

**Anthocyanin-rich Fractions of Blackberry Extracts Reduce
UV-induced Free Radicals and Oxidative Damage in
Keratinocytes**

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6 **Oxidative Damage in Keratinocytes**
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45 Running title: Blackberry Extracts Reduce UV Oxidative Damage in Keratinocytes
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ABSTRACT

Hull blackberries were purified using solid phase extraction to obtain anthocyanin-rich methanol fractions. The ability of these fractions to protect primary keratinocytes against UV-induced oxidative damage was assessed. Anthocyanin-rich methanol fractions derived from either blackberry powder or puree exhibited strong anti-oxidant properties, protecting against UV-induced ROS nearly as efficiently as N-acetyl cysteine. Furthermore, the fractions up-regulated expression of catalase, MnSOD, Gpx1/2 and Gsta1 antioxidant enzymes. Thus, we conclude that blackberry extracts may protect keratinocytes against UV-mediated oxidative damage.

KEYWORDS: antioxidant, blackberries, skin, ultraviolet

INTRODUCTION

Naturally-occurring UV radiation is an abundant environmental mutagen responsible for acute and chronic skin pathologies such as age-related degeneration and malignancy (Elwood and Jopson, 1997). UV promotes damage by direct interaction with DNA to cause formation of mutagenic photodimers as well as by generation of free radicals which subsequently damage a range of macromolecules including lipids, proteins and DNA (Marrot and Meunier, 2008). Mutagenesis, carcinogenesis and photoaging can all occur by UV-mediated oxidative DNA adduct formation (Nishigori, 2006).

Dietary phenolics and anthocyanins are multifunctional antioxidants (Seeram and Nair, 2002) that can act as reducing agents, hydrogen-donating antioxidants or single oxygen (free-radical) quenchers (Rice-Evans et al., 1996). Anthocyanins are among the most important natural antioxidant compounds (Prior and Wu, 2006). Blackberries rank among the highest of all natural foods in anthocyanins (Halvorsen et al., 2006) and blackberry extracts have been reported to scavenge peroxynitrite (Bagchi et al., 2004). We previously identified at least six distinct anthocyanins in such extracts (cyanidin-3-glucoside, cyanidin-3-arabinoside, delphinidin-3-xyloside, cyanidin-3-xyloside, cyanidin-3-malonylglucoside, and cyanidin-3-dioxalylglucoside) (Dai et al., 2009; Dai et al., 2007). Here, we describe the further isolation and characterization of anthocyanin-rich fractions of blackberry extracts and report their ability to mitigate UV-induced free radical and oxidative injury in primary epidermal keratinocytes.

MATERIALS AND METHODS

Plant material, preparation and fractionation of blackberry extracts. Hull blackberries (*Rubus eubatus* cv. "Hull") were grown at WindStone Farms (Paris, Kentucky). Seeds and skin were removed using a Langsenkamp type 161 Colossal Pulper and the resultant puree was either lyophilized and ground into a powder or processed without lyophilization. Ethanol extracts were obtained from the powder or puree (Dai et al., 2007) and fractionated by solid phase extraction (Skrede et al., 2000). Puree-derived (pH 2.8) or powder-derived (pH 2.0) extracts were applied to Discovery DSC-18 tubes (Supelco, Bellefonte, PA) and fractions were eluted sequentially with water, ethyl acetate and finally 50% aqueous methanol. The water fraction was lyophilized and reconstituted in PBS as a stock solution. The ethyl acetate and methanol fractions were dried by a rotary evaporator and reconstituted in DMSO as stock solutions. All fractions were stored at -80°C.

Chemical Characterization of Blackberry Extracts and Their Fractions. Monomeric anthocyanin content (Giusti and Wrolstad, 2001) and total anthocyanin content were calculated using an extinction coefficient of $26900 \text{ L cm}^{-1} \text{ mg}^{-1}$ and a molecular weight of 449.2 g/mol of cyanidin 3-glucoside (dominant anthocyanin). Total phenolic content was estimated using the Folin-Ciocalteu method for total phenols (Singleton, 1965). The Trolox equivalent antioxidant capacity (TEAC) assay was carried out as described (Re et al., 1999) and a standard curve was generated based on the percentage of inhibition of the blank absorbance by Trolox versus concentration. Total antioxidant capacity was calculated as Trolox equivalent (TE) based on percentage inhibition by samples.

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3 **Cell Culture.** Primary keratinocytes were isolated from the epidermises of neonatal C57Bl/6
4 mice (Tong and Marcelo, 1983). Briefly, skin harvested from sacrificed pups was incubated
5 overnight (4°C) in a solution of dispase (5%). Epidermal sheets were separated and put into
6 Minimum Eagles medium with earl's BSS without calcium (EMEM) supplemented with
7 chelexed FBS, Pen/Strep/Fungizone, Glutamax, and 0.06 mM CaCl₂, and were trituted with a
8 sterile pipette. Cells were centrifuged and resuspended in fibroblast-conditioned LoCal media
9 supplemented with epidermal growth factor (2 ng/mL), hydrocortisone (0.4 µg/mL),
10 aminoguanidine nitrate (0.75 mM) and cholera toxin (10 nM).
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24 **Cell viability assay.** C57BL/6 primary keratinocytes were treated with indicated
25 concentrations of either the methanol fraction of puree- or powder-derived blackberry extract and
26 cell viability was assessed (48h) using the CellTiter-Glo Luminescent Cell Viability assay
27 (Promega Corp., Madison, WI; (Crouch et al., 1993) using a Biotek Synergy 2 Microplate
28 Reader. Cell viability was calculated as percentage of the luminescence of corresponding vehicle
29 controls.
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41 **UV irradiation and measurement of intracellular ROS.** Primary keratinocytes were
42 irradiated with the indicated doses of UVB (15W T8.UVB mercury lamps; UV Products).
43 Immediately after irradiation cells were washed, fresh media was added and cells were incubated
44 (37°C 5% CO₂) for the indicated amount of time with the indicated amount of methanol fractions
45 of blackberry extracts.
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Measurement of free radicals and oxidative species. Intracellular ROS and superoxide ion production were measured by 5-(and-6)- Chloromethyl-2', 7'-dichlorodihydrofluorescein-diacetate acetyl ester (CM-H₂DCFDA) and Dihydroethidium (DHE) molecular probes respectively (Promega) per manufacturer's instructions. After loading with 10 μM of CM-H₂DCFDA or 5 μM DHE (30-45 min, 37°C), cells were washed in PBS and analyzed via flow cytometry (Becton Dickinson FACSCalibur). ROS and Superoxide levels were measured by mean fluorescence intensity at 519 nm and 565 nm respectively (Curtin et al., 2002).

qPCR. RNA was isolated from the cells (RNeasy kit; Qiagen). For each reaction, 1 μg RNA was converted to cDNA using qScript cDNA Supermix from QuantaBiosciences Inc. Multiplex quantitative PCR was run on a Lightcycler 480 machine (Roche) using β-actin as a reference gene. UPL probes and primers for catalase (F-ccttcaagttggtaatgcaga, R-caagttttgatgccctggt); Gpx1/2 (F-tttcccgtgcaatcagttc, R-tcggacgtacttgaggaat), MnSOD (F-gaccattgcaaggaacaa and R-gtagtaagcgtgctcccac) and Gsta1 (F-cttctgaccctttccctct, R-gctgccaggctgtagaac) were used. To calculate fold change of the gene over untreated sample, a comparative analysis method was used ($\delta C_T = C_T (\text{target}) - C_T (\text{reference})$ and $2^{-(\delta C_T (\text{treatment}) - \delta C_T (\text{untreated}))}$).

Statistical analysis. Mean ± standard error (SE) were calculated in triplicate for each assay. Statistical analysis was done using the student t-test (Analyze-it software). Differences were considered significant if $p \leq 0.05$.

Results and Discussion

A solid phase extraction (SPE) method was developed to isolate phenolic compounds from non-phenolic compounds in both puree and powder-derived extracts of Hull blackberries. Fractions were characterized in terms of total anthocyanin and total phenolic content, as well as total antioxidant capacity (TAC) by Trolox equivalent antioxidant capacity (TEAC) method. The efficiency of the SPE method employed was also evaluated by the percentages of solid recoveries. The water fraction, which contained the majority of total substances in blackberry extracts, was comprised mainly of non-phenolic compounds (Table 1). In contrast, most phenolics were concentrated in the ethyl acetate and methanol fractions with most of the anthocyanins obtained in the methanol fraction ($97.33 \pm 3.16\%$ of yield). Correspondingly, these fractions possessed significantly higher TAC than either the water fraction or the original extracts (Table 1). Similar results were also found in fractions of powder-derived extracts (Table 2). Thus, we conclude that blackberry extracts can be fractioned using water, methanol and ethyl acyate, and that ethyl acetate concentrates phenolics whereas methanol concentrates anthocyanins.

We were next interested in the ability of the anthocyanin-rich methanol fractions to protect epidermal keratinocytes against UV-mediated oxidative injury. To begin, we compared the toxicity of each of the methanol fractions (obtained either from the blackberry puree or powder) on primary keratinocytes derived from C57Bl/6 mice both by direct observation (Fig. 1A) and by the CellTiter-Glo® Luminescent Cell Viability assay (Promega Corp., Madison, WI) (Fig. 1B) which quantifies number of metabolically active cells by generating a luminescent signal proportional to the amount of ATP present (Crouch et al., 1993). Between these two methods, we calculated the median lethal dose LD_{50} for the methanol fractions of puree and powder-derived

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3 extracts to be approximately 125 and 275 $\mu\text{g}/\text{mL}$ respectively. Primary murine keratinocytes
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5 may be much more resistant to these agents than certain cancer cell lines (e.g. HL-60 human
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7 promyelocytic leukemia cells), which were found to have LD_{50} 's in the range of 50 $\mu\text{g}/\text{mL}$ (Dai
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9 et al., 2009). Whether this difference is inherently due to dissimilar sensitivities between cell
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11 types (keratinocyte vs. promyelocyte), between neoplastic status (benign vs. malignant) or
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13 between species (mouse vs. human) is unknown and remains to be determined. Nonetheless,
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15 others have reported that anthocyanins may have differential effects and toxicities in different
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17 cell types (Halliwell, 2008).
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22 UV exposure causes oxidative stress in skin by increasing generation of free radicals and
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24 reactive oxygen species (Hanson and Clegg, 2002). Since anthocyanins and phenols have each
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26 been reported to have high antioxidant capacities. (Garzon et al., 2009) we investigated the
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28 ability of the methanol fractions of blackberry extracts to reduce UV-mediated free radical
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30 formation in primary keratinocytes. To that end, primary keratinocytes were exposed to either 0
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32 or 1000 J/m^2 UV-B, a sub-lethal dose of UV-B known to induce free radical formation in
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34 primary keratinocytes as detected by CM- H_2DCFDA fluorescence (Fig. 2). CM- H_2DCFDA is a
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36 cell-permeable indicator of ROS that remains non-fluorescent until oxidation occurs within the
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38 cell and the acetate groups are removed by intracellular esterases (Curtin et al., 2002). We
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40 found that 1000 J/m^2 caused a significant ($p < 0.05$) increase in the amount of cellular ROS in
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42 the keratinocytes (Fig. 2). Furthermore, we found that methanol fraction of puree-derived
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44 blackberry extract (30 $\mu\text{g}/\text{mL}$ total which contained 5.0 $\mu\text{g}/\text{mL}$ anthocyanins and 15.4 $\mu\text{g}/\text{mL}$
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46 phenols) added to the cells either at 2 h or at 6 h post UV-B significantly reduced ROS in UV-
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48 irradiated primary keratinocytes (Fig. 2). When the blackberry extract was added at 24 h post-
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50 irradiation, its protective effect seemed to be lost, suggesting that its ability to scavenge free
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3 radicals occurs soon after irradiation. Nonetheless, the data reproducibly showed a reduction in
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5 UV-induced free radical accumulation in primary keratinocytes treated with methanol fractions
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7 of blackberry extracts.
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10 We next tested the ability of the blackberry extracts to reduce UV-induced ROS in a dose
11 dependent manner. For these experiments, N-acetyl cysteine (NAC), a thiol with potent reductive
12 capacity, was used as a positive anti-oxidative control. Since prior kinetic trials (Fig. 2)
13 suggested that the maximal protection occurred by adding the fraction 6 h after irradiation, we
14 chose that time point for each of the methanol fractions. Primary keratinocytes were exposed to
15 sub-lethal UV-B radiation, blackberry fractions (or NAC) were added after 6 h and ROS were
16 quantified by CM-H₂DCFDA fluorescence. Again, UV exposure prompted a significant increase
17 in cellular ROS accumulation ($p < 0.05$), and the methanol fraction of puree-derived blackberry
18 extract significantly reduced ROS levels, essentially to the same level as 5 μ M NAC (Fig. 3A).
19 The methanol fraction of powder-derived extract also reduced ROS caused by UV radiation (Fig.
20 3C), though not to the same degree as that derived from the puree. To investigate the effect of the
21 methanol fractions on a specific free radical ion, the superoxide ion, we used DHE staining.
22 Similar to CM-H₂DCFDA whose fluorescence correlates with total cellular ROS, DHE is a cell-
23 permeable molecular probe that fluoresces upon exposure to the superoxide ion. In contrast to
24 the previous results with total cellular ROS, we found that the methanol fraction did not
25 significantly affect levels of UV-induced superoxide formation (Fig. 3B, D). Interestingly, NAC
26 also showed a modest ability to affect superoxide levels, consistent with prior reports (Aruoma et
27 al., 1989). Thus, we conclude that the methanol fractions of both blackberry puree and powder-
28 derived blackberry extracts inhibited ROS other than the superoxide ion.
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3 To test the hypothesis that the blackberry extracts might exert antioxidative effects by
4 inducing antioxidant enzymes, the extracts were tested for their ability to induce mRNA of
5 catalase, manganese superoxide dismutase (MnSOD), glutathione S-transferase A1 (Gsta1) and
6 glutathione peroxidase (Gpx1/2) (Steenvoorden and Beijersburgen van Henegouwen, 1998).
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8 Primary keratinocytes were exposed to the methanol fractions for 6 h or 18 h and mRNA levels
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10 of each of these enzymes were measured by qPCR (Fig. 4). Up-regulation of each of these
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12 enzymes by the methanol fraction of puree-derived (Fig. 4A, C, E, G) or of powder-derived (Fig.
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14 4B, D, F, H) blackberry extract was detected at one or both time points. These results agree with
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16 published reports that suggest anthocyanin-mediated enhancement of catalase, MnSOD, GSTp1,
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18 glutathione reductase and glutathione peroxidase (Hou et al, 2010).
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29 Together, these data suggest that components present in blackberry extracts can reduce UV-
30 mediated oxidative injury in skin keratinocytes and suggest a novel UV-photoprotective
31 approach. There is precedent for berry extracts mitigating the effects of UV in the skin. Topical
32 treatment of mice with raspberry extracts, for example, protected against UV-induced
33 inflammation, oxidative DNA damage and carcinogenesis (Duncan et al., 2009). Gingerol,
34 another plant phenol, reduced UV-induced ROS production and inhibited TPA-mediated tumor
35 promotion in mouse skin (Kim et al., 2007). Lastly, *Grewia asiatica* fruit decreased lipid
36 peroxidation in gamma-irradiated mice (Sharma and Sisodia, 2009). We conclude that
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38 blackberries contain potent antioxidant natural compounds with functional capacity in skin-
39 derived keratinocytes. Our data provide rationale for the development of anthocyanin-based
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41 compounds designed to protect the skin from the harmful oxidative effects of ultraviolet
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For Peer Review

Figure Legends

Figure 1. Determination of cytotoxicity of methanol fractions of blackberry extracts on primary murine keratinocytes. Primary murine keratinocytes were purified and cultured. Cells were treated with the indicated doses of methanol fraction of puree-derived (Puree MF) or powder-derived (Powder MF) extract. (A) Representative photomicrographs of cellular morphology (phase contrast, 10x) after 24h. (B) Mean cellular survival assay (CellTiter-Glo Luminescent Cell Viability assay) \pm SE.

Figure 2. Effect of methanol fractions of blackberry extracts on UV-mediated free radical levels in primary keratinocytes. Primary murine keratinocytes were irradiated with UVB (1000 J/m²) and methanol extracts of puree-derived blackberry extract (30 ug/mL) were added 2, 6 or 24 h after irradiation. At 48 h post-irradiation, cells were loaded with CM-H₂DCFDA and mean fluorescence intensity (MFI \pm SE) was quantified by flow cytometry. The figure is representative three independent experiments. * = $p < 0.05$.

Figure 3. Methanol fractions of blackberry extracts reduce UV-induced reactive oxygen species. Primary keratinocytes were exposed to either 0 or 1000 J/m² UV radiation. After 6 h, cells were treated either vehicle control, NAC or the indicated concentrations of methanol fractions of puree-derived (Puree MF) or powder-derived (Powder MF) blackberry extract. Total reactive oxygen species (A, C) and superoxide ion (B, D) were quantified by flow cytometry (at 48h) using CM-H₂DCFDA and DHE respectively. Results are shown as MFI \pm SE, * = $p < 0.05$. The figure is representative of two independent experiments.

Figure 4. Effect of methanol fractions of blackberry extracts on antioxidant enzymes.

Primary keratinocytes were treated with the indicated concentrations of methanol fractions of puree-derived (Puree MF) or powder-derived (Powder MF) extract for either 6 h (A, C, E, G) or 18 h (B, D, F, H). Vehicle control and UV irradiated (1000 J/m^2) controls were included. mRNA levels for the antioxidant enzymes catalase (A, B), manganese superoxide dismutase (MnSOD; C, D), glutathione peroxidase (GPX1/2; E, F) and glutathione S-transferase A1 (Gsta1; G, H) were calculated. GAPDH expression was used to normalize values, and results are expressed as mean fold induction (over control) \pm SE, * = $p < 0.05$. This figure is representative of two independent experiments with three replicates per experiment.

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16 **Conflict-of-Interest Statement.** Four Tigers LLC. has an exclusive license from the
17 University of Kentucky to parts of the technology described in the manuscript.
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Table 1. Composition and Characterization of Fractions Isolated from Puree-Derived Blackberry Extract by solid phase extraction ^a

Fraction	Water	Ethyl acetate	Methanol	Total
Total Anthocyanin ^b				
Weight of total solid recovered (mg)	953.07 ± 9.86	8.06 ± 0.50	28.46 ± 0.63	989.59 ± 11.32
Total anthocyanin in recovered fraction (mg)	ND	0.12 ± 0.01	4.90 ± 0.16	5.02 ± 0.17
Anthocyanin level in recovered fraction (mg/g solid)	ND	14.77 ± 0.75	165.59 ± 5.07	-
Yield (%) [#]	-	2.53 ± 0.28	97.33 ± 3.16	99.86 ± 3.40
Fold concentrated in recovered fraction [*]	-	2.94	32.92	-
Total Phenolics ^c				
Weight of total solid recovered (mg)	953.07 ± 9.86	8.06 ± 0.50	28.46 ± 0.63	989.59 ± 11.32
Total phenolics in recovered fraction (mg)	1.85 ± 0.42	3.79 ± 0.46	14.91 ± 0.62	20.55 ± 0.96
Phenolics level in recovered fraction (mg/g solid)	1.93 ± 0.31	440.35 ± 27.92	512.83 ± 4.22	-
Yield (%) [#]	9.19 ± 2.10	18.87 ± 2.29	74.20 ± 3.09	102.67 ± 4.78
Fold concentrated in recovered fraction [*]	0.09	21.89	25.51	-
Total Antioxidant Capacity				
Total antioxidant capacity Level in recovered fraction (μmol TE/g solid)	14.98 ± 3.12	2734.61 ± 96.77	4838.20 ± 236.96	-
Fold concentrated in recovered fraction [*]	0.07	13.60	24.06	-

^a The solid phase extraction separation was repeated (3x) with the same ethanol extract sample. Volume of ethanol extract stock solution applied to the solid phase extraction cartridge contained 1g dried extract. All assays carried out in triplicate. Data expressed as mean ± SE (N = 3).

^b Total anthocyanin expressed as cyanidin 3-glucoside equivalent.

^c Total phenolics expressed as gallic acid equivalent.

[#] Calculated as percentage of total anthocyanin (phenolics) in ethanol extract added to solid phase extraction cartridge.

^{*} Calculated as fold change that anthocyanin (phenolics, Total antioxidant capacity) are concentrated in recovered solid fraction as compared to original level in ethanol extract added to the solid phase extraction cartridge.

ND: not detected.

Table 2. Composition and Characterization of Fractions Isolated from Puree-Derived Blackberry Extract by solid phase extraction ^a

Fraction	Water	Ethyl acetate	Methanol	Total
Total Anthocyanin ^b				
Weight of total solid recovered (mg)	763.56 ± 43.10	7.12 ± 0.93	13.09 ± 1.05	783.77 ± 43.01
Total anthocyanin in recovered fraction (mg)	ND	0.036 ± 0.006	3.57 ± 0.38	3.60 ± 0.37
Anthocyanin level in recovered fraction (mg/g solid)	ND	5.65 ± 1.36	305.74 ± 33.56	-
Yield (%) [#]	-	0.77 ± 0.13	76.02 ± 8.03	76.79 ± 7.91
Fold concentrated in recovered fraction [*]	-	1.19	65.18	-
Total Phenolics ^c				
Weight of total solid recovered (mg)	763.56 ± 43.10	7.12 ± 0.93	13.09 ± 1.05	783.77 ± 43.01
Total phenolics in recovered fraction (mg)	0.64 ± 0.32	1.67 ± 0.22	5.32 ± 0.52	7.63 ± 0.37
Phenolics level in recovered fraction (mg/g solid)	0.95 ± 0.053	253.29 ± 33.59	455.01 ± 27.92	-
Yield (%) [#]	4.50 ± 3.67	16.91 ± 2.27	53.87 ± 5.30	77.22 ± 4.57
Fold concentrated in recovered fraction [*]	0.10	25.66	46.10	-
Total antioxidant capacity				
Total antioxidant capacity Level in recovered fraction (μmol TE/g solid)	16.61 ± 5.60	1614.26 ± 303.38	4739.90 ± 624.78	-
Fold increase in recovered fraction [*]	0.22	21.61	63.45	-

^a The solid phase extraction separation was repeated (3x) with the same ethanol extract sample. Volume of ethanol extract stock solution applied to the solid phase extraction cartridge contained 1g dried extract. All assays carried out in triplicate. Data expressed as mean ± SE (N = 3).

^b Total anthocyanin expressed as cyanidin 3-glucoside equivalent.

^c Total phenolics expressed as gallic acid equivalent.

[#] Calculated as percentage of total anthocyanin (phenolics) in ethanol extract added to the solid phase extraction cartridge.

^{*} Calculated as fold change that anthocyanin (phenolics, Total antioxidant capacity) are concentrated in the recovered solid fraction as compared to original level in the ethanol extract added to solid phase extraction cartridge.

ND: not detected.

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Murapa and Dai, et. al. Fig. 1

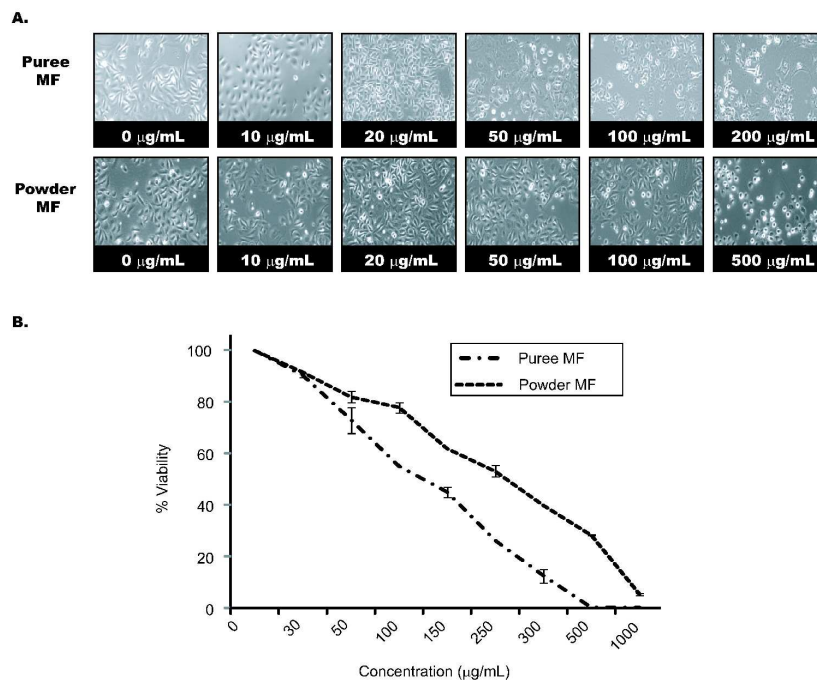


Figure 1. Determination of cytotoxicity of methanol fractions of blackberry ethanol extracts on primary murine keratinocytes. Primary murine keratinocytes were purified and cultured. Cells were treated with the indicated doses of methanol fraction of puree-derived (Puree MF) or powder-derived (Powder MF) extract. (A) Representative photomicrographs of cellular morphology (phase contrast views, magnification 10x) at 24 h of exposure. (B) Mean cellular survival assay (CellTiter-Glo Luminescent Cell Viability assay, Promega) \pm SE.

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Murapa and Dai, et. al. Fig. 2

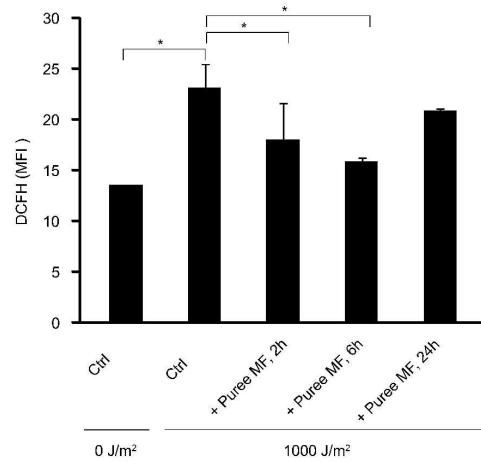


Figure 2. Effect of methanol fractions of blackberry ethanol extracts on UV-mediated free radical levels in primary keratinocytes. Primary murine keratinocytes were grown until 80-90% confluent before being irradiated with UVB 1000 J/m². Methanol extracts of puree-derived blackberry extract (Puree MF, 30 µg/mL) were added 2 h, 6 h or 24 h after irradiation. At 48 h post-irradiation, cells were loaded with CM-H₂DCFDA and mean fluorescence intensity (MFI) which measures cellular free radical level was quantified by flow cytometry. Results are shown as mean MFI ± SE. The figure is representative three independent experiments. * = $p < 0.05$.

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Murapa and Dai, et. al. Fig. 3

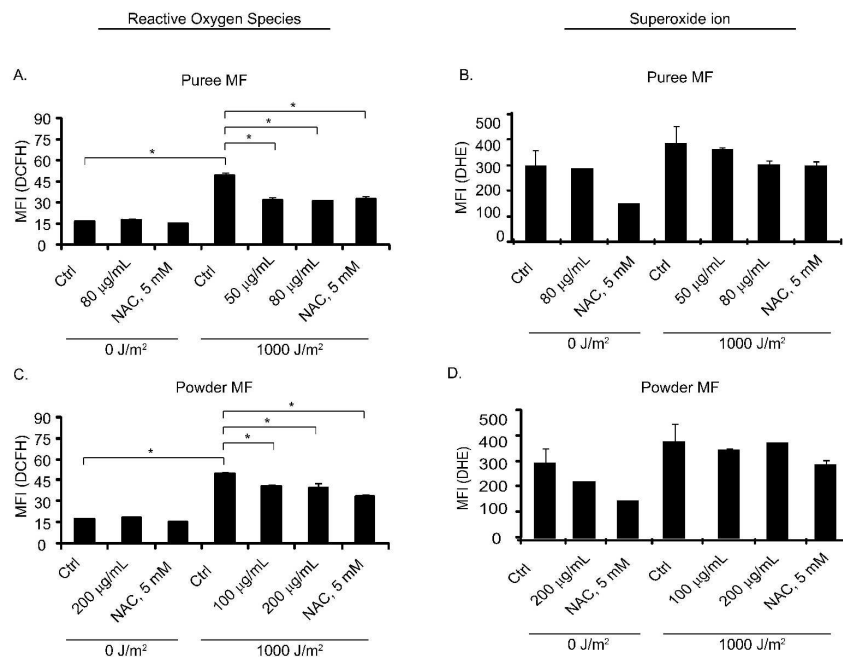


Figure 3. Methanol fractions of blackberry extracts reduce UV-induced reactive oxygen species. Primary keratinocytes were exposed to either 0 or 1000 J/m² UV radiation. After 6 h, either vehicle control, NAC or the indicated concentrations of methanol fractions of puree-derived (Puree MF) or powder-derived (Powder MF) blackberry extract were added to the cells. Total reactive oxygen species (A, C) and superoxide ion (B, D) were quantified at 48 h by flow cytometry using CM-H₂DCFDA (10 µM) and DHE (5 µM) respectively 48 h post irradiation. Results are shown as mean MFI ± SE, * = *p* < 0.05. The figure is representative of two independent experiments.

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Murapa and Dai, et. al. Fig. 4

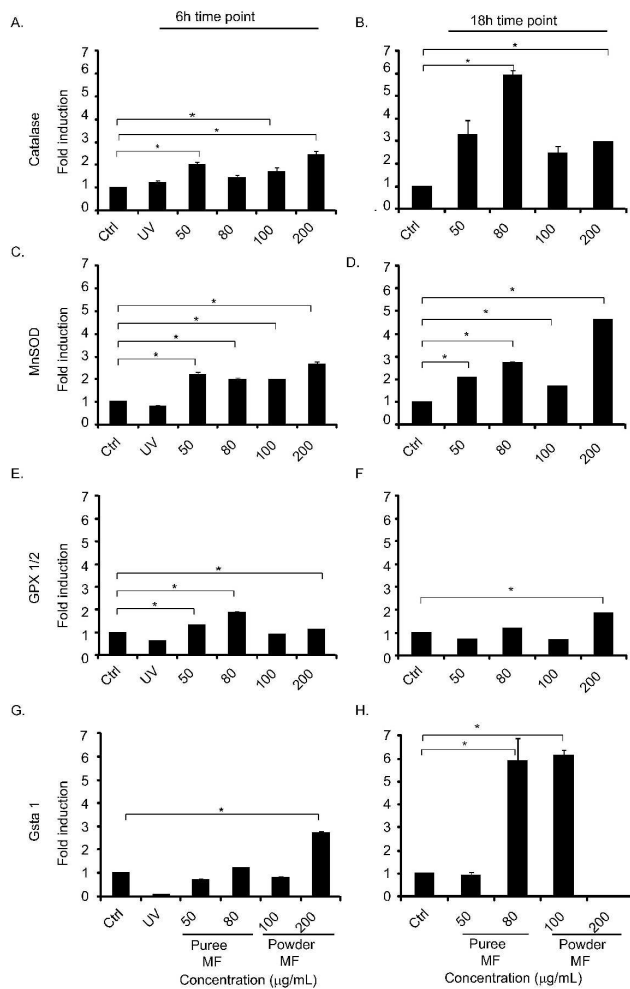


Figure 4. Effect of methanol fractions of blackberry ethanol extracts on antioxidant enzymes. Primary keratinocytes in logarithmic growth phase were exposed to the indicated concentrations of methanol fractions of puree-derived (Puree MF) or powder-derived (Powder MF) extract for either 6 h (A, C, E, G) or 18 h (B, D, F, H). Vehicle control and UV irradiated (1000 J/m²) controls were included. mRNA levels for the antioxidant enzymes catalase (A, B), manganese superoxide dismutase (MnSOD; C, D), glutathione peroxidase (GPX1/2; E, F) and glutathione S-transferase A1 (Gsta1; G, H) were calculated. GAPDH expression was used to normalize values, and results are expressed as mean fold induction (over control) ± SE. * is p < 0.05. This figure is representative of two independent experiments with three replicates within each experiment.

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