Field Chemical Characterization of *G. bucharica* Collected in Tajikistan

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One triterpene and five triterpene glycosides, including four new compounds, have been identified in the underground parts of *Glycyrrhiza bucharica*, which was shown to be closely related to Glycyrrhizin-producing *Glycyrrhiza* species, *G. uralensis*, *G. glabra* and *G. inflata*, based on their chloroplast *rbcL* sequences. Two known compounds were identified squasapogenol and macedonoside C. The structures of four new compounds, bucharosides A, B, C, and D, were determined to be 3-O-α-L-rhamnopyranosyl-(1→2)-β-D-glucuronopyranosyl-(1→2)-β-D-glucuronopyranosyl squasapogenol, 3-O-α-L-rhamnopyranosyl-(1→2)-β-D-glucuronopyranosyl squasapogenol, 3-O-α-L-rhamnopyranosyl-(1→2)-β-D-glucuronopyranosyl-macedonic acid, and 22-O-α-L-rhamnopyranosyl squasapogenol, respectively. Contents of these triterpene glycosides were less than 0.5% of dry weight, and no main saponin, like glycyrrhizin or macedonoside C found in other *Glycyrrhiza* species, was found in the underground parts of *G. bucharica*.

**Key words** *Glycyrrhiza bucharica*; triterpene; triterpene glycoside

**Introduction**

Licorice, the roots and stolons of *Glycyrrhiza* plants (Fabaceae), is one of the most important crude drugs in the world. Its main triterpene glycoside, glycyrrhizin, is used as a natural sweetener and a pharmaceutical agent because of its anti-inflammatory and hepatoprotective properties. 3 Three *Glycyrrhiza* species, *G. glabra* L., *G. uralensis* Fisch., and *G. inflata* Batal., are the major glycyrrhizin-producing species in the world, and other *Glycyrrhiza* species, such as *G. macedonica* Boiss. et Orph., *G. echinata* L., *G. pallidiflora* Maxim., *G. lepidota* (Nutt.) Pursh., and *G. yunnanensis* S. H. Cheng et L. K. Dai, produce other triterpene saponins as major constituents. 2–20

Tajikistan, a landlocked country in Central Asia, is one of the world’s main habitats of *Glycyrrhiza* species. Six *Glycyrrhiza* species (*G. glabra*, *G. uralensis*, *G. aspera* Pall., *G. kulabensis* T. Mast., *G. gontscharovii* T. Mast., and *G. bucharica* Rgl.) are distributed in Tajikistan. 5 In our previous report, two *Glycyrrhiza* species, *G. glabra* and *G. bucharica*, were collected in field surveys in Tajikistan. 3 *G. bucharica* is an endemic plant of Tajikistan 6 and is alternatively classified into genus *Meristotropis* (Meristotropis *bucharica*). 3 *G. bucharica* is also known to hybridize with *G. glabra*, 8 and *G. gontscharovii*, another endemic *Glycyrrhiza* species in Tajikistan, is suggested to be a hybrid between *G. glabra* and *G. bucharica*. 4 Notably, the phylogenetic tree constructed from the chloroplast *rbcL* sequences indicates that *G. bucharica* is closely related to *G. uralensis* and *G. inflata* mentioned in our previous report. 7 However, HPLC analysis indicated that glycyrrhizin was not detected in the underground parts of *G. bucharica*. Although some triterpene saponins were reported from *G. bucharica*, 3 triterpene glycoside has not yet been found in this plant. It is of interest to characterize triterpene glycosides of *G. bucharica* from a viewpoint of the evolution of glycyrrhizin biosynthesis. Thus, the characterization of triterpene saponins from the underground parts of *G. bucharica* was examined in the present study.

**Results and Discussion**

Isolation of Triterpene and Triterpene Glycosides from Underground Parts of *Glycyrrhiza bucharica* Collected in Tajikistan

Roots and stolons of *G. bucharica* were collected at two collection sites in Tajikistan (Fig. 1). These roots and stolons were extracted with ethyl acetate. The dried residue of ethyl acetate extraction was further extracted with acetone–water (2:8). The ethyl acetate soluble fraction was subjected to a series of reverse-phase column and silica gel column chromatography to afford a known triterpene, squa-
Compound 2 was obtained as a colorless amorphous powder and showed an ion [M + HCOO]⁻ at m/z 631.4175 in high-resolution electrospray ionization (HR-ESI)-MS, which corresponds to the molecular formula C₄₈H₇₂O₂₀. Acid hydrolysis of compound 2 with water containing 4M trifluoroacetic acid yielded L-rhamnose. The sugar configuration was determined using a previously described method. The ¹H- and ¹³C-NMR spectra were similar to those of squasapogenol (1), except compound 2 had a rhamnopyranosyl moiety (Table 1). Its connectivity with aglycone in compound 2 was confirmed by a heteronuclear multiple bond connectivity (HMBC) experiment. Long-range correlations were observed between the signals of H-1‴ of L-rhamnose and C-22 of squasapogenol, and of H-22 of squasapogenol and C-1‴ of L-rhamnose. The anomeric configuration of L-rhamnose was determined as α from the ¹³C-¹H coupling constant (167Hz) of the anomeric carbon signal. Therefore, the structure of compound 2, called bucharoside D, was determined to be 22-O-α-L-rhamnopyranosyl-squasapogenol (Fig. 2).

Compound 4 was obtained as a colorless amorphous powder and showed an ion [M–H]⁻ at m/z 1083.5380 in HR-ESI-MS, which corresponds to the molecular formula C₅₄H₈₄O₂₂. Acid hydrolysis of compound 4 with water containing 4M trifluoroacetic acid yielded L-rhamnose and D-glucuronolactone. The sugar configuration was determined using a previously described method. The ¹H- and ¹³C-NMR spectra were similar to those of bucharoside D (2), except that compound 4 had two rhamnopyranosyl moieties and two glucuronopyranosyl moieties (Table 1). The oligoglycoside structure and its connectivity with aglycone in compound 4 were confirmed by an HMBC experiment. Long-range correlations were observed between the signals of H-1‴ and C-3, H-1‴ and C-2″, H-1‴ and C-2‴, and H-1‴ and C-22. The anomeric configuration was determined as β for two D-glucuronic acids from the coupling constants of anomeric proton signals. The anomeric configuration of L-rhamnose was determined as α from the ¹³C-¹H coupling constants of the C-1‴ signal (176Hz) and C-1‴ signal (169Hz). Therefore, the structure of compound 4, called bucharoside A, was determined to be 3-O-α-L-rhamnopyranosyl-(1→2)-β-D-glucuronopyranosyl-(1→2)-β-D-glucuronopyranosyl-22-O-α-L-rhamnopyranosyl-squasapogenol (Fig. 2).

Compound 5 was obtained as a colorless amorphous powder and showed an ion [M–H]⁻ at m/z 967.4510 in HR-ESI-MS, which corresponds to the molecular formula C₆₁H₉₀O₃₀. Acid hydrolysis of compound 5 with water containing 4M trifluoroacetic acid yielded L-rhamnose and D-glucuronolactone. The sugar configuration was determined using a previously described method. The ¹H- and ¹³C-NMR spectra were similar to Table 1. ¹³C-NMR Data for Bucharosides A (4), B (5), C (6), D (2), (in Pyridine-d₅).  

|   | 4       | 5       | 6       | 2       | 4       | 5       | 6       | 2       |
|---|---------|---------|---------|---------|---------|---------|---------|---------|
| 1 | 38.1    | 38.1    | 38.1    | 38.4    | 105.3   | 105.3   | 105.3   |         |
| 2 | 26.5    | 26.5    | 26.5    | 28.0    | 2'      | 79.3    | 79.3    | 79.4    |
| 3 | 90.1    | 90.1    | 90.1    | 78.0    | 3'      | 78.8    | 78.8    | 78.8    |
| 4 | 39.8    | 39.8    | 39.8    | 39.5    | 4'      | 73.6    | 73.6    | 73.6    |
| 5 | 55.4    | 55.4    | 55.5    | 55.3    | 5'      | 77.5    | 77.6    | 77.5    |
| 6 | 18.6    | 18.5    | 18.6    | 18.9    | 6'      | 172.7   | 172.3   | 172.6   |
| 7 | 32.6    | 32.5    | 32.7    | 32.7    | GlcUA 1' | 102.8   | 102.8   | 102.8   |
| 8 | 40.6    | 40.7    | 41.1    | 40.7    | 2'      | 78.3    | 78.2    | 78.2    |
| 9 | 54.4    | 54.6    | 54.5    | 54.6    | 3'      | 78.9    | 79.0    | 78.9    |
| 10| 36.6    | 36.6    | 36.6    | 37.0    | 4'      | 73.6    | 73.6    | 73.6    |
| 11| 126.2   | 126.5   | 126.5   | 126.3   | 5'      | 77.5    | 77.6    | 77.6    |
| 12| 127.0   | 126.7   | 126.4   | 127.0   | 6'      | 172.3   | 172.8   | 172.2   |
| 13| 136.0   | 134.5   | 135.6   | 136.0   | Rha 1"  | 102.3   | 102.3   | 102.3   |
| 14| 42.2    | 42.5    | 42.4    | 42.2    | 2"     | 72.4    | 72.4    | 72.4    |
| 15| 24.4    | 24.6    | 24.6    | 24.4    | 3"     | 72.7    | 72.8    | 72.8    |
| 16| 33.7    | 36.2    | 33.8    | 33.8    | 4"     | 74.4    | 74.4    | 74.4    |
| 17| 40.2    | 36.8    | 40.7    | 40.7    | 5"     | 69.6    | 69.7    | 69.7    |
| 18| 136.5   | 135.2   | 137.5   | 136.7   | 6"     | 19.0    | 19.1    | 19.0    |
lar to those of macedonoside C (2), except that compound 5 had a rhamnopyranosyl moiety (Table 1). The oligoglycoside structure and its connectivity with aglycone in compound 5 were confirmed by an HMBC experiment. Long-range correlations were observed between the signals of H-1' and C-3, H-1" and C-2', and H-1" and C-2". The anomeric configuration was determined as β for two α-glucuronic acids from the coupling constants of anomeric proton signals. The anomeric configuration of L-rhamnose was determined as α from the 13C–1H coupling constant (173 Hz) of the anomeric carbon signal. Therefore, the structure of compound 5, called bucharoside B, was determined to be 3-O-α-L-rhamnopyranosyl-(1→2)-β-D-glucuronopyranosyl-(1→2)-β-D-glucuronopyranosyl-squasapogenol (Fig. 2).

Compound 6 was obtained as a colorless amorphous powder and showed an ion [M–H]− at m/z 937.4754 in HR-ESI-MS, which corresponds to the molecular formula C_{48}H_{74}O_{18}. Acid hydrolysis of compound 6 with water containing 4 M trifluoroacetic acid yielded L-rhamnose and D-glucuronolactone. The sugar configuration was determined using a previously described method. The 1H- and 13C-NMR spectra were similar to those of bucharoside A (4), except that compound 6 had only one rhamnopyranosyl moiety (Table 1). The oligoglycoside structure and its connectivity with aglycone in compound 6 were confirmed by an HMBC experiment. Long-range correlations were observed between the signals of H-1' and C-3, H-1" and C-2', and H-1" and C-2". The anomeric configuration was determined as β for two α-glucuronic acids from the coupling constants of anomeric proton signals. The anomeric configuration of L-rhamnose was determined as α from the 13C–1H coupling constant (173 Hz) of the anomeric carbon signal. Therefore, the structure of compound 6, called bucharoside C, was determined to be 3-O-α-L-rhamnopyranosyl-(1→2)-β-d-glucuronopyranosyl-(1→2)-β-d-glucuronopyranosyl-squasapogenol (Fig. 2).

The aglycone of bucharosides A (4), C (6) and D (2) is squasapogenol (1, olean-11(12),13(18)-diene-3β,22β-diol), which was reported from Glycyrrhiza squamulosa and Hedysarum gmelinii. The aglycone of bucharoside B (5) and macedonoside C (3) is macedonic acid (olean-11(12),13-(18)-diene-3β,21α-diol-29-ic acid), which was isolated from many Glycyrrhiza species as an aglycone of triterpene glycosides. These olean-11(12),13(18)-diene triterpenes are major aglycones of triterpene glycosides isolated from glycyrrhizin-non-producing Glycyrrhiza species, such as G. macedonica, G. echinata, G. pallidiflora and G. yunnanensis. Although G. bucharica is closely related to G. uralensis and G. inflata based on their chloroplast rbcL sequences, the structures of the triterpene glycosides of G. bucharica have been shown to be similar to those of glycyrrhizin-non-producing Glycyrrhiza species by the present study.

HPLC Analysis of Triterpene Glycosides in Underground Parts of G. bucharica HPLC analysis of roots and stolons of G. bucharica collected at two collection sites was performed to determine the contents of these triterpene glycosides. The content of bucharosides A–D and macedonoside C in the underground parts of G. bucharica was less than 0.5% of dry weight (Table 2). As shown in Fig. 3, there is no single main triterpene glycoside in the underground parts of G. bucharica, like glycyrrhizin or macedonoside C in other Glycyrrhiza species. Glycyrrhizin was not detected in the underground parts of G. bucharica. Although the structure of the sugar moiety of glycyrrhizin and macedonoside C was β-d-GlcA-(1→2)-β-d-GlcA, bucha-
Rosidi A (4), B (5) and C (6) have the sugar moiety α-1-Rha-(1→2)β-3-D-GlcA-(1→2)β-3-D-GlcA. The same sugar moiety was observed in triterpene glycosides isolated from other Glycyrrhiza species.\(^{14,15}\) In contrast, triterpene glycosides, having the sugar moiety of α-1-Rha-(1→2)β-3-D-GlcA-(1→2)β-3-D-GlcA, were major constituents of G. bucharica.

**Experimental**

**General Methods** \(^{1}\)H- and \(^{13}\)C-NMR spectra were recorded using an ECA500 (JEOL, Tokyo, Japan) spectrometer. Chemical shifts are given on a δ (ppm) scale with tetramethylsilane as an internal standard. TLC aluminum sheets (20 cm × 20 cm) Silica gel 60 F 254 (Merck, Darmstadt, Germany) were used for TLC analysis. Biotage\® SP system (Biotage AB, Uppsala, Sweden) was used for flash column chromatography. Prominence HPLC system (Shimadzu, Kyoto, Japan) was used for preparative HPLC. Specific rotation was measured on a Biotage SNAP Ultra 10g column, and eluted with a gradient of isopropanol–water (from 3 : 7 to 10 : 0) in 21-mL fractions (fractions 1–32), repeatedly. Fractions 15–19 (305 mg) of the KP-C18-HS 60g column (E1) were applied on Biotage SNAP Ultra 10g column, and eluted with a gradient of n-hexane–ethyl acetate (10 : 0–0 : 10) in 21-mL fractions (fractions 1–43) to isolate squasarapogenol\(^{11}\) (36 mg, fractions 35–37), named bucharoside D. Fractions 20–25 (242 mg) of the KP-C18-HS 60g column (E1) were applied on Biotage SNAP Ultra 10g column, and eluted with a gradient of n-hexane–ethyl acetate (10 : 0–0 : 10) in 21-mL fractions (fractions 1–43) to isolate a new triterpene glycoside 2 (36 mg, fractions 35–37), named bucharoside D. Fractions 20–25 (242 mg) of the KP-C18-HS 60g column (E1) were applied on Biotage SNAP Ultra 10g column, and eluted with a gradient of n-hexane–ethyl acetate (10 : 0–0 : 10) in 21-mL fractions (fractions 1–43) to isolate squasarapogenol\(^{11}\) (1, 20 mg, fractions 14–16).

**Bu夏roside D (2):** Colorless amorphous powder. \(^{1}\)H-NMR (pyridine-d\(_5\)) δ: 0.81 (3H, s, H-29), 0.86 (3H, s, H-26), 0.96 (3H, s, H-30), 0.99 (3H, s, H-25), 1.07 (3H, s, H-24), 1.10 (3H, s, H-27), 1.17 (3H, s, H-28), 1.27 (3H, s, H-23), 1.72 (3H, d, J = 5.8 Hz, rhamnosyl methyl-6\(^{-}\)), 2.04 (1H, brs, H-9), 2.48 (1H, d, J = 14.3 Hz, H-19a), 3.51 (1H, brs, J = 10.9 Hz, H-11), 6.50 (1H, dd, J = 10.9 Hz, H-19a), 3.51 (1H, brd, J = 10.9 Hz, H-11), 6.50 (1H, dd, J = 2.9 Hz, 10.3 Hz, H-12). \(^{13}\)C-NMR (pyridine-d\(_5\)) δ: Table 1. UV λ\(_{max}\) (MeOH) nm (ε): 242 (9200), 250 (10390), 260 (6730). HR-ESI-MS m/z: 631.4175 (M + HCOO\(^{+}\)) (Calcd for C\(_{34}\)H\(_{46}\)O\(_{14}\): 631.4210). [\(\alpha\)]\(_D\)\(^{22}\) = −46.3° (c = 0.04, MeOH).

The dried residue of ethyl acetate extraction was further extracted twice with 2L of acetonitrile–water–formic acid purchased from Nacalai Tesque Inc. (Kyoto, Japan). Acetonitrile for separation was of Special Grade (Wako Pure Chemical Industries, Ltd.), and acetonitrile for LC-MS analysis was of LC-MS Grade (Thermo Fisher Scientific, Waltham, U.S.A.). Pyridine-d\(_5\)\(^{1}\) was purchased from Cambridge Isotope Laboratories (Andover, U.S.A.).

**Plant Materials** The underground parts of Glycyrrhiza bucharica Rgl. (Fabaceae) used in the present study were collected at two collection sites in Tajikistan (Fig. 1). These G. bucharica plants were identified based on phenotypic change of fruits and leaves by Hiroaki Hayashi. All plant specimens used in the present study were deposited into the Herbarium of the Institute of Botany, Plant Physiology and Genetics, Academy of Science of Tajikistan.

**Isolation of Triterpene and Triterpene Glycosides** Dried underground parts (240 g) of G. bucharica were extracted twice with 1200 mL of ethyl acetate by ultrasonication for 2h. The dried ethyl acetate extract (1.8 g) was dissolved in 2L of acetonitrile–water–formic acid 2% (v/v). After centrifugation, the supernatant was evaporated to dryness. The dried residue of ethyl acetate extraction was further extracted twice with 2L of acetonitrile–water–formic acid purchased from Nacalai Tesque Inc. (Kyoto, Japan). Acetonitrile for separation was of Special Grade (Wako Pure Chemical Industries, Ltd.), and acetonitrile for LC-MS analysis was of LC-MS Grade (Thermo Fisher Scientific, Waltham, U.S.A.). Pyridine-d\(_5\)\(^{1}\) was purchased from Cambridge Isotope Laboratories (Andover, U.S.A.).

### Table 2. Contents of Bucharosides A (4), B (5), C (6), D (2), and Macedonoside C (3) in the Roots and Stolons of Glycyrrhiza bucharica Collected in Tajikistan

| Collection site | Plant number | Root/Stolon | Diameter (mm) | Contents (% of dry weight) of |
|-----------------|--------------|-------------|---------------|-------------------------------|
|                 |              |             | 4             | 5                            | 6 | 2 | 3 |
| Dangara         | 13A04        | Stolon      | 9.4–9.7       | 0.06                         | 0.10 | 0.01 | 0.00 | 0.08 |
|                 | 13A05        | Root        | 11.6–12.1     | 0.05                         | 0.07 | 0.01 | 0.00 | 0.05 |
|                 | 13A06        | Root        | 8.1–9.5       | 0.22                         | 0.41 | 0.03 | 0.00 | 0.20 |
| Khuroson        | 13A24        | Stolon      | 18.3–18.6     | 0.21                         | 0.30 | 0.08 | 0.02 | 0.10 |
|                 | 13A24        | Stolon      | 17.3–19.9     | 0.40                         | 0.44 | 0.27 | 0.03 | 0.17 |
|                 | 13A24        | Stolon      | 8.4–8.7       | 0.15                         | 0.18 | 0.05 | 0.00 | 0.09 |
|                 | 13A25        | Stolon      | 15.5–18.1     | 0.25                         | 0.27 | 0.14 | 0.01 | 0.09 |
|                 | 13A25        | Root        | 18.1–21.5     | 0.32                         | 0.30 | 0.16 | 0.00 | 0.07 |

**Fig. 3.** HPLC Profile of the Underground Parts of G. bucharica (13A24) Collected in Tajikistan
(20:80:1) at 60°C for 1 h. The extract (3.6 mL) was applied on Biogel SNAP cartridge KP-C18-HS 120g column (A1), and eluted with a gradient of acetonitrile–water containing 0.2% formic acid (from 2:8 to 9:1) in 21-mL fractions (fractions 1–48). Fractions 20–25 (879 mg) of the KP-C18-HS 120g column (A1) were dissolved in 50 mL of acetonitrile–water containing 0.2% formic acid (3:7). The solution was applied on Sephadex LH-20 column (180 mL), and eluted with acetonitrile–water containing 0.2% formic acid (3:7) in 50-mL fractions (fractions 1–25). Fractions 14–17 (59 mg) of the Sephadex LH-20 column were dissolved in 12 mL of acetonitrile–water (4:6) containing 0.2% formic acid. The solution was repeatedly chromatographed using preparative HPLC, as mentioned above, to isolate a compound 6 (14 mg, t_R: 44 min).

Bucharaoside C (6): Colorless amorphous powder. ¹H-NMR (pyridine-d₅) δ: 0.78 (3H, s, H-26), 0.82 (3H, s, H-25), 0.90 (3H, s, H-29), 1.02 (3H, s, H-30), 1.08 (3H, s, H-27), 1.24 (3H, s, H-24), 1.33 (3H, s, H-28), 1.47 (3H, s, H-23), 1.85 (3H, d, J = 6.5 Hz, rhamnosyl methyl-6“), 1.98 (1H, br s, H-9), 2.48 (1H, d, J = 14.2 Hz, H-19a), 3.35 (1H, dd, J = 4.0 Hz, 11.5 Hz, H-3), 3.80 (1H, dd, J = 5.0 Hz, 12.0 Hz, H-22), 5.13 (1H, d, J = 7.3 Hz, H-1”), 5.60 (1H, brd, J = 10.7 Hz, H-11), 5.97 (1H, d, J = 7.7 Hz, H-1”), 6.46 (1H, br s, H-1”), 6.47 (1H, dd, J = 2.0 Hz, 10.7 Hz, H-12). ¹³C-NMR (pyridine-d₅) δ: Table 1. UV max (MeOH) nm (ε): 242 (7060), 250 (7830), 260 (5400). HR-ESI-MS m/z: 937.4754 (M–H) (Calcd for C₄₀H₇₀O₂₁H₂O, 937.4797). [α]_D²⁰ = –34.1° (c = 0.04, MeOH).

Acid Hydrolysis of Saponins Each saponin (1 mg) was dissolved in 1 mL of water containing 4 M trifluoroacetic acid, and the solution was reflushed for 2 h. After the reaction mixture was cooled, it was dried in vacuo. The residue was dissolved in 50% acetonitrile–water, and sugars were analyzed by TLC (n-propanol–water–acetic acid, 170:30:5). The spots were detected by spraying of 50% H₂SO₄ followed by heating.

Determination of Sugar Configuration The sugar configuration was determined using a modified method described previously.¹² Each saponin (1 mg) was dissolved in 1 mL of water containing 4 M trifluoroacetic acid, and the solution was reflushed for 2 h. After cooling the reaction mixture, it was dried in vacuo. The residue was dissolved in 0.2 mL of pyridine containing L-cysteine methyl ester hydrochloride (1 mg) and heated at 60°C for 1 h, following which 0.2 mL of pyridine containing o-tolylisothiocyanate (1 mg) was added to the mixture and heated further at 60°C for 1 h. The reaction mixture (2 μL) was directly analyzed by HPLC; column: Inertsil ODS-SP (3 μm, 2.1 mm i.d. × 250 mm, GL Sciences, Japan); solvent: 25% acetonitrile–water containing 0.1% formic acid (isocratic elution); column temp.: 40°C; detector: UV absorption at 250 nm. The peaks were compared with those of derivatives prepared from d-glucuronolactone and L-rhamnose. The t_R of L-glucuronolactone, obtained by reaction of d-glucuronolactone with L-cysteine methyl ester hydrochloride, was different from that of d-glucuronolactone.

HPLC Analysis of Underground Parts Dried underground parts were powdered with a mortar and pestle, and 50 mg of each powdered sample was extracted with 5 mL of 80% methanol at 60°C for 2 h. An aliquot (2 μL) of the extract was analyzed by LC-MS; column: Inertsil ODS-3 (3 μm, 2.1 mm i.d. × 250 mm, GL Sciences, Japan); solvent: gradient of H₂O containing 0.1% formic acid (solvent A) and acetonitrile containing 0.1% formic acid (solvent B); flow rate: 0.1 mL/min; column temp.: 40°C; detector: photodiode array detector. The quantities of the constituents were determined on the basis of their peak area of UV absorption.
at 250 nm. Each constituent was identified by comparing its retention time, UV spectrum, and HRESI-MS with a respective authentic sample.

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Conflict of Interest The authors declare no conflict of interest.

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