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Flavonoids with hepatoprotective activity from the leaves of *Cleome viscosa* L.

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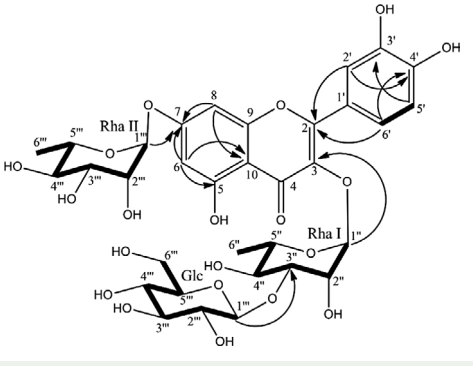
ABSTRACT

One new flavonol glycoside named visconoside C (**1**), together with seven known flavonol glycosides, quercetin 3-O-β-D-glucopyranoside 7-O-α-L-rhamnopyranoside (**2**), quercetin 7-O-α-L-rhamnopyranoside (**3**), astragalin (**4**), kaempferol 3-*O*-(4-*O*-acetyl)-*α*-L-rhamnopyranoside (**5**), kaempferol 7-*O*-*α*-L-rhamnopyranoside (**6**), kaempferitrin (**7**) and kaempferol 3-*O*-*β*-D-glucopyranoside 7-*O*-*α*-L-rhamnopyranoside (**8**) were isolated by various chromatography methods from the leaves of *Cleome viscosa* L. Their structures were elucidated by IR, UV, HR-ESI-MS and NMR (1D & 2D) experiments. The cytotoxicity and hepatoprotective activities using HepG2 human hepatoma cell line of **1** were measured by MTT assay. At the concentration of 25 µM and 50 µM, **1** showed cytotoxic activity against HepG2 cells (cell viability was decreased to 22.2 and 23.0%, respectively, compared with doxorubicin control), while at the concentration of 100 µM, **1** showed hepatoprotective activity against CCl_4-induced hepatotoxicity on HepG2 cells (34.3%, compared with quercetin control).



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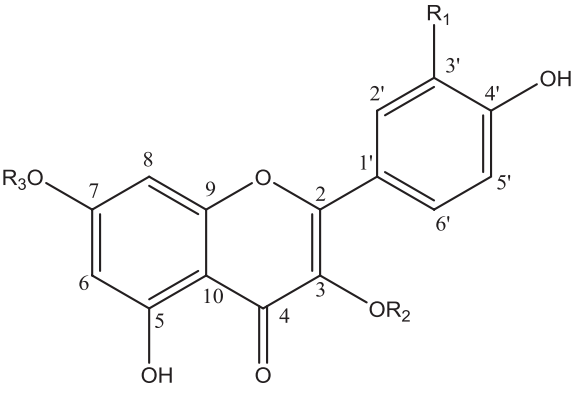

(**1**): R_1 = OH, R_2 = Rha, R_3 = [Rha(1→3)]Glc
(**2**): R_1 = OH, R_2 = Glc, R_3 = Rha
(**3**): R_1 = OH, R_2 = H, R_3 = Rha
(**4**): R_1 = H, R_2 = Glc, R_3 = H
(**5**): R_1 = H, R_2 = 4-AcRha, R_3 = H
(**6**): R_1 = H, R_2 = H, R_3 = Rha
(**7**): R_1 = H, R_2 = Rha, R_3 = Rha
(**8**): R_1 = H, R_2 = Glc, R_3 = Rha

Figure 1. Structure of compounds **1**–**8**.

1. Introduction

Cleome viscosa L. is used as drugs in the traditional medicine for the treatment of diarrhea, fever, inflammation, liver diseases, bronchitis, skin diseases and malarial fever (Mali 2010). This plant has been scientifically screened for various pharmacological activities such as antimicrobial, anti-inflammatory, hepatoprotective (Gupta & Dixit 2009; Mali 2010), antifibrotic (Kumar et al. 2009), anticonvulsant (Mishra et al. 2010), antioxidant (Gupta et al. 2011), antinociceptive, antibacterial (Bose et al. 2011), antitumor (Gopal et al. 2012) and antibacterial activities (Donkor et al. 2014). Phytochemical studies on *C. viscosa* led to the isolation of flavanone (Srivastava et al. 1979), flavonol (Chauhan et al. 1979; Senthamilselvi et al. 2012), sterol (Srivastava 1980), coumarinolignoid (Ray et al. 1985; Kumar et al. 1988), diterpene (Jente et al. 1990), lactam derivative (Jana & Biswas 2011), dipyridodiazepinone derivative (Chatterjee et al. 2013) and triterpenoid (Farimani et al. 2016). In our previous study, two new flavonol glycosides from the leaves of this species were reported (Phan et al. 2016). This paper described the isolation and structure elucidation of flavonoids (**1**–**8**) and the evaluation of cytotoxicity and hepatoprotective activity of new compound from *C. viscosa* L. collected in Ben Cat, Binh Duong province, Viet Nam.

2. Results and discussion

The MeOH extract from the dried leaves of *C. viscosa* L. was subjected to column chromatography over silica gel normal-phase and reversed-phase RP-18 to give two new flavonol glycosides named visconoside C (quercetin 3-*O*-[*β*-D-glucopyranosyl-(1 → 3)]-*α*-L-rhamnopy

ranoside 7-*O*-*α*-L-rhamnopyranoside) (**1**) and seven known flavonol glycosides (Figure 1), quercetin 3-O-β-D-glucopyranoside 7-O-α-L-rhamnopyranoside (**2**) (Kerhoas et al. 2006), quercetin 7-O-α-L-rhamnopyranoside (**3**) (Ailian et al. 2006), astragalin (**4**) (Wei et al. 2011), kaempferol 3-*O*-(4-*O*-acetyl)-*α*-L-rhamnopyranoside (**5**) (Masuda et al. 1991), kaempferol 7-*O*-*α*-L-rhamnopyranoside (**6**) (Mobiya et al. 2010), kaempferitrin (**7**) (Olszewska & Wolbis 2002) and kaempferol 3-*O*-*β*-D-glucopyranoside 7-*O*-*α*-L-rhamnopyranoside (**8**) (Song et al. 2007).

1 was obtained as yellow amorphous powder. The molecular formula was established as $C_{33}H_{40}O_{20}$ by HR-ESI-MS data ($[M + Na]^+$ *m/z* 779.1986). The aglycone of **1** was identified as quercetin, according to observation of 15 carbons in ^{13}C NMR and DEPT spectrum, including 1 carbonyl carbon at δ_C 177.9 (C-4), 7 oxygenated aromatic carbons, 2 quaternary aromatic carbons and 5 non-oxygenated aromatic carbons together with 2 AX-type aromatic protons at δ_H 6.44 (*d*, 2.0, H-6) and 6.76 (*d*, 2.0, H-8); 3 ABX-type aromatic protons at δ_H 6.92 (*d*, 8.5, H-5′), 7.31 (*dd*, 2.0 and 8.0, H-6′) and 7.38 (*br s*, H-2′) in ^1H NMR data. Moreover, three anomeric carbons at δ_H 5.32 (*br s*, H-1″), 4.36 (*d*, 7.5, H-1‴), 5.56 (*br s*, 1.0, H-1⁗) corresponded to three anomeric carbons at δ_C 101.6 (C-1″), 104.8 (C-1‴), 98.4 (C-1⁗) were assigned to *α*-L-rhamnose (Rha I), *β*-D-glucose (Glc) and *α*-L-rhamnose (Rha II) units, respectively. The COSY and HSQC spectrum allowed analysis of their spin systems and assignment of their proton resonances to determine every sugar unit clearly. Beside, the sugar moiety was identified as L-rhamnose and D-glucose by the acidic hydrolysis and using TLC to compare the hydrolysate with the authentic sugars (see Experimental). The HMBC spectrum (Figure 1), showed correlations between anomeric proton at δ_H 5.32 (H-1″) of Rha I and carbon at δ_C 134.2 (C-3) of aglycone; between anomeric proton at δ_H 4.36 (H-1‴) of Glc and carbon at δ_C 81.2 (C-3″) of Rha I; between anomeric proton at δ_H 5.56 (*br s*, 1.0, H-1⁗) of Rha II and carbon at δ_C 161.7 (C-7). Based on data of HR-ESI-MS, 1D, 2D NMR and compared with previous published data (Phan et al. 2015 & 2016), the structure of **1** was determined as quercetin 3-*O*-[*β*-D-glucopyranosyl-(1→3)]-*α*-L-rhamnopyranoside 7-*O*-*α*-L-rhamnopyranoside, and named visconoside C (Figure 1).

The cytotoxicity and hepatoprotective activities were evaluated for **1** using HepG2 cell (Table S1). At the concentration of 25 μM and 50 μM, **1** showed cytotoxic activity against HepG2 cells (cell viability was decreased to 22.2 and 23.0%, respectively, compared with doxorubicin control), while at the concentration of 100 μM, **1** showed hepatoprotective activity against CCl_4-induced hepatotoxicity on HepG2 cells (34.3%, compared with quercetin control).

One new flavonol glycoside (**1**) together with seven known flavonol glycosides (**2–8**) were isolated from the methanol extract of the dried leaves of *C. viscosa* L. For the first time, compounds **4** and **5** were reported from the genus *Cleome*. Their structures were elucidated by intensive spectroscopic and spectrometric analyses.

3. Experimental

3.1. General experimental procedures

The high-resolution electrospray ionisation mass spectroscopy (HR-ESI-MS) was recorded on a Bruker MicrOTOF-QII spectrometer. The ^1H NMR (500 MHz), ^{13}C NMR (125 MHz), DEPT, COSY, HSQC and HMBC spectra were recorded on a Bruker AM500 FT-NMR spectrometer using tetramethylsilane (TMS) as internal standard. Column chromatography was carried

out using Merck Silica gel normal-phase (230–240 mesh) and reversed-phase C_{18} (Merck). Analytical TLC was carried out in silica gel plates (Merck DC-Alufolien 60 F_{254}). Compounds were visualised by spraying aqueous 10% H_2SO_4 and heating for 3–5 min.

3.2. Chemicals

Eagle's Minimum Essential Medium (EMEM), fetal calf serum (FCS), trypsin-EDTA were purchased from Gibco, USA; L-glutamine, penicillin-streptomycin, phosphate buffer, 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT), doxorubicin, quercetin, carbon tetrachloride (CCl_4) from Sigma–Aldrich, USA; dimethylsulfoxide (DMSO), isopropanol from Merck, Germany. All chemicals met cell culture standards.

3.3. Plant material

The leaves of *C. viscosa* were collected from Ben Cat, Binh Duong province, Viet Nam, in May 2015; and identified by Prof. Vo Van Chi. A voucher specimen (No. VH/MINH-0515) was deposited in the Institute of Chemical Technology, Vietnam Academy of Science and Technology.

3.4. Extraction and isolation

Powdered leaves of *C. viscosa* (8 kg) were extracted with 95% EtOH for three times (3 × 30 L, total amount 90 L) at room temperature, residue was filtered , solvents were removed under low pressure and the crude extract (980 g) was obtained. Then, crude extract was applied to solid-phase extraction procedures and successively partitioned into *n*-hexane (70 g), $CHCl_3$ (150 g), EtOAc (260 g) and MeOH (450 g). The MeOH extract was subjected to silica gel column chromatography and eluted with gradient solvent system of chloroform–methanol (95:5–5:95) to afford seven fractions: M1 (25 g), M2 (30 g), M3 (86 g), M4 (75 g), M5 (60 g), and M6 (72 g).

Fraction M2 (5 g) was chromatographed on silica gel and eluted with $CHCl_3$–MeOH (10:1) to obtain compounds **3** (72 mg) and **7** (68 mg). Fraction M3 (5 g) was separated by silica gel chromatographic column using $CHCl_3$-MeOH (5:1), and further separated by RP-18 using gradient mixtures of MeOH–H_2O (5:1, v/v) to affrord compound **8** (268 mg). The same manner was applied to fraction M4 (75 g), eluted $CHCl_3$-MeOH (6:1 → 3:1) to give four subfractions (M4.1–M4.4). Subfraction M4.3 (25 g) was further purifed by RP-18 with MeOH-H_2O (4:1, v/v) to give compounds **5** (16 mg) and **6** (72 mg). Subfraction M4.2 (20 g) was done in the same manner**,** further separated by RP-18 with MeOH-H_2O (3:1, v/v) to affrord **1** (72 mg) and **3** (7 g). Fraction M5 (5 g) was applied on a silica gel chromatographic column and eluted with $CHCl_3$-MeOH (2:1) in silica gel column chromatography to yield compound **2** (40 mg).

Visconoside C (1): pale yellow needles (MeOH); $[\alpha]_D^{25}$ − 0.63 (*c* 0.01, MeOH); HR-ESI-MS *m/z* 779.1986 $[M + Na]^+$ (Calcd for $C_{33}H_{40}O_{20}Na$, 779.2011). ^1H NMR (DMSO-d_6, 500 MHz, *J* in Hz): 6.44 (1H, *d*, *J* = 2.0 Hz, H-6), 6.76 (1H, *d*, *J* = 2.0 Hz, H-8), 7.38 (1H, *br s*, H-2′), 6.92 (1H, *d*, *J* = 8.5 Hz, H-5′), 7.31 (1H, *dd*, *J* = 2.0 and 8.0 Hz, H-6′), 5.32 (1H, *br s*, H-1″), 4.20 (1H, *br s*, H-2″), 3.63–3.68 (1H, *m*, H-3″), 3.28–3.51 (1H, *m*, H-4″), 3.06–3.09 (1H, *m*, H-5″), 0.86 (3H, *d*, *J* = 5.0 Hz, H-6″), 4.36 (1H, *d*, *J* = 7.5 Hz, H-1‴), 3.06–3.09 (1H, *m*, H-2‴), 3.20–3.22 (1H, *m*, H-3‴), 3.28–3.51 (1H, *m*, H-4‴), 3.20–3.22 (1H, *m*, H-5‴), 3.63–3.68 (1H, *m*, H-6‴a), 3.50–3.53 (1H, *m*, H-6‴b), 5.56 (1H, *br s*, H-1⁗), 3.85 (1H, *br s*, H-2⁗), 3.63–3.68 (1H, *m*, H-3⁗), 3.28–3.51 (1H, *m*, H-4⁗),

3.50–3.53 (1H, *m*, H-5′′′′), 1.12 (3H, *d*, *J* = 6.0 Hz, H-6′′′′). ^{13}C NMR (DMSO-d_6, 125 MHz): 157.8 (C-2), 134.2 (C-3), 177.9 (C-4), 160.9 (C-5), 99.5 (C-6), 161.7 (C-7), 94.5 (C-8), 156.1 (C-9), 105.7 (C-10), 120,4 (C-1′), 115.8 (C-2′), 145.3 (C-3′), 148.8 (C-4′), 115.7 (C-5′), 121.3 (C-6′), 101.6 (C-1′′), 69.2 (C-2′′), 81.2 (C-3′′), 71.6 (C-4′′), 70.1 (C-5′′), 17.5 (C-6′′), 104.8 (C-1′′′), 73.9 (C-2′′′), 76.5 (C-3′′′), 69.9 (C-4′′′), 76.3 (C-5′′′), 60.8 (C-6′′′), 98.4 (C-1′′′′), 69.8 (C-2′′′′), 70.4 (C-3′′′′), 71.8 (C-4′′′′), 70.3 (C-5′′′′), 17.9 (C-6′′′′).

3.5. Acid hydrolysis

Each new compounds (2 mg) was refluxed with 2N aq. CH_3COOH (5 ml) for 2 h at 100°C. After extraction with $CHCl_3$ (3 × 5 ml), the aqueous layer was repeatedly evaporated to dryness with MeOH until neutral, and then analysed by TLC over silica gel (MeCOEt–isoPrOH–Me_2CO–H_2O 20:10:7:6) by comparison with authentic samples (L-rhamnose *Rf* 0.65; D-glucose *Rf* 0.40) (Nguyen et al. 2015; 2016).

3.6. The cytotoxicity and hepatoprotective assay

HepG2 cells were seeded and cultured in EMEM containing 10% FCS, 2 mM L-glutamine, 100 IU/ml penicillin, 100 µg/ml streptomycin at 5% CO_2 at 37°C to attain confluency, then harvested and seeded in 96-well plates at 2.5×10^4 cells/cm^2. Then, cells were treated with EMEM containing 2 mM CCl_4 and compound **1** alone or combined at different concentrations. Cell viability was evaluated as mitochodrial succinate dehydrogenase activity, a marker of viable cells using MTT test [Yao et al. 2011; Pareek et al. 2013]. Doxorubicin was used as a positive control for cytotoxicity and quercetin for hepatoprotective effect.

Disclosure statement

No potential conflict of interest was reported by the authors.

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