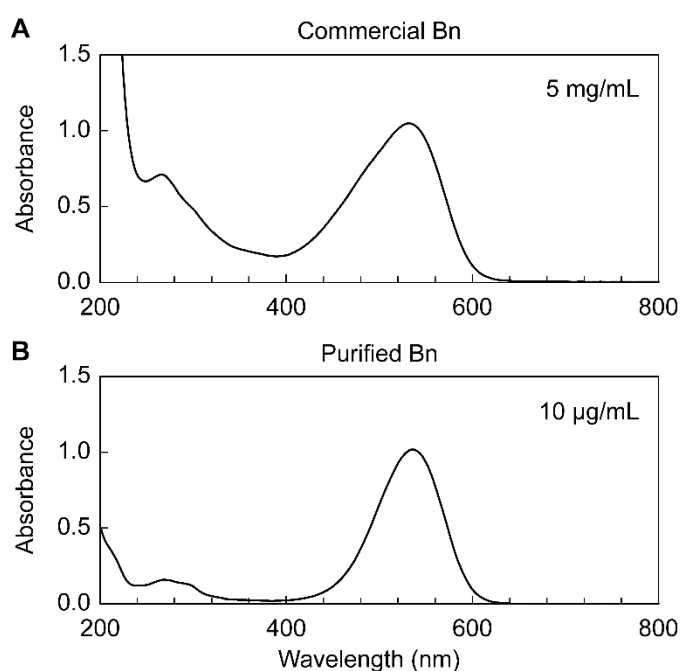


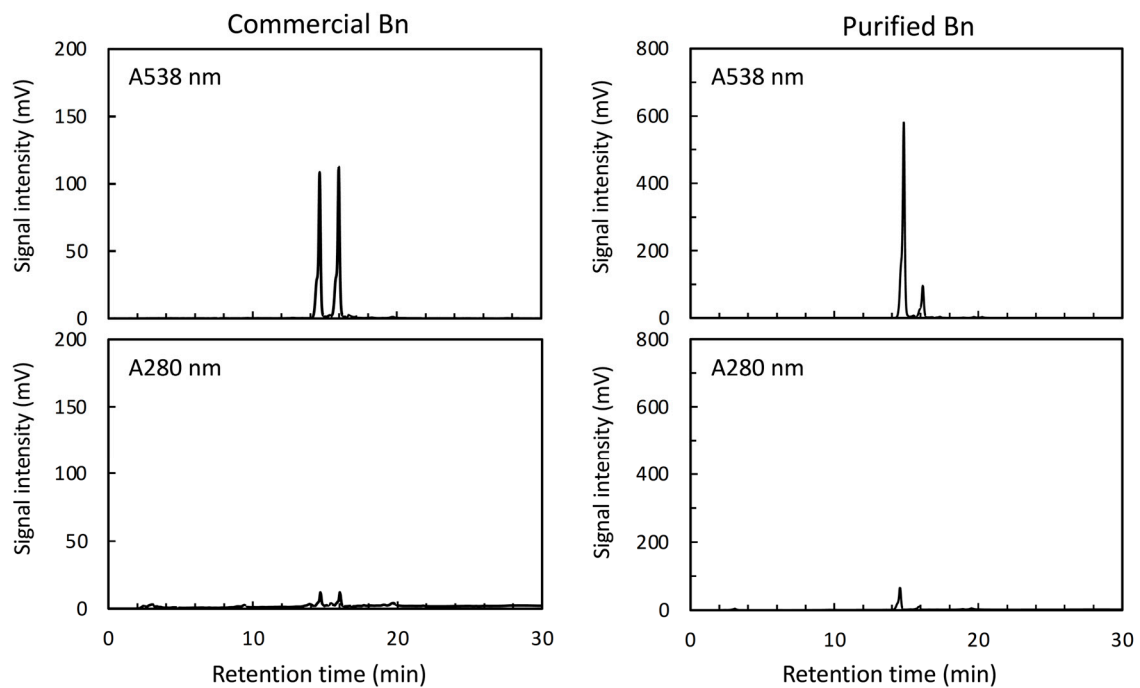
# Isolation of High-Purity Betanin from Red Beet and Elucidation of Its Antioxidant Activity against Peroxynitrite: An in vitro Study

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## Supplementary Material

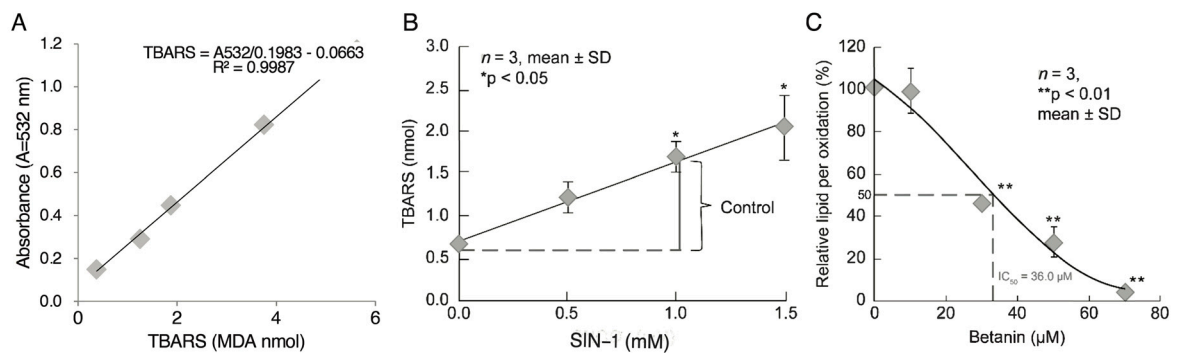


**Figure S1.** Ultraviolet–visible spectrum of betanin powders. (A) the absorption spectrum of a 5 mg/mL aqueous solution of commercial betanin powder. (B) the absorption spectrum of purified betanin powder 10 µg/mL aqueous solution. Ten mg of commercial betanin powder or 1 mg of purified betanin powder was dissolved in 1 mL of ultrapure water; diluted 2-fold and 100-fold, respectively; and used for spectrophotometric measurements.

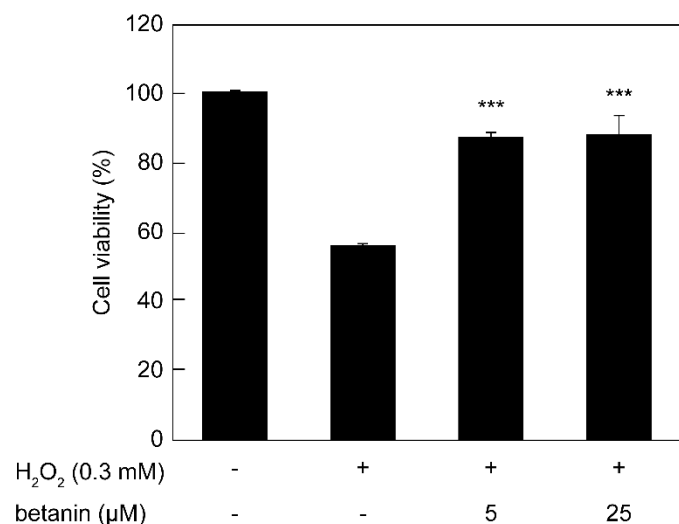


**Figure S2.** HPLC analysis of commercial and purified betanin.

10  $\mu$ L each of (A) commercial betanin (65  $\mu$ M) and (B) purified betanin (170  $\mu$ M) solutions were used for analysis and monitored at 538 and 280 nm.



**Figure S3.** ONOO<sup>-</sup>-dependent lipid peroxidation. (A) thiobarbituric acid reactive substances (TBARS) calibration curve using malondialdehyde (MDA). (B) TBARS formed in the presence of SIN-1 (1 mM) is used as the control (100%) in Figure S3C. (C) suppression of ONOO<sup>-</sup>-dependent liposome oxidation by betanin ( $n=3$ , mean  $\pm$  standard deviation).



**Figure S4.** Cytoprotective effects of betanin against H<sub>2</sub>O<sub>2</sub>. Cell viability of NIH3T3 cells exposed to H<sub>2</sub>O<sub>2</sub> (0.3 mM) after pre-treatment with betanin (5, 25 μM) for 24 h. Data are presented as mean ± standard error of the mean (*n*=3). Data were statistically assessed using one-way analysis of variance (ANOVA) followed by Dunnett's test. \*\*\**p* < 0.001 compared with the H<sub>2</sub>O<sub>2</sub> group

**Table S1.** Sequences of the primers for real-time quantitative polymerase chain reaction

Gene	Forward Sequence (5' to 3')	Reverse Sequence (5' to 3')
<i>HO-1</i>	ATGGCGTCACTTCGTCAGAG	GCTGATCTGGGGTTTCCCT
<i>NQO1</i>	GGCTGGTTTGAGAGAGTGCT	TCTGGAAAGGACCGTTGTCG
<i>γGCS</i>	AACACAGACCCAACCCAGAG	GTCTGGCTGAGAAGCCTTTG
<i>GAPDH</i>	AGAGAGGCCCTATCCCAACT	CCCTAGGCCCTCCTGTTAT

*HO-1*: Heme oxygenase-1, *NQO1*: NAD(P)H quinone oxidoreductase 1, *γGCS*: γ-glutamylcysteine synthetase, *GAPDH*: Glyceraldehyde 3-phosphate dehydrogenase