Isolation of an Angiotensin Converting Enzyme Inhibitor from *Oenanthe javanica*

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Angiotensin converting enzyme (ACE, peptidyldipeptide hydrolase, EC3.4.15.1) converts angiotensin I into angiotensin II by cleaving C-terminal dipeptide (His-Leu) of angiotensin I, and inactivates bradykinin, which decreases the blood pressure. Various food sources were screened for ACE inhibitors.[1-4] *Oenanthe javanica*, an oriental medicine for the treatment of hypertension in Korea, was used to isolate ACE inhibitors and to develop a new functional food ingredient.

Oenanthe javanica was freshly harvested, from which crude extracts of *Oenanthe javanica* were obtained by disrupting the cells using a homogenizer. The crude water extracts were then successively filtered using PM-10 and YM-3 membranes (Millipore Co., Bedford, MA, USA). The membrane-filtered solution was concentrated and loaded onto Sephadex G-15 column (1.8 cm × 75 cm) equlibrated with 20 mM phosphate buffer (pH 7.0). The eluate was monitored by measuring the absorbance at 214 nm. Six fractions were obtained from the column (Fig. 1). Each fraction was assayed through the ACE assay method of Cushman and Cheung with modifications.[5] The reaction mixture contained 150 μl of 5 mM Hip-His-Leu as a substrate, 50 μl of rabbit lung ACE powder (5 munit, Sigma Chemical Co., St. Louis, MO, USA) in a 50 mM sodium borate buffer (pH 8.3), and 50 μl of the sample solution. The reaction was carried out at 37°C for 30 min, and terminated by adding 250 μl of 1 N HCl, and 1 ml of ethylacetate was added. After centrifugation, the absorbances of the supernatants were measured at 228 nm.

Assay results (F4, 24.6%; F5, 7.9%) show that the sixth fraction, F6, had the highest inhibitory activity (53%). Therefore, F6 was pooled and reloaded onto the Sephadex G-15 column. After obtaining a single peak from the column, the fraction was loaded onto the reversed-phase HPLC having a C_{18} column (Waters, Spherisorb ODS2, 4.6 × 250 mm). HPLC was performed on the condition of solution A (0.1% tri-

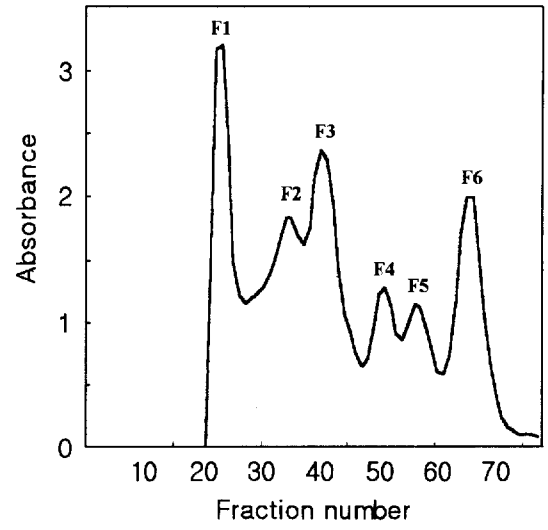

Fig. 1. Elution profile of Sephadex G-25 column chromatography using *Oenanthe javanica* extracts.

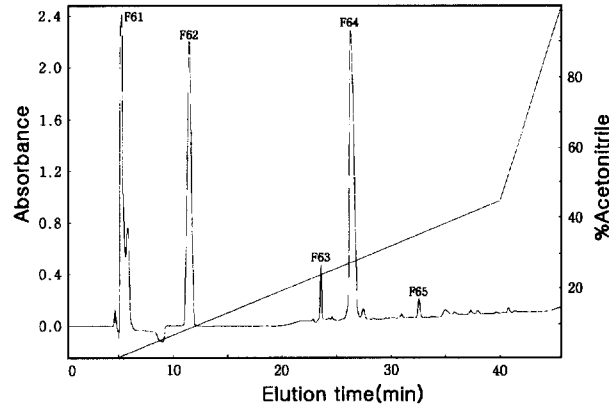

Fig. 2. Elution profile of reversed-phase HPLC using the fraction F6 in Fig. 1.

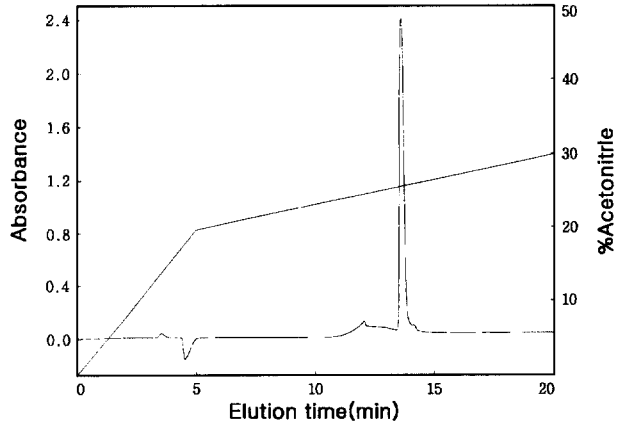

Fig. 3. The reversed-phase HPLC profile of the peak F64 in Fig. 2.

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Abbreviations: HPLC, High performance liquid chromatography.

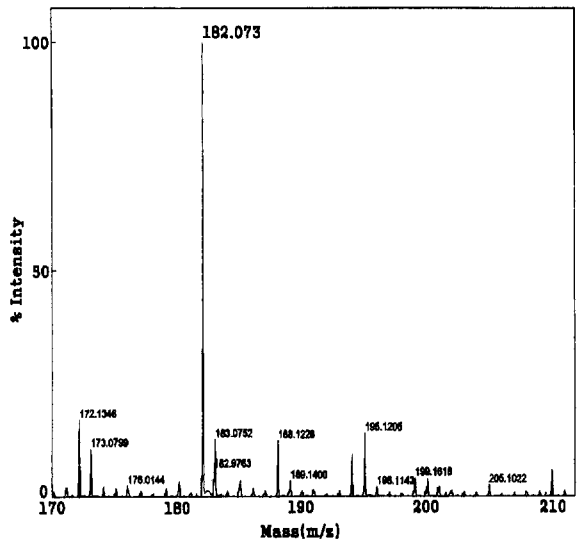

Fig. 4. Mass spectrum of the purified ACE inhibitor.

fluroacetic acid, TFA) and solution B (acetonitrile containing 0.1% TFA), having gradient of 0% of B to 100%. F6 was further separated into five sub-fractions through HPLC (Fig. 2), among which, the fraction F64 had the highest inhibitory activity (74%). Therefore, F64 was reloaded onto the HPLC on the condition of solution A and solution B, having gradient of 0% of B to 30%. A single peak was detected at 25% acetonitrile gradient (Fig. 3). The ACE inhibitor had a molecular mass of 181 as determined through ESI Tendem mass spectrometer (JMS HX-110A, JEOL, Japan) (Fig. 4) and IC_{50} of 120 μM based on the molecular weight. This is the first report regarding the isolation of a small molecular weight ACE inhibitor and the development of a functional food product from *Oenanthe javanica* extracts through simple processing.

Although the chemical nature of the inhibitor has not yet been fully characterized and *in vivo* experiments using spontaneously hypertensive rats (SHR) are needed, this small molecular-weight inhibitor is quite promising in manufacturing of drink products since it contains functional components regulating blood pressure. In particular, simple processing such as membrane filtration of molecular weight 1000 cut-off could produce products having functional components. Further characterization of the inhibitor and development of processing of drink products is currently under study.

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