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Identification of Flavonoid Glycosides in *Rosa chinensis* Flowers by Liquid Chromatography-tandem Mass Spectrometry in Combination with ^{13}C Nuclear Magnetic Resonance

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Abstract

Flowers of *Rosa chinensis* are widely used in traditional Chinese medicine as well as in food industry. Flavonoid glycosides are believed to be the major components in *R. chinensis* that are responsible for its antioxidant activities. In this work, a liquid chromatography-tandem mass spectrometry (HPLC- MS/MS) method was developed for analysis of flavonoid glycosides presented in ethyl acetate extract of dried *R. chinensis* flowers. Twelve flavonoid glycosides were separated and detected. By comparing the retention times, UV spectra, and tandem MS fragments with those of respective authentic compounds, eight flavonoid glycosides were unequivocally identified. Although the other four were also identified as flavonoid glycosides, the glycosylation positions could not be determined due to lack of authentic compounds. Fortunately, the glycosylation effects were clearly observed in the ^{13}C NMR spectrum of the extract. The detailed structural information was, therefore, obtained to identify the four flavonoid glycosides as quercetin-3-*O*-D-glucoside, quercetin-3-*O*-D-xyloside, kaempferol-3-*O*-D-xyloside and quercetin-3-*O*-D-(6''-coumaroyl)-galactoside. These flavonoid glycosides were detected and identified for the first time in this botanic material. This work reports on the first use of ^{13}C NMR of a mixture to enhance a rapid HPLC-MS/MS analysis. The proposed analytical protocol was validated with a mixture of authentic flavonoid glycosides.

Keywords

Rosa chinensis; flavonoid glycoside; HPLC-MS/MS; ^{13}C NMR

Introduction

Rosa chinensis is originated and widely cultivated in China. It is considered to be an important ancestor of modern roses. It is not only a well-known ornamental plant, but also

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heavily utilized in cosmetic and food industry, such as essential oil refining, spice preparation, natural pigment extraction, and food processing. In traditional Chinese medicine *R. chinensis* flowers are used to treat menstrual disorder, menstrual colic, etc. medical conditions as described in Chinese Pharmacopoeia [1].

Oxidation induced by free radicals has drawn a great attention in recent years because it causes serious problems in food industry such as rancid odors and flavors, texture and color deterioration, etc. [2]. It is also associated with various medical conditions, including atherosclerosis, chronic renal failure, and diabetes mellitus [3]. It was reported that extracts obtained from *R. chinensis* flowers exhibited strong radical scavenging effects due to the presence of high levels of flavonoids and hydrolyzable tannins in the material [4]. Another study indicated that the antioxidant activity of *R. chinensis* was helpful for prevention and treatment of cardiovascular and cerebrovascular diseases [5]. Further studies revealed that the ethyl acetate fraction showed the strongest antioxidant activity among all the crude extracts of *R. chinensis* flowers [6]. These findings promoted the efforts to profile flavonoids in the ethyl acetate extract of *R. chinensis* flowers.

Investigation on chemical components present in *R. chinensis* flowers has been inadequate. Several antioxidant flavonoids and their glycosides were isolated from the extract by column chromatography [7–8]. Using a facile solid-phase extraction (SPE) procedure two flavonoids as well as six glycosides were isolated and identified later by MS [9]. In analysis of phenolic antioxidants in *R. chinensis* flowers by using HPLC-MS and MALDI-TOF-MS [4], conclusions regarding to the structures of some flavonoid glycosides remained tentative. It is, therefore, significant to establish an effective analytical protocol to identify the antioxidant flavonoid glycosides present in this useful plant.

HPLC is the separation method of choice for natural product analysis. HPLC-DAD UV coupled with MSⁿ technique generates data on HPLC retention behavior, UV absorption, and MS fragmentation characteristics, which is usually very effective for rapid identification of unknown compounds. Tandem MS with collision-induced dissociation (CID) was applied to acquire detailed structural information in natural product analysis such as: (a) the aglycone moiety, (b) the types of carbohydrates (i.e. mono-, di-, tri- or tetrasaccharides and hexoses, deoxyhexoses or pentoses) or other subsistent presence, (c) the attachment of the substituents to the aglycone, etc. [10–12]. However, in many cases questions such as isomeric identities and substitution patterns on skeletons (e.g. aromatic ring systems) can not be answered by using MS-based techniques alone. An effective approach to enhance the chemical identification is based on the use of tandem MS in combination with nuclear magnetic resonance (NMR). The chemical shifts of either ¹H or ¹³C obtained in NMR reflect the chemical environment of the atoms, providing detailed structural information. In the analysis of flavonoid glycosides, the glycosylation effect in ¹³C NMR spectra was investigated to determine the attachment point of sugar moiety to aglycone [13].

HPLC-MS based methods have been reported for structural elucidation for flavonoid glycosides [14–17]. Although it was shown that HPLC–NMR technique provided more structural information [18–19] compared with HPLC-MS, the HPLC-MNR instrument is not readily available [20]. Actually, as we noted, structural elucidation for some flavonoid glycosides detected often become tentative due to the lack of support from either NMR data or authentic standards [4, 15–17]. In the case of *R. chinensis*, given the facts that only limited phytochemical investigation has been carried out so far and many standard compounds are not commercially available, to unequivocally identify the compounds of interest remains a challenge.

The aim of the present work was to establish a facile analytical protocol to identify flavonoid glycosides in *R. chinensis* flowers without needing to purify each compound of interest. Due to the complex composition of flavonoids in this plant, particularly the presence of many isomeric structures and various substitution patterns, the identification was difficult. An analytical method based on a hyphenated instrumental technique, i.e. HPLC-DAD-MSⁿ was, therefore, developed to analyze the ethyl acetate extract of *R. chinensis* flowers. From many compounds eluted, eight flavonoid glycosides were identified by comparing the HPLC retention times, UV absorption, MS fragmentation characteristics with authentic standards. Further, ¹³C NMR was deployed in support of the HPLC-DAD-MSⁿ identification in order to discover flavonoid glycosides either whose authentic standards were not available or that were unknown to this useful plant.

2. Experimental

2.1. Materials and methods

Dried flowers were obtained from Sichuan province, China. Ellagic acid was purchased from Alfa Aesar (Lance, France). Eight authentic flavonoid glycosides standards (quercetin-3-*O*-D-(2''-gallate)-glucoside (**1**), quercetin-3-*O*-D-galactoside (**3**), quercetin-3-*O*-L-arabinoside (**6**), quercetin-3-*O*-L-rhamnoside (**7**), kaempferol-3-*O*-L-arabinoside (**10**), kaempferol-3-*O*-L-rhamnoside (**11**), quercetin-3-*O*-D-(6''-coumaroyl)-glucoside (**12**), kaempferol-3-*O*-D-(6''-coumaroyl)-glucoside (**14**)) were prepared in house from *R. chinensis* (**6**), and four other flavonoid glycosides standards (myricetin-3-*O*-L-rhamnoside (**15**), kaempferol-3-*O*-D-glucoside (**16**), isorhamnetin-3-*O*-D-glucoside (**17**), rhamnetin-3-*O*-D-glucoside (**18**)) were kindly provided by Dr. Chu Chen of Sichuan Academy of Chinese Medicine Sciences. Their structures (Fig. 2) were confirmed by MS, ¹H NMR, and ¹³C NMR spectral analyses. Silica gel TLC plates (F₂₅₄, Merck) were used for flavonoid aglycons analysis. TLC plates prepared in house with silica gel G, NaH₂PO₄ and CMC-Na were used for sugars analysis. HPLC grade acetonitrile (Fisher, USA) and Milli-Q water (Millipore Corp., Bedford, MA, USA) were used through out the work. Other chemicals and solvents were of analytical reagent grade.

2.2. Preparation of ethyl acetate extract of *R. chinensis* Flowers

Dried *R. chinensis* flowers (200 g) were powdered and extracted with ethanol by reflux to give a crude ethanol extract. The residue suspended in water was extracted successively with chloroform and ethyl acetate. The solvents were removed under reduced pressure to yield 1.9 g of ethyl acetate extract.

2.3. HPLC-DAD-MSⁿ analysis of the extract

An accurately weighed 10 mg ethyl acetate extract was dissolved in 50 mL methanol for HPLC-DAD-MSⁿ analysis.

A TSP HPLC system consisting of a vacuum degasser, quaternary pump, autosampler and DAD detector (Thermo Separation Products Inc., USA) was used for acquiring chromatograms and UV spectra. For chromatographic analysis, a Tianhe kromasil C18 column (5 μm, 250 mm × 4.6 mm) with a guard column (C18, 5 μm, 4.0 mm × 3.0 mm) was used. HPLC separation was performed using a linear gradient with a flow rate of 1.4 mL/min, while column was kept in an insulated compartment at 35 °C. The mobile phase consisted of water containing 0.5 % formic acid (A) and acetonitrile (B) using a gradient of 13–30% B for 0–60 min. The injection volume was 20 μL. DAD detector was set to scan from 200 nm to 400 nm. A ThermoQuest Finnigan LCQ^{DECA} system equipped with an electrospray ionization source (ThermoQuest LC/MS Division, USA) was used for mass spectrometric measurements. The ESI-MS spectra were acquired in negative ion modes. The

mass spectrometry detector (MSD) parameters were as follows: nebulizer sheath gas, N₂ (80 uit); nebulizer auxiliary gas, N₂ (20 uit); capillary temperature, 300 °C; spray voltage, 4.5 KV; capillary voltage, -13 V; lens voltage, 18 V. All data were processed by Finnigan Xcalibur core data system Rev. 2.0.

2.4. TLC analysis of the extract

Acid hydrolysis was performed to remove the sugar moieties from flavonoid glycosides [21]. An aliquot of ethyl acetate extract (0.5 g) was refluxed with 40 mL of 1.2 M HCl in 50% aqueous methanol for 1 h. The hydrolysate was evaporated to dryness, suspended in water, and then extracted with ethyl acetate and n-butanol, successively. TLC analysis were respectively performed for the ethyl acetate fraction, standards of quercetin and kaempferol using CHCl₃/MeOH/formic acid (4:1:0.1) visualized with AlCl₃ spray reagent, and for the aqueous fraction, standards of L-rhamnose, D-xylose, L-arabinose, D-glucose and D-galactose using acetone/*n*-BuOH/H₂O (5:4:1) visualized with aniline phthalate spray reagent.

2.5. ¹³C NMR analysis of the extract

An accurately weighed 60 mg ethyl acetate extract was dissolved in 1 mL dimethyl sulfoxide-*d*₆ for ¹³C NMR analysis with a Bruker 600 MHz system.

3. Results and discussion

It was reported that ethyl acetate fraction had the strongest antioxidant activity among all the crude extracts of *R. chinensis* flowers [6]. It is believed that phenolic compounds in the extract, including flavonoids, are responsible for the antioxidant activity observed. Accordingly, in the present study, the ethyl acetate extract of *R. chinensis* flowers was analyzed to obtain the profile of flavonoids by HPLC-DAD-MSⁿ in combination with ¹³C NMR.

3.1. Detection of known flavonoid glycosides in *R. chinensis* flowers

In our previous phytochemical study on the chemical constituents of *R. chinensis* flowers, eight flavonoid glycosides were isolated by using a lengthy column chromatographic procedure [7]. These pure and well characterized flavonoid glycosides were used as authentic compounds for the identification in this work. As can be seen from Fig. 1, many compounds were separated and detected by the proposed HPLC-DAD-MS analysis. The eight flavonoid compounds could be unambiguously identified (data listed in Table 1 and structures shown in Fig. 2) by comparing the retention times, UV adsorption, and tandem-MS spectra of the peaks with those of authentic standards. They were: quercetin-3-*O*-D-(2''-gallate)-glucoside (**1**), quercetin-3-*O*-D-galactoside (**3**), quercetin-3-*O*-L-arabinoside (**6**), quercetin-3-*O*-L-rhamnoside (**7**), kaempferol-3-*O*-L-arabinoside (**10**), kaempferol-3-*O*-L-rhamnoside (**11**), quercetin-3-*O*-D-(6''-coumaroyl)-glucoside (**12**), and kaempferol-3-*O*-D-(6''-coumaroyl)-glucoside (**14**).

3.2. Identification of unreported compounds

Based on the CID fragments from MSⁿ experiments (shown in Table 1) it was obvious that the compounds for peaks **4**, **5**, **9** and **13** in the HPLC-DAD-MS chromatogram (Fig. 1) were flavonoid glycosides (see below for more details), but the compounds for peaks **2** and **8** were not. Considering the detection of a high level of gallic acid in *R. chinensis* flowers in our previous work [7], the *m/z* value of [M-H]⁻, and the fact that ellagic acid is a dimer of gallic acid, peak **2** was likely ellagic acid. Further study showed the UV and tandem-MS spectrum data of peak **2** matched those of ellagic acid reported in literature [22]. Peak **8** was,

therefore, easily identified as ellagic acid-rhamnose conjugate. Its sugar moiety was determined to be rhamnose by the fragment of 146 (m/z 447– m/z 301) collided from the pseudo-molecular peak. Its aglycone was ellagic acid as indicated by the MS³ fragments of m/z 257, 229, which were characteristic of ellagic acid. Although it was safe to say that the compounds for peaks **4**, **5**, **9**, and **13** were flavonoid glycosides based on the detection of m/z 255 and 271 ions in MS³, our effort to determine their identities by matching their retention times, UV absorption, and MSⁿ data with those of authentic flavonoid glycosides available to us failed. Thirteen authentic flavonoid glycosides were studied, but no match was found. The major uncertainty was associated with the glycosylation positions in these flavonoid glycosides. Therefore, further identification was carried out using the combined MSⁿ and ¹³C NMR data as described below.

3.3. Identification of aglycones and sugar moieties by TLC

Thin-layer chromatography (TLC), due to its inherent simplicity of operation, is very favourable for the qualitative analysis of flavonoids [23] and sugars [24]. Hence, the ethyl acetate extracts of *R. chinensis* flowers was hydrolysed by refluxing in 1.2 M HCl in 50% aqueous methanol [25] to obtain the flavonoid aglycones and sugars for TLC analysis. The TLC results showed that two flavonoid aglycones (i.e. kaempferol (R_f = 0.82) and quercetin (R_f = 0.71)), as well as five sugars (i.e. L-rhamnose (R_f = 0.77), D-xylose (R_f = 0.60), L-arabinose (R_f = 0.41), D-glucose (R_f = 0.25) and D-galactose (R_f = 0.18)) were present in the extract.

3.4. Structure elucidation of unreported flavonoid aglycones (peaks **4**, **5**, **9**, and **13**) by HPLC-DAD-MSⁿ

The UV and tandem-MS data of each peak are listed in Table 1. The characteristics of flavonoid glycosides were summarized from the results of the reported ones (peaks **1**, **3**, **6**, **7**, **10–12**, **14**). The UV absorption maximum (λ_{max} , nm) of flavonoid glycosides was found to be dependent upon the aglycone types: quercetin type was at ~255 nm and kaempferol type at ~265 nm (Fig. 3). These results were consistent with those reported in literature [25–26].

The relative abundance of the radical aglycone ion (Y_0-H)^{•−} and aglycone ion (Y_0^-) of flavonoid mono-*O*-glycoside in MS had a close correlation with the glycosylation position. For example, flavonoid-3-*O*-glycosides had some common characteristic CID fragments of tandem-MS [27]. Quercetin-3-*O*-L-rhamnoside (quercetin type) was studied as an example in this work. Both aglycone ion (Y_0^- m/z 301) induced by heterolytic cleavage and radical aglycone ion (Y_0-H)^{•−} (m/z 300) induced by homolytic cleavage were detected. Fig. 4 shows the proposed fragmentation pathways. The abundance of m/z 300 (Y_0-H)^{•−} was higher than that of m/z 301 (Y_0^-) as can be seen in Fig. 5(A1). Furthermore, [$Y_0-H-CO-H$]^{•−} ion at m/z 271 and [$Y_0-H-CO-OH$]^{•−} ion at m/z 255 were observed in the MS³ experiments as shown in Fig. 5(A2). The similar MS characteristics of kaempferol type flavonoid-3-*O*-glycoside were also observed. As shown in Fig. 5(B1), (Y_0-H)^{•−} at m/z 284 is more abundant than Y_0^- at m/z 285, and MS³ characteristic fragment of m/z 255 [$Y_0-H-CO-H$]^{•−} and m/z 227 [$Y_0-H-CO-CO$]^{•−} were also observed in Fig. 5(B2).

The compounds for peaks **4**, **5**, **9** and **13** could be identified based on the findings stated above. Take peak **4** as an example. The data of λ_{max} 255 nm and MS² m/z 301 indicated that the flavonoid aglycone was quercetin. Sugar fragment, 162 (m/z 463– m/z 301), collided from the pseudo molecular peaks suggested that the sugar moiety was hexose, which should be either D-glucose or D-galactose as TLC analysis indicated. The attachment point of hexose on aglycone were determined as C-3 according to the relative abundance of m/z 300 (Y_0-H)^{•−} and m/z 301 (Y_0^-) as well as MS³ characteristic fragment of m/z 271 ([Y_0-H-

CO-H][−]) and m/z 255 ([Y₀-H-CO-OH][−]). Since quercetin-3-*O*-D-galactoside was already recognized as peak **3**, peak **4** was identified as quercetin-3-*O*-D-glucoside. Similarly, peaks **5**, **9** and **13** were identified as quercetin-3-*O*-D-xyloside, kaempferol-3-*O*-D-xyloside and quercetin-3-*O*-D-galactoside-6'-coumaroyl, respectively. It is also worth noting that from the biogenetic points of view the structures identified were in agreement with the eight flavonoid glycosides detected previously in *R. chinensis* flowers.

3.5. Confirmation of the glycosylation position by ¹³C NMR

The effect of glycosylation in ¹³C NMR spectra of flavonoid glycosides was examined to ascertain the glycosylation position. Glycosylation at C-3 produces large "ortho" effect on C-2, causing the chemical shift of C-2 to go significantly downfield compared with when no sugar chain is attached at C-3 (11). The chemical shift of C-2 is typically around δ_C 148 when no sugar is attached to C-3, and around δ_C 157 when it is. In the present work, the ¹³C NMR spectrum of the ethyl acetate extract of *R. chinensis* flowers (Fig. 6) showed strong peaks near δ_C 158, indicating the presence of flavonoid glycosides with sugars attached to C-3 of the aglycones.

Further, to validate the proposed analytical protocol another set of experiments were carried out with a mixture solution of four flavonoid glycosides standards, i.e. myricetin-3-*O*-L-rhamnoside (**15**), kaempferol-3-*O*-D-glucoside (**16**), isorhamnetin-3-*O*-D-glucoside (**17**), and rhamnatin-3-*O*-D-glucoside (**18**). None of them were detected in the ethyl acetate extract of *R. chinensis* flowers. Following the steps of HPLC-DAD-MSⁿ, TLC, and ¹³C NMR analysis described above, the results were as expected. Fig. 7 shows the HPLC-DAD-MS chromatogram and ¹³C NMR spectrum. As can be seen from the HPLC chromatogram, all these flavonoid glycosides were well separated from each other. MS and MSⁿ studies of each peak determined the molar mass and structural characteristics. TLC results from the mixture indicated two sugars present in the sample, i.e. L-rhamnose and D-glucose. Finally, from the ¹³C NMR spectrum all of these four flavonoid glycosides have the sugar moiety attached to C-3 of the aglycones because only the chemical shift of C-3 at δ_C 157 was observed. These results confirmed that the protocol proposed in this work was accurate and effective to identify flavonoid glycosides in botanic samples.

4. Conclusions

In conclusion, antioxidant flavonoid glycosides in ethyl acetate extract of *R. chinensis* flowers, a botanic material widely used in food industry as well as in traditional herbal medicine, were identified using HPLC-DAD-MSⁿ in combination with TLC and ¹³C NMR. In the protocol, HPLC was deployed to separate the extract into individual compounds so that their MS and MSⁿ spectra were acquired in line. Based on the MS fragmentation patterns of each compound and also the R_f results from TLC analysis the nature of aglycones and sugars was identified. ¹³C-NMR spectrum revealed the glycosidation positions. Twelve flavonoid glycosides were detected in the extract. They were identified as quercetin-3-*O*-D-(2''-gallate)-glucoside, quercetin-3-*O*-D-galactoside, quercetin-3-*O*-D-glucoside, quercetin-3-*O*-D-xyloside, quercetin-3-*O*-L-arabinoside, quercetin-3-*O*-L-rhamnoside, kaempferol-3-*O*-D-xyloside, kaempferol-3-*O*-L-arabinoside, kaempferol-3-*O*-L-rhamnoside, quercetin-3-*O*-D-(6''-coumaroyl)-glucoside, quercetin-3-*O*-D-(6''-coumaroyl)-galactoside, and kaempferol-3-*O*-D-(6''-coumaroyl)-glucoside. Among them, eight were known to the plant, and four were detected and identified for the first time in this work. These results improve our knowledge about the chemical compositions in the antioxidant fraction of *R. chinensis* flowers. It was also demonstrated in this work that HPLC-DAD-MSⁿ used in combination with ¹³C NMR was very effective and accurate to identify the chemical components directly from complex botanic extracts, which was especially useful when authentic compounds were not available to investigators.

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Highlights

- An HPLC-MS/MS method for separating flavonoid glycosides
- Profile of flavonoid glycosides in flowers of *Rosa chinensis*
- Chemical identification using HPLC-MS/MS, TLC, and ^{13}C NMR data in combination
- MS/MS CID fragmentation pathways for flavonoid glycosides
- ^{13}C NMR characteristics of flavonoid glycosides

Fig. 1. HPLC-DAD-MS analysis of ethyl acetate extract of *R. chinensis* flowers: (A) HPLC-UV chromatogram monitored at 280 nm; (B) HPLC-negative ion ESI-MS total ion current (TIC) chromatogram

	No.	R ₁	R ₂	R ₃	R ₄
1	OH	H	-glc-gallate	OH
	3	OH	H	-gal	OH
4	OH	H	-glc	OH
	5	OH	H	-xyl	OH
	6	OH	H	-ara	OH
	7	OH	H	-rha	OH
	9	H	H	-xyl	OH
	10	H	H	-ara	OH
	11	H	H	-rha	OH
	12	OH	H	-glc-coumaroyl	OH
	13	OH	H	-gal-coumaroyl	OH
	14	H	H	-glc-coumaroyl	OH
	15	OH	OH	-rha	OH
	16	H	H	-glc	OH
	17	OCH ₃	H	-glc	OH
	18	OCH ₃	H	-glc	OCH ₃

Fig. 2.
Chemical structures of the compounds of interest in this work

Fig. 3. UV spectra of flavonoid glycosides (190–400 nm): (A) quercetin type; and (B) kaempferol type.

Fig. 4. Proposed fragmentation pathways producing the radical aglycone ion at m/z 300 ($Y_o-H]^{-\bullet}$) and the aglycone ion m/z 301 (Y_o^-)

Fig. 5.

Typical negative ion CID mass spectra of flavonoid glycosides: (A1) MS² of *m/z* 447 (quercetin-3-*O*-L-rhamnoside, peak **7**); (A2) MS³ of *m/z* 300; (B1) MS² of *m/z* 431 (kaempferol-3-*O*-L-rhamnoside, peak **11**); (B2) MS³ of *m/z* 284

Fig. 6.
 ^{13}C NMR spectrum of the ethyl acetate extract of *R. chinensis* flowers

Fig. 7.
Validation of the proposed protocol by analysis of a mixture of four flavonoid glycoside standards

Table 1

HPLC-DAD-MSⁿ analytical results of phenolic compounds in ethyl acetate extract of *R. chinensis* flowers

Peak No	t _R (min)	Reported compounds [*]	λ _{max} (nm)	MS (m/z)	MS/MS (m/z)	MS/MS/MS (m/z)	Unreported compounds
1	16.60	Q-3- <i>O</i> -D-glc-2''-gallate	255	615	463 (100) [#] , 301 (45), 300 (81)	271, 255	
2	19.82		253	301	271, 257, 229	--	ellagic acid [*]
3	20.38	Q-3- <i>O</i> -D-gal	254	463	301 (42), 300 (100)	271, 255	
4	21.38		255	463	301 (39), 300 (100)	271, 255	<i>O</i> -D-glc [*] Q-3-
5	23.57		256	433	301 (52), 300 (100)	271, 255	<i>O</i> -D-xyl Q-3-
6	26.70	Q-3- <i>O</i> -L-ara	255	433	301 (43), 300 (100)	271, 255	
7	27.90	Q-3- <i>O</i> -L-rha	255	447	301 (45), 300 (100)	271, 255	
8	30.50		265	447	301, 285	257, 229	ellagic acid-rha
9	31.27		264	417	285 (31), 284 (100)	255, 227	K-3- <i>O</i> -D-xyl
10	34.00	K-3- <i>O</i> -L-ara	264	417	285 (28), 284 (100)	255, 227	
11	35.88	K-3- <i>O</i> -L-rha	263	431	285 (64), 284 (100)	255, 227	
12	42.72	Q-3- <i>O</i> -D-glc-6''-coumaroyl	258	609	463 (72), 301 (52), 300 (100)	271, 255	
13	43.87		259	609	463 (34), 301 (66), 300 (100)	271, 255	Q-3- <i>O</i> -D-gal-6''-coumaroyl
14	50.54	K-3- <i>O</i> -D-glc-6''-coumaroyl	266	593	447 (10), 285 (75), 284 (100)	255, 227	

Q: quercetin; K: kaempferol; glc: glucoside; gal: galactoside; ara: arabinoside; rha: rhamnoside; xyl: xyloside

^{*} Confirmed by comparison with the authentic standards.

[#] Relative abundance