

The Protective Effects of Unripe Apple (*Malus pumila*) Extract on Ultraviolet B-Induced Skin Photoaging Mouse Model

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Supplementary materials and methods

1. High-Performance Liquid Chromatography (HPLC) Analysis

HPLC analysis was performed using Agilent HPLC system (Agilent, Waldbronn, Germany). Samples were dissolved with 70% methanol and water mixture and 10 mL of the samples were injected into the HPLC instrument, equipped with Phenomenex Luna phenyl-hexyl HPLC C18 columns (5mm, 4.6×250 mm, Torrance, CA, USA). The column temperature was 25°C, the flow rate was 0.8 mL/min, and running time was 40 min. The mobile phase consisted of 0.2% acetic acid in distilled water (A) and 0.2% acetic acid in acetonitrile (B). HPLC gradient conditions were as follows: 0 min (A 95%, B 5%), 30 min (A 60%, B 40%), and 40 min (A 5%, B 95%). The detection wavelength was set at 280 nm.

2. Real-time polymerase chain reaction (PCR)

Dorsal back skin tissues were homogenized by using taco™ Prep Bead Beater (GeneReach Biotechnology Corp.). Isolation of total RNAs, cDNA synthesis, real-time PCR, and relative quantification were performed as previously described[1,2]. Primer sequences are listed in Supplementary Table 1. Data were represented mean ± SD of ten mice group, relative to intact control/ β -actin.

Supplementary references

1. Jegal, K.H.; Kim, E.O.; Kim, J.K.; Park, S.M.; Jung, D.H.; Lee, G.H.; Ki, S.H.; Byun, S.H.; Ku, S.K.; Cho, I.J.; et al. Luteolin prevents liver from tunicamycin-induced endoplasmic reticulum stress via nuclear factor erythroid 2-related factor 2-dependent sestrin 2 induction. *Toxicol Appl Pharmacol* **2020**, *399*, 115036, doi:10.1016/j.taap.2020.115036.
2. Kim, Y.I.; Oh, W.S.; Song, P.H.; Yun, S.; Kwon, Y.S.; Lee, Y.J.; Ku, S.K.; Song, C.H.; Oh, T.H. Anti-Photoaging Effects of Low Molecular-Weight Fucoidan on Ultraviolet B-Irradiated Mice. *Mar Drugs* **2018**, *16*, doi:10.3390/md16080286.

Table S1. UAE preparation procedure.

Manufacture process		phloridzin (mg/g)	weight (kg)
raw material	unripe apple (immature fruit of <i>Malus pumila</i> Mill., harvested at 55~65 day after full bloom)	0.007	100
↓			
squeezing	15.5 brix	0.03	23.75
↓			
filtering			
↓			
concentrating	70 brix	0.13	5.25
↓			
drying	spray dry, add dextrin	0.06	12
↓			
test material	Unripe apple extract (UAE)	0.06	12

Table S2. Oligonucleotides sequences used in the present study.

Gene name	5' – 3'	Sequence
<i>COL1A1</i>	Sense	GCGGTAACGATGGTGCTGTT
	Antisense	CTTCACCCTTAGCACCAAC
<i>COL1A2</i>	Sense	ATTGTCGCCAGTGAG
	Antisense	CTGGTCCTGCTGGT
<i>HAS1</i>	Sense	GCATGGGCTATGCTACCAAGTAT
	Antisense	AGGAGGGCGTCTCCGAGTA
<i>HAS2</i>	Sense	GACCCTATGGTTGGAGGTGTTG
	Antisense	ACGCTGCTGAGGAAGGAGATC
<i>HAS3</i>	Sense	AGACCGAGCTAGCCTTCCTAGT
	Antisense	TAATGGCCAGATACAGCATGAG
<i>MMP1</i>	Sense	AAGGTTAGCTTACTGTCACACGCTT
	Antisense	CGACTCTAGAAACACAAGAGCAAGA
<i>MMP9</i>	Sense	CCCGGACCAAGGATACAG
	Antisense	GGCTTTCTCTCGGTACTG
<i>MMP13</i>	Sense	CATCCATCCCGTGACCTTAT
	Antisense	GCATGACTCTCACAATGCGA
<i>Glutathione reductase</i>	Sense	TGCGTGAATGTTGGATGTGTACCC
	Antisense	CCGGCATTCTCCAGTTCCTCG
<i>Nox2</i>	Sense	AGCTATGAGGTGGTGATGTTAGTGG
	Antisense	CACAATATTTGTACCAGACAGACTTGAG
<i>TGF-β1</i>	Sense	GCAACATGTGGA ACTCTACCAGAA
	Antisense	GACGTCAAAAGACAGCCACTCA
<i>p38 MAPK</i>	Sense	CGTTGTTTCCTGGTACAGACC
	Antisense	CCATTCTTCTTGGTCAAGGG
<i>Protein kinase B (AKT)</i>	Sense	TACTCATTCCAGACCCACGA
	Antisense	GAGGTTCTCCAGCTTCAGGT
<i>β-actin</i>	Sense	AGCTGCGTTTTACACCCTTT
	Antisense	AAGCCATGCCAATGTTGTCT

Nox2, *gp91phox* subunit of the phagocyte NADPH oxidase; TGF, Transforming growth factor; MAPK, Mitogen-activated protein kinase.

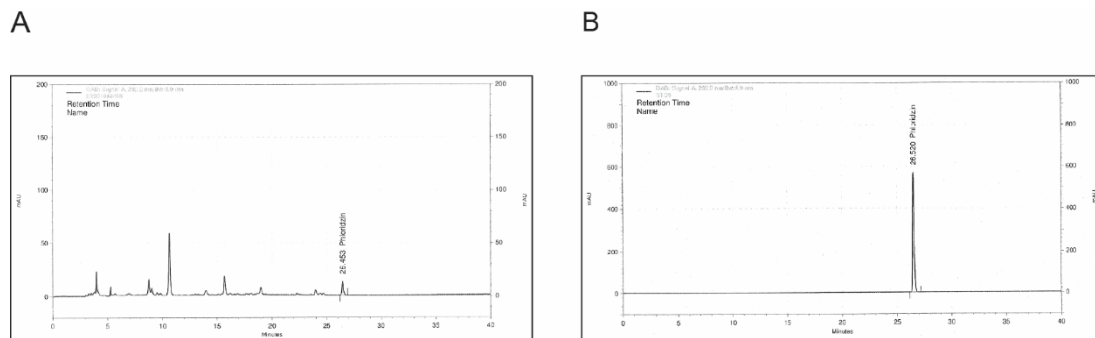


Figure S1. Identification of UAE by high-performance liquid chromatography (HPLC). (A) UAE chromatogram, (B) phloridzin standard chromatogram. HPLC analysis showed that one peak of UAE matched with phloridzin at retention times of approximately 26.453 min.

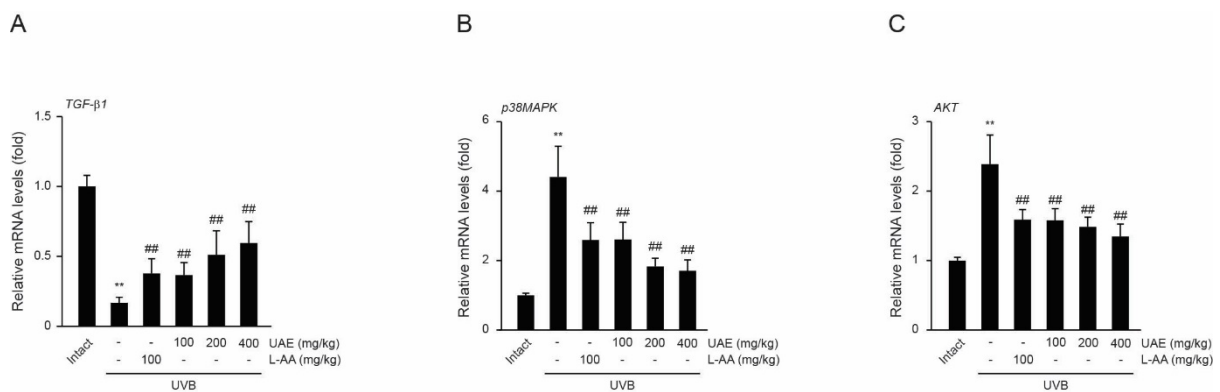


Figure S2. (A) *TGF-β1*, (B) *p38 MAPK*, and (C) *AKT* mRNA expression levels in dorsal back skin tissues were quantified by the real-time PCR. Data were expressed relative to intact/b-actin. Results are presented as mean \pm SD (n=10, significant difference *vs.* intact control; ** $p < 0.01$, *vs.* UVB-irradiated control mice; ## $p < 0.01$).