

## Isolation and Identification of Anthocyanins in the Black Adzuki Bean (*Vigna angularis*, cv. Geomguseul) by UPLC/Orbitrap-MS

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### [Introduction]

Adzuki bean (*Vigna angularis*) is indigenous to the tropical region of Asia; currently, it is one of the important crops in Korea, Japan, and China. To our knowledge, the anthocyanins of its black seeds have still not been fully characterized. This prompted us to isolate and identify the anthocyanins by reversed-phase C-18 multiple-preparative liquid chromatography (MPLC) and ultra-high-performance liquid chromatography coupled to Orbitrap high-resolution mass spectrometry (UPLC/Orbitrap-MS).

### [Materials and Methods]

Adzuki bean (*Vigna angularis*, cv Geomguseul) with black seed coat cultivar were cultivated within an experimental field at the Department of Southern Area Crop Science, NICS, RDA at Miryang, in 2017. The crude anthocyanins extracted with acidic 40% methanol (0.1% TFA) for 2 day at 4 °C in darkness. The concentrated crude extract was further purified by MPLC. The injection volume was 4 mL and the flow rate was 5.0 mL/min. The mobile phase were made of water(0.1% TFA) and methanol (0.1% TFA) by linear gradient program. Chemical structures were determined by <sup>1</sup>H, <sup>13</sup>C and 2D NMR spectroscopy. A single-stage Orbitrap mass spectrometer was equipped with Heated Electrospray Ionization Source (HESI). The HESI parameters in positive polarity were as follows: sheath gas flow rate: 50; aux gas flow rate: 15; spray voltage: 2.5 kV; capillary temp.: 270 °C; heat temp.: 400 °C. Mass range in full scan mode was set at *m/z* 150-1,000 and MS<sup>2</sup> scan of the two precursor-ion in the first scan.

### [Results and Discussions]

The aim of this study was to isolate and identify the anthocyanins in the black adzuki bean (cv. Geomguseul, *Vigna angularis*) using reverse phase MPLC and UPLC/Orbitrap-MS analysis, respectively. Anthocyanins were extracted from the coat of black adzuki bean with acidic 40% methanol, isolated by ODS MPLC column chromatography, and their structures elucidated by 1D and 2D NMR spectroscopy. The isolated anthocyanins were characterised as delphinidin-3-galactoside and delphinidin-3-glucoside. Furthermore, eight minor anthocyanins were detected and identified as delphinidin-diglucosede, delphinidin-3,5-diglucoside, delphinidin-3-rutinoside, delphinidin-3-coumaroylglucoside, cyanidin-3-glucoside, petunidin-3-galactoside, petunidin-3-glucoside and petunidin-3-(6'-coumaroyl)glucoside based on the fragmentation patterns of UPLC/Orbitrap-MS analysis.

### [Acknowledgements]

This work was supported by a grant from AGENDA Program (No. PJ013204012018), Rural Development Administration, Republic of Korea.

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