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Chemotaxonomic Significance of Taxifolin-3-O-Arabinopyranoside in *Rhododendron* Species Native to Korea

TaeHee Kim[1], Ye Eun Kwon[1], Sun Min Park[1], Min Seok Kim[1], Young Hwan Jeong[1], Se Yeong Park[1], Young-Soo Bae[1], Eun Ju Cheong[2], Yi-Chang He[3], Chun Gong[3], Wei Gao[3], Hee Kyu Kim[4], Yeon Ho Ham[4], Jin-Kyu Kim[5] and Sun Eun Choi[1,*]

[1]*Department of Forest Biomaterials Engineering, College of Forest & Environmental Sciences, Kangwon National University, Chuncheon 24341, Republic of Korea*
[2]*College of Forest and Environmental Science, Kangwon National University, Chuncheon 24341, Republic of Korea*
[3]*Jiangxi Academy of Forestry, Jiangxi Province, Nanchang 330032, China*
[4]*Gangwondo Forest Science Institute, Chuncheon 24207, Republic of Korea*
[5]*Gyeonggido Business and Science Accelerator, Suwon 16229, Republic of Korea*

Abstract

Genus of *Rhododendron* has been used in traditional medicine since ancient times and is known to be effective in immune function, inflammation, and cold symptoms. And the reason for this activity is the flavanonol type among flavonoids in the genus of *Rhododendron*. Among the flavanonol types, Taxifolin-3-O-arabinopyranoside was isolated from the root of native *R. mucronulatum* in Korea, and the structure was finally identified through HPLC, LC-MS/MS, ^{1}H-NMR, and ^{13}C-NMR. Taxifolin-3-O-arabinopyranoside is a compound mainly found in *R. mucronulatum*, a representative species of the genus of *Rhododendron*, and exhibits antioxidant, anti-inflammatory, and anti-atopic activities. In this study, Taxifolin-3-O-arabinopyranoside was chemotaxonomic significant in 5 species of the genus *Rhododendron* native to Korea (*R. mucronulatum*, *R. mucronulatum* var. *ciliatum*, *R. schlippenbachii*, *R. yedoense* var. *Poukhanense*, *R. japonicum* for. *Flavum*). Compared with the existing literature, Taxifolin-3-O-arabinopyranoside was identified for the first time in 4 species of *Rhododendron* except for the *R. mucronulatum*.

Key Words: *Rhododendron* species, chemotaxonomy, NMR, LC-MS/MS, HPLC

Introduction

Genus of *Rhododendron* is a plant belonging to the Ericaceae family, ranging in size from 2.5 cm to large trees up to 40 m tall, and grows in well-ventilated, acidic soils (Paul et al. 2018). The genus of *Rhododendron* has about 1,200 species distributed all over the world except Australia, and in the temperate and sub-alpine and alpine regions of the eastern Himalayas, the genus of *Rhododendron* is the dominant group of plants (Kim et al. 2006; Paul et al. 2018). According to the Korea National Arboretum of the Korea Forest Service, there are 19 species of *Rhododendron* native to Korea, including *R. mucronulatum* Turcz., *R. mucronulatum* Turcz. var. *ciliatum* Nakai and *R. schlip-*

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Corresponding author: Sun Eun Choi
Department of Forest Biomaterials Engineering, College of Forest & Environmental Sciences, Kangwon National University, Chuncheon 24341, Republic of Korea
Tel: +82-33-250-8324, Fax: +82-33-259-5621, E-mail: oregonin@kangwon.ac.kr

penbachii Maxim. (Korea National Arboretum 2021). Plants of the genus of *Rhododendron* are known to be widely used in folk remedies or traditional medicines in various countries for the treatment of inflammation, cold symptoms, skin diseases, gastrointestinal disorders, diabetes, pain relief, liver and heart protection, antioxidants, and immune action (Popescu et al. 2013). For example, in China, dried leaves of *R. mucronulatum* and *R. dauricum* are used to treat acute and chronic bronchitis such as fever, asthma, and coughing (Iwata et al. 2004; Fu et al. 2012). Native Americans of North America used *R. tomentosum* to treat cold symptoms, toothache, stomach pain, snow blindness, and stomatitis (Black et al. 2011). The species *R. mucronulatum* has reported its use in oriental medicine to improve blood circulation, hemostasis, and pain relief (Jang et al. 2005; Kim et al. 2018) reported that *R. brachycarpum* was used to treat various diseases such as hypertension, hypotension, diabetes, headache, menstrual irregularity, hepatitis, liver cirrhosis, sinusitis, heart failure, and kidney disease. The leaves of *R. schlippenbachii* were used to excrete strong heart medicine or phlegm (Sancheti et al. 2011), and the leaves of *R. aureum* were used to treat pain and swelling in inflammatory diseases such as rheumatoid arthritis (Kim et al. 2011).

In addition, according to a previous study by this research team, it was reported that the root extract of *R. mucronulatum* is effective in improving immunomodulatory effects and chronic inflammatory diseases such as atopic dermatitis treatment (Kim et al. 2008; Ahn et al. 2010). The physiological activity of plants is due to secondary metabolites, such as phenolic compounds (Yang et al. 2021). Phenolic compounds include flavonoids, which constitute most of the secondary metabolites of the genus of *Rhododendron* and are known as major substances that cause various activities (Qiang et al. 2011; Yang et al. 2021). The flavonoids derived from the genus of *Rhododendron* are divided into flavone, flavanone, flavanonol, and flavan-3-ol types. The flavone type includes Juglanin, Myricetin, Myricitrin, Myricitrin-5-methyl ether, Astragalin, Afzelin, Quercetin, Quercitrin, Isoquercitrin, Quercetin-5-O-β-D-glucopyranoside isolated from *R. mucronulatum*, *R. schlippenbachii*, and *R. yedoense* var. *Poukhanense*. Flavanone and flavanonol types include (+)-Taxifolin, (+)-Taxifolin-3-O-α-L-arabinopyranoside, and Ampelopsin isolated from *R. mucronulatum*. The flavan-3-ol type includes (+)-Catechin, (-)-Catechin, and (+)-Epicatechin isolated from *R. mucronulatum* (Lee et al. 2005; Kim et al. 2006; Hong et al. 2007; Jung et al. 2007).

Previous studies related to the activity of flavonoids in genus of *Rhododendron* are as follows.

First, it has been reported that (+)-Taxifolin and (+)-Epicatechin isolated from *R. mucronulatum* stems and Quercetin, Quercitrin, and Myricitrin-5-methyl ether isolated from *R. yedoense* var *Poukhanense* had excellent antioxidant activity (Lee et al. 2005; Jung et al. 2007). Second, it was reported that Isoquercitrin and Quercitrin isolated from the leaves of *R. ponticum* L. are effective in anti-inflammatory and pain relief (Erdemoglu et al. 2008). According to previous studies, Taxifolin glycoside, which exhibits antioxidant, anti-inflammatory, and anti-atopic activities, was isolated from *R. mucronulatum* in many cases (Kim et al. 2008; Ahn et al. 2010; Kang et al. 2010; Choi et al. 2011; Kim et al. 2018). However, few cases were found in other species of *Rhododendron* except *R. mucronulatum*, so we tried to investigate the presence or absence of Taxifolin glycoside from other species of *Rhododendron*.

In this study, Taxifolin glycoside was used as a standard sample. Afterwards, for chemotaxonomy research, extracts of *R. mucronulatum* (stems, branches, leaves, and flowers), *R. mucronulatum* var. *ciliatum* (stems, leaves), *R. schlippenbachii* (stems, branches, leaves, and flowers), *R. yedoense* var. *Poukhanense* (stems, branches, and leaves), and *R. japonicum* for. *Flavum* (branches, leaves), among the species of *Rhododendron* native to Korea confirmed the presence of Taxifolin glycoside. In addition, when classifying the Korean native *Rhododendron*, most of them check the appearance of flowers, germination characteristics and dormancy types of seeds (Kim et al. 2021), availability of medicinal products, distribution status, and efficacy (Min et al. 2013), but the basic data for chemical classification are still insufficient. Therefore, based on the results of this study, it was intended to be used as basic data for the chemical phylogenetic classification of the Korean native *Rhododendron*.

Materials and Methods

Plant extracts of Rhododendron species

The root of *R. mucronulatum* was purchased from Cheonji Herbs (Seoul, Korea), certificated by Prof. Choi (Wood Natural Products Functional Materials Lab, Department of Forest Biomaterials Engineering, College of Forest & Environmental Sciences, Kangwon National University). And, a voucher specimen is stored in the Wood Natural Products Functional Materials Lab, Department of Forest Biomaterials Engineering, College of Forest & Environmental Sciences, Kangwon National University. The MeOH extracts of *R. mucronulatum* (stem, branch, leave, flower, bar code; PB4095.2-3, 7-8), *R. mucronulatum* var. *ciliatum* (stem, leave, bar code; PB4097.2-3), *R. schlippenbachii* (stem, branch, leave, flower, bar code; PB4100A.4-6, 8-9, 11-12), *R. yedoense* var. *Poukhanense* (stem, branch, leave, bar code; PB4102.2-7), *R. japonicum* for. *Flavum* (branch, leave, bar code; PB4109B.1-2) were purchased from the Korea Plant Extract Bank (Cheongju, Korea). Samples used in the experiment is shown in Table 1, and each was dissolved in MeOH to prepare a stock solution of 20000 ppm, which was then diluted and used.

Separation of active compounds in the root of R. mucronulatum

After extracting the raw material of the root of *R. mucronulatum* with 60% alcohol, it was concentrated under reduced pressure and freeze-dried to recover the extract powder. Then, the extract powder was dissolved in methanol, and thin-layer chromatography (TLC) was performed.

Table 2. Conditions of HPLC analysis

Analytical-HPLC equipment	Waters 2,695 separation module 2,487 Dual λ Absorbance Detector
Guard column	Phenomenex KJ0-4282 guard column
Column	SkyPak C18 analytical column (5 μm)
Temperature	RT
Flow rate	1 mL/min
Wavelength	280 nm
Injection volume	20 μL
Run time	60 min.

RT, room temperature.

Table 1. Sample list

Number	Species	Part	Habitat
1	Rhododendron mucronulatum	Stem	Gyeongsangnam-do
2		Flower	Gyeongsangnam-do
3		Branch	Gangwon-do
4		Leave	Gangwon-do
5	Rhododendron mucronulatum var. ciliatum	Leave	Jeju
6		Stem	Jeju
7	Rhododendron schlippenbachii	Flower	Gangwon-do
8		Leave	Gangwon-do
9		Stem	Gangwon-do
10		Branch	Gyeongsangbuk-do
11		Leave	Gyeongsangbuk-do
12		Branch	Gangwon-do
13		Leave	Gangwon-do
14	Rhododendron yedoense var. Poukhanense	Leave	Jeju
15		Stem	Jeju
16		Branch	Jeju
17		Leave	Jeju
18		Leave	Chungcheongnam-do
19		Branch	Chungcheongnam-do
20	Rhododendron japonicum for. Flavum	Branch	Jeollanam-do
21		Leave	Jeollanam-do

For, TLC, a plate coated with silica gel was used, and a ratio of chloroform, methanol, and water was prepared at 70:30:4 and used as a developing solvent. After drying the developed silica gel plate, the spot was checked at a wavelength of 254 nm, and 10% sulfuric acid, *ρ*-anisaldehyde sulfuric acid, and $FeCl_3$ were used as color development reagents.

The extract powder from the root of *R. mucronulatum* was dissolved in methanol at a concentration of 1,000 ppm, and HPLC analysis was performed. The HPLC equipment and mobile phase conditions used for the analysis are shown in Table 2, 3.

After dissolving the extract of the root of *R. mucronulatum* in distilled water, ethyl acetate (EA) fractionation was performed. First, the fraction was concentrated under reduced pressure and freeze-dried to recover in powder form. Then, the active compound was confirmed with TLC monitoring, and separated and purified from the EA fraction using Prep LC and MPLC. The equipment and mobile phase conditions used for Prep LC and MPLC analysis are shown in Table 4-7.

Table 3. HPLC method of solvent system

Gradient	0 min.	40 min.	45 min.	50 min.	60 min.
Solvent A	90	40	0	90	90
Solvent B	10	60	100	10	10

Solvent A, 1% formic acid in H_2O; Solvent B, Acetonitrile (CH_3CN).

Table 4. Conditions of Prep LC analysis

Prep LC equipment	Waters Prep LC controller 2487 Dual λ absorbance detector
Column	Universal™ Column Silica Gel (40 μm, 60 Å, 3.0×16.5 cm, 40 g)
Flow rate	20 mL/min
Run time	20 min.

Table 5. Prep LC method of solvent system

Isocratic	0-20 min.
Solvent A (CMW)	100

Solvent A: Chloroform (70%), Methanol (30%), Water (4%).

Structural identification of active compound isolated from an extract of the root of R. mucronulatum

The active compound separated and purified from the extract of the root of *R. mucronulatum* was recovered in powder form, and the structure was identified through LC-MS/MS analysis and 1D nuclear magnetic resonance (NMR) analysis after HPLC analysis. For LC-MS/MS analysis, QTRAP 4500 (AB SCIEX, USA) was used, and the conditions used for HPLC analysis and LC-MS/MS analysis are shown in Table 2, 3. In addition, for NMR analysis, Avance III 700 (Bruker) was used, ^{1}H NMR and ^{13}C NMR spectra were obtained at 700 MHz and 176 MHz, respectively, and MeOH-*d*4 was used as a solvent.

Quantitative analysis of Taxifolin-3-O-arabinopyranoside using HPLC of several species of the genus of Rhododendron

Taxifolin-3-O-arabinopyranoside obtained directly from the extract of the root of *R. mucronulatum* was used as a standard material and dissolved in methanol to prepare 1,000 ppm. After that, a standard calibration curve was prepared by diluting the concentrations 1,000, 500, 250, 125, 62.5, 20, 10, and 2.5 ppm, and the limit of detection

Table 6. MPLC analysis conditions

MPLC equipment	Yamazen smart flash
Column	UniversalTM Column ODS-SM (50 μm, 120 Å, 3.0×16.5 cm, 37 g)
Flow rate	20 mL/min
Wavelength	280 nm
Inject volume	2,000 μL
Run time	70 min.

Table 7. MPLC method of solvent system

Gradient	0 min.	10 min.	15 min.	50 min.	55 min.	60 min.	70 min.
Sol. A	100	70	65	55	50	100	100
Sol. B	0	30	35	45	50	0	0

Solvent A, H_2O; Solvent B, Methanol.

(LOD) and limit of quantization (LOQ) was calculated. The following formula was used to calculate the detection limit and quantization limit. After that, the stock solutions of samples 1-21 were diluted to 2,000 ppm with methanol, respectively, and HPLC analysis was performed, and the analysis conditions are shown in Table 2, 3. After the analysis, the samples' retention time and the standards were compared, and quantitative analysis was performed according to the LOD and LOQ.

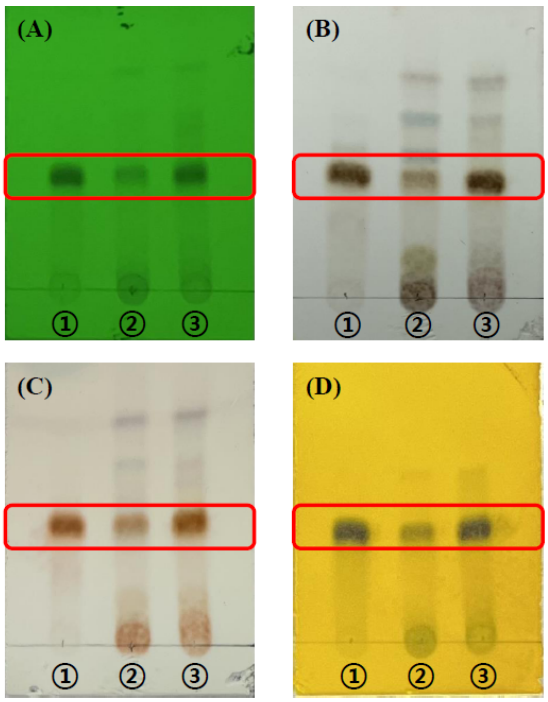

Fig. 1. TLC monitoring data of root extract of *R. mucronulatum* 1,000 ppm. (A) UV lamp 254 nm (B) 10% H_2SO_4 (C) ρ-Anisaldehyde H_2SO_4 (D) $FeCl_3$. And each sample is ① Taxifolin-3-O-arabinopyranoside, ② root extract of *R. mucronulatum*, ③ ethyl acetate solvent fraction of root extract of *R. mucronulatum*.

LOD (Limit Of Detection, ppm)=3.3×SE/S
'SE'=Standard Error
'S'=Slope of the calibration curve
LOQ (Limit Of Quantization, ppm)=10×SE/S
'SE'=Standard Error
'S'=Slope of the calibration curve

Molecular weight confirmation using LC-MS/MS of several species of the genus of Rhododendron

After confirming the molecular weight of 5 species of the genus of *Rhododendron* through LC-MS/MS analysis, it was compared with that of Taxifolin-3-O-arabinopyranoside used as a standard compound. QTRAP 4500 (AB SCIEX, USA) was used, and the conditions used for the analysis are shown in Table 2, 3, and the molecular weight was confirmed under negative conditions.

Results and Discussion

After spreading the extract of the root of *R. mucronulatum* and ethyl acetate solvent fraction on a silica gel plate, 10% sulfuric acid, *ρ*-Anisaldehyde sulfuric acid, and $FeCl_3$ were sprayed, and the heat was applied to confirm the brown spot of the active compound. And the result is shown in Fig. 1.

HPLC chromatogram of the active compound isolated from the extract of the root of *R. mucronulatum* is shown in Fig. 2, and the retention time is 12.208±0.03 min. Then, through LC-MS/MS analysis, it was confirmed that the molecular weight of the active compound was 435.1 under negative conditions, shown in Fig. 3.

The NMR spectrum is shown in Fig. 4, 5, and the corresponding spectral data are as follows.

^{1}H-NMR (700 MHz, MeOH-d4, ppm): δ6.97 (1H,

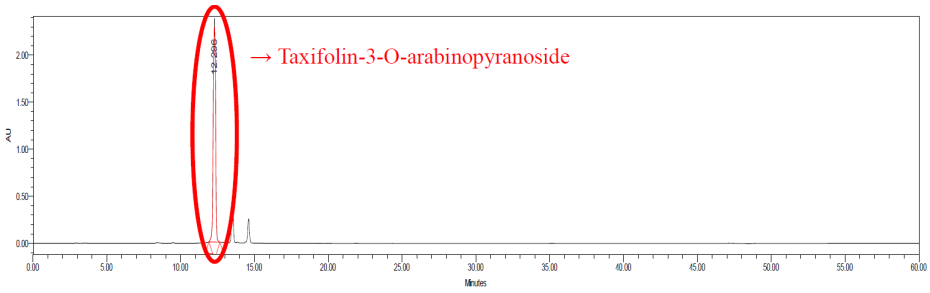

Fig. 2. HPLC chromatogram of active compound isolated from root extract of *R. mucronulatum* 1,000 ppm.

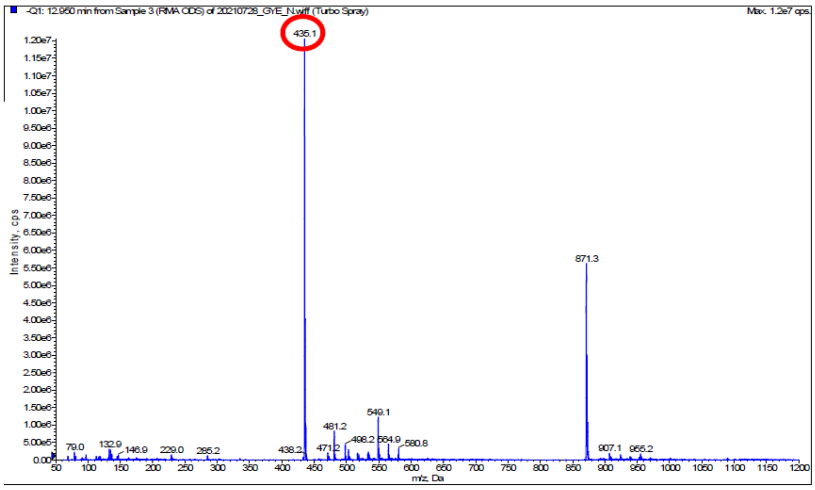

Fig. 3. Molecular weight of active compound isolated from root extract of *R. mucronulatum.*

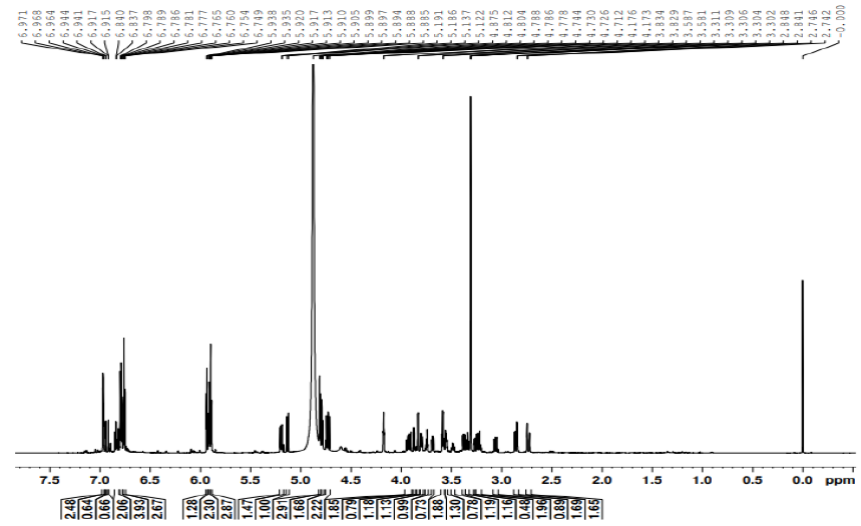

Fig. 4. ^{1}H-NMR spectrum of active compound isolated from extract of root of *R. mucronulatum.*

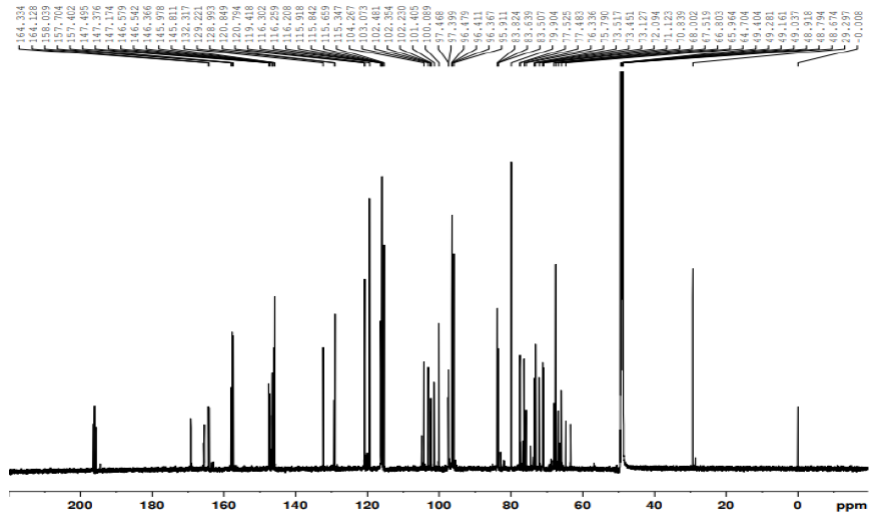

Fig. 5. ^{13}C-NMR spectrum of active compound isolated from extract of root of *R. mucronulatum.*

d, J=2.1 Hz, H-2'), δ6.84 (1H, dd, J=2.1, 8.4 Hz, H-6'), δ6.80 (1H, d, J=8.4 Hz, H-5'), δ5.94 (1H, d, J=2.1 Hz, H-6), δ5.91 (1H, d, J=2.1Hz, H-8), δ5.12 (1H, d, J=10.5 Hz, H-2), δ4.80 (1H, d, J=11.2 Hz, H-3), δ3.93 (1H, dd, J=7.0, 11.2 Hz, H-5"), δ3.83 (1H, d, J=3.5 Hz, H-1"), δ3.80 (1H, m, H-4"), δ3.59 (1H, m, H-2"), δ3.56 (1H, m, H-3"), δ3.38 (1H, dd, J=3.5, 11.2 Hz, H-5"); ^{13}C-NMR (176 MHz, MeOH-d4, ppm): δ196.11 (C-4), δ169.27 (C-7), δ165.49 (C-5), δ 164.13 (C-9), δ147.50 (C-4'), δ146.58 (C-3'), δ128.99 (C-1'), δ120.79 (C-6'), δ115.92 (C-5'), δ115.66 (C-2'), δ 102.54 (C-1"), δ101.41 (C-10), δ97.40 (C-6), δ96.41 (C-8), δ83.82 (C-2), δ76.34 (C-3), δ73.13 (C-2"), δ71.12 (C-4"), δ66.80 (C-3"), δ63.37 (C-5").

In the ^{1}H-NMR spectrum, at δ 6.80 to 6.97, a peak presumed to have the ABX type 3,4-dihydroxylation pattern of the B ring appeared. And it was confirmed that each of these showed meta-coupled aromatic signal [δ 6.97 (1H, d, J=2.1 Hz, H-2')], ortho-coupled aromatic signal [δ 6.80 (1H, d, J=8.4 Hz, H-5')], and ortho-meta-coupled aromatic signal [δ 6.84 (1H, dd, J=2.1, 8.4 Hz, H-6')]. The signals of H-8 and H-6, which are meta-coupled through δ5.94 (1H, d, J=2.1 Hz, H-6), and δ5.91 (1H, d, J=2.1Hz, H-8) were confirmed. Therefore, it was confirmed that the structure of the A ring was a 5,7-dihydroxylatioin pattern. Then, through δ5.12 (1H, d, J=10.5 Hz, H-2), and δ4.80 (1H, d, J=11.2 Hz, H-3), it was flavanonol structure that had no double bond in the C ring, and at δ3.83 (1H, d, J=3.5 Hz, H-1"), the anomeric proton of the sugar appeared, which was assumed to be 3',4',5,7-tet-

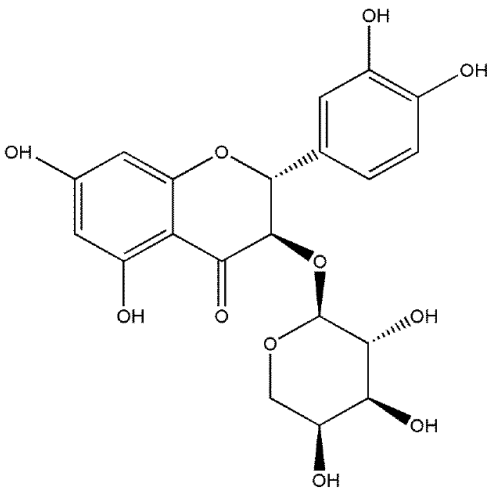

Fig. 6. Chemical structures of Taxifolin-3-O-arabinopyranoside isolated from root extract of *R. mucronulatum*.

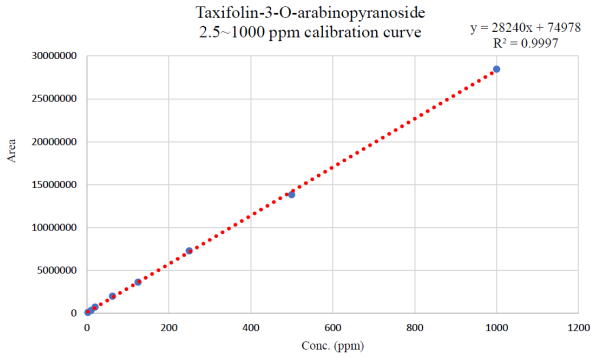

Fig. 7. Calibration curve of Taxifolin-3-O-arabinopyranoside.

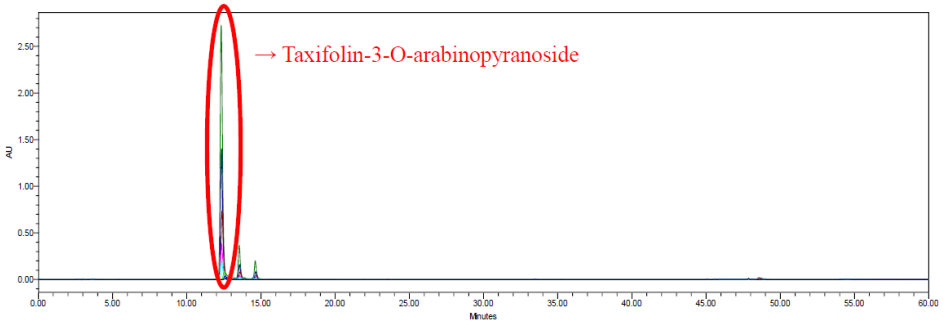

Fig. 8. HPLC chromatogram of Taxifolin-3-O-arabinopyranoside. Sample 1, Taxifolin-3-O-arabinopyranoside 1,000 ppm (green color line); Sample 2, Taxifolin-3-O-arabinopyranoside 500 ppm (purple color line); Sample 3, Taxifolin-3-O-arabinopyranoside 250 ppm (brown color line); Sample 4, Taxifolin-3-O-arabinopyranoside 125 ppm (pink color line); Sample 5, Taxifolin-3-O-arabinopyranoside 62.5 ppm (sky blue color line); Sample 6, Taxifolin-3-O-arabinopyranoside 20 ppm (light green color line); Sample 7, Taxifolin-3-O-arabinopyranoside 10 ppm (blue color line); Sample 8, Taxifolin-3-O-arabinopyranoside 2.5 ppm (black color line).

rahydroxy flavanonol arabinoside. In the ^{13}C-NMR spectrum, when compared with taxifolin, the parent nucleus, C-3 is 2.63 ppm downfield shift, C-2 is 1.34 ppm upfield shift, and C-4 is 2.36 ppm upfield shift. Through this, it was confirmed that the sugar was attached to C-3, and from δ102.54 (C-1"), δ73.13 (C-2"), δ71.12 (C-4"), δ66.80 (C-3"), δ63.37 (C-5") signals, it was confirmed that the sugar was arabinopyranoside.

By comparing and confirming that the above analysis results are consistent with the literature (Hong et al. 2007; Usman et al. 2016), the active compound separated and purified from the root extract of *R. mucronulatum* was identified as Taxifolin-3-O-arabinopyranoside, which is a Taxifolin glycoside (Fig. 6).

For the HPLC analysis of 21 extracts of 5 species *R. mucronulatum*, *R. mucronulatum* var. *ciliatum*, *R. schlippenbachii*, *R. yedoense* var. *Poukhanense*, and *R. japonicum* for. *Flavum*, Taxifolin-3-O-arabinopyranoside was diluted by concentration to obtain a standard calibration curve (y=28240x+74978; R^2=0.9997), and Fig. 7, 8 and Table 8 are shown. Accordingly, the limit of detection (LOD) was 9.760 ppm, and the limit of quantization (LOQ) was 29.575 ppm. Then, the HPLC chromatogram of 21 extracts is shown in Fig. 9-13 are shown.

Table 8. Chromatogram area value according to concentration

Conc. (ppm)	Area
2.5	81,121
10	303,467
20	710,062
62.5	1,982,119
125	3,612,477
250	7,265,170
500	13,817,416
1,000	28,460,462

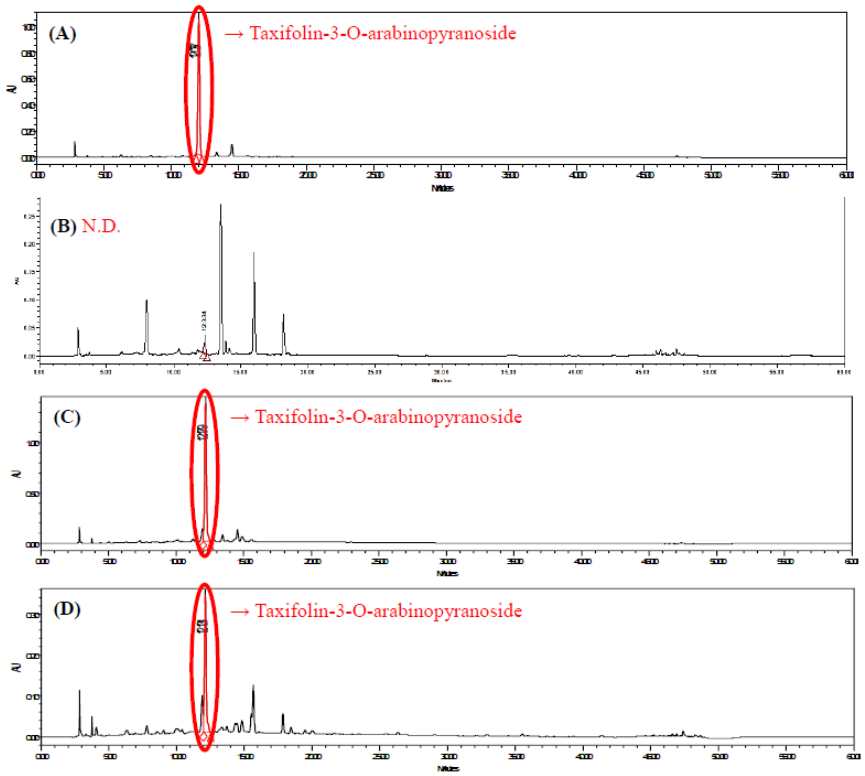

Fig. 9. HPLC chromatogram of the extracts of *R. mucronulatum* (Gn, Gw). Gn is Gyeongsangnam-do, Gw is Gangwon-do. N.D. is no detection. (A) Sample 1, stem extracts of *R. mucronulatum* 1,000 ppm (Gn). (B) Sample 1, flower extracts of *R. mucronulatum* 1,000 ppm (Gn). (C) Sample 1, branch extracts of *R. mucronulatum* 1,000 ppm (Gw). (D) Sample 1, leave extracts of *R. mucronulatum* 1,000 ppm (Gw).

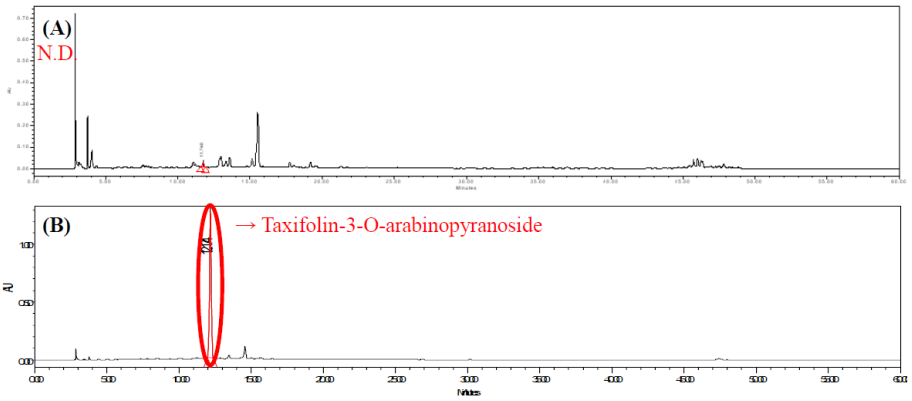

Fig. 10. HPLC chromatogram of the extracts of *R. mucronulatum* var. *ciliatum* (Jj). Jj is Jeju. N.D. is no detection. (A) Sample 1, leave extracts of *R. mucronulatum* var. *ciliatum* 1,000 ppm (Jj). (B) Sample 1, stem extracts of *R. mucronulatum* var. *ciliatum* 1,000 ppm (Jj).

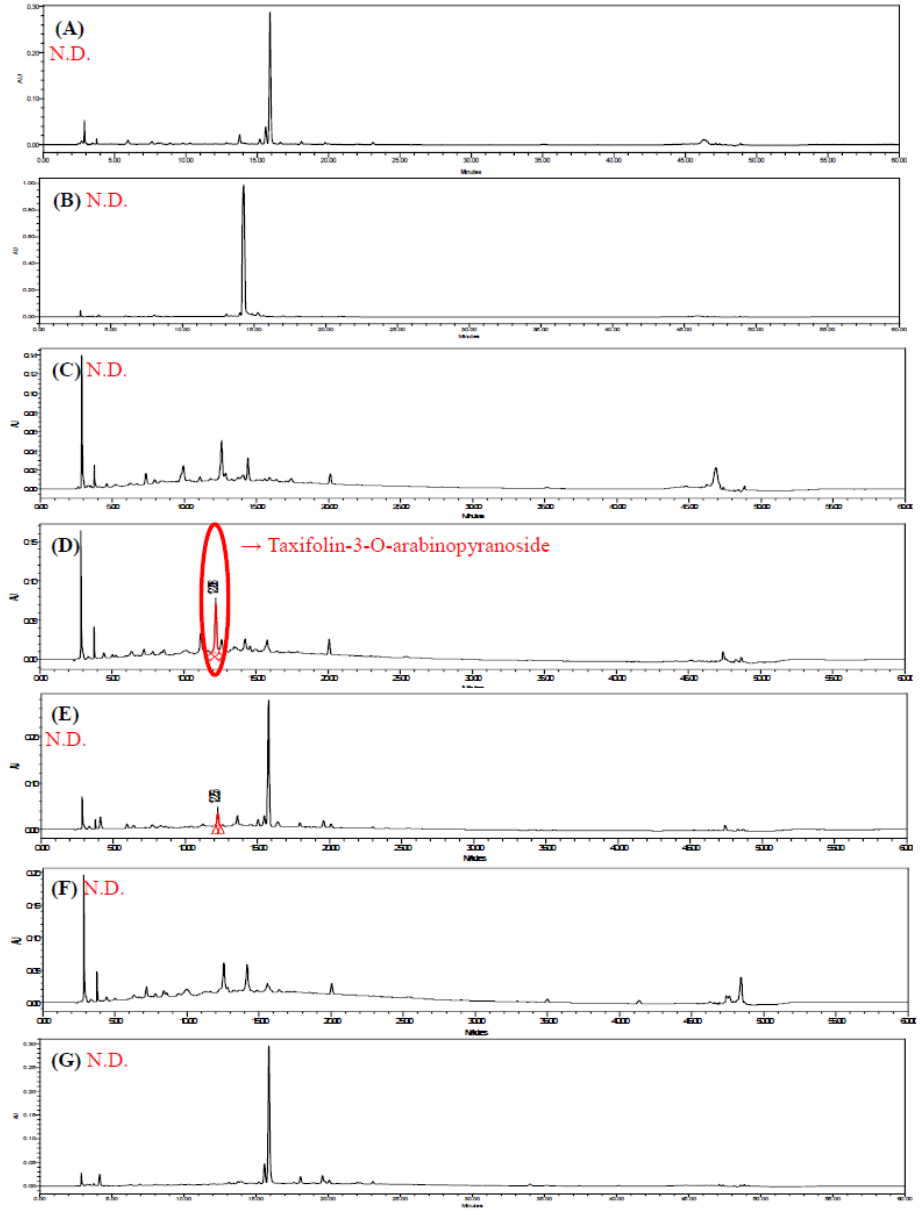

Fig. 11. HPLC chromatogram of the extracts of *R. schlippenbachii* (Gw, Gb). Gw is Gangwon-do, Gb is Gyeongsangbuk-do. N.D. is no detection. (A) Sample, flower extracts of *R. schlippenbachii* 1,000 ppm (Gw). (B) Sample 1, leave extracts of *R. schlippenbachii* 1,000 ppm (Gw). (C) Sample 1, stem extracts of *R. schlippenbachii* 1,000 ppm (Gw). (D) Sample 1, branch extracts of *R. schlippenbachii* 1,000 ppm (Gb). (E) Sample 1, leave extracts of *R. schlippenbachii* 1,000 ppm (Gb). (F) Sample 1, branch exrtracts of *R. schlippenbachii* 1,000 ppm (Gw). (G) leave extracts of *R. schlippenbachii* 1,000 ppm (Gw).

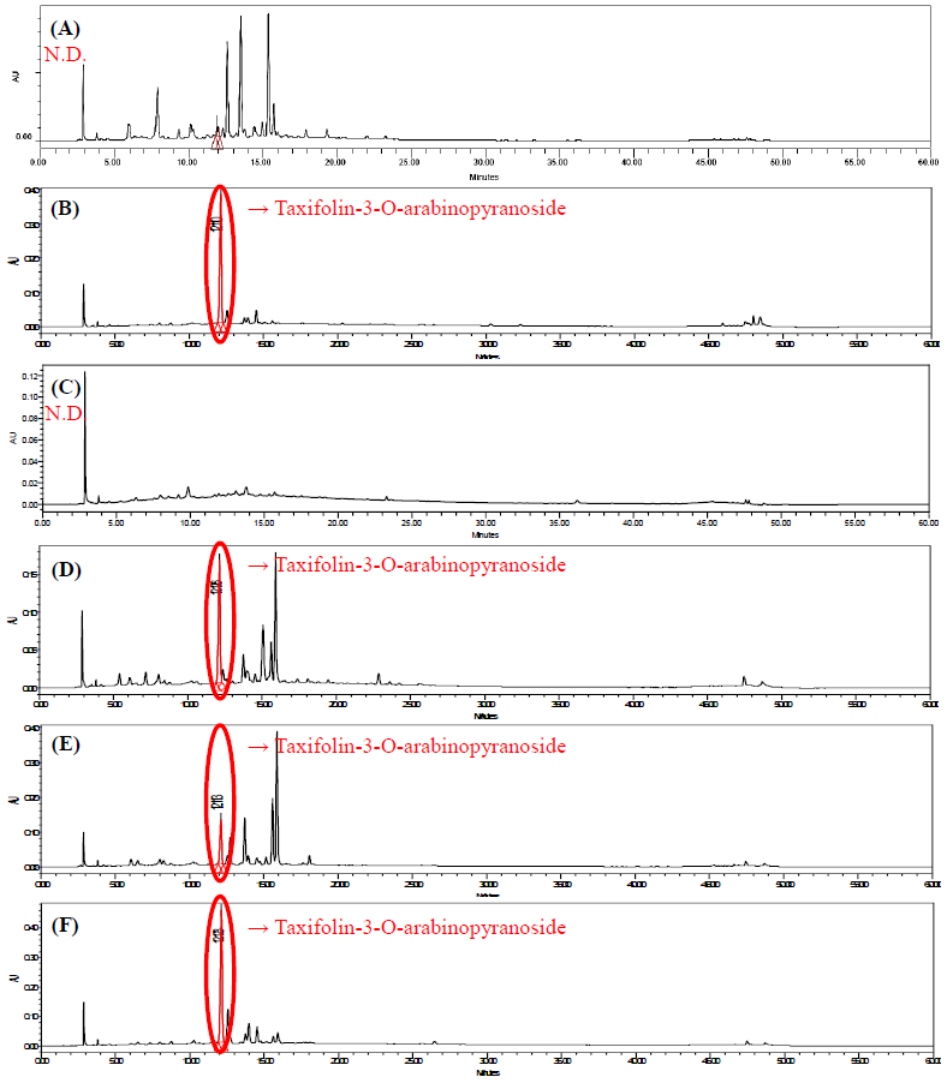

Fig. 12. HPLC chromatogram of the extracts of *R. yedoense* var. *Poukhanense* (Jj, Cn). Jj is Jeju, Cn is Chungcheongnam-do. N.D. is no detection. (A) Sample 1, leave extracts of *R. yedoense* var. *Poukhanense* 1,000 ppm (Jj). (B) Sample 1,stem extracts of *R. yedoense* var. *Poukhanense* 1,000 ppm (Jj). (C) Sample 1, stem extracts of *R. yedoense* var. *Poukhanense* 1,000 ppm (Jj). (D) Sample 1, leave extracts of *R. yedoense* var. *Poukhanense* 1,000 ppm (Jj). (E) Sample 1, leave extracts of *R. yedoense* var. *Poukhanense* 1,000 ppm (Cn). (F) Sample 1, branch extracts of *R. yedoense* var. *Poukhanense* 1,000 ppm (Cn).

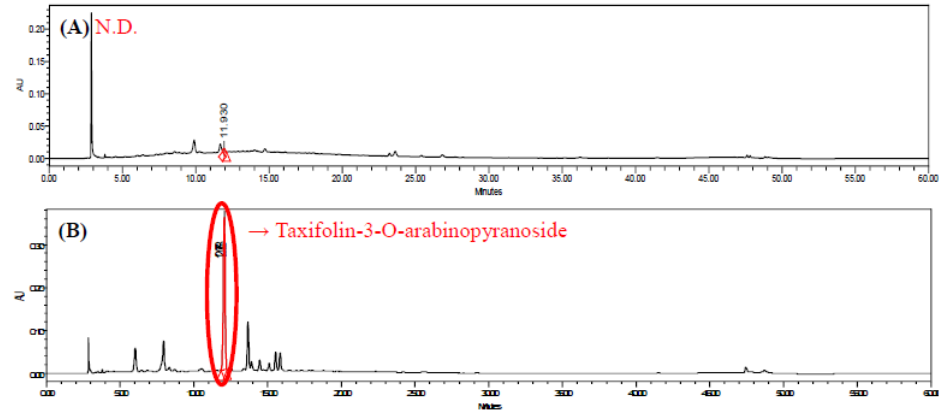

Fig. 13. HPLC chromatogram of the extracts of *R. japonicum* for. *Flavum* (Jn). Jn is Jeollanam-do. N.D. is no detection. (A) Sample 1, branch extracts of *R. japonicum* for. *Flavum* 1,000 ppm (Jn). (B) Sample 1, leave extracts of *R. japonicum* for. *Flavum* 1,000 ppm (Jn).

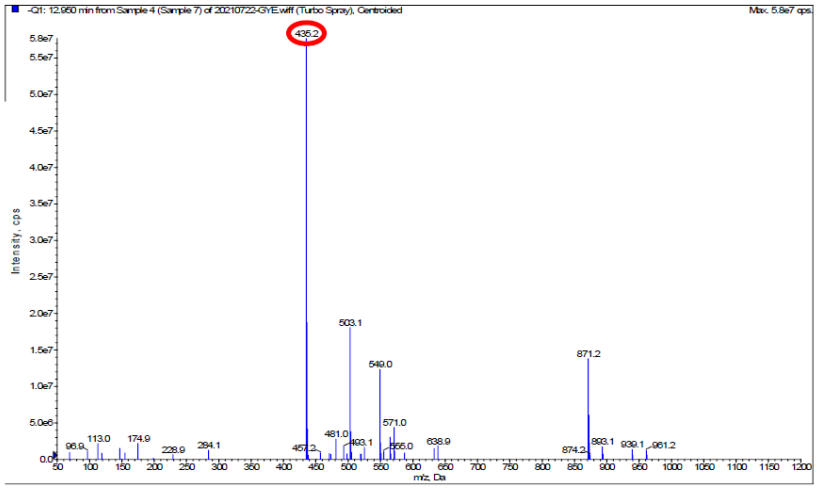

Fig. 14. Molecular weight of the stem extracts of *R. mucronulatum* (Gn). Gn is Gyeongsangnam-do.

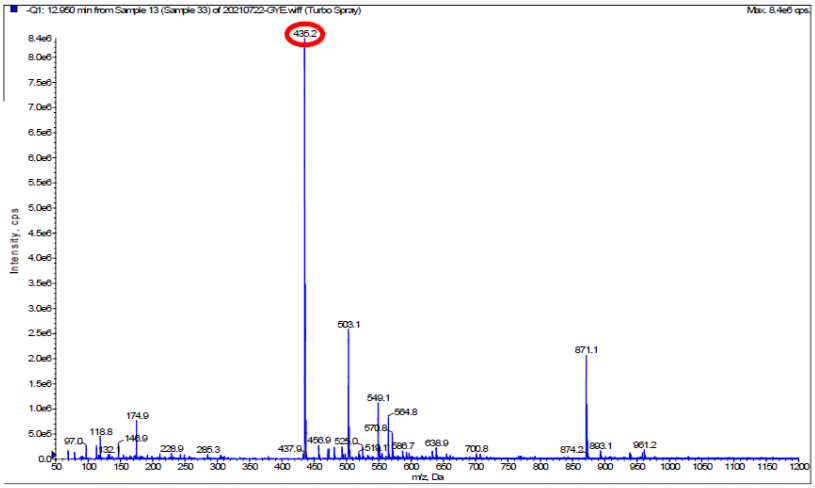

Fig. 15. Molecular weight of the leave extracts of *R. mucronulatum* (Gw). Gw is Gangwon-do.

Afterward, extracts with different retention times or peak shapes were selected for each detected chromatogram, and LC-MS/MS analysis was performed. The molecular weight was confirmed under negative conditions. As a result of analysis of stem and flower extracts from Gyeongsangnam-do and leaf extracts from Gangwon-do among *R. mucronulatum* species, a molecular weight of 435.2 was confirmed in stem and leaf extracts but not in flower extracts. As a result of analysis of stem extracts from Jeju-do, a species of *R. mucronulatum* var. *ciliatum*, confirmed a molecular weight of 435.1. As a result of analysis of branch extracts from Gyeongsangbuk-do, a species of *R. schlippenbachii*, confirmed a molecular weight of 435.1. As a result of analysis of stem and leaf extracts from Jeju Island among *R. yedoense* var. *Poukhanense* species, and molecular weights of 435.2 and 435.1 were confirmed, respectively. Finally, a molecular weight of 435.1 was confirmed as a result of the analysis of leaf extracts from Jeollanam-do among *R. japonicum* for. *Flavum* species. In addition, LC-MS/MS data of the extract having the same molecular weight as Taxifolin-3-O-arabinopyranoside, a standard material, are shown in Fig. 14-20.

Table 9 summarizes the HPLC retention time, content, and molecular weight according to the limit of detection (LOD) of 21 extracts of 5 species of genus of *Rhododendron* based on the results of HPLC and LC-MS/MS analysis

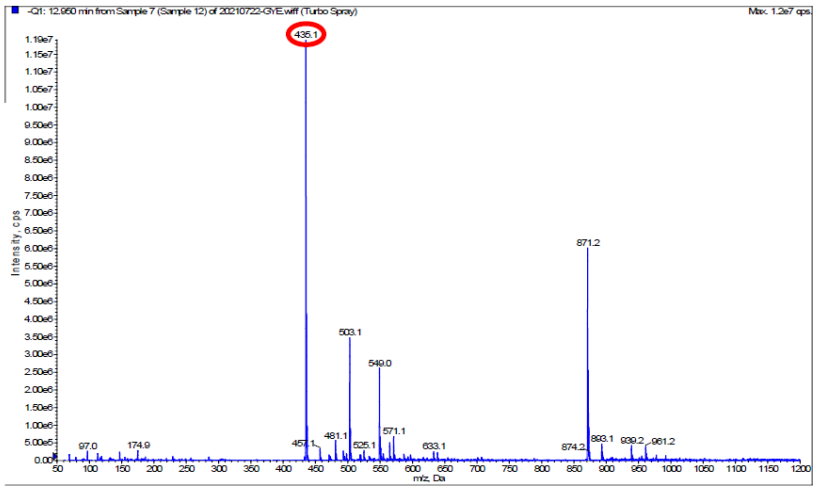

Fig. 16. Molecular weight of the stem extracts of *R. mucronulatum* var. ciliatum (Jj). Jj is Jeju.

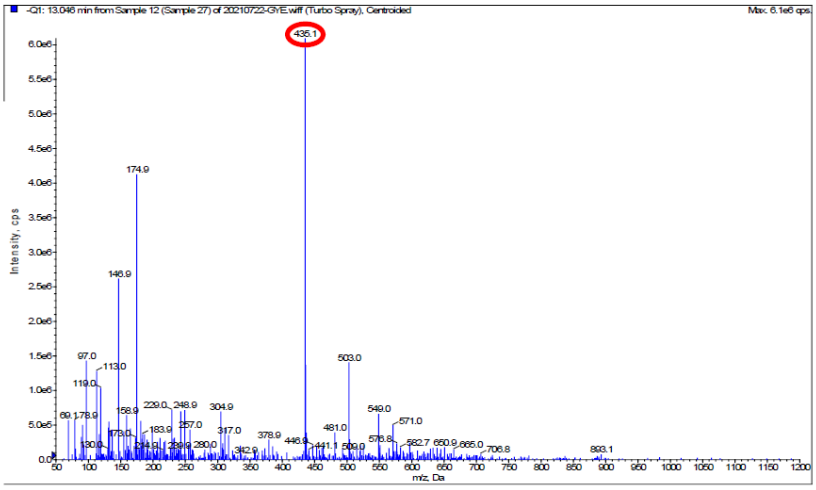

Fig. 17. Molecular weight of the branch extracts of *R. schlippenbachii* (Gb). Gb is Gyeongsangbuk-do.

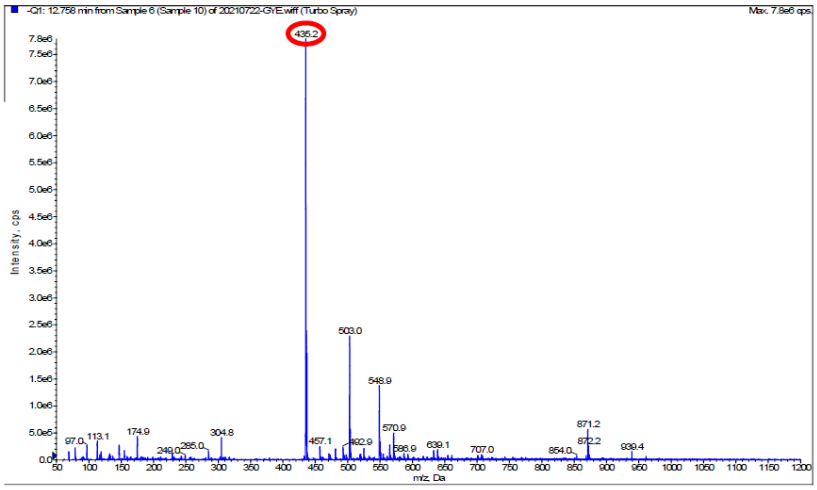

Fig. 18. Molecular weight of the stem extracts of *R. yedoense* var. *Poukhanense* (Jj). Jj is Jeju.

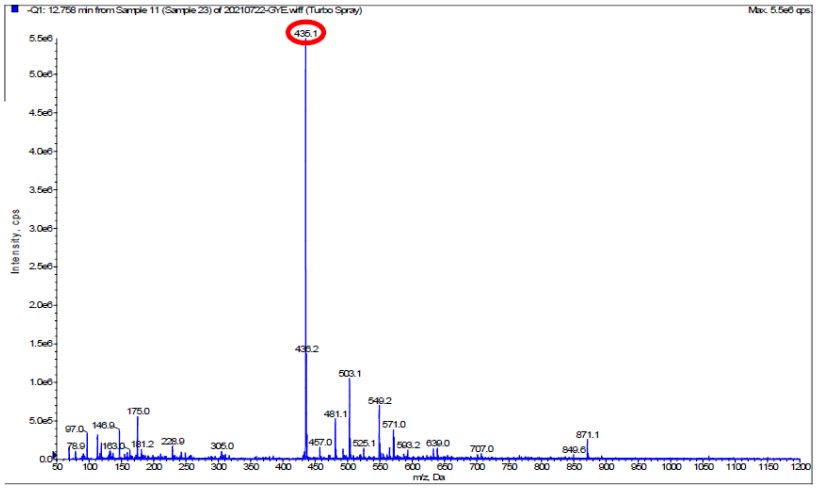

Fig. 19. Molecular weight of the leave extracts of *R. yedoense* var. *Poukhanense* (Jj). Jj is Jeju.

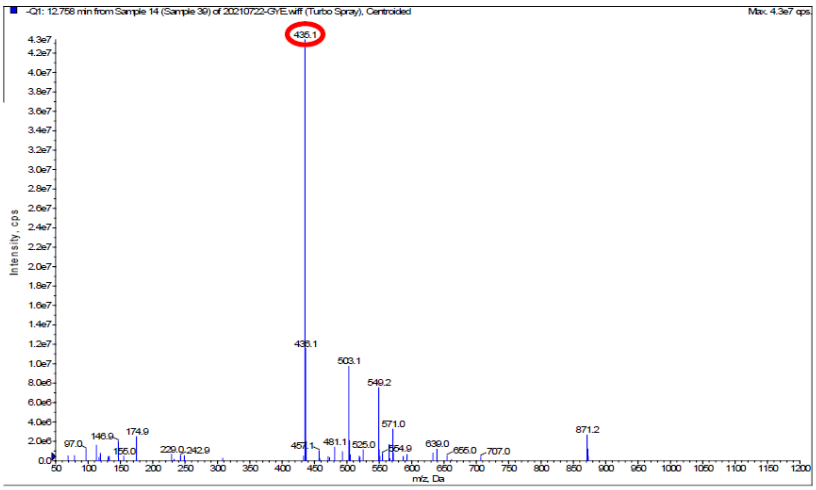

Fig. 20. Molecular weight of the leave extracts of *R. japonicum* for. *Flavum* (Jn). Jn is Jeollanam-do.

above. Then finally, the presence or absence of Taxifolin-3-O-arabinopyranoside was determined and shown in Table 10. In the case of 4 kinds of extracts of *R. mucronulatum*, Taxifolin-3-O-arabinopyranoside peaks were found in stems, branches, and leaves, and the molecular weights were also consistent. However, a trace amount of the component was present in flowers but did not reach the detection limit. Two types of extract of *R. mucronulatum* var. *ciliatum*, Taxifolin-3-O-arabinopyranoside peak were found in the stem extract, and the molecular weight was also consistent. However, a trace amount was present in the leaf extract but did not reach the detection limit. Finally, in the case of 7 kinds of extracts of *R. schlippenbachii*, Taxifolin-3-O-arabinopyranoside peak was found in branch extracts from Gyeongsangbuk-do, and the molecular weight was the same. Still, it was not found, or a trace amount was present in the extracts of the remaining parts, so it did not reach the detection limit. In the case of 6 kinds of extracts of *R. yedoense* var. *Poukhanense*, Taxifolin-3-O-arabinopyranoside peak was found in stem and leaf extracts from Jeju Island, and branch and leaf extracts from Chungcheongnam-do, and the molecular weights were also consistent. However, it was not found in branch extracts from Jeju Island. Although it is an extract from the same branch, it is judged that additional research is needed on the fact that it was detected in extracts from Chungcheongnam-do and not from extracts from Jeju-do. In the case of two types of extracts of *R. japonicum* for. *Flavum*, Taxifolin-3-O-arabinopyrano-

Table 9. Data of HPLC analysis and LC-MS/MS of extracts of genus of Rhododendron

species	Part	Habitat	Retention time (min.)	Content (ppm)	Molecular weight
R. mucronulatum	Stem	Gn	11.995±0.21	135.60±2.57	435.2
	Flower	Gn	-	-	-
	Branch	Gw	12.291±0.10	73.15±4.61	*
	Leave	Gw	12.265±0.12	19.86±1.28	435.2
R. mucronulatum var. *ciliatum*	Leave	Jj	-	-	-
	Stem	Jj	12.275±0.09	74.37±7.00	435.1
R. schlippenbachii	Flower	Gw	-	-	-
	Leave	Gw	-	-	-
	Stem	Gw	-	-	-
	Branch	Gb	12.299±0.09	14.63±2.08	435.1
	Leave	Gb	-	-	-
	Branch	Gw	-	-	-
	Leave	Gw	-	-	-
R. yedoense var. *Poukhanense*	Leave	Jj	-	-	-
	Stem	Jj	12.025±0.09	64.24±4.42	435.2
	Branch	Jj	-	-	-
	Leave	Jj	12.033±0.09	31.52±1.94	435.1
	Leave	Cn	12.032±0.09	10.95±1.63	*
	Branch	Cn	12.026±0.10	39.99±0.73	*
R. japonicum for. Flavum	Branch	Jn	-	-	-
	Leave	Jn	12.017±0.07	42.16±10.01	435.1

*It has the same molecular weight as that of a sample with the same retention time among 21 samples.
Gn, Gyeongsangnam-do; Gw, Gangwon-do; Jj, Jeju; Gb, Gyeongsangbuk-do; Cn, Chungcheongnam-do; Jn, Jeollanam-do.
-, none of the compounds is detected.

Table 10. Existence of Taxifolin-3-O-arabinopyranoside in genus of *Rhododendron*

Species	Plant part	Habitat1	Taxifolin-3-O-arabinopyranoside
R. mucronulatum	Stem	Gn	O
	Flower	Gn	X
	Branch	Gw	O
	Leave	Gw	O
R. mucronulatum var. *ciliatum*	Leave	Jj	X
	Stem	Jj	O
R. schlippenbachii	Flower	Gw	X
	Leave	Gw	X
	Stem	Gw	X
	Branch	Gb	O
	Leave	Gb	X
	Branch	Gw	X
	Leave	Gw	X
R. yedoense var. *Poukhanense*	Leave	Jj	X
	Stem	Jj	O
	Branch	Jj	X
	Leave	Jj	O
	Leave	Cn	O
	Branch	Cn	O
R. japonicum for. Flavum	Branch	Jn	X
	Leave	Jn	O

Gn, Gyeongsangnam-do; Gw, Gangwon-do; Jj, Jeju; Gb, Gyeongsangbuk-do; Cn, Chungcheongnam-do; Jn, Jeollanam-do.
O, exists; X, none.

side peak was found in the leaf extract, and the molecular weight was the same, but it was present in a trace amount in the eggplant extract, which did not reach the detection limit.

Conclusion

This research team conducted a chemotaxonomy study to determine whether Taxifolin-3-O-arabinopyranoside, a flavonoid compound directly isolated and structurally identified from the extract of the root of the *R. mucronulatum*, can be judged as an indicator compound for species of the genus of *Rhododendron*. As a result, Taxifolin-3-O-arabinopyranoside was detected in all five species of *R. mucronulatum*, *R. mucronulatum* var. *ciliatum*, *R. schlippenbachii*, *R. yedoense* var. *Poukhanense*, and *R. japonicum* for. *Flavum*. It was confirmed that the molecular weight of each extract was also consistent with that of Taxifolin-3-O-arabinopyranoside. In particular, compared with the existing literature, this is the first report on Taxifolin-3-O-arabinopyranoside detection in the other four species (*R. mucronulatum* var. *ciliatum*, *R. schlippenbachii*, *R. yedoense* var.

Poukhanense, and *R. japonicum* for. *Flavum*).

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