Supporting Information

Gut Metabolism of Furanocoumarins: Proposed Function of Co O-Methyltransferase

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1. Composition analysis of *Angelica dahurica* extract

A. Extraction

The dried powder of *Angelica dahurica* Roots (Voucher specimen # PBNV201812310001) was obtained from Phytobean Agricultural Corporation Co., Ltd. (Yecheon-gun, South Korea). Plant sample (100 g) was extracted by supercritical CO$_2$ fluid extraction (SCE) method. Supercritical fluid system (Msys Engineering, Korea) was used, with extraction conditions as follows: temperature, 50 °C; pressure, 200 bar; extraction time, 90 min; extraction mode, dynamic. The extracted residue was collected from the collection chamber and dried under the flow of nitrogen gas. It was further dried inside vacuum desiccator with phosphorus pentoxide as the desiccant, yielding 2.14 g of dried matter. Concentration of the extract was adjusted to 10 mg/mL for TLC analysis, or to 0.2 mg/mL for HPLC-DAD analysis with HPLC-grade methanol. Prior to injection into HPLC system, the solution was passed through a 0.2 µm PTFE filter (Advantec, Japan).

B. Analysis

Identification of the furanocoumarins in the *A. dahurica* extract was conducted by comparison with the reference standards. Xanthotoxin (1), bergapten (2), and imperatorin (3) reference standards with purities of > 98.0% by HPLC were purchased from TCI (Tokyo, Japan). Isoimperatorin (4) and oxypeucedanin (5) reference standards with purities of > 98.0% by HPLC were obtained from Korean Ministry of Food and Drug Safety (MFDS, Ochang, Korea).

Analysis of the *A. dahurica* extract was performed by conducting TLC and HPLC-DAD analysis. TLC analysis was performed on TLC Silica gel 60 F$_{254}$ plates (Merck, Germany). Spotted plate was developed using the solvent system consisting of hexanes and ethyl acetate,
with a ratio of 2 to 1. Developed plates were observed under the illumination of UV light, both short (254 nm) and long-wavelength UV light (365 nm).

HPLC-DAD analysis was performed with a Dionex UltiMate 3000 UHPLC system (Thermo Fisher Scientific, USA), equipped with a pump, an autosampler, a thermostatted column compartment, and a diode array detector (DAD). Program setup, data collection and analysis were conducted using Chromelone™ Chromatography Data System (CDS) software version 6.80 (Thermo Fisher Scientific, USA). Separation was carried out by Kinetex C$_{18}$ column (1.7 µm particle size, 100 x 2.1 mm i.d., Phenomenex) protected by SecurityGuard ULTRA (for 2.1 mm i.d., Phenomenex). Multi-step gradient was programmed with a mobile phase consisting of 0.1% (v/v) acetic acid in water (A) and acetonitrile (B) at a flow rate of 0.2 mL/min. The gradient started at 10% B was changed to 30% B for 1 min, to 40% B for 11 min, to 50% B for 15 min, to 60% B for 3 min, to 80% B for 2 min, to 90% B for 1 min and held for 7 min. Post-equilibration was performed prior to the next sample injection by returning to the mobile phase composition to 10% B and holding at it for 10 min. Injection volume of analyte was set at 1 µL and column temperature was maintained at 25 °C. Detector was set to record at 250 nm, simultaneously with UV spectrum monitoring in the range of 190 – 380 nm.

Identification of the compounds in the *A. dahurica* extract was conducted by HPLC-ESI/MS$^n$ analysis with a Dionex UltiMate 3000 UHPLC system (Thermo Fisher Scientific, USA) connected to LCQ Fleet Ion Trap mass spectrometer (Thermo Fisher Scientific, USA). Program setup, data collection and analysis were conducted using Xcalibur™ software (Thermo Fisher Scientific, USA). Separation was carried out by Hypersil GOLD™ column (3 µm particle size, 100 x 2.1 mm i.d., Thermo Fisher Scientific) with Uniguard™ guard column (for 2.1 mm i.d., Thermo Fisher Scientific). The mobile phase consisted of 0.1% (v/v) formic acid in water (A) and 0.1% formic acid (v/v) in acetonitrile (B). Multi-step gradient for HPLC-UV-ESI/MS$^n$ analysis was programmed, starting at 20% B and holding at it for 1 min, increased to 40% B
for 4 min, to 50% B for 5 min, to 70% B for 5 min, and to 80% B for 5 min. Injection volume of analyte was set at 2 µL. Helium (He) was used as collision gas, while nitrogen (N₂) was used as both sheath and auxiliary gas. Analysis was performed in positive ion detection mode and the conditions were adjusted as follows: ion spray voltage, 5 kV; capillary temperature, 275 °C; capillary voltage, 19 V; tube lens offset, 90 V. Both full-scan and multi-stage mass spectrometry (MSⁿ) analysis mode were operated. Full-scan mode was programmed to perform scanning in the range of \( m/z \) 150-500. For MSⁿ analysis, a data-dependent program was used so that the most abundant ions in each scan were selected and subjected to MSⁿ analysis (up to \( n = 3 \)), with collision energy of 35.

**C. Assignment of major furanocoumarins**

Five major furanocoumarins, 1 – 5, in the extract of *A. dahurica* were identified from the comparison of the HPLC retention times and UV spectra with reference compounds, and further confirmed by MS spectra.
Figure S1. HPLC chromatograms of the SFE extract (A) and furanocoumarin reference standards (B and C). 1: Xanthotoxin; 2: Bergapten; 3: Imperatorin; 4: Isoimperatorin; and 5: Oxypeucedanin.

Figure S2. HPLC-MS analysis of *A. dahurica*. UV (top) and TIC (bottom).
The other furanocoumarin at the retention time of 16.8 min was identified as byakangelicol (6), based on ESI-MS\textsuperscript{n} analysis.\textsuperscript{S1}

**Figure S3.** ESI(+)\textregistered-MS spectra of byakangelicol (6). The second spectrum shows the MS\textsuperscript{2} spectrum of 316.88 m/z peak ionization.

Finally, major dietary furanocoumarins in *A. dahurica* were assigned in the HPLC chromatogram, as shown at Figure S4.

**Figure S4.** HPLC analysis of the supercritical CO\textsubscript{2} extract obtained from the root of *A. dahurica*. 1: Xanthotoxin; 2: Bergapten; 3: Imperatorin; 4: Isoimperatorin; 5: Oxypeucedanin; 6: Byakangelicol.
2. Biotransformation of oxypeucedanin (5) and byakangelicol (6) by *Blautia* sp. MRG-PMF1

**A. Experimental**

Biotransformation was performed under anaerobic condition (5% CO$_2$; 10% H$_2$; 85% N$_2$). *Blautia* sp. MRG-PMF1 was grown in Gifu Anaerobic Medium (GAM) broth at 35°C until the optical density at 600 nm (OD$_{600}$) of bacterial culture reached ~0.6. Substrate was added into culture (final concentration of substrate in medium: 0.1 mM), mixed well and incubated at 35°C for a given period of time. Aliquots (300 µL) were collected at scheduled times and extracted twice with 1 mL of ethyl acetate. Mixture was vortexed for 1 min and centrifuged at 10,770 g for 10 min. Supernatant was transferred into a microcentrifuge tube and dried under reduced pressure using vacuum centrifugal concentrator (N-BIOTEK, Korea) at 35°C. The dried residue was dissolved in 300 µL MeOH and passed through 0.2-µm PTFE filter prior to chromatography analysis. For control, sterile GAM broth containing substrate was incubated in the absence of bacterial culture for same duration with the biotransformation assay, then collected and extracted as described above.

**B. Results**

In the presence of MRG-PMF1, oxypeucedanin (5) of which peak found at the retention time of 17.0 min on the chromatogram, converted to one observable metabolite at retention time of 9.4 min (Figure S5).
**Figure S5.** HPLC analysis of oxypeucedanin (5) biotransformation by MRG-PMF1 strain.

The metabolite was also analyzed by ESI-MS and assigned as oxypeucedanin hydrate (9) (Figure S7). From the full-scan MS spectrum, the molecular ion peak, [M+H]+, at m/z 305 was found and its fragmentation pattern also matched to the assignment.\[^{82}\]

**Figure S6.** ESI(+) - MS spectra of oxypeucedanin hydrate (9). The second spectrum shows the MS\(^2\) spectrum of 305.29 m/z peak ionization, and the third spectrum shows the MS\(^3\) spectrum of 203.16 m/z peak ionization.
However, it was found that oxypeucedanin hydrate (9) was also produced from oxypeucedanin (5) in the absence of MRG-PMF1. When the substrate was incubated with GAM broth medium, production of oxypeucedanin hydrate (9) was observed, as can be seen in Figure S7 A. In the presence of MRG-PMF1 cells (Figure S7 B), the rate of conversion was faster, and which suggested the effect of the bacterium in the conversion of oxypeucedanin (5) to oxypeucedanin hydrate (9). It is noteworthy that biotransformation of oxypeucedanin (5) did not yield any other metabolite, such as bergaptol (8).

**Figure S7.** HPLC analysis of oxypeucedanin transformation in the absence (A) and presence (B) of *Blautia* sp. MRG-PMF1 in GAM broth medium.

Chromatograms obtained from the biotransformation of byakangelicol (6) revealed three metabolites, designated as M2, M3 and M4, were formed throughout the process (Figure S8). The UV spectra of metabolites were also recorded to aid the structural identification (Figure S9).
Figure S8. HPLC analysis of byakangelicol (6) biotransformation products by human intestinal bacteria MRG-PMF1. Responses were recorded at wavelength of 250 nm.

Figure S9. UV spectra of metabolites formed from byakangelicol (6) biotransformation by human intestinal bacteria MRG-PMF1. A: byakangelicol (6); B: M2; C: M3 and D: M4.
The metabolite $\mathbf{M}_3$ was confirmed as byakangelicin (11) from the UV and MS analysis (Figure S9 and S10). In the HPLC chromatographic analysis, the metabolite $\mathbf{M}_2$ was the intermediate species, as it disappeared in 24hr. Therefore, metabolite $\mathbf{M}_2$ was assigned as desmethylbyakangelicol (10). Finally, the metabolite $\mathbf{M}_4$ was assigned as desmethylbyakangelicin (12) as shown at Figure S11.

**Figure S10.** MS spectra of metabolite $\mathbf{M}_3$.

**Figure S11.** Biotransformation pathway of byakangelicol (6) by MRG-PMF1.
3. Biotransformation of 7-allyloxy coumarin

A. Experimental

7-allyloxy coumarin (13) was prepared from the reaction between 7-hydroxy coumarin (14, Acros Organics, Fair Lawn, NJ) and allyl bromide (Alfa Aesar, Haverhill, MA), according to the published method. For the analysis of 7-allyloxy coumarin (13) biotransformation products, solution B was started at 10% and increased to 30% for 10 min, to 40% for 2 min, to 70% for 8 min, to 90% for 2 min, and hold for 3 min. Detector was set to record at 323 nm for 7-allyloxy coumarin (13) biotransformation product analysis.

B. Result

From HPLC analysis (Figure S8), it was clear that 7-allyloxy coumarin (13) was transformed to 7-hydroxy coumarin (14) without other metabolic intermediates.

Figure S12. HPLC analysis of 7-allyloxy coumarin (13) biotransformation by the MRG-PMF1 strain.

References
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