

## Quantitative Analysis of the Flavonoid Content in the Leaves of *Boehmeria nivea* and Related Commercial Products

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**Abstract** – Content analysis of flavonoids (epicatechin, epicatechin gallate, and rutin) present in the leaves of *Boehmeria nivea* (originating from Geumsan-myeon, Biin-myeon, Hansan-myeon, and Baeksu-eup) and their commercial products (ramie tteok, ramie songpyeon, ramie bory-tteok, and ramie tea) was conducted by HPLC. The content of epicatechin, epicatechin gallate, and rutin was highest in the leaves of *B. nivea* from Geumsan-myeon (0.138 mg/g), Baeksu-eup (1.654 mg/g) and Geumsan-myeon (12.205 mg/g), respectively. With respect to commercial products, the content of epicatechin and epicatechin gallate was highest in ramie tea, with concentrations of 1.879 and 1.090 mg/g, respectively. Given these flavonoid concentrations, *B. nivea* leaf extracts have the potential to be used as additives in natural medicinal products, health supplements, and beverages.

**Keywords** – *Boehmeria nivea*, Epicatechin, Epicatechin gallate, Rutin, HPLC

### Introduction

*Boehmeria nivea*, which belongs to the family Urticaceae, is distributed throughout Asia including the Philippines, India, China, Korea, and Thailand. It is a herbaceous perennial plant with broad (6 - 12 cm) and long (7 - 15 cm) heart-shaped leaves that appear silvery due to the dense small hairs on the underside.<sup>1</sup> *B. nivea* is commonly referred to as China grass, white ramie, green ramie, and rhea, and has been used as far back as 5,000 BC in Egyptian mummy cloths. Furthermore, the leaves of this plant have been used as sources of tea, cloths, and tteok (traditional Korean rice cakes).<sup>2,3</sup>

*B. nivea* leaves are also commonly used in folk remedies as a diuretic and anti-pyretic, and has are thought to possess hepatoprotective, anti-oxidant, and anti-inflammatory properties.<sup>4</sup> Previous studies have indicated that these leaves contain kiwiionoside, rutin, uracil, 3-hydroxy-4-methoxy-benzoic acid, cholesterol,  $\alpha$ -amyirin, nonacosanol, emodin, emodin-8-*O*- $\beta$ -glucoside, physcion, polydatin, catechin, potassium nitrate,  $\beta$ -sitosterol, epicatechin, and

epicatechin gallate.<sup>5-7</sup> Furthermore, the leaves of *B. nivea* contain a large amount of phenolic compounds, which were able to inhibit angiotensin I-converting enzyme.<sup>6</sup> In the roots of a related species, *B. tricuspis* contains several epicatechin dimers such as (–)-epiafzelechin-(–)-epicatechin-4,8(or 6)-dimer, and (–)-epicatechin-(–)-epicatechin-4,8-(or 6)-dimer.<sup>8</sup> Among them, epicatechin, epicatechin gallate, and rutin are polyphenolic compounds known as flavonoids, which are known for their anti-oxidant, anti-inflammatory, anti-tumorigenic, anti-bacterial, anti-viral, and anti-allergenic properties.<sup>9-16</sup>

This study utilized HPLC to analyze the concentrations of epicatechin, epicatechin gallate, and rutin in the leaves of *B. nivea* originating from four different locations (Geumsan-myeon, Biin-myeon, Hansan-myeon, and Baeksu-eup), and in commercial products (ramie tteok, ramie songpyeon, ramie bory-tteok, and ramie tea).

### Experimental

**Plant material** – The leaves of *B. nivea* were cultivated and collected by Yeonggwang Agricultural Technology & Extension Center, Korea. The collection areas for *B. nivea* were Geumsan-myeon, Biin-myeon, Hansan-myeon, and Baeksu-eup, Korea.

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**Commercial products** – Commercial products (tteok, songpyeon, bory-tteok, and ramie tea) containing *B. nivea* leaves were purchased from Yeonggwang-gun, Korea. Among them, tteok, songpyeon, and bory-tteok were made up of *B. nivea* leaves and wheat flour. Ramie tea was made up of dried *B. nivea* leaves.

**Instruments and reagents** – Methanol (MeOH), *n*-hexane, dichloromethane (CH<sub>2</sub>Cl<sub>2</sub>), ethyl acetate (EtOAc), and *n*-butanol (*n*-BuOH) were purchased from SamChun Pure Chemical Co. (Pyeongtaek, Korea). Epicatechin (**1**), epicatechin gallate (**2**), and DMSO were purchased from Sigma-Aldrich (St. Louis, MO, USA). Electron ionization-mass spectrometry (EI-MS) was conducted with a Jeol JMS-600W mass spectrometer (Tokyo, Japan). NMR spectra were recorded with a Bruker AVANCE 500 NMR spectrometer (Bremen, Germany) using TMS as the internal standard. Chemical shifts are reported in parts per million ( $\delta$ ), and coupling constants ( $J$ ) are expressed in Hertz. An Eyela rotary evaporator system (Tokyo, Japan) under reflux *in vacuo* was used for evaporation. Thin-layer chromatography was conducted with Kiesel-gel 60 F<sub>254</sub> plates (silica gel, 0.25 mm layer thickness; Art. 5715, Merck Co., Darmstadt, Germany), and compounds were visualized by spraying with 10% H<sub>2</sub>SO<sub>4</sub> in MeOH, followed by heating to 100 °C. Sephadex LH-20 (20 - 100  $\mu$ m) was purchase from Sigma-Aldrich. HPLC chromatograms of flavonoids were recorded with a Waters Breeze system (Massachusetts, USA) equipped with a Waters 1525 binary HPLC pump and a 2489 system UV/VIS detector. Water and MeOH used in this study were of HPLC grade, and all other reagents were of analytical grade.

**Isolation and identification of rutin (**3**)** – *B. nivea* leaves (2 kg) were dried finely powdered and immersed in MeOH for 3 h (4 L  $\times$  8) under reflux at 65 °C-75 °C. The solvent was evaporated *in vacuo* to produce the MeOH extract (294.6 g). This extract was then suspended in distilled water and partitioned with *n*-hexane (106.9 g), CH<sub>2</sub>Cl<sub>2</sub> (4.1 g), EtOAc (2.4 g), and *n*-BuOH (23.8 g), successively. The EtOAc fraction (2.4 g) was separated on an LH-20 column ( $\phi$  2.0  $\times$  50 cm) using MeOH/water (gradient: 1 : 3  $\rightarrow$  1 : 0, v/v). Nine fractions were obtained by combining those with similar R<sub>f</sub> on TLC behavior (1  $\rightarrow$  9). Among them, compound **3** was isolated from fraction 7 by recrystallization with MeOH (Fig. 1).

Compound **3**: FAB-MS  $m/z$ : 611 [M + H]<sup>+</sup>; <sup>1</sup>H-NMR (500 MHz, DMSO-*d*<sub>6</sub>):  $\delta$  1.00 (3 H, d,  $J$  = 6.0 Hz, Rha CH<sub>3</sub>), 3.07-3.69 (12 H, m,  $J$  = 2 Hz, sugar H), 4.38 (1 H, s, Rha H-1), 5.34 (1H, d,  $J$  = 7.5 Hz, Glc H-1), 6.19 (1 H, d,  $J$  = 1.2 Hz, H-6 ), 6.38 (1 H, d,  $J$  = 1.2 Hz, H-8), 6.84

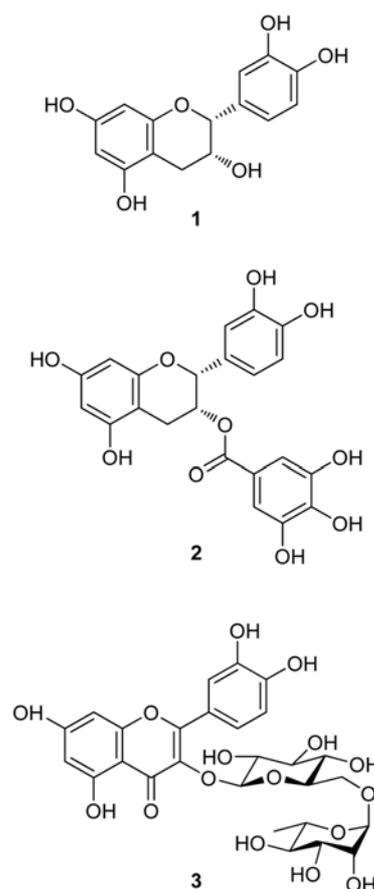


Fig. 1. Structures of compounds 1 - 3.

(1 H, d,  $J$  = 8.5 Hz, H-5'), 7.54 (1 H, d,  $J$  = 2.5 Hz, H-2'), 7.55 (1 H, dd,  $J$  = 2.5, 8.5 Hz, H-6'), 12.58 (1 H, s, 5-OH); <sup>13</sup>C-NMR (125 MHz, DMSO-*d*<sub>6</sub>):  $\delta$  156.5 (C-2), 133.2 (C-3), 177.3 (C-4), 161.1 (C-5), 98.6 (C-6), 164.1 (C-7), 93.5 (C-8), 156.5 (C-9), 103.9 (C-10), 121.5 (C-1'), 115.2 (C-2'), 133.2 (C-3'), 148.4 (C-4'), 116.2 (C-5'), 121.1 (C-6'), 101.1 (Glc C-1), 74.0 (Glc C-2), 76.4 (Glc C-3), 70.5 (Glc C-4), 75.9 (Glc C-5), 67.0 (Glc-C6), 100.7 (Rha C-1), 70.3 (Rha C-2), 70.0 (Rha C-3), 72.0 (Rha C-4), 68.2 (Rha C-5), 17.7 (Rha C-6).

**Sample preparation** – To determine the content of **1**, **2**, and **3** in *B. nivea* leaves and in the commercial products (mentioned above), 50 g of either leaves or commercial products were dried finely powdered and immersed in 50% MeOH (3  $\times$  100 mL) by reflux and evaporation *in vacuo*. The residue was dissolved in 1 mL of MeOH and filtered through a 0.45  $\mu$ m filter. The resulting solution was used for HPLC analysis.

**HPLC conditions**- HPLC separation of **1**, **2**, and **3** for qualitative and quantitative analysis was performed using a reverse phase system. A Waters Spherisorb<sup>®</sup> ODS2 (4.6