Quantitative Determination of Bioactive Compounds in Some *Artemisia capillaris* by High-Performance Liquid Chromatography

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Abstract – In order to facilitate the quality control of *Artemisia capillaris*, a simple, accurate and reliable HPLC method was developed for the simultaneous determination of the six bioactive compounds: scopolin (1), chlorogenic acid (2), 2,4-dihydroxy-6-methoxyacetophenone 4-glycoside (3), hyperoside (4), isorhamnetin 3-O-robinobioside (5), and scoparone (6), which were selected as the chemical markers of *A. capillaris*. Separation was achieved on an Agilent Eclipse XDB-C18 column with a gradient solvent system of 0.1% trifluoroacetic acid aqueous-acetonitrile at a flow-rate of 1.0 mL/min and detected at 254 nm. All six calibration curves showed good linearity ($R^2 > 0.998$). A simple reversed phase HPLC method was developed for extracting pharmacologically active compounds scopolin, chlorogenic acid, 2,4-dihydroxy-6-methoxyacetophenone 4-glycoside, hyperoside, isorhamnetin 3-O-robinobioside, and scoparone from *A. capillaris* using a binary gradient of acetonitrile : 0.1% trifluoroacetic acid with UV detection at 254 nm. The scopolin (1), chlorogenic acid (2), 2,4-dihydroxy-6-methoxyacetophenone 4-glycoside (3), hyperoside (4), isorhamnetin 3-O-robinobioside (5), and scoparone (6) contents of the herb of *A. capillaris* collected from fifteen district markets in Korea were 0.00~0.90 mg/g, 0.06~7.29 mg/g, 0.06~0.91 mg/g, 0.07~5.05 mg/g, 0.42~13.11 mg/g, and 1.11~29.82 mg/g, respectively. The results demonstrated that this method is simple and reliable for the quality control of *A. capillaris*.

Keywords – *Artemisia capillaris*, HPLC, Isorhamnetin 3-O-robinobioside, Scoparone

Introduction

*Artemisia capillaris* Thunb. (Compositae) is a medicinal plant, which is distributed in worldwide. The whole part of *A. capillaris* has been used for the food additives and traditional medicines, particularly treatment of hepatitis, inflammation, malaria, cancer, and microbial infections (Cha et al., 2009; Aniya et al., 2000; Mase et al., 2010). On biological studies of principles from this plant, a few coumarins have been elucidated as anticarcinogenic and anti-inflammatory agents (Kim et al., 2008; Jang et al., 2005). Isolation of many classes of biological active metabolites such as coumarins, essential oils, flavonoids, polyacetylenes, and sterol glycosides were reported to have a wide range of activities, including hepatoprotective, anti-oxidative, anti-malarial, anti-cancer, anti-viral, anti-fungal, anti-complementary, and interferon inducing (Tan et al., 1998). Furthermore, a variety of biological studies, the *Artemisia* genus has emerged as a potent candidates of natural occurring therapeutic agents for diabetes due to their compounds, including coumarins, flavonoids, and caffeic acids (Cui et al., 2009; Logendra et al., 2006; Okada et al., 1995). Generally, coumarins were believed to be the beneficial components and were chosen as marker compounds for the quality evaluation, standardization of *Artemisia* genus, and their preparation. However, due to multiple compounds that might be associated with the therapeutic functions, a single or a few marker compounds could not be responsible for the overall pharmacological activities of the *Artemisia*. Therefore, it is urgently needed to establish a comprehensive quality evaluation method based on analysis of a variety of structural active compounds in order to accurately reflect the quality of these herbal drugs. Our present study aims to develop a simple and validated HPLC method for the simultaneous determination of active coumarins, phenolic compounds, and flavonoids in *A. capillaris*, namely scopolin (1), chlorogenic acid (2), 2,4-dihydroxy-6-methoxyacetophenone 4-glycoside (3), hyperoside (4), isorhamnetin 3-O-robinobioside (5), and scoparone (6) (Fig. 1).
Experimental

**General**—Acetonitrile HPLC grade was purchased from SK Chemicals Company (Ulsan, Korea). Distilled and deionized water were obtained from the instrument center (Catholic University of Daegu, Daegu, Korea) and used throughout the study. Trifluoroacetic acid (TFA) was obtained from Sigma-Aldrich (Germany). Other solvents and reagents were of analytical grade. The reference compounds 1 - 6 were supplied from Prof. Jae-Sue Choi, Pukyung National University, Korea. The purities of compounds were determined to be greater than 98% by normalization of the peak areas detected by HPLC analyses. The internal standard of caffeic acid was purchased from the Sigma-Aldrich (Germany). The 15 batches of the aerial part of *A. capillaris* were collected from Korea and China markets: ACH-1 (collected on Autumn, cultivated in Korea), ACH-2 (collected on Autumn, cultivated in Korea), ACH-3 (collected on Autumn, cultivated in Korea), ACH-4 (collected on Autumn, cultivated in Korea), ACH-5 (collected on Autumn, cultivated in Korea), ACH-6 (collected on Autumn, cultivated in Korea), ACH-7 (collected on Autumn, cultivated in Korea), ACH-8 (collected on Autumn, cultivated in Korea), ACH-9 (collected on Autumn, cultivated in Korea), ACH-10 (collected on Autumn, cultivated in China), ACH-11 (collected on Autumn, cultivated in Korea), ACH-12 (collected on Spring, cultivated in Korea), ACH-13 (collected on Spring, cultivated in Korea), ACH-14 (collected on Spring, cultivated in China), and ACH-15 (collected on Spring, cultivated in China). The origin of sample was identified by Prof. Je Hyun Lee, Dongguk University, Korea and voucher specimens were deposited in Catholic University of Daegu, Korea.

**HPLC apparatus and chromatographic conditions**—The chromatographic system for quantitative analysis consisted of a 306 pump (Gilson, USA), 811C dynamic mixer (Gilson, USA), UV/VIS-156 detector (Gilson, USA), 231 XL sample injector (Gilson, USA), and GILSON UniPoint data processor (Gilson, USA). The chromatographic separation of analyses was performed on an Agilent Eclipse XD8-C18 (Agilent Technologies, USA; 5 µm, 4.6 x 150 mm) performed at ambient temperature using a MetaTherm (Varian, USA). The auto-sampler was also set at ambient temperature. Data was collected and analyzed using Gilson Millennium software. The mobile phase consisting of 0.1% TFA in water (A) and acetonitrile (B) was run with gradient elution at a flow rate of 1.0 mL/min. The linear gradient elution was set as follows: 0–10 min, 10% B; 10–40 min, 10% → 40% B. The injection volume was 10 µL. UV absorption was monitored at 254 nm. The column temperature was maintained at 30 °C (Fig. 2). Quantification was conducted using an internal standard method based on the peak area ratio of the analyte/IS versus the amount of each analyte.

**Preparation of standard solutions**—Based on the solubility of each component in DMSO, a stock standard solution was prepared by dissolving 1.00 mg of each

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**Fig. 1.** Structure of scopolin (1), chlorogenic acid (2), 2,4-dihydroxy-3-methoxy-acetophenone 4-glycoside (3), hyperoside (4), isorhamnetin 3-O-robinobioside (5), and scoparone (6) isolated from *A. capillaris*. 