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Content Analysis of Rutin in the Leaves of *Boehmeria nivea* Harvested in Different Regions of South Korea by HPLC-UV

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Abstract – Phytochemical analysis of *Boehmeria nivea* (Bn) leaves by medium pressure liquid chromatography led to the isolation of a flavonoid glycoside identified by spectroscopic analysis as rutin. The amount of rutin in the leaves of Bn harvested from nine regions in South Korea (Bn 1-9) which were collected on the months of June, July, August, and September was determined by HPLC-UV analysis. A gradient elution program that utilizes a Discovery® C18 (4.6 × 250 mm, 5 μm) column and mobile phase composed of 1% acetic acid-water: acetonitrile (90:10 to 60:40 for min) was followed. The injection volume and flow rate were 10 μl and 1 mL/ min, respectively. UV detection was set at 350 nm. Results show that Bn-8 harvested in September reported the highest content of rutin among the samples analyzed. This study provides a basis for the optimal harvest time of Bn which maximizes the yield of rutin.
Keywords – *Boehmeria nivea*, HPLC-UV, Rutin, Content analysis

Introduction

Boehmeria nivea (Urticaceae) is an herbaceous plant indigenous to many Asian countries such as China, Japan, Philippines, and Korea. It is commonly known as "ramie" and is primarily cultivated as a raw material for many industries such as in textile, paper, and fiber-reinforced composite material production due to its fine and strong fibers.[1-3] *B. nivea* is also consumed as food and used in traditional herbal medicine preparations as a treatment for fever, common cold, infections of the urinary tract, and edema.[4] Several studies have provided scientific evidence regarding the biological activities of *B. nivea* which showed that it exhibits antioxidant, hepatoprotective, antihyperlipidemic, antidiabetic, and anti-inflammatory effects.[5-7] Moreover, phytochemical analysis revealed that it contains many bioactive compounds such polyphenols, flavonoids, sterols, and fatty acids which contributes to the medicinal properties of its extracts.[8-9]

This study focuses on rutin, a flavonoid glycoside we isolated from *B. nivea* leaves in our previous study.[9] Rutin is a commonly consumed dietary polyphenol that is present in various plant species which includes *Fagopyrum esculentum, Sophora japonica, Amaranthus paniculatus,* and *Eucaplyptus* spp. as major sources.[10-13] It is an economically important phytochemical due to its pharmacological activities. In fact, it has been used as a component of many multivitamins and in over 130 herbal medicines which sales in 1998 was estimated as $430 million in the US.[14-15]

The aim of this study is to determine the amount of rutin present in the leaves of *B. nivea* collected from different regions of South Korea and harvested in different months by HPLC-UV analysis to assess the potential of the plant as a source of rutin. The results of this study will also provide a basis for the optimal harvest time of *B. nivea* which maximizes the yield of rutin from the plant.

Experimental

Plant materials – Dried leaves of *B. nivea* were provided by Yeonggwang Agricultural Technology and Extension Center, Korea. The samples were collected from

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Table 1. Collection areas for *B. nivea*

Sample	Collection area
Bn-1	Seobang variety in Hansan, Seocheon
Bn-2	Taiwan variety in Duwon, Goheung
Bn-3	Ramie in Mangun, Muan
Bn-4	Local variety in Hakgyo, Hampyeong
Bn-5	Local variety in Seocheon-4
Bn-6	White Peel variety in Biin, Seocheon-2
Bn-7	Improved variety in Gwangju, Taiwan variety in Goheung
Bn-8	Natural cross of White Peel variety
Bn-9	Local variety in Baeksu, Yeonggwang

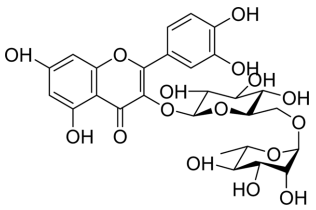


Fig. 1. Structure of rutin.

different regions in South Korea and were harvested on the months of June, July, August, and September (Table 1).

Apparatus and chemicals – Spectroscopic analysis was performed with a JEOL JMS-AX505WA FAB-MS (Japan) and a Bruker Avance 500 NMR (Rheinstetten, Germany). HPLC analysis utilized a Waters Breeze HPLC system (MA, USA) provided with a binary pump and a UV/VIS detector. HPLC water and acetonitrile were obtained from J.T. Baker Chemicals (PA, USA).

Isolation of rutin from *B. nivea* – Dried and powdered *B. nivea* leaves were extracted with methanol (MeOH) at 65 - 75 ºC for 3 hours and the resulting extract was then filtered and evaporated *in vacuo* to obtain the MeOH extract. Identification was performed by spectroscopic analysis and is presented in our previous study on the phytochemical analysis of *B. nivea* leaves.[9] The structure of rutin (purity: 97%) is shown in Fig. 1.

Preparation of standard and sample solutions – A stock solution of the standard compound was prepared by dissolving 1 mg of rutin in 50% MeOH. The working solutions used for the calibration curve was prepared by diluting the stock solution to desired concentrations. *B. nivea leaves* collected from different regions of South Korea and harvested in different months were analyzed for their rutin content. Dried and powdered leaves were extracted with MeOH under a reflux system at 65 - 75 ºC for 3 h. The resulting extract was filtered and evaporated *in vacuo* to obtain a concentrated MeOH extract of the samples. Twenty milligrams of the MeOH extract of *B. nivea* leaves were dissolved in 1 mL 50% MeOH and filtered using a 0.45 μm filter prior to HPLC analysis.

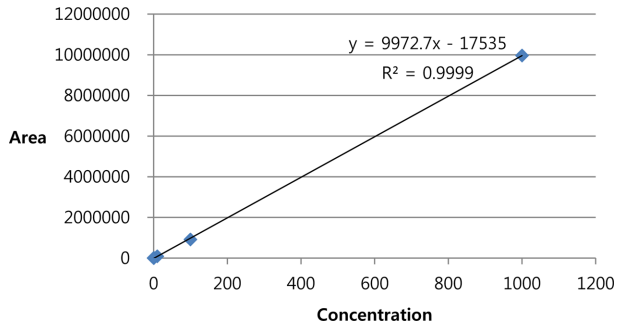


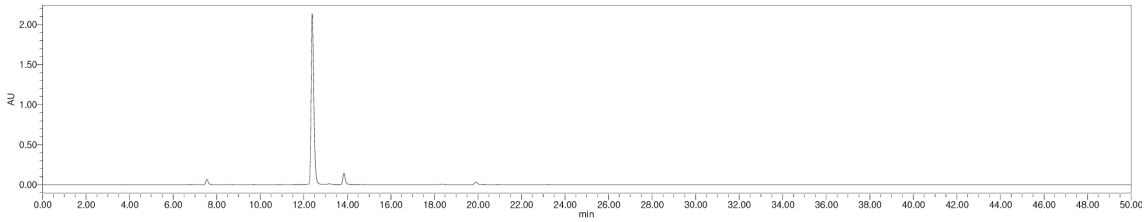
Fig. 2. Calibration curve of rutin.

HPLC conditions – A reverse phase system utilizing a Discovery® C18 (4.6 × 250 mm, 5 μm) column and a gradient elution of 1% acetic acid in water and acetonitrile (90:10 to 60:40 for 60 min) were followed for the HPLC analysis. The flowrate and injection volume were 1 mL/min and 10 μL, respectively. The UV detector was set at 350 nm.

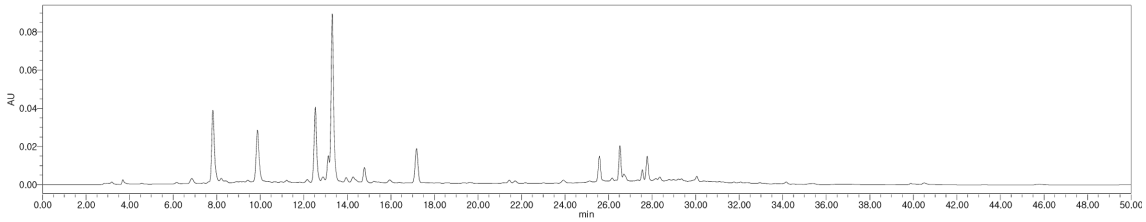
Calibration curve – Five concentrations of the standard compound were prepared. The calibration curve was constructed by plotting the peak area of each solution against their corresponding concentration. Linearity was then determined based on the correlation coefficient (r^2). The amount of the analyte present in each sample was determined using the constructed calibration curve where (Y) corresponds for the peak area and (X) for the concentration of the reference compound (μg/10 μl).

Results and Discussion

The use of *B. nivea* in traditional medicine to remedy various illnesses has prompted several studies to determine the bioactive components responsible for its healing properties. In our previous research, the flavonoid glycoside, rutin, was isolated from the leaves of Bn together with other organic compounds.[9] Particularly, the content of rutin in the leaves of Bn was investigated in this study by HPLC-UV analysis. Rutin is a pharmacologically important plant utilized in the preparations of many herbal medicines, hence, the evaluation of potential plant sources rich in this compound will prove useful in commercial production of rutin. The analytical method used showed good linearity with a correlation coefficient (r^2) greater than 0.9999 (Fig. 2). The linear calibration equation was $Y = 9972.7X - 17535$, where (Y) corresponds to the peak area and (X) corresponds to concentration of rutin. The amount of rutin present in



(A)



(B)

Fig. 3. HPLC chromatograms of rutin (A) and MeOH extract of *B. nivea* (B).

Table 2. Rutin content of the MeOH extracts of *B. nivea* (Bn) collected from different regions and harvest time

Harvest Time	Bn-1	Bn-2	Bn-3	Bn-4	Bn-5	Bn-6	Bn-7	Bn-8	Bn-9
June	2.79 ± 0.05	3.37 ± 0.00	3.36 ± 0.00	3.64 ± 0.01	2.78 ± 0.14	2.02 ± 0.00	3.05 ± 0.03	4.06 ± 0.00	4.29 ± 0.05
July	4.47 ± 0.07	4.24 ± 0.08	5.69 ± 0.01	8.89 ± 0.05	2.84 ± 0.04	1.93 ± 0.00	7.60 ± 0.18	6.51 ± 0.01	7.22 ± 0.08
August	1.88 ± 0.01	8.04 ± 0.00	5.96 ± 0.13	5.34 ± 0.13	3.56 ± 0.11	6.52 ± 0.01	2.33 ± 0.00	2.58 ± 0.00	3.15 ± 0.03
September	1.63 ± 0.04	8.28 ± 0.57	14.78 ± 0.12	14.77 ± 0.04	16.68 ± 0.10	19.49 ± 0.17	2.86 ± 0.14	27.03 ± 0.05	4.71 ± 0.00
Total	10.76	23.93	29.79	32.64	25.86	29.96	15.84	40.18	19.37

Data is represented as the mean ± SD (n = 3) in mg/g DW.

each sample was calculated from the calibration curve. The chromatographic separation of rutin and the MeOH extracts of Bn is shown in Fig. 3 and the results of the quantitative analysis is summarized in Table 2. Results show that the content of rutin in Bn leaves vary according to the regions and time they were collected. The rutin content of Bn leaves ranges from 1.63 - 27.03 mg/g which is comparable with other plant species used as commercial sources of rutin such as *F. esculentum* (24.6 - 36.1 mg/g) and *S. japonica* (44 - 287 mg/g).[16-17] It can also be observed that rutin content generally increases on the month of September. Bn-8 collected on September showed the highest amount of rutin (27.03 mg/g). Moreover, Bn-8 displayed the highest total rutin content in all four months of sampling period with a total of 40.18 mg/g (Table 2).

The results of this study show that the rutin content of *B. nivea* leaves is affected by the geographical location and harvest time. Particularly, Bn-8 samples record the highest content of the compound showing that collecting the plants on the month of September increases the yield of rutin. Ultimately, *B. nivea* is an abundant source of dietary rutin and can be employed as a source of the compound as a food additive and in the preparation of herbal medicines.

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