# New phenolic derivatives from *Vernonia mapirensis* Gleason

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**Abstract**
Six new phenolic derivatives, including four flavonoids and two benzofuranones, were isolated from the aerial part extracts of *Vernonia mapirensis* Gleason (synonymous *Lepidaploa mapirensis* (Gleason) H. Robinson, *Vernonia trichoclada* Gleason, Asteraceae family), together with four known flavonoids. Their structural elucidation was achieved by extensive spectroscopic methods, 1D- ($^{1}$H, $^{13}$C, $^{13}$C DEPT, TOCSY, ROESY) and 2D-NMR experiments (DQF-COSY, HSQC, HMBC) as well as ESI-MS analysis.

**Keywords**: *Vernonia mapirensis*, Asteraceae, flavonoids, benzofuranones

## Introduction

*Vernonia* genus (Asteraceae family) comprises tropical and sub-tropical species widespread through both the hemispheres.[1] Previous phytochemical studies on this genus led to the isolation and characterization of flavonoids, steroidal glycosides, and sesquiterpenes.[2-5] In our continuing studies on the chemistry of *Vernonia* species, we selected *V. mapirensis* Gleason (synonymous *Lepidaploa mapirensis* (Gleason) H. Robinson, *Vernonia trichoclada* Gleason), a species native to Bolivia where is used traditionally for the preparation of anti-inflammatory remedies.

The aim of our work was to carry out the phytochemical investigation of *V. mapirensis* aerial parts and herein we report the isolation and structural characterization of six new phenolic derivatives, including four flavonoids (**1-4**) and two benzofuranones (**5-6**), from the methanol and chloroform-methanol extracts of the title plant, on the basis of extensive spectroscopic and spectrometric analysis (1D-NMR, 2D-NMR, ESI-MS).

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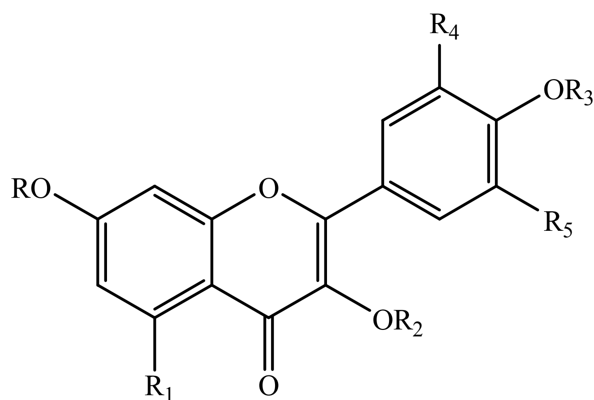
## Results and Discussion

Compound **1** was isolated as a yellow amorphous powder. Its molecular formula was established as $C_{36}H_{36}O_{19}$ by means of ESI-MS ($[M-H]^-$ peak at *m/z* 771), $^{13}C$, $^{13}C$-DEPT NMR, and elemental analysis. Analysis of 600 MHz NMR spectra suggested a flavonoid skeleton for compound **1**. The $^1H$-NMR spectrum (Table 1) indicated a 5,7-dihydroxylated pattern for ring A (two *meta*-coupled doublets at δ 6.16 and 6.33, $J$ = 1.5 Hz) and a 3′,4′-dihydroxylation pattern for ring B (ABX system signals at δ 6.97, d, $J$ = 8.5 Hz; 7.84, dd, $J$ = 8.5, 2.0 Hz; 7.65, d, $J$ = 2.0 Hz), allowing the aglycon to be recognized as quercetin.[6] The $^1H$-NMR spectrum of **1** also showed signals ascribable to sugar moieties and a *p*-coumaroyl residue (Table 1). Two anomeric protons arising from the sugar moieties appeared at δ 5.26 and 4.88 each (1H, d, $J$ = 7.5 Hz), which correlated respectively with signals at δ 103.4 and 104.7 ppm in the HSQC spectrum. All the $^1H$- and $^{13}C$-NMR signals of **1** were assigned using 1D-TOCSY, DQF-COSY, HSQC, and HMBC experiments. Complete assignments of proton and carbon chemical shifts of the sugar portion were accomplished by DQF-COSY and 1D-TOCSY experiments and allowed the identification of the sugars as two β-D-glucopyranosyl units, one terminal and one esterified. The configurations of the sugar units were assigned after hydrolysis of **1** with 1 N HCl. The hydrolysate was trimethylsilylated, and GC retention times compared with those of authentic sugar samples prepared in the same manner. The lower field shifts of $H_2$-6''' (δ 4.32 and 4.23) of one glucosyl unit suggested the substitution site of the *p*-coumaroyl moiety. Unequivocal information could be obtained by 2D-NMR spectra; the HMBC experiment indicated correlations between δ 5.26 (H-1''') and 135.6 (C-3), δ 4.88 (H-1'') and 148.0 (C-3'), δ 4.32 and 4.23 ($H_2$-6''') and 168.5 (COO). Thus, the structure of **1** was determined as quercetin 3-*O*-(6''-*p*-coumaroyl)-β-D-glucopyranoside-3'-*O*-β-D-glucopyranoside.




**1** $R = R_3 = R_5 = H$ $R_1 = OH$ $R_2$ = (6''-*p*-coumaroyl)glc $R_4$ = *O*-glc
**2** $R = R_4 = R_5 = H$ $R_1 = OH$ $R_2$ = (6''-*p*-coumaroyl)glc $R_3$ = Me
**3** $R = R_2$ = Me $R_1$ = OMe $R_3 = R_5 = H$ $R_4$ = *O*-glc
**4** $R = R_1 = H$ $R_2$ = glc $R_3$ = Me $R_4 = R_5 = OH$

**Table 1.** $^{1}$H- and $^{13}$C-NMR data of compounds **1-2** ($CD_3OD$, 600 MHz)[a]

| position | 1 | | | 2 | |
|---|---|---|---|---|---|
| | $\delta_H$ | $\delta_C$ | | $\delta_H$ | $\delta_C$ |
| 2 | | 159.0 | 2 | | 159.3 |
| 3 | | 135.6 | 3 | | 136.0 |
| 4 | | 179.0 | 4 | | 179.8 |
| 5 | | 163.5 | 5 | | 163.5 |
| 6 | 6.16 d (1.5) | 100.0 | 6 | 6.50 d (2.0) | 99.4 |
| 7 | | 166.3 | 7 | | 165.8 |
| 8 | 6.33 d (1.5) | 94.2 | 8 | 6.68 d (2.0) | 95.0 |
| 9 | | 159.0 | 9 | | 158.9 |
| 10 | | 105.8 | 10 | | 106.0 |
| 1' | | 123.1 | 1' | | 122.0 |
| 2' | 7.65 d (2.0) | 117.2 | 2' | 7.95 d (8.0) | 129.6 |
| 3' | | 148.0 | 3' | 7.08 d (8.0) | 115.7 |
| 4' | | 145.0 | 4' | | 159.0 |
| 5' | 6.97 d (8.5) | 118.0 | 5' | 7.08 d (8.0) | 115.7 |
| 6' | 7.84 dd (2.0, 8.5) | 123.2 | 6' | 7.95 d (8.0) | 129.6 |
| | | | 4'-OMe | 3.90 s | 58.5 |
| 3'-*O*-Glc 1" | 4.88 d (7.5) | 104.7 | 3-*O*-Glc1" | 5.27 d (7.8) | 103.5 |
| 2" | 3.58 dd (7.5, 9.0) | 74.8 | 2" | 3.54 dd (7.8, 9.5) | 74.0 |
| 3" | 3.52 t (9.0) | 77.3 | 3" | 3.50 t (9.5) | 77.8 |
| 4" | 3.42 t (9.0) | 71.2 | 4" | 3.39 t (9.5) | 71.6 |
| 5" | 3.53 m | 78.4 | 5" | 3.60 m | 75.4 |
| 6"a | 3.95 dd (5.0, 12.0) | 62.4 | 6"a | 4.34 dd (12.0, 4.5) | 64.5 |
| 6"b | 3.79 dd (3.5, 12.0) | | 6"b | 4.24 dd (12.0, 3.0) | |
| 3-*O*-Glc1"' | 5.26 d (7.5) | 103.4 | *p*-coumaroyl 1 | | 125.0 |
| 2"' | 3.56 dd (7.5, 9.0) | 73.6 | 2,6 | 7.95 d (8.0) | 129.7 |
| 3"' | 3.50 t (9.0) | 77.7 | 3,5 | 6.83 d (8.0) | 116.2 |
| 4"' | 3.40 t (9.0) | 71.8 | 4 | | 159.5 |
| 5"' | 3.59 m | 75.2 | α | 6.42 d (16.0) | 116.1 |
| 6"'a | 4.32 dd (12.0, 5.0) | 64.2 | β | 7.65 d (16.0) | 146.0 |
| 6"'b | 4.23 dd (12.0, 3.5) | | COO | | 168.3 |
| *p*-coumaroyl 1 | | 125.2 | | | |
| 2,6 | 8.00 d (8.5) | 130.0 | | | |
| 3,5 | 6.90 d (8.5) | 116.5 | | | |
| 4 | | 159.0 | | | |
| α | 6.40 d (16.0) | 116.0 | | | |
| β | 7.68 d (16.0) | 146.2 | | | |
| COO | | 168.5 | | | |

[a] Coupling pattern and coupling constants (*J* in Hertz) are in parentheses.

**Table 2**: $^{1}$H- and $^{13}$C-NMR data of compounds **3-4** ($CD_3OD$, 600 MHz)[a]

| Position | 3 | | 4 | |
|---|---|---|---|---|
| | $\delta_H$ | $\delta_C$ | $\delta_H$ | $\delta_C$ |
| 2 | | 158.2 | | 158.1 |
| 3 | | 142.0 | | 134.5 |
| 4 | | 179.0 | | 176.0 |
| 5 | | 166.0 | 8.02 d (8.0) | 128.3 |
| 6 | 6.51 d (2.0) | 97.0 | 6.95 dd (8.0, 2.0) | 116.9 |
| 7 | | 162.2 | | 164.7 |
| 8 | 6.85 d (2.0) | 93.4 | 6.93 d (2.0) | 103.3 |
| 9 | | 158.5 | | 158.3 |
| 10 | | 110.0 | | 103.0 |
| 1' | | 122.3 | | 122.0 |
| 2' | 8.04 d (2.0) | 118.3 | 7.30 s | 100.9 |
| 3' | | 148.0 | | 146.8 |
| 4' | | 145.3 | | 141.0 |
| 5' | 6.97 d (8.5) | 118.0 | | 146.8 |
| 6' | 7.83 dd (2.0, 8.5) | 126.5 | 7.30 s | 100.9 |
| 3-OMe | 3.80 s | 60.0 | | |
| 5-OMe | 3.95 s | 56.5 | | |
| 7-OMe | 3.92 s | 56.3 | | |
| 4'-OMe | | | 3.90 s | 56.7 |
| 3'-*O*-Glc 1'' | 4.88 d (7.5) | 104.7 | | |
| 2'' | 3.58 dd (7.5, 9.0) | 74.8 | | |
| 3'' | 3.52 t (9.0) | 77.3 | | |
| 4'' | 3.42 t (9.0) | 71.2 | | |
| 5'' | 3.53 m | 78.4 | | |
| 6''a | 3.95 dd (12.0, 5.0) | 62.4 | | |
| 6''b | 3.79 dd (12.0, 3.5) | | | |
| 3-*O*-Glc1'' | | | 5.31 d (7.5) | 102.9 |
| 2'' | | | 3.52 dd (7.5, 9.5) | 74.0 |
| 3'' | | | 3.47 t (9.5) | 78.1 |
| 4'' | | | 3.39 t (9.5) | 71.3 |
| 5'' | | | 3.56 m | 77.7 |
| 6''a | | | 3.90 dd (12.0, 5.0) | 62.9 |
| 6''b | | | 3.87 dd (12.0, 3.5) | |

[a] Coupling pattern and coupling constants (*J* in Hertz) are in parentheses.

To compound **2** was assigned the molecular formula $C_{31}H_{28}O_{13}$ by ESI-MS ($[M-H]^-$ peak at *m/z* 607), $^{13}$C NMR (Table 1), and $^{13}$C DEPT data. The $^{1}$H NMR of **2** (Table 1) was very similar to **1** except for signals of ring B of the aglycon moiety that in **2** were typical of a kaempferol 4′-methyl ether.[6] The $^{1}$H NMR and $^{13}$C NMR exhibited signals which can be ascribed to a kaempferol, *p*-coumaroyl, and methoxyl moieties along with those of one anomeric proton identified with the help

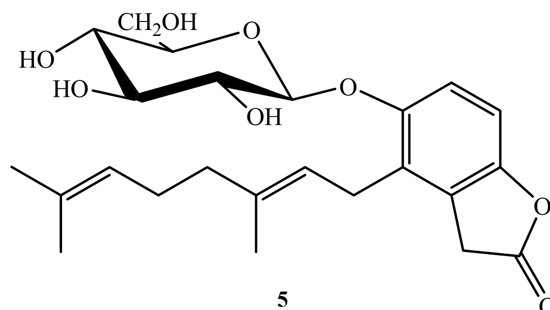
of DQF-COSY and 1D-TOCSY as one glucose (δ 5.27, *J* = 7.8 Hz). The configuration of the glucose unit was determined as reported for compound **1**. The assignments of all protonated carbons were accomplished by interpretation of the HSQC NMR spectrum while HMBC experiment correlations indicated connections between δ 5.27 (H-1'') and 136.0 (C-3), δ 4.24 and 4.34 ($H_2$-6'') and 168.3 (COO), δ 3.90 (OMe) and 159.0 (C-4'). From these results, the structure of **2** was concluded to be kaempferol 4'-methyl ether 3-*O*-(6''-*p*-coumaroyl)-β-D-glucopyranoside.

The molecular formula $C_{24}H_{26}O_{12}$ for compound **3** was determined by ESI-MS ($[M+H]^+$ at *m/z* 507), $^{13}C$, $^{13}C$-DEPT NMR analyses and was supported also by elemental analysis. Its $^1H$- and $^{13}C$-NMR spectra (see Table 2) indicated that it was a quercetin 3,5,7-trimethyl ether derivative.[7] Its $^1H$-NMR spectrum further displayed signals for one sugar residues that were easily clarified with the help of 1D-TOCSY and DQF-COSY experiments, leading to the identification of one β-D-glucopyranosyl residue. The configuration of the glucose unit was determined as reported for compound **1**. HMBC correlations [δ 4.88 (H-1'') and 148.0 ppm (C-3')] established the substitution sites of the glucose moiety and the three methoxyl groups, allowing compound **3** to be identified as quercetin 3,5,7-trimethyl ether 3′-*O*-β-D-glucopyranoside.

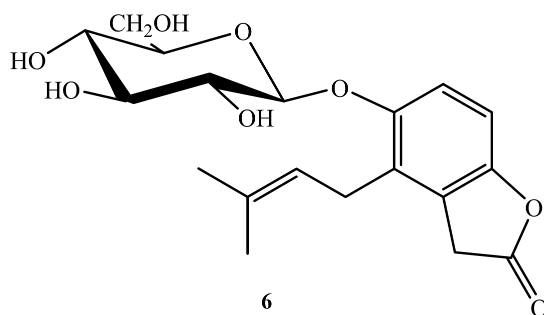
Compound **4** was assigned the molecular formula $C_{22}H_{22}O_{12}$ ($[M-H]^-$ peak at *m/z* 477). Analysis of its MS, $^{13}C$, and $^{13}C$ DEPT NMR data indicated that it was a flavonoid with 16 carbon atoms assigned to the aglycon and 6 carbons to the sugar moieties. The 600 MHz $^1H$ NMR spectrum indicated an unusual 7-hydroxylated pattern for ring A (ABX system signals at δ 6.93, d, *J* = 2.0 Hz; 6.95, dd, *J* = 8.0, 2.0 Hz; 8.02, d, *J* = 8.0 Hz) and a 3′,4′,5′-trihydroxylation pattern for ring B (two-proton singlet at δ 7.30), allowing the aglycon to be recognized as 3,7,3′,4′,5′-pentahydroxyflavone or robinetin.[8,9] Except for the aglycon signals, the $^1H$ NMR spectrum of **4** revealed the presence of one-proton doublet at δ 5.31 (*J* = 7.5 Hz) representative of one anomeric proton and of a three-proton singlet at δ 3.90 attributable to a methoxyl group. Selected 1D TOCSY data, when compared with those obtained from the $^{13}C$ NMR and HSQC experiments, allowed the identification of the sugar as glucose with a β-configuration. The relative postions of the β-D-glucopyranose and methoxyl units were established from the HMBC correlations [δ 5.31 (H-1′′) with 134.5 ppm (C-3) and δ 3.90 (OMe) with 141.0 ppm (C-4′)]. Compound **4** was therefore identified as 3,7,3′,5′-tetrahydroxy-4′-methoxyflavone 3-*O*-β-D-glucopyranoside.

Compound **5** displayed the molecular formula $C_{24}H_{32}O_8$. Its ESI-MS spectrum revealed a molecular ion at *m/z* 447, together with ion at *m/z* 285, corresponding to the loss of one hexose unit. In the $^1H$ NMR spectrum of **5** (Table 3), two *ortho*-coupled aromatic protons (each d, δ 6.95 and 6.66, *J* = 8.5 Hz) and an isolated methylene group (s, δ 3.56) were evident, suggesting the presence of an *ortho*-disubstituted 2(3*H*)-benzofuranone.[10] Moreover the $^1H$ NMR data showed signals for three tertiary methyl (δ 1.82, 1.63, and 1.58), two olefinic protons (δ 5.11 and 5.09), and three methylene groups (δ 3.62 and 3.54, 2.09 and 1.99). A 1D-TOCSY subspectrum obtained by irradiating signal at δ 5.11 showed a set of coupled protons at δ 3.62 and 3.54 ($CH_2$) and δ 1.82 ($CH_3$), while irradiating signal at δ 5.09 the set of coupled protons at δ 2.09 and 1.99 (both $CH_2$) and δ 1.63 and 1.58 (both $CH_3$) was observed. Analysis of the correlated $^{13}C$ NMR signals in the HSQC spectrum and DQF-COSY led to the identification of a geranyl side chain. One sugar residue, easily recognizable as β-D-glucopyranose by 1D-TOCSY and DQF-COSY experiments, was also present. The D configuration of the glucose residue was established as reported for compound **1**. All carbon and proton signals of the molecule **5** were assigned from the HSQC and HMBC experiments: long-range correlations between δ 3.56 ($H_2$-3) and 132.6 (C-4), 153.0 (C-7a),

and 180.8 (C-2), between δ 3.62 and 3.54 ($H_2$-1′) and 126.0 (C-3a), 132.6 (C-4), 135.2 (C-3'), and 150.8 (C-5), and between δ 4.79 (H-1″) and 150.8 ppm (C-5), restricted the location of the geranyl side chain and glucose moiety to C-4 and C-5, respectively. Thus, the new compound **5** was characterized as 4-geranyl-5-*O*-β-D-glucopyranosyl-2(3*H*)-benzofuranone. This is the first report of a 2(3*H*)-benzofuranone from a *Vernonia* species. The aglycon of compound **5** was previously identified from *Mimulus clevelandii*.[10]




Compound **6** gave molecular formula $C_{19}H_{24}O_8$ as determined from $^{13}$C, $^{13}$C DEPT NMR, and ESI-MS analyses ($[M-H]^-$ peak at *m/z* 379). The analysis of the NMR data of **6** and the comparison with those of **5** revealed signals completely superimposable, except for those of the side chain, consisting in five carbon skeleton in **6** instead of ten in **5**. Particularly, in the $^1$H NMR spectrum of **6** were evident signals for two tertiary methyl (δ 1.81 and 1.66), one olefinic protons (δ 5.13), and one methylene group (δ 3.56) ascribable to an emiterpene unit. The structure elucidation of **6** was achieved by 1D-TOCSY, DQF-COSY, HSQC, and HMBC experiments allowing to identify **6** as 4-isoprenyl-5-*O*-β-D-glucopyranosyl-2(3*H*)-benzofuranone.




Four known flavonoids, quercetin, rutin, acacetin 7-*O*-rutinoside, and quercetin 3-*O*-(6"-caffeoyl)-β-D-glucopyranoside were identified by means of 1D- and 2D-NMR spectroscopy, ESI-MS analysis, and by comparison of their data with those reported in the literature.[6,11,12]

**Table 3.** $^{1}H$- and $^{13}C$-NMR data of compounds **5-6** ($CD_3OD$, 600 MHz)[a]

| position | 5 | | 6 | |
|---|---|---|---|---|
| | $\delta_H$ | $\delta_C$ | $\delta_H$ | $\delta_C$ |
| 2 | | 180.8 | | 181.2 |
| 3 | 3.56 s | 38.4 | 3.58 s | 38.4 |
| 3a | | 126.0 | | 126.0 |
| 4 | | 132.6 | | 132.0 |
| 5 | | 150.8 | | 150.0 |
| 6 | 6.95 d (8.5) | 116.4 | 6.69 d (8.0) | 115.9 |
| 7 | 6.66 d (8.5) | 115.6 | 6.97 d (8.0) | 116.8 |
| 7a | | 153.0 | | 153.0 |
| 1'a | 3.54 dd (15.0, 7.0) | 26.7 | 3.52 dd (14.8, 7.0) | 27.0 |
| 1'b | 3.62 dd (15.0, 7.0) | | 3.62 dd (14.8, 7.0) | |
| 2' | 5.11 m | 125.1 | 5.13 br t (7.0) | 125.3 |
| 3' | | 135.2 | | 135.2 |
| 4' | 1.82 s | 16.7 | 1.81 s | 24.0 |
| 5' | 1.99 t (7.5) | 41.0 | 1.66 s | 17.5 |
| 6' | 2.09 dd (7.5, 1.5) | 27.8 | | |
| 7' | 5.09 m | 125.6 | | |
| 8' | | 132.1 | | |
| 9' | 1.63 s | 25.9 | | |
| 10' | 1.58 s | 17.8 | | |
| *O*-Glc 1" | 4.79 d (7.5) | 104.0 | 4.81 d (7.5) | 104.7 |
| 2" | 3.46 dd (7.5, 9.0) | 75.2 | 3.51 dd (7.5, 9.0) | 75.9 |
| 3" | 3.44 t (9.0) | 78.2 | 3.38 t (9.0) | 78.8 |
| 4" | 3.38 t (9.0) | 71.5 | 3.28 t (9.0) | 72.0 |
| 5" | 3.36 m | 78.2 | 3.49 m | 78.8 |
| 6"a | 3.88 dd (12.0, 5.0) | 62.7 | 3.91 dd (12.0, 5.0) | 63.0 |
| 6"b | 3.71 dd (12.0, 3.5) | | 3.74 dd (12.0, 3.5) | |

[a] Coupling pattern and coupling constants (*J* in Hertz) are in parentheses.

## Experimental Section

**General Procedures.** Optical rotations were measured on a Perkin-Elmer 241 polarimeter equipped with a sodium lamp (589 nm) and a 10 cm microcell. Elemental analysis was obtained from a Carlo Erba 1106 elemental analyzer. UV spectra were recorded on a Perkin-Elmer-Lambda 12 spectrophotometer. A Bruker DRX-600 NMR spectrometer using the UXNMR software package was used for NMR experiments. ESIMS (positive and negative mode) were obtained using a Finnigan LC-Q Advantage Termoquest spectrometer, equipped with Xcalibur software. TLC was performed on precoated Kieselgel 60 $F_{254}$ plates (Merck, Darmstadt, Germany); compounds were detected by spraying with $Ce(SO_4)_2/H_2SO_4$ (Sigma-Aldrich, St. Louis, Mo, USA) and NTS (Naturstoffe reagent)-PEG (Polyethylene glycol 4000) solutions. Column chromatography was

performed over Sephadex LH-20 (Pharmacia); reversed-phase (RP) HPLC separations were conducted on a Waters 515 pumping system equipped with a Waters R401 refractive index detector and Waters U6K injector, using a $C_{18}$ μ–Bondapak column (30 cm x 7.8 mm) and a mobile phase consisting of MeOH-$H_2O$ mixtures at a flow rate of 2 mL/min. GC analyses were performed using a Dani GC 1000 instrument.

**Plant material.** The aerial parts of *Vernonia mapirensis* Gleason were collected in Cotapata, Bolivia, in 2001. A voucher specimen (Michel R de y Morale L. No. 2991 HNB) is deposited at the Herbario Nacional de Bolivia.

**Extraction and isolation.** The dried powdered leaves of *V. mapirensis* (500 g) were extracted with *n*-hexane, $CHCl_3$, $CHCl_3$-MeOH (9:1), and MeOH, by Accelerated Solvent Extraction (ASE), to give 19.5, 18.0, 13.0, and 18.5 g of the respective residues. The methanol extract (18.5 g) was partitioned between *n*-BuOH and $H_2O$ to give a *n*-BuOH soluble portion (3.6 g) which was chromatographed over a Sephadex LH-20 column (100 cm x 3 cm) with MeOH as eluent. A total of 50 fractions were collected (8 mL each) and combined according to TLC analysis [silica 60 $F_{254}$ gel-coated glass sheets with *n*-BuOH-AcOH-$H_2O$ (60:15:25) and $CHCl_3$-MeOH-$H_2O$ (40:9:1)] to give five pooled fractions (A-E) together with pure quercetin 3-*O*-(6''-caffeoyl)-β-D-glucopyranoside and quercetin. Fraction C (270 mg) was purified by RP-HPLC on a $C_{18}$ μ-Bondapak column (30 cm x 7.8 mm, flow rate 2.0 mL $min^{-1}$) using MeOH-$H_2O$ (4.7:5.3) to give rutin (20 mg, $t_R$ = 16 min), compounds **1** (10 mg, $t_R$ = 19 min), and **2** (6 mg, $t_R$ = 29 min). Fraction D (150 mg) was purified by RP-HPLC on a $C_{18}$ μ-Bondapak column (30 cm x 7.8 mm, flow rate 2.0 mL $min^{-1}$) using MeOH-$H_2O$ (5.5:4.5) to yield compounds **3** (12 mg, $t_R$ = 29 min), **4** (7 mg, $t_R$ = 33 min), and acacetin 7-*O*-rutinoside (4 mg, $t_R$ = 40 min). A portion of the $CHCl_3$-MeOH residue (4.0 g) was chromatographed on Sephadex LH-20 using MeOH as eluent; fractions of 8 mL were collected and grouped into nine major fractions (A-I) by TLC results on silica 60 $F_{254}$ gel-coated glass sheets with *n*-BuOH-AcOH-$H_2O$ (60:15:25) and $CHCl_3$-MeOH-$H_2O$ (40:9:1). Fraction D (100 mg) was purified by RP-HPLC on a $C_{18}$ μ-Bondapak column (30 cm x 7.8 mm, flow rate 2.0 mL $min^{-1}$) using MeOH-$H_2O$ (2:3) as eluent, to yield pure compounds **6** (4 mg, $t_R$ = 9 min) and **5** (15 mg, $t_R$ = 12 min). Fraction E was purified by RP-HPLC on a $C_{18}$ μ-Bondapak column (30 cm x 7.8 mm, flow rate 2.0 mL $min^{-1}$) using MeOH-$H_2O$ (1:1) as eluent to obtain rutin (20 mg, $t_R$ = 12 min).

**Quercetin 3-*O*-(6''-*p*-coumaroyl)-β-D-glucopyranoside-3'-*O*-β-D-glucopyranoside (1).** Yellow amorphous powder, $[\alpha]_D$: -21° (*c* 0.1, MeOH), $^1H$ and $^{13}C$ NMR (600 MHz, $CD_3OD$): see Table 1, ESIMS: *m/z* 771 $[M-H]^-$, Anal. Calcd for $C_{36}H_{36}O_{19}$: C, 55.96; H, 4.70. Found C, 56.00; H 4.72.

**Kaempferol 4'-methyl ether 3-*O*-(6''-*p*-coumaroyl)-β-D-glucopyranoside (2).** Yellow amorphous powder, $[\alpha]_D$: -44° (*c* 0.1, MeOH), $^1H$ and $^{13}C$ NMR (600 MHz, $CD_3OD$): see Table 1, ESIMS: *m/z* 607 $[M-H]^-$, Anal. Calcd for $C_{31}H_{28}O_{13}$: C, 61.18; H, 4.64. Found C, 61.14; H 4.67.

**Quercetin 3,5,7-trimethyl ether 3′-*O*-β-D-glucopyranoside (3).** Yellow amorphous powder, $[\alpha]_D$: +32° (*c* 0.1, MeOH), $^1H$ and $^{13}C$ NMR (600 MHz, $CD_3OD$): see Table 2, ESIMS: *m/z* 507 $[M+H]^+$, 345 $[M+H-162]^+$, 330 $[M+H-162-15]^+$, Anal. Calcd for $C_{24}H_{26}O_{12}$: C, 56.92; H, 5.17. Found C, 57.00; H 5.13.

**3,7,3′,5′-Tetrahydroxy-4′-methoxyflavone 3-*O*-β-D-glucopyranoside (4).** Orange amorphous powder, $[\alpha]_D$: +36° (*c* 0.1, MeOH), $^1H$ and $^{13}C$ NMR (600 MHz, $CD_3OD$): see Table 2, ESIMS: *m/z* 477 $[M-H]^-$, Anal. Calcd for $C_{22}H_{22}O_{12}$: C, 55.23; H, 4.64. Found C, 55.20; H 4.61.

**4-Geranyl-5-*O*-β-D-glucopyranosyl-2(3*H*)-benzofuranone (5).** Amorphous powder, UV/Vis $\lambda_{max}$ (MeOH) nm (log $\varepsilon$): 218 (4.09), 295 (3.50), $^1$H and $^{13}$C NMR (600 MHz, $CD_3OD$): see Table 3, ESIMS: *m/z* 447 [M-H]$^-$, 285 [M-H-162]$^-$, Anal. Calcd for $C_{24}H_{32}O_8$: C, 64.27; H, 7.19. Found C, 64.24; H 7.20.

**4-Isoprenyl-5-*O*-β-D-glucopyranosyl-2(3*H*)-benzofuranone (6).** Amorphous powder, UV/Vis $\lambda_{max}$ (MeOH) nm (log $\varepsilon$): 216 (4.15), 290 (3.71), $^1$H and $^{13}$C NMR (600 MHz, $CD_3OD$): see Table 3, ESIMS: *m/z* 379 [M-H]$^-$, 217 [M-H-162]$^-$, Anal. Calcd for $C_{19}H_{24}O_8$: C, 59.99; H, 6.36. Found C, 60.05; H 6.37.

**Acid hydrolysis of compounds 1-6.** A solution of each compound (**1**-**6**, 2.0 mg each) in 1 N HCl (1 mL) was stirred at 80 °C in a stoppered reaction vial for 4 h. After cooling, the solution was evaporated under a stream of $N_2$. Each residue was dissolved in 1-(trimethylsilyl)imidazole and pyridine (0.2 mL), and the solution was stirred at 60 °C for 5 min. After drying the solution, the residue was partitioned between water and $CHCl_3$. The $CHCl_3$ layer was analyzed by GC using an L-CP-Chirasil-Val column (0.32 mm x 25 m). Temperatures of the injector and detector were 200 °C for both. A temperature gradient system was used for the oven, starting at 100 °C for 1 min and increasing up to 180 °C at a rate of 5 °C/min. Peaks of the hydrolysate were detected by comparison with retention times of authentic sample of D-glucose (Sigma Aldrich) after treatment with 1-(trimethylsilyl)imidazole in pyridine.

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