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Triterpene saponins of *Genista ulicina* Spach

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Abstract

From the *n*-BuOH extract of the aerial parts of *Genista ulicina*, six triterpene saponins, 3-*O*-β-D-glucopyranosyl-olean-12-ene-3β,27,28,30-tetraol, 3-*O*-β-D-glucopyranosyl-olean-12-ene-3β,27,28,29-tetraol, 3,29-di-*O*-β-D-glucopyranosyl-olean-12-ene-3β,27,28,29-tetraol, 3-*O*-β-D-glucopyranosyl-olean-12-ene-3β,28,29-triol-27-oic acid, 3-*O*-β-D-glucopyranosyl-olean-12-ene-3β,27,28-triol-29-oic acid, and 3-*O*-β-D-glucopyranosyl-14-*H*-27-nor-olean-12-ene-3β,28,29-triol, were isolated together with eight seven known triterpene saponins and six flavonoids. Their structures were established mainly by means of spectroscopic methods (1D and 2D-NMR as well as HR-ESI-MS). The *n*-BuOH extract, investigated for its antitumor growth inhibition of human colon cancer HT-29 cells, presented no significant activity (IC₅₀ > 100 μg).

Keywords:

Genista ulicina

Fabaceae

Triterpenoid saponins

Cytotoxic activity

1. Introduction

Fabaceae is the second largest family of medicinal plants, containing over 490 medicinal plant species most of which have been used as traditional medicines (Ting et al., 2010). Many genera of this family such as *Genista* have recently attracted the attention of various authors for their phytochemical and biological properties, particularly antidiabetic (Rauter et al., 2005, 2009), antiinflammatory, antiulcer (Ilarionov, 1979; Rainova et al., 1988) spasmolytic, antioxidant, phytoestrogenic (Luczkiewicz and Glod, 2003; Garritano et al., 2005) and cytotoxic activity against different human cancer cell lines (Rigano et al., 2009, 2010), and effects on the thyroid gland (Korpachov et al., 1995).

The genus *Genista* (Fabaceae = Leguminosae) consists of 100 species predominantly distributed in the Mediterranean area and in Western Asia (Noccioli et al., 2011). Among these species, 23 grow in Algeria including 11 endemics distributed particularly in the Northern parts of the country (Quezel and Santa, 1963). Previous phytochemical investigations on the *Genista* genus indicated the presence of alkaloids (Martins et al., 2005; Pistelli et al., 2001) flavonoids and isoflavonoids (Pistelli et al., 1998, 2000; Giachi et al., 2002; Tosun et al., 2009), which are chemotaxonomic markers of the genus (Harborne, 1994). As a part of our ongoing research on new bioactive compounds from endemic plants of Algeria, we have studied the aerial parts of *Genista ulicina* Spach. In the present paper, we describe the isolation of fourteen saponins among which six (**1-6**) are isolated for the first time, and six known flavonoids. All compounds were identified by extensive spectroscopic methods including 1D- (^1H and ^{13}C) and 2D-NMR (COSY, HSQC, HMBC, ROESY and TOCSY) experiments as well as HR-ESI-MS analysis and by comparison of their spectral data with the literature values for the known compounds. To our best knowledge, this is the first report of saponins in *Genista* genus.

2. Results and discussion

2.1. Isolation and identification of new compounds 1–6

The *n*-butanol-soluble part partitioned from the methanol extract of *G. ulicina* was submitted to vacuum liquid chromatography (VLC) on reversed phase C₁₈ (RP-18) to afford six subfractions. Purification by a series of column chromatographies over silica gel and reverse phase C₁₈ and finally by HPLC afforded fourteen saponins from which six are new (**1–6**) along with six known flavonoids. The known compounds were identified as: 3-*O*- β -D-galactopyranosyloleanolic 28-*O*- β -D-glucopyranosyl ester (Sha et al., 2008), soyasaponin I (Kitagawa et al., 1976, Rao et al., 1985), soyasaponin III (Kitagawa et al., 1976, Kudou et al., 1993), sandosaponin A (Yoshikawa et al., 1997), dehydrosoyasaponin I (Miyao et al., 1996; Konoshima et al., 1991), kaikasaponin I (Kitagawa et al., 1988), kaikasaponin III (Kitagawa et al., 1988, Miyao et al., 1996), phaseoside IV (Miyao et al., 1996), biochanin A (Santos et al., 1995), kaempferol 3-*O*- β -D-apiofuranosyl-(1 \rightarrow 2)-[α -L-rhamnopyranosyl-(1 \rightarrow 6)]- β -D-glucopyranoside (Piccinelli et al., 2007), rutin (Bourama et al., 2004), isovitexin (Krafczyk et al., 2008), isorhamnetin-3-*O*-rutinoside (Shibano et al., 1996), 6,8-di-*C*- β -D-glucopyranosylapigenin (Krafczyk et al., 2008). The sugar composition of all compounds in BuOH extract was identified as glucose, galactose, glucuronic acid and rhamnose by TLC after acid hydrolysis of the *n*-butanol-soluble fraction. Their absolute configuration was determined to be D for glucose, galactose, and glucuronic acid and L for rhamnose, after preparative TLC and measurement of the optical rotation of each sugar.

The positive HR-ESI-MS of the saponin **1** showed a pseudo-molecular ion peak [M+Na]⁺ at *m/z*. 659.4144 in accordance with a C₃₆H₆₀O₉Na formula indicating that it contained one hexose unit. The presence of one sugar moiety in **1** was evidenced by the ¹H NMR spectrum which displayed one anomeric proton at δ_{H} 4.34 (d, *J* = 7.8 Hz) and identified after analysis of COSY and HSQC spectra as a glucose (Table 1). The β anomeric configuration of glucose

was defined from its $^3J_{H_1,H_2}$ coupling constant as well as from rOe information (Jia et al., 1998; Zhang et al., 1999). The 1H NMR spectrum of **1** showed signals of an olean-12-ene type aglycone (Mahato et al., 1994), five methyl singlets, one olefinic proton signal at δ_H 5.64 (t, $J = 3.3$ Hz, H-12), one oxygen-bearing methine proton signal at δ_H 3.20 (dd, $J = 11.3, 5.3$ Hz, H-3), three primary alcoholic functions at δ_H 3.20 and 3.57 (d, $J = 11.1$ Hz, each, H-28a, H-28b), 3.45 and 3.49 (d, $J = 11.0$ Hz, each, H-30a, H-30b), and 3.55 and 3.82 (d, $J = 12.3$ Hz, each, H-27a, H-27b). The assignments of the proton and carbon signals and the positions of primary alcoholic functions of **1** were established by analysis of COSY, HSQC and HMBC spectra. The protons at δ_H 3.55 and 3.82 showed HMBC correlations with C-8 at δ_C 40.2, C-13 at δ_C 138.4, C-14 at δ_C 46.9 and C-15 at δ_C 21.1, which revealed the location of the first alcoholic function at position 27. The ROESY spectra showed a correlation between an axial proton at δ_H 2.05, assigned to H-18 (dd, $J = 13.8, 4.2$ Hz), and axial protons at δ_H 3.45 (H-30a) and 3.49 (H-30b). This indicated the position of the second hydroxyl group at C-30. Furthermore, based on HMBC correlations between protons at δ_H 3.20 and 3.57 and C-18 at δ 41.4, C-16 at δ 22.0, and C-21 at δ 28.8, the third primary alcoholic function were assigned at C-28 position. The full assignments of the protons and carbons of **1** were achieved by combination of 1H , ^{13}C , DEPT, COSY, HSQC, HMBC and ROESY experiments. The linkage of the glucose at C₃ of aglycone was established from the HMBC correlation between Glc- H-1' (δ_H 4.34) with aglycone C-3 (δ_C 89.4). Based on the above evidence, the structure of **1** was established as 3-*O*- β -D-glucopyranosyl-olean-12-ene-3 β ,27,28,30-tetraol.

A comparison of NMR data of saponin **1** with the new isolated saponins (**2-6**) showed the presence of a glucose moiety linked at position C-3 of their aglycone (Tables 1 and 2).

Saponin **2** was an isomer of saponin **1** (C₃₆H₆₀O₉), as deduced from the HR-ESI-MS positive ion mode which showed a [M+Na]⁺ ion peak at m/z 659.4128. The analysis of the 2D-NMR (COSY, HSQC, HMBC and ROESY) data of compound **2** displayed the signals characteristic

of a Δ^{12} oleanene skeleton derivative with three primary alcoholic functions and β -D-glucose as in **1**. Significant differences between these two isomers observed in the ^{13}C NMR spectrum were the upfield shift of C-29 (Δ 46.6 ppm), and the downfield shift of C-30 (Δ 47.1 ppm) compared to the corresponding signals in **1**. The chemical shifts of C-29 (δ_{C} 73.2) was found shifted to higher field suggesting a free primary alcoholic function at C-29 in **2**, instead of C-30 as in **1**. This was confirmed by the rOe correlation observed between an axial proton at δ_{H} 2.12, assigned to H-18, and H-30. Accordingly, the structure of **2** was elucidated as 3-*O*- β -D-glucopyranosyl-olean-12-ene-3 β ,27,28,29-tetraol.

Saponin **3** exhibited in the HR-ESI-MS a quasi molecular ion peak at m/z 821.4671 [$\text{M}+\text{Na}$] $^{+}$ corresponding to the molecular formula of $\text{C}_{42}\text{H}_{70}\text{O}_{14}+\text{Na}$, suggesting that this compound possessed a supplementary hexose unit in comparison with saponins **1** and **2**. This hypothesis was confirmed by the ^1H NMR spectrum which displayed two anomeric protons at δ_{H} 4.23 (d, $J = 7.8$ Hz) and 4.34 (d, $J = 7.8$ Hz), giving correlations in the HSQC spectrum with two anomeric carbons at δ_{C} 103.5 and 105.4, respectively. The evaluation of spin-spin couplings and chemical shifts allowed the identification of two β -glucopyranosyl moieties. The ^1H and ^{13}C NMR signals of the aglycone moiety was superimposable to saponin **2** except for signals of C-29 deshielded at δ_{C} 80.8, suggesting the same aglycone as in **2** with substitution of the primary alcohol in C-29 by the second glucopyranosyl moiety. The HMBC correlation between anomeric proton at δ_{H} 4.23 (Glc H-1'') and δ_{C} 80.8 (C-29) proved the location of the supplementary glucose at C-29, and this is confirmed by the ROESY cross peak δ_{H} 4.23/3.17 (Glc H-1''/ H-29). On the basis of above results, the structure of saponin **3** is 3,29-di-*O*- β -D-glucopyranosyl-olean-12-ene-3 β ,27,28,29-tetraol.

Saponin **4** gave a [$\text{M}+\text{Na}$] $^{+}$ pseudomolecular ion peak at m/z 673.4671 in positive HR-ESI-MS, 14 mass units higher than that of **2**. Detailed comparison of the NMR data of **4** and **2** concluded that both compounds shared a common sugar with a difference for the aglycone

moieties. In the ^{13}C NMR of **4**, the signal due to primary alcoholic function at C-27 in **2** (δ_{C} 63.2) was replaced by a resonance at δ_{C} 178.8. This, together with the MS data, clearly indicated that compound **4** had a carboxylic function in C-27 position. The change in chemical shifts of C-14 [δ_{C} 46.9 in **2** and 55.9 in **4**] and C-7 [δ_{C} 32.7 in **2** and 36.5 in **4**] supported this assignment (Table 1). The structure of **4** was thus determined as 3-*O*- β -D-glucopyranosyl-olean-12-ene-3 β ,28,29-triol-27-oic acid.

Saponin **5** had the same molecular formula of $\text{C}_{36}\text{H}_{58}\text{O}_{10}$ as **4**, as indicated by HR-ESI-MS. The ^1H NMR and ^{13}C NMR signals of **5** assigned from extensive 2D NMR analysis showed that **5** is very similar to **4**, since it possessed the same constitutive elements, an aglycone of Δ^{12} oleanene derivative, two primary alcoholic and one carboxylic functions. The compounds **5** and **4** differ only by the position of the carboxylic function in the aglycone part (Tables 1 and 2). Comparison of the ^{13}C NMR data of **5** with that of **4** indicated the change in chemical shifts of C-27 [δ_{C} 178.8 in **4** and 63.3 in **5**] and C-29 [δ_{C} 73.0 in **4** and 181.5 in **5**]. Furthermore the correlation in the HMBC spectrum between δ_{H} at 1.24 (CH_3 -30) and δ_{C} at 181.5 revealed the location of the carboxylic function at C-29 in **5**. From the above discussion, the structure of **5** was assigned as 3-*O*- β -D-glucopyranosyl-olean-12-ene-3 β ,27,28-triol-29-oic acid.

Saponin **6** was assigned a molecular formula of $\text{C}_{35}\text{H}_{58}\text{O}_8$, as deduced from its positive ion in HR-ESI-MS which exhibited a quasi molecular ion peak at m/z 629.4033 $[\text{M}+\text{Na}]^+$, 44 mass units lower than that of **4**, implying the absence of the carboxylic function in **6**. Among the 35 carbons in ^{13}C NMR spectrum, 29 were assigned to the triterpenoid aglycone and 6 of the sugar moiety. Extensive NMR spectroscopic analysis indicated that **6** and **4** are structurally different only in the substituent in C-14 position of the aglycone. The lack of a substitution in this C-14 position in **6** was supported by the presence of HMBC correlation between C-13 and proton at δ_{H} 1.93 (1H, m, H-14), and by the significant chemical shift difference for C-14 [Δ

7.8] between **6** and olean Δ^{12} ene type (Table 2) (Mahato et al., 1994). Accordingly, the structure of **6** was elucidated as 3-*O*- β -D-glucopyranosyl-14-*H*-27-nor-olean-12-ene-3 β ,28,29-triol.

2.2. Cytotoxicity results

On the basis of the activity reported for the triterpene saponin derivatives isolated from the Fabaceae family (Verrota et al., 2001), the cytotoxic activity of the *n*-butanol extract was tested against the HT-29 human colon cancer cell line using a MTT assay (Mosmann, 1983). No significant activity was found in these bioassays ($IC_{50} > 100 \mu\text{g/mL}$).

3. Conclusions

Six new triterpenoid saponins (**1-6**), and 14 known compounds were isolated from the *n*-BuOH extract of the aerial parts of *G. ulicina*. Their structures were elucidated by extensive spectroscopic examinations. To our knowledge this is the first report of triterpene saponins in *Genista* genus which is known to contain alkaloids and flavonoids. The new saponins (**1-6**) contained glucose in position 3 of the aglycone, whereas the oleanene triterpenoid skeleton contained primary alcoholic function in C-28 and primary alcoholic or carboxylic functions in C-27, C-29 or C-30, it is also worthwhile to mention that the compound **6** had 27-noroleanene aglycone. The seven known isolated saponins exhibit a glucuronic acid but not glucose attached at C-3 and are characteristic of Fabaceae plants. Thus, the finding of oleanane-type triterpene saponin may provide a significant chemotaxonomic proof for *Genista* genus. The results of the present phytochemical study are in agreement with the flavonoidic patterns in the tribe Genisteae reported by Harborne (Harborne, 1994).

4. Experimental

4.1. General experimental procedures

Optical rotations were measured in MeOH or CHCl₃ with a Perkin-Elmer 241 polarimeter. ¹H and ¹³C NMR spectra were recorded on a Bruker Avance III 500 spectrometer (¹H at 500 MHz and ¹³C at 125 MHz) or on a Bruker Avance III 600 spectrometer equipped with a cryoplatfom (¹H at 600 MHz and ¹³C at 150 MHz). 2D-NMR experiments were performed using standard Bruker microprograms (Xwin-NMR version 2.1 software). ESIMS and HRESIMS experiments were performed using a Micromass Q-TOF micro instrument (Manchester, UK). The samples were introduced by direct infusion in a solution of MeOH at a rate of 5 μL min⁻¹. TLC was carried out on precoated silica gel 60 F₂₅₄ (Merck), and spots were visualized by heating after spraying with 50% H₂SO₄. Kieselgel 60 (63-200 mesh) Merck or LiChroprep RP-18 (40-63 μm) Merck were used for Column chromatography (CC). HPLC was performed on a Dionex apparatus equipped with an ASI-100 autosampler, a P580 pump, a UVD 340S diode array detector, and Chromeleon[®] software. A prepacked RP-18 column (201SP510, 250 × 10 mm, 5 μm, 90 Å, Dionex, Vydac, France) was used for semi preparative HPLC of the flavonoids with a binary gradient elution (solvent A: H₂O with 0.0025% TFA, solvent B: MeCN) at 25 °C and a flow rate of 3.5 mL min⁻¹; the chromatogram was monitored at 254 and 365 nm.

4.2. Plant material

The aerial parts of *Genista ulicina* Spach were collected during the flowering stage at Taza national park (Jijel, Algeria) in May 2009 and identified by Pr. Gerard De Bélair (University of Annaba, Algeria). A voucher specimen (ZKGu05/09) was deposited in the Herbarium of Biology Department (Mentouri University, Constantine).

4.3. Extraction and isolation of new compounds

Powdered air-dried aerial parts of *G. ulicina* (1000g) were macerated in MeOH-H₂O (8:2, 10L). This extract was concentrated to dryness, and the residue was suspended in H₂O (600

ml), and partitioned successively with CHCl₃, EtOAc and *n*-BuOH (3x 300ml each), then exhaustively concentrated to yield a CHCl₃ fraction (15 g), EtOAc (7 g), and *n*-BuOH fraction (20 g). 12g of the *n*-BuOH fraction were submitted to VLC over RP-18, eluted with MeOH-H₂O (4:6, 6:4, 8:2 and 0:10, each 450ml) to give six fractions [F₁: 6.64g, F₂: 2.18g, F₃: 0.45g, F₄: 0.49g, F₅: 1.1g, F₆: 0.21g].

Fraction F₅ (1.1g) was subjected to RP-18 CC using a gradient of MeOH-H₂O (4:6–8:2). Fractions [220-264] (70 mg), eluted with MeOH-H₂O (55:45) were purified by silica gel CC, eluted with CHCl₃-MeOH-H₂O [95:5:0-30:70:5], to give (4.5 mg) of **5**. Fraction [29-47] (36 mg), eluted with CHCl₃-MeOH (85:15), were purified by semi prep HPLC using an isocratic gradient of 30% B during 20 min to yield **3** (2.6 mg) and **5** (1.5 mg). Fractions [265-284] (138 mg), eluted MeOH-H₂O (6:4), were purified by silica gel CC, using a gradient of CHCl₃-MeOH [95:5-85:15]. Fractions [39-47] (20.6 mg) and [86-112] (18.8 mg) eluted, respectively with CHCl₃-MeOH [93:7] and [9:1], were purified by semi preparative HPLC using isocratic elution with 30% B for 30 min to give **1** (4.3 mg), **2** (4.6 mg), **5** (5 mg) and **4** (2.7 mg). Fractions [351-357] (40.3 mg), eluted with MeOH-H₂O (7:3), contained 2.1 mg of **6**, which were purified by semi prep HPLC using an isocratic gradient at 40% B for 15 min.

4.4. Saponin 1

White amorphous powder; [α]_D +18.9 (*c* 0.28, MeOH); ¹H and ¹³C NMR (CD₃OD), see Table 1. HRESIMS (positive-ion mode) *m/z* 659.4144 [M+Na]⁺ (calcd for C₃₆H₆₀O₉Na, 659.4135).

4.5. Saponin 2

White amorphous powder; [α]_D +18.6 (*c* 0.57, MeOH); ¹H and ¹³C NMR (CD₃OD), see Table 1. HRESIMS (positive-ion mode) *m/z* 659.4128 [M+Na]⁺ (calcd for C₃₆H₆₀O₉Na, 659.4135).

4.6. Saponin 3

White amorphous powder; [α]_D +14.8 (*c* 0.25, MeOH); ¹H and ¹³C NMR (CD₃OD), see Table 1. HRESIMS (positive-ion mode) *m/z* 821.4671 [M+Na]⁺ (calcd for C₄₂H₇₀O₁₄Na, 821.4663).

4.7. Saponin 4

White amorphous powder; $[\alpha]_D +24$ (*c* 0.75, MeOH); ^1H and ^{13}C NMR (CD_3OD), see Table 1. ESIMS (positive-ion mode) m/z 673.6 $[\text{M}+\text{Na}]^+$; HRESIMS (positive-ion mode) m/z 673.3921 $[\text{M}+\text{Na}]^+$ (calcd for $\text{C}_{36}\text{H}_{58}\text{O}_{10}\text{Na}$, 673.3928).

4.8. Saponin 5

White amorphous powder; $[\alpha]_D + 6.3$ (*c* 0.35, MeOH); ^1H and ^{13}C NMR (CD_3OD), see Table 2. HRESIMS (positive-ion mode) m/z 673.3933 $[\text{M}+\text{Na}]^+$ (calcd for $\text{C}_{36}\text{H}_{58}\text{O}_{10}\text{Na}$, 673.3928).

4.9. Saponin 6

White amorphous powder; $[\alpha]_D +15.8$ (*c* 0.19, MeOH); ^1H and ^{13}C NMR (CD_3OD), see Table 2. HRESIMS (positive-ion mode) m/z 629.4033 $[\text{M}+\text{Na}]^+$ (calcd for $\text{C}_{35}\text{H}_{58}\text{O}_8\text{Na}$, 629.4029).

4.10. Acid hydrolysis

A part of the *n*-BuOH fraction (1g) was refluxed in 2 N HCl (30 mL) for 4 h. The reaction mixture was neutralized with 0.5N KOH and freeze-dried. Four sugars were identified and compared with authentic samples by TLC using MeCOEt-*iso*-PrOH-Me₂CO-H₂O (20:10:7:6) as D-glucuronic acid, D-glucose, D-galactose and L- rhamnose. After the purification of these sugars by preparative TLC in the same solvent, the optical rotation of each purified sugar was measured.

4.11. MTT cytotoxicity assay

The bioassay was performed according to the [3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide] MTT (Sigma, St. Quentin Fallavier, France) assay method as described by Mosmann, 1983 with human colorectal cancer cell line (HT-29).

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References

- Bourama, N., Byung-Hun, U., Lobstein, A., Weniger, B., Koné, M., Anton, R., 2004. Flavonoides de *Tephrosia deflexa* et *Tephrosia albifoliolis*. C. R.C. 7, 993–996.
- Garritano, S., Pinto, B., Giachi, I., Pistelli, L., Reali, D., 2005. Assessment of estrogenic activity of flavonoids from Mediterranean plants using an *in vitro* short-term test. Phytomedicine. 12, 143–147.
- Giachi, I., Manunta, A., Morelli, I., Pistelli, L., 2002. Flavonoids and isoflavonoids from *Genista morisii*. Biochem. Syst. Ecol. 30, 801–803.
- Harborne, J.B., 1994. In: Harborne, J.B. (Ed.), Phytochemical Dictionary of Leguminosae Chapman & Hall, London, pp. 313–330.
- Ilarionov, I., Rainova, L., Nakov, N., 1979. Antiinflammatory and antiulcer effect of some flavonoids isolated from the genus *Genista*. Farmatsiya. 29, 39–46.
- Jia, Z.H., Koike, K., Nikaido, T., 1998. Major triterpenoid saponins from *Saponaria officinalis*. J. Nat. Prod. 61, 1368–1373.
- Kitagawa, I., Yoshikawa, M., Yosioka, I., 1976. Saponin and sapogenol XLVI. Structures of three soybean saponins: soyasaponin I, soyasaponin II and soyasaponin III. Chem. Pharm. Bull. 24, 121–129.
- Kitagawa, I., Taniyama, T., Hong, W.W., Hori, K., Yoshikawa, M., 1988. Saponin and sapogenol XLV. Structures of Kaikasaponins I, II and III from Sophorae Flos, the Buds of *Sophora japonica* L. Chem. Pharm. Bull. 108, 538–546.

Konoshima, T., Kozuka, M., Haruna, M., Ito, K., 1991. Constituents of Leguminous Plants, XIII. New triterpenoid saponins from *Wistaria brachybotrys*. J. Nat. Prod. 54, 830–836.

Korpachov, V.V., Litvinenko, O., Paster, I.P., 1995. Effect of *Genista tinctoria* extracts on the function of thyroid gland of intact rats and rats with experimental hypothyroidism. Farm. Zh. (Kiev). 5, 82–86.

Krafczyk, N., Kötke, M., Lehnert, N., Glomb, M., 2008. Phenolic composition of rhubarb. Eur. Food. Res. Technol. 228, 187–196.

Kudou, S., Masahide, T., Tsukamoto, C., Sakabe, T., Tamura, N., Okubo, K., 1993. Isolation and structural elucidation of DDMP conjugated soyasaponins as genuine saponins from soybean seeds. Biosci. Biotechnol. Biochem. 57, 546–550.

Luczkiewicz, M., Glod, D., 2003. Callus cultures of *Genista* plants *in vitro* material producing high amounts of isoflavones of phytoestrogenic activity. Plant Sci. 165, 1101–1108.

Mahato, S.B., Kundu, A.P. 1994. ¹³C NMR Spectra of pentacyclic triterpenoids—a compilation and some salient features. Phytochemistry. 37, 1517–1575.

Martins, A., Wink, M., Tei, A., Brum-Bousquet, M., Tillequin, F., Rauter, A.P., 2005. Phytochemical study of quinolizidine alkaloids from *Genista tenera* by gas–liquid chromatography/mass spectrometry. Phytochem. Anal. 16, 264.

Miyao, H.; Sakai, Y., Takeshita, T., Kinjo, J., Nohara, T., 1996. Triterpene saponins from *Abrus cantoniensis* (Leguminosae). I. Isolation and characterization of four new saponins and a new sapogenol. Chem. Pharm. Bull. 44, 1222–1227.

Mosmann, T., 1983. Rapid colorimetric assay for cellular growth and survival: Application to proliferation and cytotoxicity assays. J. Immunol. Meth, 65, 55–63.

Noccioli, C., Meini, L., Cecilia Loi, M., Potenza, D., Pistelli, L., 2011. A new alpinumisoflavone derivative from *Genista pichisermolliana*. Phytochem Lett. 4, 342–344.

- Piccinelli, A., Veneziano, A., Passi, S., Simone, F., Rastrelli, L., 2007. Flavonol glycosides from whole cottonseed by-product. *Food Chem.* 100, 344–349.
- Pistelli, L., Bertoli, A., Giachi, I., Manunta, A., 1998. Flavonoids from *Genista ephedroides*. *J. Nat. Prod.* 61, 1404–1406.
- Pistelli, L., Giachi, I., Potenza, D., Morelli, I., 2000. A New Isoflavone from *Genista corsica*. *J. Nat. Prod.* 63, 504–506.
- Pistelli, L., Bertoli, A., Giachi, I., Morelli, I., Rubiolo, P., Bicchi, C., 2001. Quinolizidine alkaloids from *Genista ephedroides*. *Biochem. Syst. Ecol.* 29, 137–141.
- Quezel, P., Santa, S., 1963. Nouvelle flore de l'Algérie et des régions désertiques Méridionales, CNRS, Paris, Vol. 2, pp 1170.
- Rainova, L., Nakov, N., Bogdanova, S., Minkov, E., Staneva-Stoicheva, D., 1988. Ulceroprotective activity of the flavonoids of *Genista rumelica* Vel. *Phytother. Res.* 2, 137–139.
- Rao, G.V., Rao, P.S., Tomimori, T., Kizu, H., 1985. Soyasaponin I from *Rothia trifoliata*. *J. Nat. Prod.* 48, 135–138.
- Rauter, A.P., Martins A., Borges, C., Ferreira, J., Justino, J., Bronze, M. R., Coelho, A.V., Choi, Y.H., Verpoorte, R., 2005. Liquid chromatography-diode array detection-electrospray ionization mass spectrometry nuclear magnetic resonance analyses of the anti-hyperglycemic flavonoid extract of *Genista tenera*. Structure elucidation of a flavonoid-C-glycoside. *J. Chromatogr. A.* 1089, 59–64.
- Rauter, A. P., Martins, Al., Lopes, R., Ferreira, J., Serralheiro, L. M., Araujo, M., Borges, C., Justino, J., Silva, F., Goulart, M., Thomas-Oates., Rodrigues, J. A., Edwards, E., Noronha, J., Helder Mota-Filipe, R. P., 2009. Bioactivity studies and chemical profile of the antidiabetic plant *Genista tenera*. *J. Ethnopharmacol.* 122, 384–393.

Rigano, D., Cardile, V., Formisano, C., Maldinic, M. T, Piacente, S., Bevilacqua, J., Russo, A., Senatore, F., 2009. *Genista sessilifolia* DC, and *Genista tinctoria* L. inhibit UV light and nitric oxide-induced DNA damage and human melanoma cell growth. *Chem. Biol. Intera.* 18, 211–219.

Rigano, D., Russo, A., Formisano, C., Cardile, V., Senatore, F., 2010. Antiproliferative and cytotoxic effects on malignant melanoma cells of essential oils from the aerial parts of *Genista sessilifolia* and *G. tinctoria*. *Nat. Prod. Commun.* 5, 1127–1132.

Santos, S.A., Carvalho, M.G., 1995. Unambiguous ¹H and ¹³C NMR-assignments of isoflavones from *Viola caducifolia*. *J. Braz.Chem. Soc.* 4, 349–352.

Sha, Y., Yan, M.-C., Liu, J., Liu, Y., Cheng, M.-S., 2008. Facile Synthesis of Oleanolic Acid Monoglycosides and Diglycosides. *Molecules.* 13, 1472–1486.

Shibano, M., Matsumoto ,Y., Kusano, G., Shibata, T., 1996. Researches of *Glycyrrhiza* species grown at medicinal plant gardens in Japan and basic studies for selection of pharmaceutically fine races. 1. Comparative studies by HPLC patterns and constituents of aerial parts. *Nat. Med.* 50, 273–283.

Ting, G., Hui, Yao., Jingyuan, S., Chang, L., Yingjie, Z., Xinye, M., Xiaohui, P., Hongxi, X., Shilin, C., 2010. Identification of medicinal plants in the family Fabaceae using a potential DNA barcode ITS2. *J. Ethnopharmacol.* 130, 116–121.

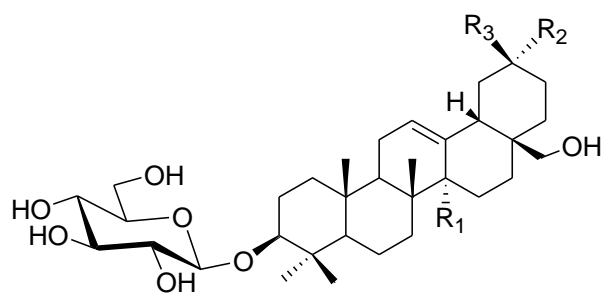
Tosun, F., Akyuz Kizilay, C., and Tosun, A. U., 2009. Flavonoids and isoflavonoids from *Genista sessilifolia* growing in Turkey. *Chem. Nat. Comp.* 45, 83–84.

Verotta, L., El-Sebakhy, N. A., 2001. In *Studies in Natural Products Chemistry*; Atta-ur-Rahman, Ed.; Elsevier: Amsterdam. 25, 179–234.

Yoshikawa, M., Shimada, H., Komatsu, H., Sakurama, T., Nishida, N., Yamahara, J. Shimoda, H., Matsuda, H., Tani, T., 1997. Medicinal foodstuffs. VI. Histamine release

inhibitors from kidney bean, the seeds of *Phaseolus vulgaris* L: chemical structures of sandosaponins A and B. Chem. Pharm. Bull. 45, 877–882.

Zhang, Z.Z., Koike, K., Jia, Z.H., Nikaido, T., Guo, D.A., Zheng, J.H., 1999. Gleditsiosides N-Q, New triterpenoid saponins from *Gleditsia sinensis*. J. Nat. Prod. 62, 877–881.



	R ₁	R ₂	R ₃
1	CH ₂ OH	CH ₃	CH ₂ OH
2	CH ₂ OH	CH ₂ OH	CH ₃
3	CH ₂ OH	CH ₂ O-Glc	CH ₃
4	COOH	CH ₂ OH	CH ₃
5	CH ₂ OH	COOH	CH ₃
6	H	CH ₂ OH	CH ₃

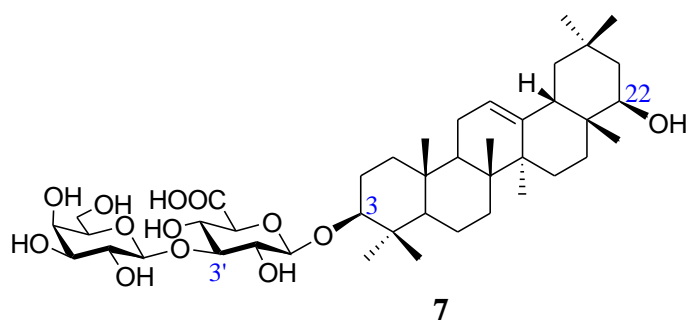


Fig. 1. Structures of compounds 1–7.

Table1 NMR spectroscopic data (CD₃OD) of compounds **1**, **3-4** at 500 MHz and **2** at 600 MHz.

	1		2		3		4	
	δ_C	δ_H (<i>J</i> in Hz)	δ_C	δ_H (<i>J</i> in Hz)	δ_C	δ_H (<i>J</i> in Hz)	δ_C	δ_H (<i>J</i> in Hz)
1	38.4	1.04, td (11.9, 3.3) 1.61, dm (11.9)	38.3	1.03, m 1.51, m	38.4	1.052, td (14.2, 3.8) 1.63, dm (14.2)	38.5	1.03, m 1.68, d (13.4)
2	25.6	1.71, m 1.94, m	25.6	1.70, m 1.95, m	25.6	1.71, td (13.6, 3.4) 1.95, m	25.7	1.70, tm (13.4) 1.95, m
3	89.4	3.20, dd (11.3, 5.3)	89.4	3.20, dd (11.9, 4.1)	89.4	3.20, m	89.3	3.16, m
4	38.8	-	38.8	-	38.8	-	38.7	-
5	55.4	0.91, dm (12.8)	55.4	0.92, m	55.4	0.91, dm (10.4)	55.6	0.79, dm (11.6)
6	17.9	1.45, td (11.8, 2.6) 1.61, dm (11.9)	17.9	1.47, td (13.2, 3.7) 1.61, m	17.9	1.46, dm (14.2) 1.63, m	17.9	1.40, m 1.56, m
7	32.8	1.39, dm (11.3) 1.71, m	32.7	1.38, dt (13.2, 2.8) 1.70, m	32.7	1.38, d (13.1) 1.68, td (12.8, 4.9)	36.5	1.32, m 1.68, dm (13.4)
8	40.2	-	40.2	-	40.2	-	39.4	-
9	48.4	1.98, dd (9.7, 7.6)	48.1	1.98, t (8.2)	48.1	2.03, t (8.6)	46.9	2.24, dd (10.7, 6.0)
10	36.6	-	36.5	-	36.5	-	36.4	-
11	23.5	1.91, m	23.6	1.92, m	23.6	1.92, m	21.5	1.94, m
12	127.9	5.64, t (3.3)	127.7	5.61, t (3.4)	127.8	5.63, t (3.4)	125.8	5.60, t (3.0)
13	138.4	-	138.3	-	138.3	-	137.1	-
14	46.9	-	46.9	-	46.7	-	55.9	-
15	21.1	1.10, m 1.68, m	21.2	1.04, m 1.71, td (13.7, 4.9)	21.4	1.00, m 1.72, td (13.6, 3.4)	21.4	1.72, t (12.8) 2.01, dm (12.4)
16	22.0	1.23, dd (13.7, 3.8) 1.83, td (14.1, 4.7)	21.6	1.20, dm (13.7) 1.85, td (13.7, 4.7)	21.5	1.19, dm (12.8) 1.88, td (13.5, 4.7)	22.6	1.19, dm (15.0) 1.97, m
17	36.5	-	37.0	-	37.1	-	37.5	-
18	41.4	2.05, dd (13.8, 4.2)	41.2	2.12, dd (13.6, 4.0)	41.2	2.12, dd (13.5, 4.8)	43.6	2.13, dd (13.5, 4.0)
19	40.6	1.36, dm (13.7) 1.53, t (13.7)	39.7	1.08, m 1.67, t (13.5)	39.9	1.26, dd (13.5, 3.8) 1.75, t (13.5)	38.2	1.00, m 1.50, t (13.5)
20	35.0	-	35.7	-	35.1	-	35.7	-
21	28.8	1.24, td (13.7, 3.8) 1.44, dm (13.7)	28.0	1.13, dm (13.7) 1.41, m	28.3	1.19, dm (12.8) 1.51, dd (13.2, 3.2)	28.1	1.16, dm (13.2) 1.35, tm (13.2)
22	30.1	1.33, dm (13.5) 1.56, td (13.5, 4.3)	29.9	1.41, dm (14.3) 1.60, m	29.8	1.41, dm (13.3) 1.60, td (13.3, 3.8)	29.7	1.38, m 1.55, td (15.3, 5.0)
23	27.1	1.09, s	27.0	1.09, s	27.1	1.09, s	27.1	1.05, s
24	15.6	0.88, s	15.6	0.88, s	15.6	0.88, s	15.6	0.87, s
25	15.2	0.98, s	15.2	0.99, s	15.2	0.99, s	15.5	1.08, s
26	17.3	0.98, s	17.3	0.99, s	17.3	1.00, s	17.3	1.05, s
27	63.2	3.55, d (12.3) 3.82, d (12.3)	63.2	3.53, d (12.2) 3.83, d (12.6)	63.2	3.55, d (12.1) 3.82, d (12.2)	178.8	-
28	67.9	3.20, d (11.1) 3.57, d (11.1)	68.2	3.21, d (11.1) 3.60, d (11.1)	68.2	3.20, d (9.4) 3.59, d (9.4)	68.4	3.20, d (11.0) 3.54, d (11.0)
29	26.6	0.90, s	73.2	3.20, brs	80.8	3.17, d (9.1) 3.61, d (9.1)	73.0	3.15, brs
30	65.3	3.45, d (11.0) 3.49, d (11.0)	18.2	0.92, s	18.5	1.00, s	17.9	0.90, s
Glc (at C-3)								
1'	105.3	4.34, d (7.8)	105.4	4.34, d (7.8)	105.4	4.34, d (7.8)	105.3	4.32, d (7.8)
2'	74.3	3.21, dd (8.6, 7.8)	74.3	3.21, dd (8.9, 7.8)	74.3	3.20, t (7.9)	74.3	3.20, dd (8.7, 7.8)
3'	76.9	3.36, t (8.6)	76.9	3.36, t (8.9)	76.9	3.36, t (8.8)	76.9	3.35, t (8.8)
4'	70.3	3.31, t (8.5)	70.3	3.31, t (8.6)	70.2	3.32, t (8.7)	70.3	3.30, t (8.5)
5'	76.3	3.26, ddd (9.4, 5.3, 2.3)	76.3	3.25, ddd (9.5, 5.3, 2.3)	76.3	3.25, m	76.3	3.26, ddd (8.5, 5.3, 2.2)
6'	61.4	3.68, dd (11.9, 2.3) 3.85, dd (11.9, 5.3)	61.4	3.68, dd (11.9, 5.5) 3.85, dd (11.9, 2.3)	61.3	3.69, dd (11.0, 5.5) 3.85, dd (10.6, 2.1)	61.4	3.68, dd (11.9, 5.3) 3.86, dd (11.9, 2.2)
Glc (at C-29)								
1''					103.5	4.23, d (7.8)		
2''					73.9	3.20, t (7.9)		
3''					76.5	3.36, t (8.8)		
4''					70.3	3.31, t (8.7)		
5''					76.5	3.25, m		
6''					61.4	3.68, dd, (10.5, 5.6) 3.87, dd, (10.5, 2.1)		

Table2 NMR spectroscopic data (CD₃OD) of compound **5** at 500 MHz and **6-7** at 600

MHz

	5		6		7	
	δ_C	δ_H (J in Hz)	δ_C	δ_H (J in Hz)	δ_C	δ_H (J in Hz)
1	38.4	1.04, tm (15.4) 1.61, m	38.4	0.99, tm (14.2) 1.71, m	38.3	1.01, m 1.66, dt (13.0, 3.0)
2	25.6	1.71, m 1.94, m	25.7	1.74, m 1.96, m	25.7	1.75, tm (13.9) 1.91, m
3	89.4	3.21, dd (11.7, 4.2)	89.4	3.19, dd (11.8, 4.6)	89.9	3.20, dd (11.7, 4.4)
4	38.8	-	38.8	-	38.9	-
5	55.4	0.92, dm (13.4)	55.7	0.86, m	55.6	0.81, dm (11.8)
6	17.9	1.45, d (12.1) 1.61, m	18.1	1.43, m 1.59, dm (13.6)	17.9	1.46, t (12.5) 1.60, m
7	32.7	1.38, dm (13.1) 1.72, m	41.5	1.16, td (13.8, 2.7) 1.96, m	32.8	1.42, m 1.58, m
8	40.3	-	35.2	-	39.4	-
9	48.1	2.01, dd (9.9, 8.8)	55.6	1.03, dd (10.4, 4.7)	47.9	1.59, dd (11.5, 6.4)
10	36.6	-	36.4	-	36.4	-
11	23.6	1.92, m	22.2	1.90, m	23.2	1.92, m
12	128.2	5.63, t (3.8)	121.1	5.37, brd (5.3)	122.4	5.27, brs
13	137.7	-	139.9	-	143.8	-
14	46.7	-	48.2	1.93, m	41.9	-
15	21.0	1.10, dm (13.9) 1.70, td (13.9, 5.2)	19.4	1.44, tm (14.2) 1.52, dm (15.0)	25.5	1.06, dm (14.0) 1.81, m
16	21.8	1.22, dm (14.6) 1.87, td (14.6, 5.6)	23.7	1.28, m 1.85, td (13.0, 4.3)	28.5	1.33, m 1.76, m
17	36.5	-	36.6	-	37.2	-
18	40.8	2.15, dm (13.7)	42.2	1.99, dm (13.6)	45.3	2.08, dm 13.7
19	39.3	1.43, dm (13.7) 2.13, t (13.7)	38.9	1.20, dm (13.6) 1.56, t (13.6)	46.1	0.98, dd (10.7, 2.7) 1.77, t (13.4)
20	42.1	-	34.9	-	29.9	-
21	28.5	1.48, dm (12.1) 1.83, td (14.6, 4.6)	28.5	1.29, m 1.45, m	40.8	1.36, dm (13.3) 1.47, m
22	29.6	1.42, m 1.63, m	29.8	1.34, dm (12.8) 1.47, tm (12.8)	75.6	3.41, dd (8.8, 3.3)
23	27.1	1.09, s	27.1	1.08, s	27.1	1.10, s
24	15.6	0.88, s	15.6	0.87, s	15.6	0.89, s
25	15.2	0.98, s	15.3	0.98, s	16.3	1.01, s
26	17.2	0.98, s	14.2	0.88, s	14.8	1.02, s
27	63.3	3.56, d (11.9) 3.82, d (11.9)	-	-	24.1	1.15, s
28	67.8	3.24, d (10.9) 3.57, d (10.9)	68.2	3.16, d (11.1) 3.50, d (11.1)	19.0	0.86, s
29	181.5	-	65.3	3.41, d (10.9) 3.51, d (10.9)	31.2	0.94, s
30	18.4	1.24, s	26.8	0.90, s	27.6	1.04, s
Glc (at C-3)					GlcA (at C-3)	
1'	105.4	4.34, d (7.8)	105.3	4.34, d (7.9)	104.1	4.53, d (7.1)
2'	74.3	3.21, dd (9.2, 7.8)	74.3	3.21, dd (8.7, 7.9)	76.3	3.61, t (8.2)
3'	76.9	3.36, t (8.6)	76.9	3.35, t (8.7)	81.2	3.58, dd (9.6, 8.2)
4'	70.3	3.31, t (8.6)	70.3	3.31, t (8.7)	71.7	3.56, t (9.6)
5'	76.3	3.26, ddd (8.6, 5.3, 3.0)	76.2	3.26, ddd (9.4, 5.5, 2.3)	75.2	3.78, m
6'	61.4	3.68, dd (11.9, 5.3) 3.85, dd (11.9, 2.7)	61.4	3.68, dd (11.9, 5.5) 3.86, dd (11.9, 2.3)	169.7	-
					Gal (at C-3')	
1''					104.7	4.57, d (7.7)
2''					72.6	3.62, dd (9.5, 7.7)
3''					73.3	3.51, dd (9.7, 3.4)
4''					68.4	3.88, d (3.1)
5''					75.4	3.49, t (6.6)
6''					60.4	3.74, dd (6.2, 3.1)

