SUPPLEMENTARY DATA SHEETS

Protection of UVB-Induced Photoaging by Fuzhuan-Brick Tea Aqueous Extract via MAPKs/*Nrf*2-Mediated Down-Regulation of MMP-1

Peijun Zhao [1,†], Md Badrul Alam [1,2,†] and Sang-Han Lee [1,2,*]

[1]Department of Food Science and Biotechnology, Graduate School, Kyungpook National University, Daegu 41566, Republic of Korea
[2]Food and Bio-Industry Research Institute, Inner Beauty/Antiaging Center, Kyungpook National University, Daegu 41566, Republic of Korea
† These authors contributed equally to this work.

Running head: Attenuation of photo-aging by Fuzhuan-brick tea

Corresponding authors:

Dr. Sang Han Lee; E-mail: sang@knu.ac.kr

2. Materials and Methods

2.1. Drugs and chemicals

2,2-Diphenyl-1-picrylhydrazyl (DPPH), neucaprine, 2,20-azino-bis(3-ethylbenzothiazoline-6-sulphonic acid) (ABTS), 2',7'-dichlorofluorescin diacetate (DCFH-DA), 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT), and dimethylsulfoxide (DMSO) were purchased from Sigma Aldrich (St. Louis, MO, USA). Dulbecco's modified Eagle's medium (DMEM), fetal bovine serum (FBS), penicillin-streptomycin mixture (P/S), and 0.25% trypsin-EDTA were purchased from Gibco-BRL Life Technologies (Grand Island, NY, USA). All antibodies anti-SOD1, anti-CAT, anti-GPx-1, anti-HO-1, anti-MMP-1, anti-type I procollagen, and anti-*Nrf2* were purchased from Santa Cruz Biotechnology (Santa Cruz, CA, USA) as described in Table S2.

2.2. High-performance liquid chromatographic (HPLC) analysis

Phytochemical characterization of FBTA, including standard molecules (gallic acid, theaflavins, theobromine, epigallocathecin, caffeine, epicatechin and epigallocatechingallate (EGCG)) was performed using an auto-sampler HPLC (HPLC-DAD) setup (Shimadzu, Japan) supported with a diode array detector (SPD-M20A) and LC solution software (ver. 1.22 SP1). A 5 μm-diameter particles packed C18 column (4.6 × 250 mm) was used for reverse-phase chromatographic analysis. The following gradient solvent system [acetonitrile (A) and 1% formic acid (B)] was employed with change in ratio at every time (min); solvent A (10%) was run up to 10 min, followed by 30, 50, 60, 90, and 20% for 15, 20, 25, 30, 35, and 40 min, respectively ($\lambda = 280$ nm). The injection volume was 20 μL, and the flow rate was maintained at 0.8 mL/min [1]. The phenolic components were identified based on the retention time compared with those of standard compounds.

Table S2: List of the primer sets used in this study.

Gene name		***Sequences***
Hmox-1	*forward*	*ACGCATATACCCGCTACCTG*
	reverse	*TCCTCTGTCAGCATCACCTG*
Nrf-2	*forward*	*ACATCCTTTGGAGGCAAGAC*
	reverse	*GGGAATGTCTCTGCCAAAAG*
Gapdh	*Forward*	*TTGTGATGGGTGTGAACCAC*
	reverse	*ACACATTGGGGGTAGGAACA*

Table S2: List of antibodies used in this study.

Name	*Catalog number*	*Company*	*Antigen*	*Host*
Anti-SOD1	sc-101523	Santa Cruz Biotechnology, Inc.	SOD1	Mouse
Anti-CAT	sc-515782	Santa Cruz Biotechnology, Inc.	CAT	Mouse
Anti-GPx-1	sc-133160	Santa Cruz Biotechnology, Inc.	GPx-1	Mouse
Anti-HO-1	sc-136256	Santa Cruz Biotechnology, Inc.	HO-1	Mouse
Anti *Nrf2*	sc-81342	Santa Cruz Biotechnology, Inc.	*Nrf2*	Mouse
Anti-p-p38	sc-166182	Santa Cruz Biotechnology, Inc.	p38	Mouse
Anti-p38	BS3567	Bioworld Technology, Inc.	p38	Rabbit
Anti-p-ERK1/2	sc-7383	Santa Cruz Biotechnology, Inc.	ERK	Mouse
Anti-ERK1/2	BS 6472	Bioworld Technology, Inc.	ERK	Rabbit
Anti-p-JNK	BS 4322	Bioworld Technology, Inc.	JNK	Rabbit
Anti-JNK	sc-7345	Santa Cruz Biotechnology, Inc.	JNK	Mouse
Anti-MMP1	sc-21731	Santa Cruz Biotechnology, Inc.	MMP-1	Mouse

Anti-type I Procollagen	sc-376350	Santa Cruz Biotechnology, Inc.	COL1A2	Mouse
Anti-Lamin B	Sc-6217	Santa Cruz Biotechnology, Inc.	Lamin B	Goat
Anti-β actin	Sc-47778	Santa Cruz Biotechnology, Inc.	β-actin	Mouse

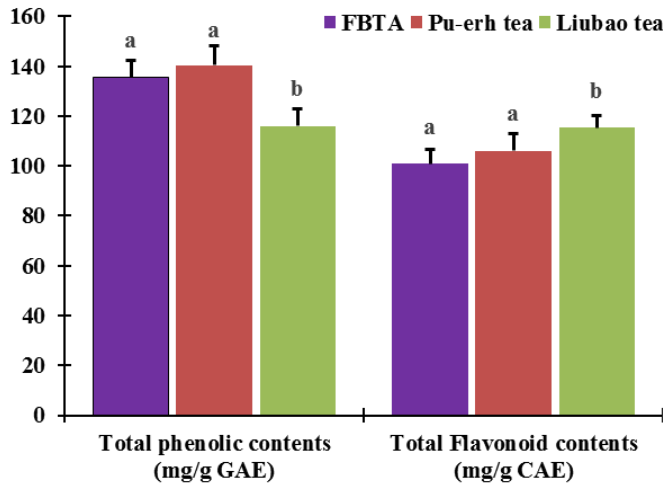

Figure S1. Total phenolic and flavonoid contents of the aqueous extract of Fuzhuan-brick tea, pu-erh tea and libao tea. The different letter in the same group indicate the difference between the teas is significant.

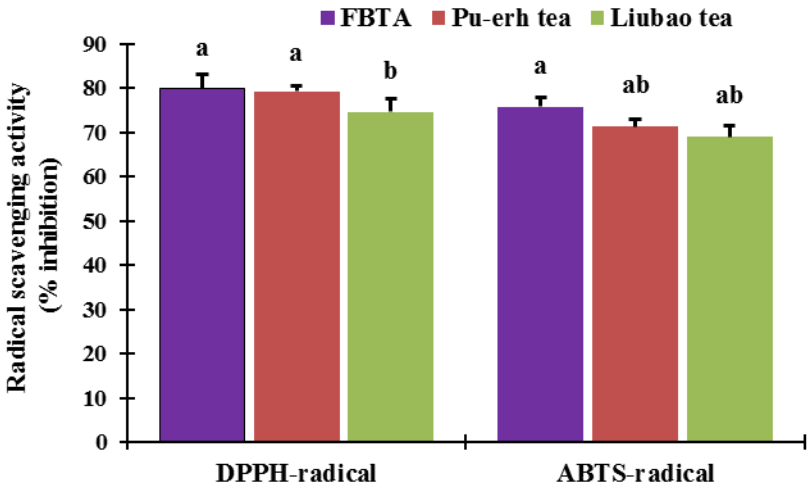

Figure S2. DPPH- and ABTS-radical scavenging activities of the aqueous extract of Fuzhuan-brick tea, pu-erh tea and libao tea. The different letter in the same group indicate the difference between the teas is significant.

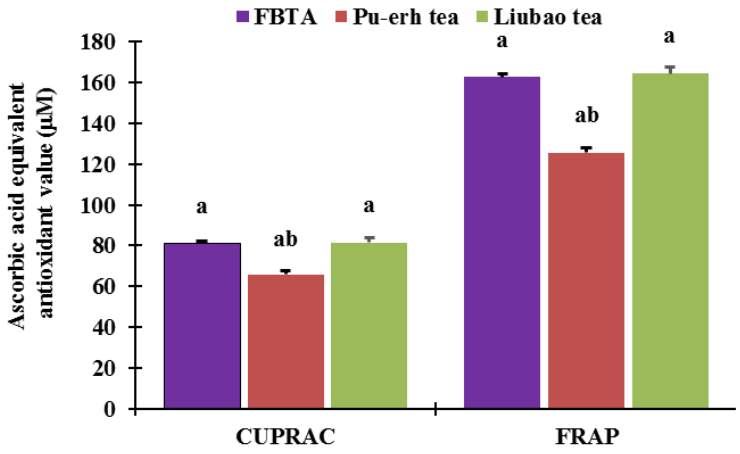

Figure S3. Reducing power activities of the aqueous extract of Fuzhuan-brick tea, pu-erh tea and libao tea in CUPRAC and FRAP assays. The different letters in the same group indicate the difference between the teas is significant.

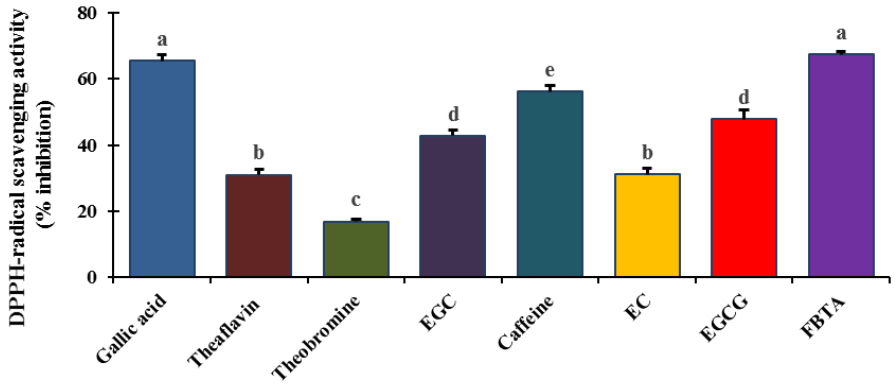

Figure S4. DPPH-radical scavenging activities of the aqueous extract of Fuzhuan-brick tea (FBTA) and the identified constituents at their putative concentrations in FBTA. The different letters indicate the difference between the constituents is significant ($p < 0.05$). The experimental concentration of gallic acid (10 μmol/L), theaflavins (2 μmol/L), theobromine (2 μmol/L), EGC (4 μmol/L), caffeine (15 μmol/L), EC (2 μmol/L), EGCG (2 μmol/L) and FBTA (100 μg/mL).

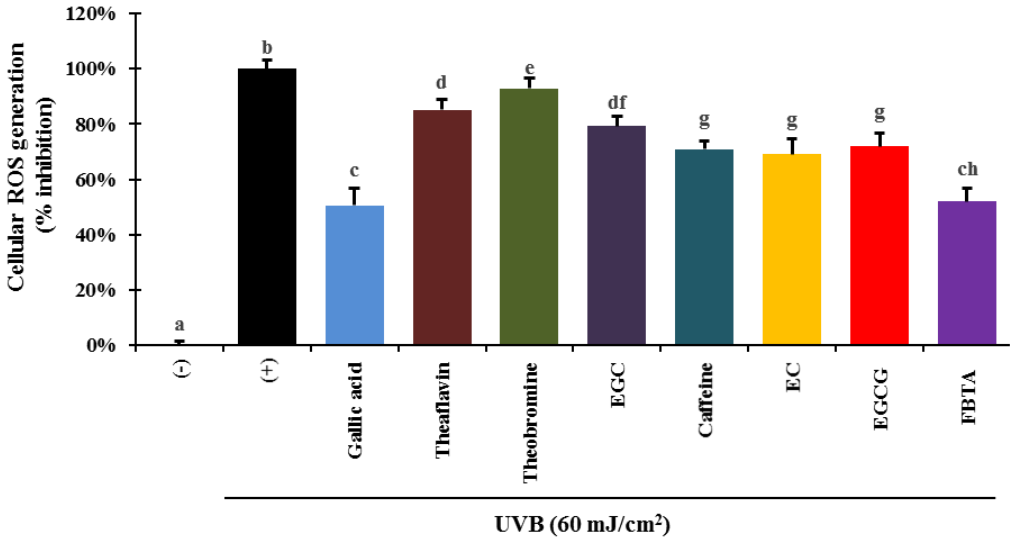

Figure S5. Cellular ROS quenching effects of the aqueous extract of Fuzhuan-brick tea (FBTA) and the identified constituents at their putative concentration in FBTA. The experimental concentration of gallic acid (10 μmol/L), theaflavins (2 μmol/L), theobromine (2 μmol/L), EGC (4 μmol/L), caffeine (15 μmol/L), EC (2 μmol/L), EGCG (2 μmol/L) and FBTA (100 μg/mL). Different letter of each column are significant ($p < 0.05$).

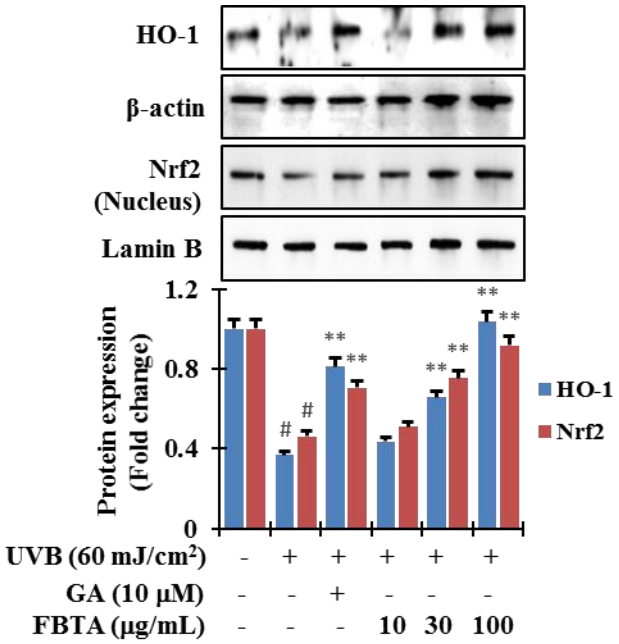

Figure S6. Effects of Fuzhuan-brick tea aqueous extract on *Nrf2* signaling. FBTA-pretreated HaCaT cells were exposed with UVB (60 mJ/cm^2), and the protein expressions of HO-1 and

Nrf2 were detected by western blotting. #$p < 0.01$, compared to the normal cells, **$p < 0.05$, compared to UVB-irradiated cells.

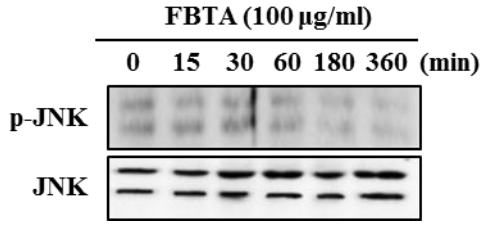

Figure S7. Effects of Fuzhuan-brick tea aqueous extract on the activation of JNK. HaCaT cells were treated with FBTA (100 μg/mL) for indicated time points and the activated and non-activated forms of JNK were identified by immunoblotting assay.

References

[1]. S.M. Brito, S.M.; Coutinho, H.D.; Talvani, A.; Coronel, C.; Barbosa, A.G.; Vega, C. Analysis of bioactivities and chemical composition of *Ziziphus joazeiro* Mart. using HPLC–DAD. Food Chem. **2015,** *186*, 185-191.