New constituents from *Gymnocarpos decander*

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* Dedicated to Professor Dr. Max Wichtl in recognition of his outstanding contribution to pharmacognosy research

Abstract

The phytochemical investigation of *Gymnocarpos decander* aerial parts extract afforded two new saponins, 3-*O*-β-D-glucuronopyranosyl-2β,3β,16α,23-tetrahydroxyolean-12-en-28-*O*-β-D-apiofuranosyl-(1→3)-β-D-xylopyranosyl-(1→4)-α-L-rhamnopyranosyl-(1→2)-α-L-arabinopyranosyl ester (**1**), 3-*O*-β-D-glucuronopyranosyl-2β,3β,16α-trihydroxyolean-12-en-28-*O*-α-L-rhamnopyranosyl-(1→3)-β-D-xylopyranosyl-(1→4)-α-L-rhamnopyranosyl-(1→2)-α-L-arabinopyranosyl ester (**2**) and three new flavonol glycosides, isorhamnetin 3-*O*-2''''-*O*-acetyl–β-D-xylopyranosyl-(1→6)-[β-D-apiofuranosyl-(1→2)]-β-D-glucopyranoside (**3**), isorhamnetin 3-*O*-2'''-*O*-acetyl–β-D-xylopyranosyl-(1→6)-β-D-glucopyranoside (**4**), and quercetin 3-*O*-2'''-*O*-acetyl–β-D-xylopyranosyl-(1→6)-β-D-glucopyranoside (**5**), together with three known compounds. Their structures were determined by spectroscopic methods including 1D and 2D NMR analysis and high resolution mass spectrometry. The new isolates were investigated for their potential cytotoxic activity on three cancer cell lines. Compounds **1** and **2** showed a moderate antiproliferative activity.

Key words

Gymnocarpos decander, Caryophyllaceae, triterpenoid saponins, flavonol glycosides, cytotoxicity

Introduction

Gymnocarpos is a genus of Caryophyllaceae family including woody and undershrub plants with succulent and mucronate leaves, native of the Canary Islands, North Africa, and Middle East [1]. *Gymnocarpos decander* Forssk. is a 30-50 cm tall undershrub, widely grown in Algeria in rocks and stony soils without sand. The species has locally an economic value since it is used as fuel wood and feed for grazing [2, 3]. The whole plant or its aerial parts are used against helmintiasis [4] and have some traditional uses, such as against kidney stones, psychosomatic diseases, and to break down evil eye and bad spirits [5, 6]. Moreover, the potential anti-malarial efficacy of a hydroalcoholic extract was reported [7]. The composition of the essential oil [8] and the polyphenols content, the antioxidant and the antimicrobial activities of the aerial parts were investigated [9]. On the contrary, no information is available on the chemical composition of polar extracts. Thus, the present investigation was designed to carry out a phytochemical study of *G. decander* aerial parts and to evaluate the cytotoxic properties of the new isolated chemical constituents.

Results and Discussion

The crude methanolic aerial parts extract of *G. decander* was partitioned between chloroform, ethyl acetate, and *n*-butanol. A portion of the *n*-BuOH soluble portion was subjected to Sephadex LH-20 column chromatography followed by RP-HPLC, to afford two new triterpenoid saponins (**1**-**2**), three new flavonol glycosides (**3**-**5**) (**Fig. 1**), along with three known compounds.

Compound **1**, obtained as white amorphous powder, was assigned the molecular formula $C_{57}H_{90}O_{28}$ as determined by its negative HRESIMS data (*m/z* 1221.5527 $[M-H]^-$). The HRESIMS/MS spectrum showed peaks at *m/z* 1089.53 $[M-H-132]^-$, 957.53 $[M-H-132-132]^-$, 811.47 $[M-H-132-132-146]^-$, 679.36 $[M-H-132-132-146-132]^-$, 503.33 $[M-H-132-132-146-132-176]^-$ due to the subsequent loss of two pentoses, one deoxyhexose, one pentose, and one uronic acid unit, respectively. The peak at *m/z* 503.33 was attributed to the aglycon moiety. Analysis of ^1H and ^{13}C

NMR data (**Tables 1** and **2**) suggested a triterpenoid saponin structure. The ^{1}H NMR spectrum of **1** revealed the presence of six singlet methyl groups (δ 0.81, 0.90, 0.98, 1.00, 1.32, and 1.40), one doublet methyl group at δ 1.30 (d, *J* = 6.5 Hz), signals of three hydroxymethines at δ 3.64 (d, *J* = 4.5 Hz), 4.36 (ddd, *J* = 13.0, 9.2, 3.9 Hz), 4.52 (br s), one hydroxymethylene at δ 3.25 and 3.66 (each, d, *J* = 12.0 Hz), a resonance for an olefinic hydrogen at δ 5.40 (t, *J* = 3.5 Hz), and five anomeric protons [δ 4.49 d (*J* = 7.8 Hz), 4.56 d (*J* = 7.5 Hz), 5.09 d (*J* = 1.8 Hz), 5.26 d (*J* = 3.0 Hz), 5.64 d (*J* = 6.8 Hz)]. The ^{13}C NMR spectrum showed, for the aglycon moiety, signals attributable to a triterpene skeleton confirmed by HSQC experiment, leading to the identification of the aglycon as 2β,3β,16α,23-tetrahydroxyolean-12-en-28-oic acid (polygalacic acid) [10]. The structures of the sugar moieties were deduced by using 1D TOCSY and 2D NMR experiments, which indicated the presence of one β-glucuronic acid, one α-arabinose in a predominant $^{4}C_{1}$ conformation (ara $J_{H\text{-}1/H\text{-}2}$ = 6.8 Hz), one α-rhamnose, one β-xylose in a predominant $^{4}C_{1}$ conformation (xyl $J_{H\text{-}1/H\text{-}2}$ = 7.5 Hz), and one β-apiose [11]. The assignments of proton and carbon chemical shifts by HSQC experiment, indicated that the glucuronopyranosyl acid and the apiofuranosyl units were the terminal units. The configuration of the sugar units was assigned after hydrolysis of **1** with 1 N HCl. The hydrolyzate was trimethylsilylated, and GC retention times of each sugar were compared with those of authentic samples prepared in the same manner. The HMBC correlations between H-1$_{ara}$-C-28, H-1$_{rha}$-C-2$_{ara}$, H-1$_{xyl}$-C-4$_{rha}$, H-1$_{api}$-C-3$_{xyl}$, and H-1$_{glc\text{-}A}$-C-3 allowed the identification of the sugar sequence. Therefore, compound **1** was identified as 3-*O*-β-D-glucuronopyranosyl-2β,3β,16α,23-tetrahydroxyolean-12-en-28-*O*-β-D-apiofuranosyl-(1→3)-β-D-xylopyranosyl-(1→4)-α-L-rhamnopyranosyl-(1→2)-α-L-arabinopyranosyl ester.

Compound **2** was purified as a white amorphous powder. Its molecular formula was determined to be $C_{58}H_{92}O_{27}$ from the pseudo molecular ion $[M+Na]^{+}$ at *m/z* 1243.5592 in the HRESIMS spectrum (positive mode). Analysis of NMR data (**Tables 1** and **2**) of compound **2** led to establish again the presence of a triterpenoid saponin structure with five sugar units. Comparison of NMR spectra with those of **1** revealed **2** to differ from **1** both in the aglycon moiety and in the sugar chain at C-28,

while the β-glucuronopyranosyl moiety at C-3 was identical. NMR data of the aglycon moiety showed the presence of seven methyl singlets, at δ 0.81, 0.90, 0.98, 1.10, 1.11, 1.27, and 1.40, three hydroxymethines at δ 3.89 (d, *J* = 4.5 Hz), 4.23 (ddd, *J* = 13.0, 9.2, 3.8 Hz), 4.51 (br s), and a resonance for an olefinic hydrogen at δ 5.40 (t, *J* = 3.5 Hz), allowing to characterize the structure of the aglycon as 2β,3β,16α-trihydroxyolean-12-en-28-oic acid (asterogenic acid) [12]. The proton-coupling network within each sugar residue was traced out using a combination of 1D TOCSY, DQF-COSY, HSQC, and HMBC experiments. These results established that the tetrasaccharide chain at C-28 contains a terminal α-rhamnopyranose in **2** instead of a β-apiofuranose in **1**. The D or L configuration of sugar moieties were determined by hydrolysis of **2**, trimethylsilylation, and GC analysis, as reported for **1**. Thus, the structure of 3-*O*-β-D-glucuronopyranosyl-2β,3β,16α-trihydroxyolean-12-en-28-*O*-α-L-rhamnopyranosyl-(1→3)-β-D-xylopyranosyl-(1→4)-α-L-rhamnopyranosyl-(1→2)-α-L-arabinopyranosyl ester was assigned to compound **2**.

Compound **3**, a yellow amorphous solid, showed a quasi molecular ion peak at *m/z* 807.1951 for $[M+Na]^+$ in the HRESIMS, allowing the assignment of molecular formula $C_{34}H_{40}O_{21}$. The UV spectrum of **3** showed two absorption maxima at 251 and 354 nm, indicating its flavonol skeleton. The ^1H and ^{13}C NMR spectra (**Table 3**) showed a typical pattern of a flavonol acyl glycoside. Compound **3** aglycon was deduced to be isorhamnetin [13] from the presence of two *meta*-coupled protons for ring A (δ 6.22 and 6.49, d, *J* = 1.8 Hz) and an ABX system signals for ring B (δ 6.95 d, *J* = 8.5 Hz, 7.74 dd, *J* = 8.5, 2.0 Hz, and 8.30 d, *J* = 2.0), together with one methoxy signal at δ 4.03. Three anomeric protons arising from the sugar moieties appeared at δ 4.24 (*J* = 7.5 Hz), 5.46 (*J* = 2.0 Hz), and 5.51 (*J* = 7.5 Hz) and correlated respectively with signals at δ 102.3, 110.4, and 101.6 in the HSQC spectrum. Moreover, a signal of an acetyl group (δ_H 1.71, δ_C 20.0) was also evident. DQF-COSY and 1D TOCSY experiments, together with HSQC cross peaks interpretation led to identify the sugars as one β-glucopyranosyl, one β-apiofuranosyl, and one β-xylopyranosyl unit. The sugar moiety configuration was established as reported for **1** and **2**. Unambiguous

determination of the substitution sites was obtained from the HMBC experiment showing correlations between H-1_{glc}—C-3, H-1_{api}—C-2_{glc}, H-1_{xyl}—C-6_{glc}, H-2_{xyl}—OCOCH$_3$. In the light of these data, the structure of **3** was elucidated as isorhamnetin 3-*O*-2''''-*O*-acetyl–β-D-xylopyranosyl-(1→6)-[β-D-apiofuranosyl-(1→2)]-β-D-glucopyranoside.

The HRESIMS of compound **4**, a yellow amorphous powder, displayed a quasi molecular ion peak at *m/z* 675.1529 $[M+Na]^+$ corresponding to $C_{29}H_{32}O_{17}$ molecular formula. Comparison of the MS and NMR data of **4** (**Table 3**) with those of **3** showed the absence of the terminal β-apiofuranosyl unit in **4**. Thus, compound **4** was established to be isorhamnetin 3-*O*-2'''-*O*-acetyl–β-D-xylopyranosyl-(1→6)-β-D-glucopyranoside.

The HRESIMS of compound **5**, obtained as a yellow amorphous powder, displayed a quasi molecular ion peak at *m/z* 661.1360, 14 uma less than that of **4**. Its NMR profile was almost identical to that of **4** (**Table 3**), being the NMR signals of flavonol B-ring the point of difference. The absence of the methoxy signal in **5** led to identify quercetin as its aglycon [13]. On the basis of this evidence, the structure of compound **5** was established to be quercetin 3-*O*-2'''-*O*-acetyl–β-D-xylopyranosyl-(1→6)-β-D-glucopyranoside.

Roseoside [14], quercetin 3-*O*-β-D-xylopyranosyl-(1→6)-β-D-glucopyranoside [15], and quercetin 3-*O*-β-D-glucopyranoside [13] were also identified by comparison with published spectroscopic data.

The antiproliferative activity of new compounds **1-5** was evaluated in HeLa, MCF7 and Jurkat cancer cell lines. Cell viability was evaluated at 48 h treatment by MTT assay. Only compounds **1** and **2** inhibited the growth of all cell lines in a dose- and time-dependent manner. Half maximal inhibitory concentration (IC_{50}) values, obtained from dose-response curves, are shown in **Table 4**. Jurkat cells were slightly more susceptible than HeLa and MCF7 cells.

Furthermore, the cytotoxic potential of compounds **1** and **2** was evaluated in PBMC from healthy donors, chosen as the normal counterpart of leukemia-derived Jurkat cell line. Compounds **1** and **2**

did not causes any significant reduction of the number of freshly isolated non-proliferating PBMC, at least in the range of doses cytotoxic in leukemia cells.

Materials and Methods

General experimental procedures

Optical rotations were measured on a Autopol IV Automatic polarimeter (Rudolph) equipped with a sodium lamp (589 nm) and a 1 dm microcell. UV spectra were recorded on an Evolution 201 (Thermo Scientific) spectrophotometer. NMR experiments were performed on a Bruker DRX-600 spectrometer (Bruker BioSpin GmBH) equipped with a Bruker 5 mm TCI CryoProbe at 300 K. Chemical shifts were expressed in δ (parts per million) referred to the solvent peaks δ_H 3.34 and δ_C 49.0 for CD_3OD. The NMR data were processed on a Silicon Graphic Indigo2 Workstation using XWINNMR software. Standard pulse sequences and phase cycling were used for DQF-COSY, TOCSY, HSQC, and HMBC experiments. HRESIMS were acquired in positive and negative ion mode on a Q-TOF premier spectrometer equipped with a nanospray source. Column chromatography was performed over Sephadex LH-20 (Pharmacia). HPLC separations were conducted on a Waters 590 series pumping system equipped with a Waters R401 refractive index detector, a Luna C_8 10 µ (250 x 10 mm) column (Phenomenex), and a U6K injector. TLC were carried out on precoated Kieselgel 60 F_{254} plates (Merck); compounds were detected by $Ce(SO_4)_2$/ H_2SO_4 (Sigma-Aldrich) solution. GC analysis was performed using a Dani GC 1000 instrument on a L-CP-Chirasil-Val column (0.32 mm × 25 m) working with the following temperature program: 100 °C for 1 min, ramp of 5 °C/min up to 180 °C; injector and detector temperature 200 °C; carrier gas N_2 (2 mL/min); detector dual FID; split ratio 1:30; injection 5 µL.

Plant material

The aerial parts of *G. decander* (Caryophyllaceae) were collected during the flowering period, in April 2013, in the region of Bechar, southwest of Algeria. The plant was identifed by Dr. Mohamed Benabdelhakem head of the Bechar National Agency of Natural Resources Preservation. A voucher specimen (n. GD149/13/VARENBIOMOL) was deposited in the Herbarium of the Department of Chemistry, University of Mentouri, Algeria.

Extraction and isolation

The aerial parts of *G. decander* (1.7 Kg) were powdered and successively extracted by exhaustive maceration in methanolic solution (80%, 10 L), filtered and the solvent was removed under vacuum at 40 °C in a rotary evaporator, obtaining respectively 97.8 g of dried extract. All the extract was partitioned with chloroform (0.22 L), ethyl acetate (0.22 L), and *n*-butanol (*n*-BuOH) (0.22 L), 3 times each, to yield 3.66, 1.94, and 10.40 g of the respective residues. A portion (2.5 g) of the *n*-BuOH-soluble fraction was separated by Sephadex LH-20 with MeOH as eluent (flow rate 1.1 mL/ min). Fractions of 10 mL were collected, analyzed by TLC on silica 60 F254 gel-coated glass sheets with *n*-BuOH-AcOH-H_2O (60:15:25 v/v/v) and grouped to obtain five fractions (A–E). Fraction A (452.1 mg, 200 mL) was purified by RP-HPLC using MeOH–H_2O (1:1) to give compounds **1** (4.4 mg, t_R 21 min) and **2** (4.0 mg, t_R 27 min). Fraction B (126 mg, 100 mL), C (129.7 mg, 110 mL), and D (219.1 mg, 150 mL) were separately purified by RP-HPLC using MeOH-H_2O (2:3) to give roseoside (2.4 mg, t_R 15 min) from fraction B, compound **3** (10.0 mg, t_R 12 min) from fraction C, and compounds **4** (7.0 mg, t_R 28 min) and **5** (15.9 mg, t_R 19 min) from fraction D. Fraction E (70.0 mg, 300 mL) was purified by RP-HPLC using MeOH-H_2O (35:65) to give quercetin 3-*O*-β-D-xylopyranosyl-(1→6)-β-D-glucopyranoside (2.2 mg, t_R 30 min) and quercetin 3-*O*-β-D-glucopyranoside (2.0 mg, t_R 35 min). All the compounds met the criteria of ≥95% purity, as inferred by HPLC and NMR analyses.

*Compound **1***: white amorphous powder; $[\alpha]_D^{25}$ -33.8 (*c* 0.13, MeOH); ^{1}H and ^{13}C NMR data for the aglycon moiety, see **Table 1**; ^{1}H and ^{13}C NMR data for the sugar moiety, see **Table 2**; HRESIMS:

m/z 1221.5527 $[M-H]^-$ (calcd. for $C_{57}H_{89}O_{28}$, *m/z* 1221.5540); MS/MS *m/z* 1089.53 [(M-132)-H]$^-$, 957.53 [(M-132-132)-H]$^-$, 811.47 [(M-132-132-146)-H]$^-$, 679.36 [(M-132-132-146-132)-H]$^-$, 503.33 [(M-132-132-146-132-176)-H]$^-$.

Compound ***2***: white amorphous powder; $[\alpha]_D^{25}$ -55.0 (*c* 0.08, MeOH); ^{1}H and ^{13}C NMR data for the aglycon moiety, see **Table 1**; ^{1}H and ^{13}C NMR data for the sugar moiety, see **Table 2**; HRESIMS: *m/z* 1243.5592 $[M+Na]^+$ (calcd. for $C_{58}H_{92}NaO_{27}$, *m/z* 1243.5724).

Compound ***3***: yellow amorphous powder; $[\alpha]_D^{25}$ -48.6 (*c* 0.25, MeOH); UV (MeOH) λ_{max} (log ε): 251 (3.90), 354 (2.25); ^{1}H and ^{13}C NMR data, see **Table 3**; HRESIMS: *m/z* 807.1951 $[M+Na]^+$ (calcd. for $C_{34}H_{40}NaO_{21}$, 807.1960).

Compound ***4***: yellow amorphous powder; $[\alpha]_D^{25}$ -27.1 (*c* 0.13, MeOH); UV (MeOH) λ_{max} (log ε): 253 (2.80), 356 (1.29); ^{1}H and ^{13}C NMR data, see **Table 3**; HRESIMS: *m/z* 675.1529 $[M+Na]^+$ (calcd. for $C_{29}H_{32}NaO_{17}$, 675.1537).

Compound ***5***: yellow amorphous powder; $[\alpha]_D^{25}$ + 4.3 (*c* 1.01, MeOH); UV (MeOH) λ_{max} (log ε): 257 (2.40), 362 (1.48); ^{1}H and ^{13}C NMR data, see **Table 3**; HRESIMS: *m/z* 661.1360 $[M+Na]^+$ (calcd. for $C_{28}H_{30}NaO_{17}$, 661.1381).

Acid hydrolysis of compounds 1-5

A solution of each compound (2.0 mg) 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. The 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 H_2O and $CHCl_3$. The $CHCl_3$ layer was analyzed by GC using a L-CP-Chirasil-Val column (0.32 mm x 25 m). Temperatures of both the injector and detector was 200 °C. 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 samples of D-

glucuronic acid, L-arabinose, L-rhamnose, D-xylose, D-apiose, and D-glucose (Sigma Aldrich) after treatment with 1-(trimethylsilyl)imidazole in pyridine.

Cytotoxicity assay

Cells and treatment

Jurkat cells (T-cell leukemia), obtained from Cell Bank in GMP-IST, were maintained in RPMI 1640 medium, 2 mML-glutamine and antibiotics at 37 °C in a humidified atmosphere of 5% CO_2. HeLa (cervical carcinoma) and MCF7 (breast carcinoma) cells were obtained from American Type Culture Collection (ATCC). Cells were maintained in DMEM, supplemented with 10% FBS, 100 mg/L streptomycin and penicillin 100 IU/mL at 37°C in a humidified atmosphere of 5% CO_2. To ensure logarithmic growth, cells were subcultured every two days. Human peripheral blood mononuclear cells (PBMC) were isolated from buffy coats of healthy donors (kindly provided by the Blood Center of the Hospital of Battipaglia, Salerno, Italy) by using a standard Ficoll–Hypaque gradient. Freshly isolated PBMC contained 90.6 ± 1.2% live cells as assessed by the manual Trypan blue exclusion method. Stock solutions (50 mM) of compounds **1**-**5** in DMSO were stored in the dark at 4 °C. Appropriate dilutions were prepared in culture medium immediately prior to use. In all experiments, the final concentration of DMSO did not exceed 0.15% (v/v).

Cell viability

Cells were seeded in 96-well plates and incubated for the established times in the absence (vehicle only) and in the presence of different concentrations of compounds. Jurkat cells were seeded at a cell density of 2×10^4/well; HeLa and MCF7 were seeded at a density of 1×10^4/well the day before treatment. The number of viable cells was quantified by MTT ([3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide]) assay. Absorption at 550 nm for each well was assessed using a

microplate reader (LabSystems). In some experiments cell viability was also checked by Trypan Blue exclusion assay using a Bürker counting chamber. Half maximal inhibitory concentration (IC_{50}) values were calculated from cell viability dose–response curves and defined as the concentration resulting in 50% inhibition in cell survival as compared to controls [16]. Etoposide was used as a positive control. Each experimental condition was tested once in quadruplicate. IC_{50} values were performed with GraphPad software.

Supporting information

HRESIMS and NMR spectra of compounds **1-5** and data for the IC_{50} determination of cytotoxic activity are available as Supporting Information.

References

1 *Petrusson L, Thulin. M.* Taxonomy and biogeography of *Gymnocarpos* (Caryophyllaceae). Edinburgh J Bot 1996; 53: 1-26

2 *Bidak LM, Kamal SA, Halmy MWA, Heneidy SZ.* Good sand services provided by native plants in desert ecosystems: Examples from the northwestern coastal desert of Egypt. Global Ecol Conserv 2015; 3: 433-447

3 *Shaltout K, Ahmed DAE.* Ecosystem services of the flora of Southern Mediterranean desert of Egypt. Ethnobot Res Appl 2012; 10: 403-422

4 *Abouri M, El Mousadik A, Msanda F, Boubaker H, Saadi B, Cherifi K.* An ethnobotanical survey of medicinal plants used in the Tata Province, Morocco. Int J Med Plant Res 2012; 1: 99-123

5 *Ahmed S, Mohtasheemul Hasan M, Alam Mahmood Z.* Antiurolithiatic plants in different countries and cultures. J Pharmacogn and Phytochemistry 2016; 5: 102-115

6 *Maiza K, Hammiche V, Maiza-benabdesselam F.* Traditional medicine in north Sahara: the "Deffi". Life Sci Leaflets 2011; 16: 551-560

7 *Kaiser J, Yassin M, Prakash S, Safi N, Agami M, Lauw S, Ostrozhenkova E, Bacher A, Rohdich F, Eisenreich W, Safi J, Galan-Goldhirsh A.* Anti-malarial drug targets: screening for inhibitors of 2C-methyl-D-erythritol 4-phosphate synthase (IspC protein) in Mediterranean plants. Phytomedicine 2007; 14: 242-249

8 *Javidnia K, Miri R, Jamalian A, Sarkarzadeh H, Nasiri A.* Composition of essential oil of *Gymnocarpos decander* Forrssk. from Iran. J Essent Oil Res 2008; 20: 477-478

9 *Bouaziz M, Dhouib A, Loukil S, Boukhris M, Sayadi S.* Polyphenols content, antioxidant and antimicrobial activities of extracts of some wild plants collected from the south of Tunisia. African J Biotechnol 2009; 8: 7017-7027

10 *Hernandez-Carlos B, Gonzalez-Coloma A, Orozco-Valencia AU, Ramirez-Mares MV, Andres-Yeves MF, Joseph-Nathan P*. Bioactive saponins from *Microsechium helleri* and *Sicyos bulbosus*. Phytochemistry 2011; 72: 743-751

11 *Matsuo Y, Takaku R, Mimaki Y*. Novel steroidal glycosides from the bulbs of *Lilium pumilum*. Molecules 2015; 20: 16255-16265

12 *Schoepke T, Wray V, Kunath A, Hiller K*. Bayogenin and asterogenic acid glycosides from *Bellis perennis*. Phytochemistry 1992; 31: 2555-2557

13 *Agrawal PK*. Carbon-13 NMR of flavonoids. Amsterdam: Elsevier; 1989: 336

14 *Kerbab K, Mekhelfi T, Zaiter L, Benayache S, Benayache F, Picerno P, Mencherini T, Sansone F, Aquino RP, Rastrelli L*. Chemical composition and antioxidant activity of a polar extract of *Thymelaea microphylla* Coss. et Dur. Nat Prod Res 2015; 29: 671-675

15 *Bah M, Gutiérrez-Avella DM, Fuentes-Ordaz R, Castañeda-Moreno R, Martínez M*. Chemical constituents of the Mexican mistletoe (*Psittacanthus calyculatus*). Molecules 2011; 16: 9397-9403

16 *Amodio G, Venditti R, De Matteis MA, Moltedo O, Pignataro P, Remondelli P*. Endoplasmic reticulum stress reduces COPII vesicle formation and modifies Sec23a cycling at ERESs. FEBS Lett. 2013; 587: 3261-3266

Figure legend:

Fig. 1 Chemical structures of compounds **1-5** isolated from *G. decander* aerial parts

Table 1 ^{1}H and ^{13}C-NMR data for aglycons of compounds **1-2** (CD_3OD, 600 MHz, *J* in Hz).

position	**1**		**2**	
	δ_H	δ_C	δ_H	δ_C
1a	2.10 m	44.6	2.06 m	45.0
1b	1.20 dd (13.5, 2.8)		1.21 dd (13.5, 2.6)	
2	4.36 ddd (13.0, 9.2, 3.9)	70.8	4.23 ddd (13.0, 9.2, 3.8)	70.8
3	3.64 d (4.5)	83.8	3.89 d (4.5)	91.0
4		48.9		39.9
5	1.34[a]	48.5	0.89[a]	57.6
6a	1.54[a]	18.8	1.59[a]	19.6
6b	1.49[a]		1.51[a]	
7a	1.66 m	33.6	1.66 m	34.3
7b	1.38 m		1.38 m	
8		43.0		43.5
9	1.65 dd (11.0, 5.3)	48.3	1.59[a]	48.8
10		37.8		39.0
11a	2.01[a]	24.6	2.00[a]	24.9
11b	1.98[a]		1.98[a]	
12	5.40 t (3.5)	123.9	5.40 t (3.5)	124.0
13		144.7		145.3
14		40.8		41.9
15a	1.93[a]	36.4	1.93[a]	36.5
15b	1.78[a]		1.78[a]	
16	4.52 br s	74.6	4.51 br s	74.5
17		47.0		47.2
18	3.09 dd (13.5, 2.6)	42.0	3.08 dd (14.0, 3.2)	42.0
19a	2.30 t (13.2)	47.6	2.30 t (13.2)	47.0
19b	1.07[a]		1.06[a]	
20		31.6		31.7
21a	1.41[a]	36.5	1.41[a]	35.7
21b	1.16[a]		1.15[a]	
22a	1.94[a]	32.0	1.92[a]	32.0
22b	1.80[a]		1.78[a]	
23a	3.66 d (12.0)	65.5	1.11 s	30.0
23b	3.25 d (12.0)			
24	0.98 s	14.0	1.10 s	18.9
25	1.32 s	18.0	1.27 s	18.9
26	0.81 s	18.0	0.81 br s	18.3
27	1.40 s	27.0	1.40 s	27.0
28		177.0		178.2
29	0.90 s	33.0	0.90 s	33.9
30	1.00 s	25.3	0.98 s	26.0

Data assignments were confirmed by DQF-COSY, 1D-TOCSY, HSQC, and HMBC experiments.
[a] Overlapped signal.

Table 2 ^{1}H and ^{13}C-NMR data for sugar moieties of compounds **1-2** (CD_3OD, 600 MHz, *J* in Hz).

position	**1**		**2**	
	δ_H	δ_C	δ_H	δ_C
GlcA[a]-1	4.49 d (7.8)	104.9	4.41 d (7.8)	102.6
2	3.35 dd (9.0, 7.8)	75.2	3.35 dd (9.5, 7.8)	75.7
3	3.40 t (9.0)	78.0	3.48 t (9.5)	78.0
4	3.44 t (9.0)	73.7	3.49 t (9.5)	71.8
5	3.68 d (9.0)	76.0	3.44 d (9.0)	76.0
6		172.0		172.0
Ara[b]-1	5.64 d (6.8)	94.1	5.64 d (7.0)	94.4
2	3.82 dd (9.0, 6.8)	75.5	3.80 dd (9.0, 7.0)	75.9
3	3.88 dd (9.0, 2.0)	72.2	3.86 dd (9.0, 3.0)	71.9
4	3.84 m	67.0	3.85 m	67.0
5a	3.93 dd (12.0, 2.0)	64.0	3.91 dd (12.0, 2.5)	63.8
5b	3.52 dd (12.0, 4.0)		3.51 dd (12.0, 4.0)	
Rha[c]-1	5.09 d (1.8)	101.4	5.04 d (1.5)	102.1
2	3.86 dd (3.0, 1.8)	72.0	3.89 dd (3.0, 1.5)	71.0
3	3.89 dd (9.0, 3.0)	71.5	3.86 dd (9.0, 3.0)	72.0
4	3.59 t (9.0)	83.6	3.57 t (9.0)	83.2
5	3.71 m	69.0	3.70 m	69.0
6	1.30 d (6.5)	18.0	1.31 d (6.5)	18.5
Xyl[d]-1	4.56 d (7.5)	106.7	4.55 d (7.5)	107.0
2	3.40 dd (9.5, 7.5)	75.5	3.40 dd (9.0, 7.5)	74.6
3	3.41 t (9.5)	86.0	3.46 t (9.0)	84.5
4	3.55 m	69.9	3.53 m	70.4
5a	3.91 dd (11.0, 3.0)	66.8	3.89 dd (10.5, 2.5)	66.8
5b	3.23 dd (11.0, 4.5)		3.22 dd (10.5, 5.0)	
Api[e]-1	5.26 d (3.0)	111.3		
2	4.01 br s	77.9		
3		80.8		
4a	4.16 d (10.0)	74.8		
4b	3.81 d (10.0)			
5	3.61 br s	65.3		
Rha-1			5.16 d (1.8)	102.8
2			3.96 dd (3.0, 1.8)	72.0
3			3.73 dd (9.5, 3.0)	71.8
4			3.44 t (9.5)	73.8
5			4.01 m	70.5
6			1.27 d (6.0)	18.0

Data assignments were confirmed by DQF-COSY, 1D-TOCSY, HSQC, and HMBC experiments.
[a] Glc-A = β-D-glucuronopyranosic acid, [b] Ara = α-L-arabinopyranose, [c] Rha = α-L-rhamnopyranose, [d] Xyl = β-D-xylopyranose, [e] Api = β-D-apiofuranose.

Table 3 ^{1}H and ^{13}C-NMR data of compounds **3-5** (CD_3OD, 600 MHz, *J* in Hz).

position	**3**		**4**		**5**	
	δ_H	δ_C	δ_H	δ_C	δ_H	δ_C
2		160.0		160.0		160.0
3		136.1		136.0		136.2
4		179.4		179.4		179.3
5		163.4		162.9		163.0
6	6.22 d (1.8)	99.6	6.23 d (1.8)	99.8	6.25 d (1.8)	99.0
7		167.2		167.6		165.0
8	6.49 d (1.8)	94.7	6.46 d (1.8)	95.5	6.43 d (1.8)	94.6
9		158.6		158.4		159.9
10		103.7		104.0		104.0
1'		124.0		124.2		123.0
2'	8.30 d (2.0)	114.3	8.26 d (2.0)	114.4	8.11 d (2.0)	117.8
3'		148.6		148.9		147.0
4'		149.5		150.0		148.5
5'	6.95 d (8.5)	115.8	6.96 d (8.5)	116.3	6.92 d (8.5)	116.0
6'	7.74 dd (8.5, 2.0)	123.5	7.73 dd (8.5, 2.0)	123.6	7.72 dd (8.5, 2.0)	122.6
OMe	4.03 s	56.9	4.00 s	57.0		
Glc[a]-1	5.51 d (7.5)	101.6	5.30 d (7.5)	104.5	5.13 d (7.5)	105.5
2	4.03 dd (9.0, 7.5)	76.0	3.87 dd (9.0, 7.5)	73.0	3.88 dd (9.0, 7.5)	73.0
3	3.56 t (9.0)	78.0	3.59 t (9.0)	77.0	3.60 t (9.0)	77.0
4	3.73 t (9.0)	71.2	3.75 t (9.0)	70.0	3.77 t (9.0)	70.0
5	3.74 m	75.0	3.60 m	75.0	3.61 m	75.0
6a	3.77 dd (12.0, 3.0)	68.6	3.83 dd (12.0, 3.0)	69.0	3.74 dd (12.0, 3.0)	68.0
6b	3.66 dd (12.0, 4.5)		3.73 dd (12.0, 4.5)		3.70 dd (12.0, 4.5)	
Api[b]-1	5.46 d (2.0)	110.4				
2	4.06 d (2.0)	77.0				
3		80.4				
4a	4.09 d (10.0)	75.6				
4b	3.69 d (10.0)					
5	3.68 br s	66.4				
Xyl[c]-1	4.24 d (7.5)	102.3	4.20 d (7.5)	103.0	4.12 d (7.5)	102.6
2	4.41 dd (9.0, 7.5)	75.0	4.44 dd (9.0, 7.5)	75.3	4.43 dd (9.0, 7.5)	74.6
3	3.10 t (9.0)	75.6	3.07 t (9.0)	75.5	3.04 t (9.0)	75.6
4	3.38 m	71.3	3.39 m	71.0	3.43 m	71.0
5a	3.83 dd (10.5, 2.0)	66.4	3.69 dd (10.5, 2.0)	65.5	3.73 dd (10.5, 2.0)	66.6
5b	3.59 dd (10.5, 5.0)		2.70 dd (10.5, 5.0)		2.84 dd (10.5, 5.0)	
$\underline{CO}CH_3$		171.0		172.0		172.0
$CO\underline{CH_3}$	1.71 s	20.0	1.71 s	20.0	1.71 s	20.0

Data assignments were confirmed by DQF-COSY, 1D-TOCSY, HSQC, and HMBC experiments.
[a] Glc = β-D-glucopyranose, [b] Api = β-D-apiofuranose, [c] Xyl = β-D-xylopyranose.

Table 4 IC_{50} (μM) of compounds **1** and **2** in different cancer cell lines.

compound	HeLa[a]	Jurkat[b]	MCF7[c]
1	57	42	59
2	71	60	67

[a]cervical carcinoma; [b]T-cell leukemia; [c]breast carcinoma