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Supplementary information

Title: Evaluation and development of a novel pre-treatment method for mulberry leave to enhance their bioactivity via enzymatic degradation of GAL-DNJ to DNJ

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HPLC analysis of GAL-DNJ and DNJ for preliminary test

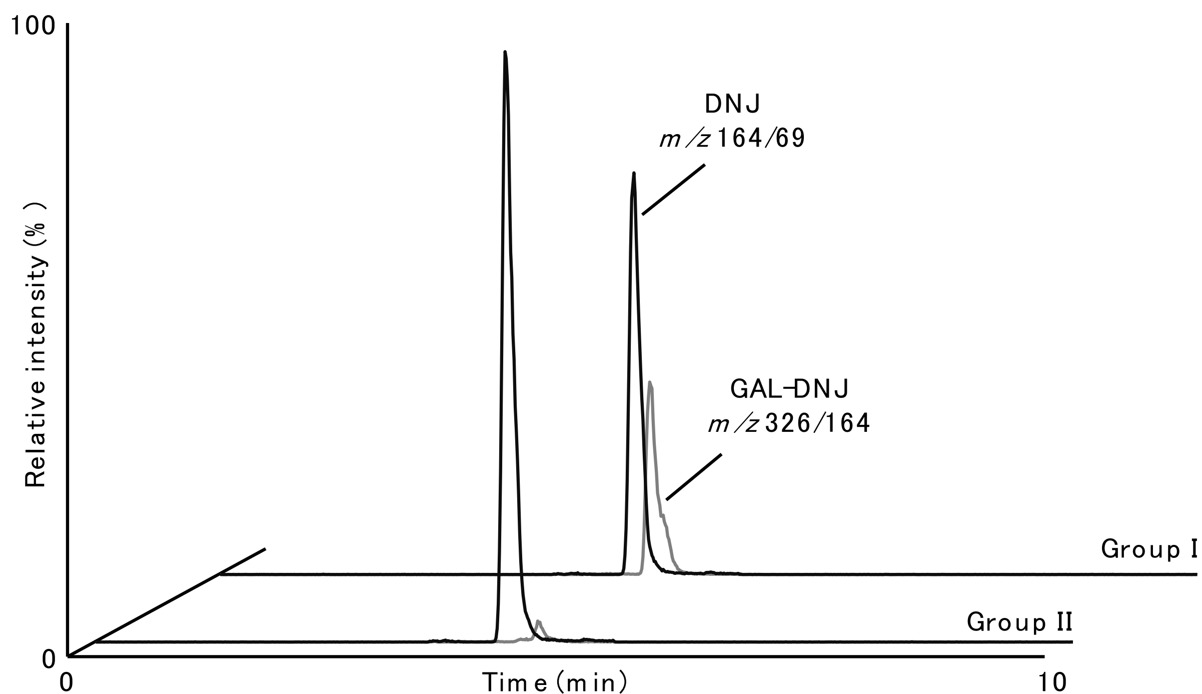

Fig. S1

The LC-MS/MS chromatograms of DNJ and GAL-DNJ from plasma sample at 30 minutes after sample oral administration. Group I was given DNJ and GAL-DNJ mixture (20 μmol/kg B.W. for each aza-sugar), and Group II was given only DNJ (40 μmol/kg B.W.).

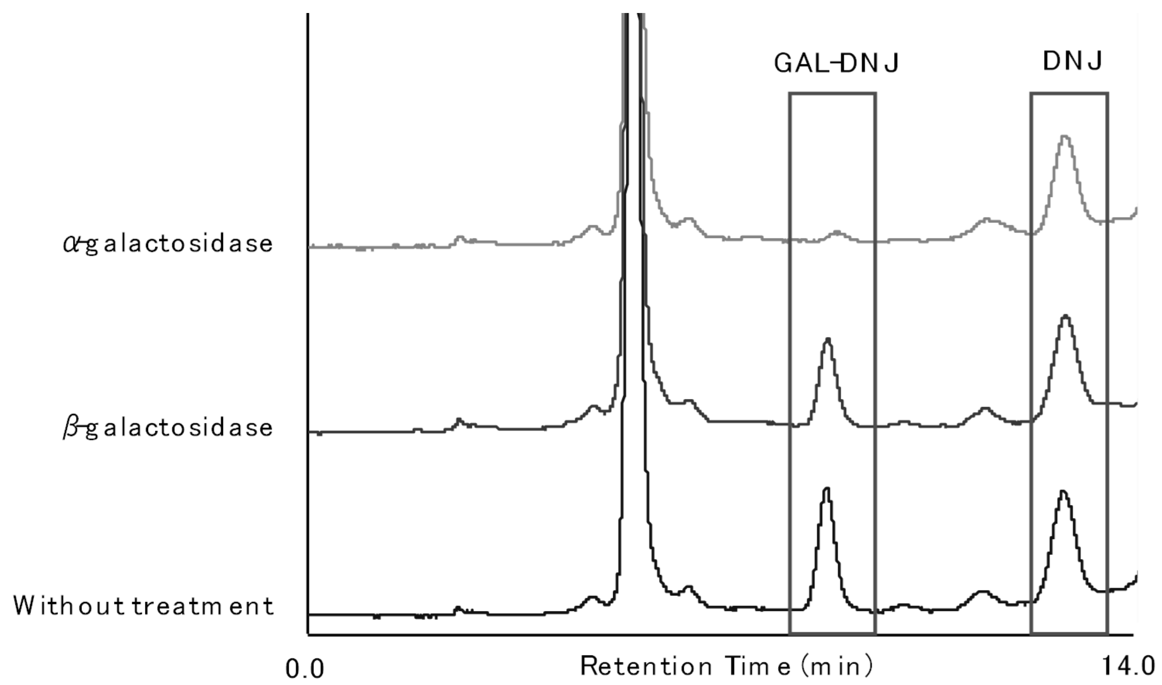

Fig. S2

The chromatogram of HPLC analysis of MLE treated with/without α-galactosidase or β-galactosidase.

HPLC analysis of GAL-DNJ and DNJ for preliminary test

In the preliminary test, GAL-DNJ and DNJ were derivatized with 9-fluorenylmethyl chloroformate (FMOC) and analyzed using an HPLC fluorescence detector according to the method of Kim *et al*.[1] Briefly, enzymatically-treated MLE were reacted with FMOC-Cl in the borate buffer (pH 8.5) and subjected to HPLC analysis. Chromatographic separations were carried out using a column (Capcell Pak C18 UG 80, 5 µm, 4.6 mm × 250 mm, Shiseido, Japan) at a flow rate of 1.0 mL/min. The column temperature was maintained at 40˚C. The analyte was eluted with 50% aqueous methanol solution. After 16.4 minutes of analysis, the column was washed with methanol containing 0.1% formic acid for 7 minutes and equilibrated for 6 minutes. Derivatized DNJ and GAL-DNJ were detected using a fluorescent detector (excitation 254 nm, emission 322 nm).

1 J.-W. Kim, S.-U. Kim, H. S. Lee, I. Kim, M. Y. Ahn and K. S. Ryu, *J Chromatogr A*, 2003, **1002**, 93–99.