Chemical Constituents from the Polar Fraction of *Rubus suavissimus*

Venkata Sai Prakash Chaturvedula*, Rafael Ignacio San Miguel and Indra Prakash

The Coca-Cola Company, Organic Chemistry Department, Global Research and Development, One Coca-Cola Plaza, Atlanta, GA 30313, USA

**Abstract**

Systematic phytochemical study of the n-BuOH fraction of the aqueous extract of *Rubus suavissimus* resulted in the isolation of three diterpene glycosides namely rubusoside, suavioside-A and sugeroside; a phenolic glycoside quercetrin; and a lignan glycoside arctiin. The structures of the isolated compounds were characterized on the basis of extensive spectral data (1D and 2D NMR; and MS) and chemical studies which has not been reported earlier. This is the first report of the isolation of quercetrin and arctiin not only from the plant *R. suavissimus* but also from the genus *Rubus*. Also, herewith we are reporting the sweetness recognition threshold and sweetness enhancement effect of rubusoside, the major constituent of *R. suavissimus*.

**Keywords:** *Rubus suavissimus*, Rosaceae, Diterpene glycosides, Phenolic glycoside, Lignan glycoside, NMR, MS, rubusoside, sweetness recognition threshold, sweetness enhancement effect

**Introduction**

*Rubus suavissimus* S. Lee belongs to *Rubus*, a large genus of flowering plants in the rose family, Rosaceae, subfamily Rosoideae. Raspberries, blackberries, and dewberries are common, widely distributed members of this genus. *R. suavissimus* is a perennial shrub grows widely grown in Guang-xi and Guang-dong, China [1]. The leaves of *R. suavissimus* are used to make beverage leaf tea by the local residents because of its intensely sweet flavor. It is generally known as tiancha in Chinese or Sweet tea in Guang-xi and Guang-dong, China [1]. The leaves of *R. suavissimus* are use to make beverage leaf tea by the local residents because of its intensely sweet flavor. It is generally known as tiancha in Chinese or Sweet tea in Guang-xi and Guang-dong, China [1].

In our continuing research to isolate natural compounds from various sweet taste plants collected from all over the World, we have isolated three diterpene glycosides rubusoside (1), suavioside-A (2) and sugeroside (3) as well as the phenolic and lignin glycosides namely quercetrin (4) and arctiin (5) respectively from the polar fraction of the aqueous extract of the leaves of *R. suavissimus* obtained from Chengdu Biopurify Phytochemicals Limited, China. This paper describes the isolation and structure elucidation of the isolated glycosides 1-5 (Figure 1) on the basis of extensive spectroscopic and chemical studies as well as in comparison of their physical and spectral properties reported from the literature. Also, we are herewith reporting the sweetness recognition threshold (SRT) and sweetness enhancement effect (SEE) of the predominant constituent of the plant, rubusoside (1).

**Results and Discussion**

Compound 1 was isolated as a white powder and its molecular formula has been deduced as C_{32}H_{50}O_{13} on the basis of its HRMS which showed [M+Na]^+ ions at m/z 660.3590 and 665.3136 respectively, and this was supported by the ^13C NMR spectral data. The ^1H NMR spectrum of 1 showed the presence of two methyl singlets at δ 1.24 and 1.26, two olefinic protons as singlets at δ 5.03 and 5.56 of an exocyclic double bond, nine methylene and two methine protons between δ 0.79-2.72 characteristic for the predominant constituent of the plant, *R. suavissimus*.

*Corresponding author: Venkata Sai Prakash Chaturvedula, The Coca-Cola Company, Organic Chemistry Department, Research and Technology, One Coca-Cola Plaza, Atlanta, GA 30313, USA, Tel: +1-404-676-9257; Fax: +1-404-598-9257; E-mail: vchaturvedula@na.co.com

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H-11/H-12) and HMBC (H-1' C-2, C-10; H-3' C-1, C-2, C-4, C-5, C-18, C-19; H-5' C-4, C-6, C-7, C-9, C-10, C-18, C-19, C-20; H-9' C-8, C-10, C-11, C-12, C-14, C-15; H-14' C-8, C-9, C-13, C-15, C-16 and H-17' C-13, C-15, C-16) correlations. The fragment ions observed at m/z 481 and 319 in the positive ESI mode MS/MS spectrum of 1 indicating the presence of two hexose sugars in its structure. This was further supported by the 1H NMR spectrum of 1 which showed the presence of two anomeric protons at δ 5.13, and 6.15. Enzymatic hydrolysis of 1 furnished an aglycone which was identified as steviol (6) by comparison of 1H NMR [10] spectral data reported in the literature and co-TLC with standard compound. Acid hydrolysis of 1 afforded D-glucose that was identified by preparing the corresponding thiocarbamoyl-thiazolidine carboxylate derivative with L-cysteine methyl ester and O-tolyl isothiocyanate, and in comparison of its retention time with the standard sugars as described in the literature and co-TLC with standard compound. Acid hydrolysis of 1 was further supported by the HMBC correlations: H-1'/C-19, C-2', C-3'; and H-1''/C-13, C-2'', C-3''. A close comparison of the NMR spectral data of 1 with the reported literature values for rubusoside confirmed its structure.

The molecular formula of compound 2 was established as C_{18}H_{26}O_{8} from its HRMS spectral data which showed [M+NH]^{+} and [M+Na]^{+} ions at m/z 502.3327 and 507.2924 respectively. The 1H NMR spectrum of 2 showed the presence of three methyl singlets at δ 0.87, 1.02, and 1.17, eight methylene and two methine protons between δ 0.89-2.43, similar to 1. The 1H NMR of 2 also showed the presence of signal at δ 4.12 as a triplet like with W_{1/2} = 2.6 Hz and a pair of doublets corresponding to an oxymethylene at δ 3.91 (J=10.6 Hz), 4.49 (J=10.2 Hz) and an anomeric proton as a doublet at δ 5.05. Acid hydrolysis of 2 afforded D-glucose that was identified by preparing its corresponding thiocarbamoyl-thiazolidine carboxylate derivative as in 1, and the coupling constant observed for the anomeric proton J=8.2 Hz suggested the β-orientation of the D-glucosyl unit. The 13C NMR spectrum of 2 showed the presence of nine oxygenated carbons between δ 63.2 and 107.2 of which six were assigned to the β-D-glucopyranosyl unit, leaving the assignment of the other three carbons.

The 13C NMR values for all the carbons were assigned on the basis of HSQC and HMBC correlations (Table 1 and Table 2). From COSY, and HMBC correlations as shown in Figure 2, 1 was found to have a steviol aglycone moiety having a β-D-glucopyranosyl unit attached C-13 hydroxyl and another β-D-glucopyranosyl moiety in the form of an ester at C-19. This was supported by the HMBC correlations: H-1'/C-19, C-2', C-3'; and H-1''/C-13, C-2'', C-3''. A close comparison of the NMR spectral data of 1 with the reported literature values for rubusoside confirmed its structure.

Table 1: 1H NMR chemical shift values for 1–3 isolated from Rubus suavissimus recorded in C_{6}D_{6}N.

| Position | 1       | 2       | 3       |
|----------|---------|---------|---------|
| 1        | 0.79 m, 1.68 m | 0.89 m, 1.86 m | 1.25 m, 1.98 m |
| 2        | 1.41 m, 1.92 m | 1.34 m, 1.94 m | 1.82 dd (13.2, 12.2), 2.54 dd (12.0, 4.8) |
| 3        | 1.06 m, 2.34 d (12.4) | 4.12 t (W_{1/2}, 2.60) | - |
| 5        | 1.32 m | 1.57 m | 1.83 m |
| 6        | 1.42 m, 1.72 m | 1.42 m, 1.64 m | 1.40 m, 1.63 m |
| 7        | 1.32 m, 1.70 m | 1.47 m, 1.66 m | 1.45 m, 1.67 m |
| 9        | 0.94 m | 0.98 m | 0.97 m |
| 11       | 1.74 m | 1.65 m | 1.68 m |
| 12       | 1.76 m, 1.95 m | 1.54 m, 1.70 m | 1.51 m, 1.73 m |
| 13       | - | 2.02 m | 1.98 m |
| 14       | 2.24 m, 2.72 d (12.8) | 1.76 m, 2.41 m | 1.76 m, 2.43 m |
| 15       | 2.08 m, 2.52 m | 1.35 m, 1.82 m | 1.35 m, 1.82 m |
| 16       | 5.03 s, 5.56 s | 3.91 (10.6), 4.49 d (10.2) | 3.95 d (10.4), 4.50 d (10.6) |
| 17       | 1.26 s | 0.87 s | 0.93 s |
| 19       | - | 1.02 s | 1.02 s |
| 20       | 1.24 s | 1.17 s | 1.10 s |
| 1''      | 6.15 d (8.5) | 5.05 d (8.2) | 5.06 d (7.8) |
| 2''      | 3.96 m | 4.04 m | 4.02 m |
| 3''      | 4.08 m | 4.46 m | 4.45 m |
| 4''      | 4.33 m | 4.25 m | 4.23 m |
| 5''      | 4.21 m | 4.63 m | 4.64 m |
| 6''      | 4.03 dd (4.2, 12.4), 4.41 dd (2.4, 9.2) | 3.61 dd (4.4, 12.2), 4.27 dd (2.5, 10.6) | 3.60 dd (4.4, 12.0), 4.24 dd (2.3, 10.2) |
| 1''      | 5.13 d (7.8) | - | - |
| 2''      | 4.19 m | - | - |
| 3''      | 4.35 m | - | - |
| 4''      | 4.23 m | - | - |
| 5''      | 3.38 m | - | - |
| 6''      | 4.22 dd (3.8, 12.2), 4.62 dd (2.2, 8.0) | - | - |

Assignments made on the basis of COSY, HSQC and HMBC correlations; Chemical shift values are in δ (ppm); Coupling constants are in Hz.
From HSQC and HMBC spectral data, it was found that the other three oxygenated carbons of 2 as one oxymethylene, one oxymethine and a tertiary hydroxyl resonating at δ 76.0, 75.6 and 81.3 respectively in its 13C NMR spectrum. From the above spectral data and chemical studies, the structure was identified as an ent-kaurane diterpenenoid skeleton having a β-D-glucopyranosyl unit and three oxygenated carbons as mentioned above. A search from the literature indicated the presence of two compounds with the above functional groups namely ent-kaurane-3α, 16β, 17-triol-17-β-D-glucoside (suavioside A) and ent-kaurane-3β, 16β, 17-triol-17-β-D-glucoside (iwayoside A) respectively. The key COSY and HMBC correlations as displayed in Figure 3 supported the basic skeleton of ent-kaurane-3α, 16β, 17-triol-17-β-D-glucoside. Since the spectral data for suavioside A and iwayoside A were reported in CDOD and CD3N respectively and in order to compare the NMR values of 2 with the isolated compounds, its 1H and 13C NMR were also recorded in C3D8 and C3D6 respectively. Acid hydrolysis confirmed the sugar anomeric proton; identical to D-glucose. A close comparison of the 1H and 13C NMR chemical shift values of 2 and 3 together with the ESI-MS data which has 2 amu difference, suggested the presence of a carboxyl group in 3 at C-3 position in place of an oxymethine proton in 2. This was further supported by the presence of the peak observed in its 13C NMR spectrum at δ 217.3, and the absence of a triplet like signal for the oxymethine proton at C-3 position and its corresponding carbon in the respective proton and carbon NMR spectral data of 3. The above spectral and chemical data suggested its structure as 3, which was reported as sugeroside earlier from Ilex sugerosa [15] and R. suavissimus [13].

Compound 4 was obtained as yellow amorphous powder and its molecular formula was established as C31H46O12 from its HRMS spectral data that showed [M+H]+ and [M+Na]+ ions at m/z 449.1075 and m/z 471.0892 respectively; this was supported by the 13C NMR spectral data.

Table 2: 13C NMR chemical shift values for 1–3 isolated from Rubus suavissimus recorded in CD3N.

| Position | 1     | 2     | 3     |
|----------|-------|-------|-------|
| 1        | 41.2  | 34.2  | 39.6  |
| 2        | 19.9  | 27.4  | 34.6  |
| 3        | 38.8  | 75.5  | 217.3 |
| 4        | 44.4  | 38.5  | 47.5  |
| 5        | 57.8  | 49.4  | 54.6  |
| 6        | 22.6  | 20.8  | 22.3  |
| 7        | 42.2  | 42.9  | 41.6  |
| 8        | 42.9  | 45.3  | 44.9  |
| 9        | 54.3  | 57.2  | 55.9  |
| 10       | 40.2  | 40.0  | 39.0  |
| 11       | 21.1  | 18.9  | 19.3  |
| 12       | 37.7  | 26.9  | 27.0  |
| 13       | 86.4  | 47.0  | 46.7  |
| 14       | 44.9  | 37.9  | 37.5  |
| 15       | 48.3  | 53.8  | 53.2  |
| 16       | 155.0 | 81.3  | 81.2  |
| 17       | 104.9 | 76.0  | 75.9  |
| 18       | 28.8  | 29.8  | 27.7  |
| 19       | 177.4 | 22.8  | 21.5  |
| 20       | 16.1  | 18.4  | 18.2  |
| 1′       | 95.4  | 107.2 | 107.1 |
| 2′       | 75.9  | 75.8  | 76.0  |
| 3′       | 79.5  | 79.0  | 79.0  |
| 4′       | 72.8  | 72.0  | 72.1  |
| 5′       | 76.5  | 79.3  | 79.2  |
| 6′       | 63.5  | 63.2  | 63.2  |
| 1″       | 100.2 |       |       |
| 2″       | 74.5  |       |       |
| 3″       | 79.3  |       |       |
| 4″       | 71.5  |       |       |
| 5″       | 79.8  |       |       |
| 6″       | 61.5  |       |       |

*Assignments made on the basis of HSQC and HMBC correlations; Chemical shift values are in δ (ppm)*

**Figure 4:** Key HMBC correlation of 4.
also showed the presence of an anomeric proton as a doublet at δ 5.69 (J = 7.2 Hz). From the above spectral data and chemical studies it was evident that the structure of 5 should contain a β-D-glucopyranosyl unit that has been attached to the aglycone moiety of arctigenin. From the HMBC correlations shown in Figure 5, the presence of β-D-glucopyranosyl unit was suggested at C-6’ position unambiguously as arctigenin; confirmed its structure completely [19,20].

This is the first report of the isolation of quercetin and arctigenin not only from the plant R. suavissimus but also from the genus Rubus. Further, the detailed NMR characterizations have not been studied on some of the isolated glycosides and herewith we have assigned the entire proton and carbon values on the basis of COSY, HSQC and HMBC correlation data as well as confirmed the sugars and their configuration by hydrolysis studies. Also, we are reporting the 1H and 13C NMR data for all the isolated five compounds (1-5) in C6D6N2 in this article.

### Experimental

#### General

Melting points were measured using a SRS Optimelt MPA 100 instrument and are uncorrected. Optical rotations were recorded using a Rudolph Autopol V at 25 °C and NMR spectra were acquired on a Varian Unity Plus 600 MHz instrument using standard pulse sequences at ambient temperature. Chemical shifts are given in δ (ppm), and coupling constants are reported in Hz. HRMS data was generated with a Thermo LTQ Orbitrap Discovery mass spectrometer in the positive ion mode electrospray. Instrument was mass calibrated with a mixture of Ultramid 1621, MRFA [a peptide], and caffeine immediately prior to accurate mass measurements of the samples. Samples were diluted with water:acetonitrile:methanol (1:2:2) and prepared a stock solution of 30 ul concentration for each sample. Each sample (25 ul) was introduced via infusion using the onboard syringe pump at a flow injection rate of 120 ul/min. Low pressure chromatography was performed on a Biotage Flash system using a C18 cartridge (40 x 1 mm, 35-70 µm). TLC was performed on Baker Si: C8-F plates and identification of the spots on the TLC plate was carried out by spraying 10% H2SO4 in EtOH and heating the plate at about 80°C. Analytical HPLC for sugar analysis was carried out with a Waters 600E multisolvent delivery system using a Phenomenex Luna C8 column (150 x 4.6 mm, 5 µm) column.

#### Plant material

The commercial sample consisting of the aqueous extract of the leaves of R. suavissimus was purchased from Chengdu Biopurify Phytochemicals, China. The plant material was identified by Professor Weiping He, Natural Plant Scientific Institute, Guangdong Ocean University, Guangxi, China and a voucher specimen is deposited at The Coca Cola Company, No. VSPC-3166-68.

#### Isolation

The aqueous extract of the leaves of R. suavissimus (10g) was suspended in 100 ml water and extracted successively with n-hexane (3 x 100 ml), CH2Cl2 (3 x 100 ml) and n-BuOH (2 x 100 ml). The n-BuOH
layer was concentrated under vacuum furnished a residue (2.5g) which was purified on a Biogate flash chromatography system using C-18 (100g) column (solvent system: gradient from 20-80 MeOH-water to 100% MeOH at 60 ml/min. detection at UV 210 nm) for 40 min. Fractions 13-20 (0.2g) were combined and further subjected to repeated flash chromatography purification with gradient from 40-80% MeOH-water at 30 ml/min for 30 min afforded suaviside A (2, Rf 18.6 min, 10.2 mg). Fractions 21-23 (1.4g) were combined and crystallized with MeOH furnished rubusoside (1, 1.1g). Purification of the combined fractions 24-27 (0.12g) using the gradient from 40-80% MeOH-water at 30 ml/min for 40 min furnished sugroside (3, Rf 16.4 min, 11.5 mg). Fractions 46-50 and 52-56 were combined to get residues 0.14 g and 0.11 g respectively, which on repeated purification using the gradient 60-90% MeOH-water at 30 ml/min for 40 min resulted qercetin (4, Rf 22.4 min, 6.8 mg), and arctin (5, Rf 19.6 min, 8.4 mg), respectively.

Identification

Rubusoside: White powder, mp 177-179 °C [reported mp 178-181 °C]; [α]23 37.20 (c 1.0, MeOH) [reported [α]23 40.30 (c 0.8, MeOH)]; 1H NMR (600 MHz, CD3CN, δ ppm) and 13C NMR (150 MHz, CD3CN, δ ppm) spectroscopic data see Table 1 and Table 2; HRMS m/z [M+Na]+ calculated for C18H29O12Na: 500.3220; found 500.3219.

Sugroside: White powder, [α]23 55.20 (c 0.35, CH3CN) [reported [α]23 55.50 (c 0.36, CH3CN)]; 1H NMR (600 MHz, CD3CN, δ ppm) and 13C NMR (150 MHz, CD3CN, δ ppm) spectroscopic data see Table 1 and Table 2; HRMS m/z [M+Na]+ calculated for C22H34O17Na: 520.2338; found 520.2336.

Determination of the configuration of sugars in 1-3: Each compound 1-3 (500 µg) was hydrolyzed with 0.5 M HCl (0.5 ml) for 1.5 h. After cooling, the mixture was diluted with 5 ml water, passed through an Amberlite IRA400 column, lyophilized and the residues obtained were converted to the corresponding thiocarbamoyl-thiazolidine carbonate derivative with L-cysteine methyl ester and O-tolyliothiocyanate as described above. The sugars were identified as L-rhamnose (Rf, 21.32 min) and D-glucose (Rf, 12.21 min) respectively from the hydrolysis experiments with 4 and 5 [authentic samples: D- rhamnose (Rf, 11.73 min) and L- rhamnose (Rf, 21.64 min); D-glucose (Rf, 12.35) and L-glucose (Rf, 11.12 min)] [12].

Sweetness Recognition Threshold (SRT) measurement of 1: The sweetness recognition threshold of 1 was measured by three experienced panelists in duplicated runs. All solutions were made in carbon-treated water and used at room temperature. Each of three subjects was asked to isosweet the random, different order blind samples against standard sugar solutions at 0.5%, 1.0% and 1.5% (w/v). The subjects were asked to focus on second sip of each sample and to rinse their mouths with water in between samples. The blind results indicated both duplicated runs yielded consistent results among samples at three different concentrations of 35, 50 and 65 ppm of 1 and the overall % sweetness equivalence (SE) averages were 0.59, 0.92 and 1.06, respectively. As a result, the SRT at 0.75% SE of sugar in water is estimated to be 42 ppm for 1. Similarly, the SRT of 1 in carbonated lemon-lime (LL) soda prototypes without sweetener was evaluated and determined to be 150 ppm.

Sweetness Enhancement Effect (SEE) measurement of 1: In order to find the SEE, test solutions of glucose, fructose and sucrose were prepared equivalent to 6% SE with carbon-treated water at room temperature at SRT of 1 in each carbohydrate solution. The same 6% SE solutions with the above three carbohydrates were prepared without 1. Experimental results indicated that 1 was found to have a slight, positive SEE (ca. 1% SE more) in glucose and fructose solutions whereas a relatively larger SEE in sucrose by ca. 2% SE compared to the solutions of glucose, fructose and sucrose without 1. Likewise, the SEE in carbonated lemon-lime (LL) soda prototypes was evaluated for sensory data in a trained, descriptive analysis of 10 descriptive analysis panelists by preparing 8% SE solution using high fructose corn syrup (HFCS), with and without 1 as described above. Each assessor evaluated all the beverage products in triplicate runs with 8 min break time between testing samples. Unsalted cracker, 0.75% saline solution and mineral grade water was used as a mouth rinse and refresher before testing each sample. Sensory data revealed that the 8% HFCS containing 1 had a SE of 9.2% which is 1.15 times as sweet as the prototype without 1.

Conclusions

Five glycosides including three diterpene, a phenolic and a lignan were isolated from the commercial extract obtained from the leaves of R. suavisissimus obtained from Chengdu Biopurify Phychochemicals Limited, China. The structures of all the isolated new compounds were identified as rubusoside (1), suavisoside-A (2), sugrososide (3), quercitin (4) and arctin (5) on the basis of spectroscopic and chemical studies as well as by comparing their physical properties reported in the literature. This is the first report of the isolation of quercetin.
and arctin from R. suavissimus in nature. The complete 1H and 13C NMR spectral assignments of all the isolated compounds are reported herewith in CD3N, based on COSY, HSQC, HMBC, and MS/MS spectroscopic data as well as chemical studies. The sensory evaluation results demonstrated that the SRT of I in water and LL soda matrixes are 50 and 150 ppm respectively. Also, I showed ca.1% SSE in glucose and fructose, and ca.2% SSE in sucrose in aqueous solutions; whereas it showed 1.15 times SEE in LL soda prototypes at its SRT.

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References
1. Koh GY, Chou G, Liu Z (2009) Purification of a water extract of Chinese sweet tea plant (Rubus suavissimus S. Lee) by alcohol preparation. J Agric Food Chem 57: 5000-5006 and references cited therein.
2. Gao F, Chen F, Tanaka T, Kasai R, Seto T, Tanaka O (1985) 19α-hydroxyursane-type triterpene glucosyl esters from the roots of Rubus suavissimus S. Lee. Chem Pharm Bull 33: 37-40.
3. Wang J, Lu H (2007) Chemical constituents of Rubus suavissimus S. Lee. Zhong yao cai 30: 800-802.
4. Sugimoto N, Kikuchi H, Yamazaki T, Maitani T (2001) Polyphenolic constituents from the leaves of Rubus suavissimus. Nat Med (Tokyo, Jpn.) 55: 219.
5. Brandle JE, Starratt AN, Gijen M (1998) Stevia rebaudiana: its agricultural, biological and chemical properties. Can J Plant Sci 78: 527-536.
6. Chaturvedula VSP, Rhea J, Milanowski D, Mocek U, Prakash I (2011) Two minor diterpene glycosides from the leaves of Stevia rebaudiana. Nat Prod Commun 6: 175-178.
7. Chaturvedula VSP, Prakash I (2011) A new diterpenoid glycoside from Stevia rebaudiana. Molecules 16: 2937-2943.
8. Chaturvedula VSP, Mani U, Prakash I (2011) Diterpene glycosides from Stevia rebaudiana. Molecules 16: 3552-3562.
9. Chaturvedula VSP, Prakash I (2011) Curcurbitane glycosides from Siraitia grosvenorii. J Carb Chem 30: 16-26.
10. Ohtani K, Aikawa Y, Kasai R, Chou W, Yamazaki K, Tanaka O (1992) Minor diterpene glycosides from sweet leaves of Rubus suavissimus. Phytochemistry 31: 1553-1559.
11. Tanaka T, Kohda H, Tanaka O, Chen FH, Chou WH, Leu JL (1981) Rubusoside (β-D-gluosyl ester of 13-O-β-D-gluosyl-steviol), a sweet principle of Rubus chinensis Hu. Agric Biol Chem 45: 2165-2166.
12. Tanaka T, Nakashima T, Ueda T, Tomii K, Kouno I (2007) Facile discrimination of aldose enantiomers by reversed-phase HPLC. Chem. Pharm Bull 55: 899-901.
13. Hiroso S, Chou WH, Kasai R, Tanaka O, Tada T (1990) Sweet and bitter diterpene glycosides from the leaves of Rubus suavissimus. Chem Pharm Bull 38: 1743-1744.
14. Liang YD, Yang SY, Kim JH, Lee YM, Kim YH (2002) A new diterpene glycoside from Artemisia wayomogi Kitamura that enhances IL-2 secretion. Bull Korean Chem Soc 31: 2422-2423.
15. Ichikawa N, Ochi M, Kubota T (1973) Bitter principles of Aquifoliaceae. II. Structure of the bitter principles of Ilex sugerokii var brevipedunculata and var longipedunculata. Nippon Kagaku Kaishi 4: 785-793.
16. Kobayashi M, Horikawa S, Degrandi IH, Ueno J, Mitsushashi H (1977) Dulcosides A and B, new diterpene glycosides from Stevia rebaudiana. Phytochemistry 16: 1405-1408.
17. Shen CJ, Chen CK, Lee SS. (2009) Polar constituents from Sageretia thea leaf characterized by HPLC-SPE-NMR assisted approaches. J Chin Chem Soc (Taipei, Taiwan) 56: 1002-1009.
18. Ma X, Tian W, Wu L, Cao X, Ito Y (2005) Isolation of queretin-3-O-L-rhamnoside from Acer truncatum Bunge by high-speed counter-current chromatography. J Chromatogr A 1070: 211-214.
19. Saklani A, Sahoo MR, Misra PD, Vishwakarma R (2011) Saussura heteromalla (D.Don) Hand-Mazz.: a new source of arctin, arctigenin and chlorojanerin. Indian J Chem 50(B): 624-626.
20. Rahman MMA, Dewick PM, Jackson DE, Lucas JA (1990) Lignans of Forsytha intermedia. Phytochemistry 29: 1971-1980.