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# 터리풀의 채집장소 및 채집시기에 따른 카테킨 함량 HPLC/UV 분석

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# HPLC/UV Quantification of (+)-Catechin in *Filipendula glaberrima* from Different Regions and Flowering Stages

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**Abstract** – *Filipendula glaberrima* (FG) is a plant endemic to South Korea. It is economically important as a food source and used as a medicine in treating ailments. *Filipendula* flowers are characterized by the presence of several polyphenolic constituents. The aim of this study is to determine the content of (+)-catechin in *Filipendula glaberrima* collected from different regions at different flowering stages. High-performance liquid chromatography with a gradient elution system (0.5% acetic acid in water : acetonitrile = 95 : 5 to 0 : 100 for 35 min) was used. A reverse-phase INNO column with UV detection at 278 nm was employed. The results revealed that *F. glaberrima* from Mt. Odae has the highest (+)-catechin content (10.600 mg/g). Furthermore, its content was the lowest in samples collected during the pre-flowering period and the highest at the early-flowering stage. This study provides a basis in establishing the optimal period and the best region for collecting *F. glaberrima* with maximized (+)-catechin yield.

**Keywords** – (+)-Catechin, Content analysis, *Filipendula glaberrima*, HPLC/UV

*Filipendula glaberrima* (FG), commonly known as meadowsweet, is a plant endemic to South Korea along with *F. formosa* and *F. koreana*.[1)] Most *Filipendula* species are native to Northeast Asian regions, including South Korea, Japan, Manchuria, and Eastern Siberia. Among the perennial herbaceous plants native to South Korea, FG grows in wet and shady surroundings. Studies have reported that it exhibits significant anti-viral and anti-bacterial properties. It is economically important as a food source and it also has medicinal and ornamental applications.[2,3)]

Several *Filipendula* species exhibit a wide spectrum of pharmacological activities, including anti-inflammatory, antimicrobial, anti-ulcerous, anti-oxidant, wound-healing, hepatoprotective, anti-cancer, anti-coagulant, and anti-diabetic activities.[4-9)] Furthermore, *Filipendula* has been traditionally used in medicine as a febrifuge to treat certain inflammatory diseases, rheumatism, arthritis, and gout. It is also acts as an antacid for stomachic remedies.[10)] *Filipendula* leaves are mainly used as a decoction to treat stomach ache and diarrhea, breathlessness, wheezing, kidney problems, congestion, sore throat, and for relieving influenza symptoms.[11)]

*Filipendula* flowers are characterized by the presence of several polyphenolic constituents, including flavonols (e.g., rutin, hyperoside, spiraeoside, and kaempferol 4′-O-glucoside), salicylates (e.g., salicylic acid, spiraein, and methyl salicylate), and ellagitannins (rugosin D and tellimagrandins I and II).[12-14)] *F. vulgaris* possesses high concentrations of phenolics, contributing to its anti-bacterial activity, salicylates, and a pharmacologically active plant heparin.[15)] Addi-

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tionally, monotropitin, (+)-catechin, and β-sitosterol 3-O-β-D-glucoside have been isolated and identified from FG,[16] and a novel flavonoid glycoside ulmarioside along with 48 compounds, including (+)-catechin, have been isolated from *F. ulmaria*.[17]

The (+)-catechin we analyzed in the present study is the main ingredient found in green tea. It is a well-known flavonoid that have many beneficial effects on human health. Here, ethanol (EtOH) extracts were used for assessing (+)-catechin distribution and quantity in FG at different flowering stages collected from various regions in South Korea. High-performance liquid chromatography (HPLC) coupled with ultraviolet-visible (UV) spectroscopy was employed for (+)-catechin analysis. To the best of our knowledge, this is the first report on the quantification and comparison of (+)-catechin content in FG at different flowering stages obtained from different regions.

## Materials and Methods

**Plant Materials** – FG at their pre-flowering, early-flowering, and full-flowering stage (Fig. 1) were collected from Yeoncheon and Mt. Odae, South Korea, in June 2020. Pre-flowering stage is when the flower stalk has not been raised yet (Fig. 1B). Early-flowering stage has a flower stalk with only buds (Fig. 1A) and full-flowering stage is when the buds of a flower stalk begin to bloom (Fig. 1C). The plant was identified by Dr. C. G. Park, National Institute of Horticultural and Herbal Science, South Korea. A voucher specimen was deposited at the Department of Plant Science and Technology herbarium, Chung-Ang University, Anseong, South Korea.

**Instruments and Reagents** – (+)-Catechin (Fig. 2) was isolated from FG[16] and acquired from the Natural Product Institute of Science and Technology (www.nist.re.kr),

(A)

(B)

(C)

**Fig. 1.** FG at different flowering stages, early-flowering (A), pre-flowering (B), and full-flowering (C).

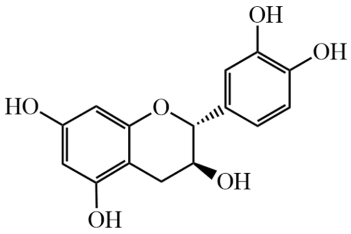



**Fig. 2.** Chemical structure of (+)-catechin.

Anseong, South Korea. Chromatographic analysis was performed using an HPLC system (Gilson 72, Rue Gambetta, BP45 95400 Villers Lebel France) equipped with a pump, auto-sampler, and UV detector. HPLC-grade solvents, including water, methanol (MeOH), and acetonitrile (ACN), were purchased from J. T. Baker (Phillipsburg, PA, USA). Acetic acid (99.7%) was purchased from Samchun Pure Chemicals (Pyeongtaek, Korea).

Preparation of Sample and Standard Solutions for HPLC – Dried aerial parts of FG (20 g) from different cultivated regions and at different flowering stages were extracted with EtOH under reflux and evaporated *in vacuo*. The EtOH extract (20 mg) was dissolved in 1 mL MeOH and filtered through a syringe filter (0.45 μm). A stock solution of the standard compound was prepared by dissolving 1 mg of (+)-catechin in 1 mL MeOH. To prepare the (+)-catechin calibration curve, working solutions were prepared by diluting the stock solution to the desired concentrations.

HPLC/UV Conditions – (+)-Catechin was quantified using a reverse-phase HPLC system with an INNO C18 column (25 cm × 4.6 mm, 5 μm) at ambient temperature. The injection volume was 20 μL and was monitored at 278 nm. The flow rate was set at 1.1 mL/min. The mobile phase consisted of 0.5% acetic acid in water (A) and ACN (B), and the gradient elution was achieved as follows: 95% A at 0 min, 89% A from 0 to 12 min, 88% A from 12 to 25 min, decreased to 0% A from 25 to 35 min and maintained as such till 45 min, increased to 95% A from 45 to 50 min and maintained as such until 60 min.

Calibration Curve – A calibration curve was prepared by plotting the concentrations of the standard solution with their respective peak areas. The linearity of the calibration curve was determined based on the correlation coefficient ($r^2$), and the (+)-catechin concentrations in the samples were then calculated from the calibration curve. The calibration functions were determined based on the peak area ($Y$), concentration ($X$, mg/mL), and mean ± standard deviation (n = 5).

## Results and Discussion

Flavonoids are polyphenolic phytochemicals widely distributed in fruits and vegetables.[18,19] Flavonoids possess a wide range of biological activities, including anti-arteriosclerotic, anti-oxidative stress, anti-proliferative, anti-inflammatory, and anti-diabetic activities.[20-24] Among these, catechin is one of the important flavonoids found in tea. It has two enantiomers, i.e., (+)-catechin and (-)-catechin. Most foods contain (+)-catechin, except chocolate, and (+)-catechin is known to be more bioavailable than (-)-catechin.[25]

(+)-Catechin, a type of flavan-3-ol phenol, is a secondary plant metabolite which acts as an anti-oxidant agent. It belongs to a representative group of polyphenols and exhibits a wide range of pharmacological activities, including anti-oxidant and anti-inflammatory properties.[26-30] This compound is abundant in various natural products, such as green tea and fruits, and its content is closely related to the cultivation region and type of green tea.[31]

We investigated the (+)-catechin content of FG using HPLC-UV analysis. Good separations were observed in the HPLC chromatogram, and the retention time was determined as 19.85 min. The HPLC conditions and results of (+)-catechin quantification are illustrated in Fig. 3. The equation for the linear calibration of the standard curve was Y = 48.367X + 50.478, where Y and X represent a given peak area and the corresponding (+)-catechin concentration, respectively. The correlation coefficient ($r^2$) was found to be greater than 0.9998, indicating good linearity of the analytical method (Table I). The amount of (+)-catechin in each sample was calculated using the calibration curve. Figs. 3-5 illustrate the chromatographic separation of (+)-catechin and the EtOH extract of FG. The results of the quantitative analyses are summarized in Table I.

Our results showed that the (+)-catechin content of FG varied depending on the region and flowering stage. In particular, the (+)-catechin content was highest in samples collected from Mt. Odae (10.600 mg/g) and the early-flowering stage presented higher (+)-catechin contents than those of the other two flowering stages (Table II).

The (+)-catechin content of *F. ulmaria* has previously been reported. (+)-Catechin was quantified in extracts from different parts of *F. ulmaria* before and after hydrolysis. (+)-Catechin was identified and quantified in extracts of the root (17.17 mg/g) and aerial parts (11.30 mg/g). It was the main component in *F. ulmaria* root extract; however, its quantity

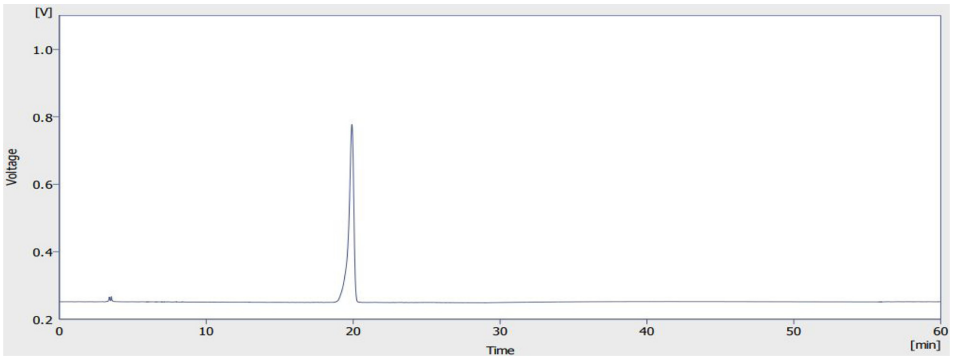
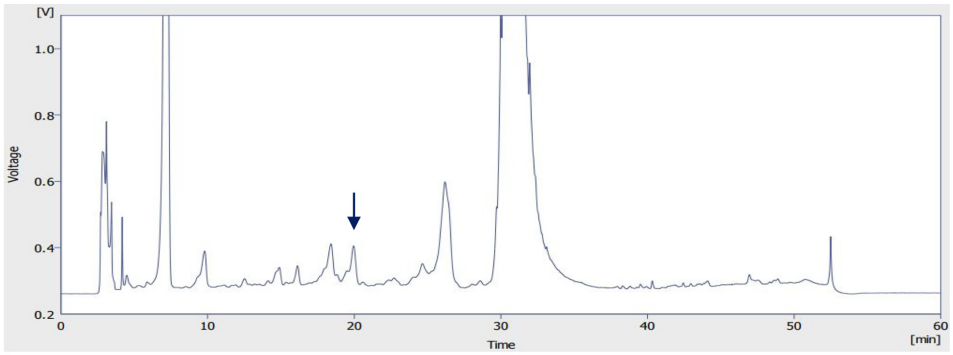
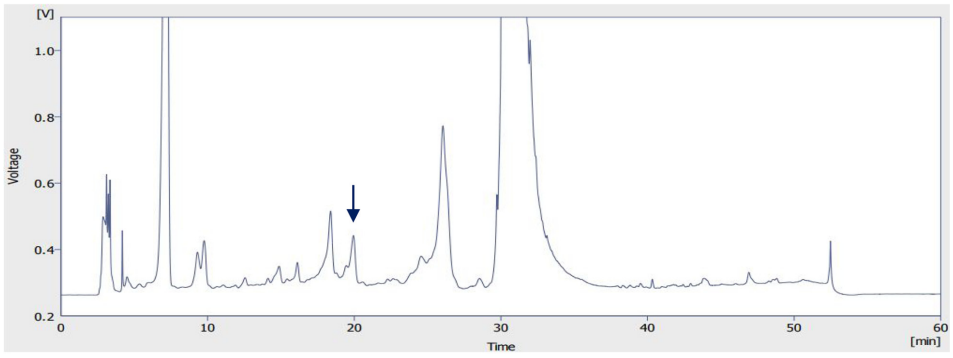


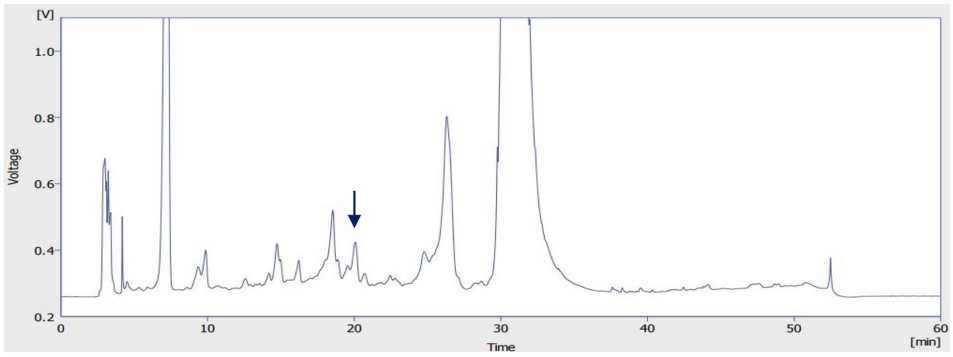

**Fig. 3**. HPLC chromatogram of (+)-catechin.




(A)




(B)




(C)

**Fig. 4**. HPLC chromatograms of the EtOH extracts of FG from Yeoncheon at the pre-flowering (A), early-flowering (B), and full-flowering (C) stages.

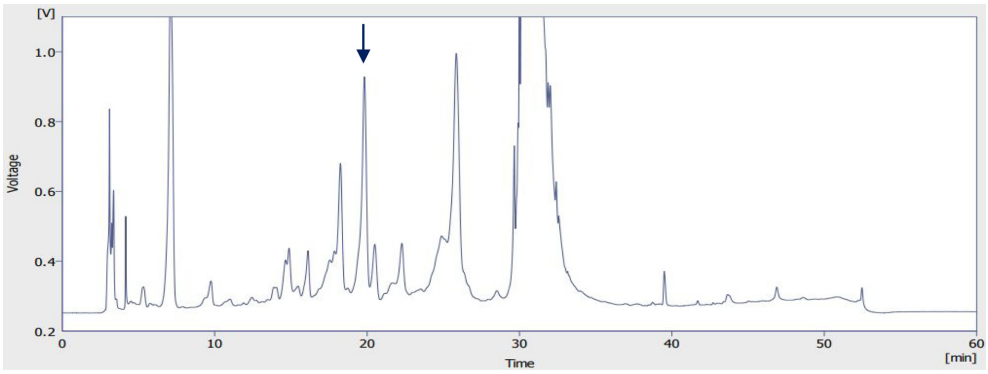



**Fig. 5.** HPLC chromatogram of the EtOH extract of FG from Mt. Odae.

**Table I.** The calibration curve for (+)-catechin

| Compound | $t_R$ | Calibration equation | Correlation factor, $r^2$ |
|---|---|---|---|
| (+)-catechin | 19.85 | Y = 48.367X + 50.478 | 0.9998 |

$t_R$ = retention time
Y = peak area, X = concentration of the standard (mg/mL)
$r^2$ = correlation coefficient for five data points on the calibration curve

**Table II.** Content of (+)-catechin in FG from Yeonchon and Mt. Odae

| Region | Yeonchon | | | Mt. Odae |
|---|---|---|---|---|
| Collection time | Pre-flowering | Early-flowering | Full-flowering | Pre-flowering |
| Content (mg/g extract) | 2.166 ± 0.022 | 2.508 ± 0.044 | 2.321 ± 0.007 | 10.600 ± 0.011 |

decreased after hydrolysis.[7)]

Our results demonstrate that the (+)-catechin content of FG is influenced by the flowering stage and geographical location. Samples collected at their early-flowering stage presented the highest (+)-catechin content, indicating that plants collected in the flowering season would have higher (+)-catechin production. These results provide the basis for further experimentation, and FG could potentially be used as a health supplement and in the preparation of herbal medicines.

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