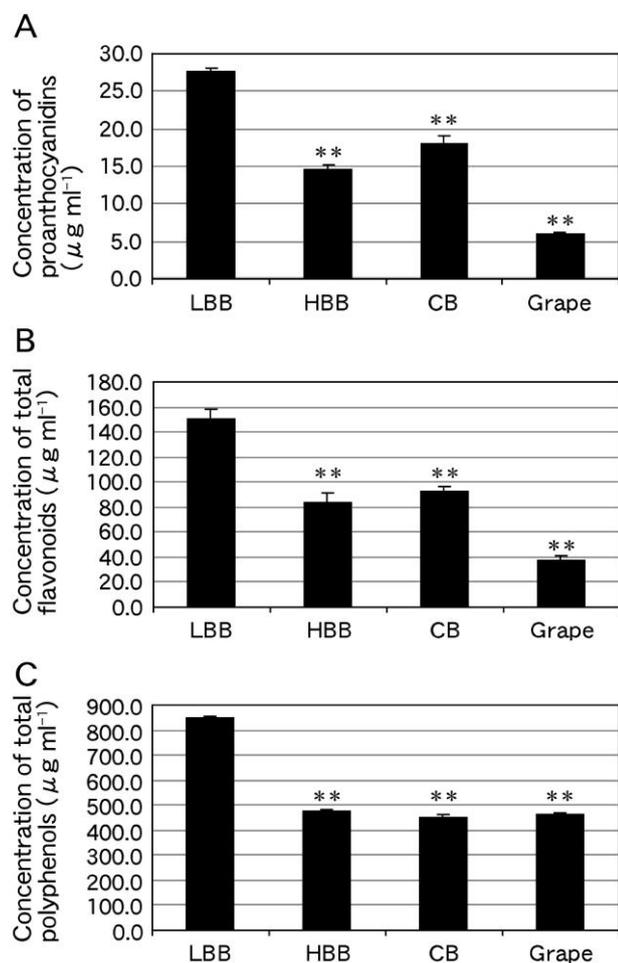
$7.9$ ,  $92.4 \pm 4.3$  and  $37.4 \pm 3.5 \mu\text{g ml}^{-1}$ , respectively (Figure 4B). Therefore, the total flavonoid concentration in LBB extract was 1.81-, 1.63- and 4.03-fold greater than those in HBB ( $P < 0.01$ ), CB ( $P < 0.01$ ) and grape ( $P < 0.01$ ) extracts, respectively.



**Figure 3.** Effect of transient UV-B (+) (80 s) on the viability of NHEKs treated with extracts of 2 % LBB (A), 10 % HBB (B), 10 % CB (C) and 10 % grape (D) 6 h before, 10 min before and 10 min after UV-B (+). The vertical axis of the graph indicates relative numbers of viable cells expressed as  $\text{OD}_{450} \text{ h}^{-1}$ . Values represent the average of four replicates (four wells in the culture plate). (a) NHEK viability under UV-B (–); (b) NHEK viability after treatment with 2 % LBB (A), 10 % HBB (B), 10 % CB (C) or 10 % Grape (D) extract under UV-B (–); (c) NHEK viability after transient UV-B (+); (d) NHEK viability after pretreatment with 2 % LBB (A), 10 % HBB (B), 10 % CB (C) or 10 % Grape (D) extract 6 h before transient UV-B (+); (e) NHEK viability after pretreatment with 2 % LBB (A), 10 % HBB (B), 10 % CB (C) or 10 % Grape (D) extract 10 min before transient UV-B (+); and (f) NHEK viability after treatment with 2 % LBB (A), 10 % HBB (B), 10 % CB (C) or 10 % Grape (D) extract 10 min after transient UV-B (+). Statistically significant differences were determined by Dunnett's test (\*\* $P < 0.01$  vs. column c).

The total polyphenol concentrations in LBB, HBB, CB and grape extracts were  $851.2 \pm 5.8$ ,  $477.4 \pm 6.9$ ,  $450.2 \pm 12.6$  and  $460.3 \pm 9.0 \mu\text{g ml}^{-1}$ , respectively (Figure 4C). Therefore, the total polyphenol concentration in LBB extract was 1.78-, 1.89- and 1.85-fold greater than those in HBB ( $P$

< 0.01), CB ( $P < 0.01$ ) and grape ( $P < 0.01$ ) extracts, respectively. This concentration of total polyphenols is consistent with that of our previous study.<sup>16</sup> These combined results indicate that the concentrations of proanthocyanidins, total flavonoids and total polyphenols in the LBB extract were all substantially higher than those in the other three berry extracts.



**Figure 4.** Concentrations of (A) proanthocyanidins, (B) total flavonoids and (C) total polyphenols in LBB, HBB, CB and grape extracts. Statistically significant differences were determined by Dunnett's test (\*\* $P < 0.01$  vs. column LBB).

## DISCUSSION

In this study, we prepared water extracts from four dry berries, LBB, HBB, CB and grape. Although the four berry extracts efficiently absorb UV-B primarily,<sup>16</sup> they showed slight toxicity against NHEKs under UV-B (-) (Figure 3Aa, Ab-Da, Db). Of the four berry extracts, pretreatment of NHEKs with LBB extract attenuated UV-B-induced damage (Figure 3A). These results indicate that LBB extract confers protection against UV-B-induced damage to human epidermal cells despite its cell toxicity.

We showed that concentrations of proanthocyanidins, total flavonoids and total polyphenols in the LBB extract were higher than those in the other three berry extracts (Figure 4). Therefore, the concentrations of proanthocyanidins, total flavonoids and total polyphenols are positively correlated

with protection against UV-B-induced damage to NHEKs. In higher plants, flavonoids and proanthocyanidins are major UV-absorbing compounds.<sup>13-15</sup> Therefore, it is possible that flavonoids and proanthocyanidins in the LBB extract absorb UV-B irradiation, thereby protecting NHEKs from UV-B-induced damage. Proanthocyanidins also have high antioxidant and radical scavenging activity,<sup>20-22</sup> which is usually higher than that of vitamins C and E, the antioxidant and radical scavenging gold standards. It is conceivable that UV-B-induced ROS oxidize proanthocyanidins and this protects NHEKs from excess ROS, thereby attenuating UV-B-induced damage. To confirm the exact roles of proanthocyanidins and total flavonoids in protecting NHEKs from UV-B-induced damage, it will be necessary to analyze the effect of UV-B irradiation on NHEKs treated with these substances at concentrations similar to those found in LBB extract.

Alternatively, if the exact concentrations of proanthocyanidins and total flavonoids found in LBB extract are the cause to protect NHEKs from UV-B-induced damage, pretreatment of NHEKs with diluted LBB extract containing these substances at concentrations similar to those found in HBB, CB or Grape extract would not attenuate UV-B-induced damage. Such analysis might also provide useful information.

The LBB extract may contain other unidentified polyphenol compounds that attenuate UV-B-induced damage in NHEKs. We previously conducted an HPLC analysis of 51 polyphenols in the four berry extracts and showed that the most abundant compounds in LBB extract were caffeic acid, protocatechuic acid, syringic acid, vanillic acid and quercetin.<sup>16</sup> It is known that these compounds efficiently absorb UV-B light.<sup>23-25</sup> Caffeic acid is a nonflavonoid catecholic compound and is present in many plants.<sup>26</sup> Catecholic acids are reported to have anti-inflammatory, antimutagenic, antioxidant and anticarcinogenic activities.<sup>26</sup> Syringic acid and vanillic acid have been identified as antioxidants in medicinal mushroom (*Inonotus obliquus*).<sup>27</sup> Quercetin is one of the most abundant natural flavonoids and is a powerful antioxidant and metal ion chelator.<sup>24</sup> Thus, caffeic acid, syringic acid, vanillic acid and quercetin have verified antioxidant activities. It is possible that these five compounds contribute to the attenuation of UV-B-induced damage in NHEKs by a mechanism similar to that of proanthocyanidins as described above.

Of the four berry extracts, we showed that pretreatment of NHEKs with LBB extract attenuated UV-B-induced damage, although LBB extract has NHEK cell toxicity (Figure 3A). We also showed that pretreatment of cucumber (*Cucumis sativus* L.) cotyledons with LBB, HBB and CB extracts attenuated the damage induced by UV-B (+) without causing any side effects.<sup>16</sup> Thus, LBB extract was the most effective protector of NHEKs against UV-B(+), whereas LBB, HBB and CB extracts were the most effective protectors of cucumber cotyledons against UV-B(+). These results suggest that the compounds involved in protection from UV-B radiation might differ in plants and humans. Further work is required to identify the specific compounds involved in attenuating UV-B-induced damage and to determine the underlying mechanisms. Also, the precise nature of the compounds causing toxicity to NHEKs in LBB extract should be clarified. Alternatively, because the

ingestion of LBB and LBB extracts is not known to have toxic side effects, a patch test of LBB extract on the human skin should be conducted. This work has potential applications for developing a topical treatment to protect against UV-B radiation in humans.

## CONCLUSION

To search for natural materials that protect human skin from damage caused by UV-B irradiation, we prepared water extracts of the following four dry berries: LBB, HBB, CB and grape. Pretreatment of NHEKs with LBB extract significantly attenuated UV-B-induced damage. The concentrations of proanthocyanidins, total flavonoids and total polyphenols in LBB extract were higher than those in the other three berry extracts. These compounds might mediate the protection of NHEKs from UV-B-induced damage.

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