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Two new triterpenoid saponins: telephiifoliosides A and B from the roots of *Corrigiola litoralis* subsp. *telephiifolia* (Pourr.) Briq

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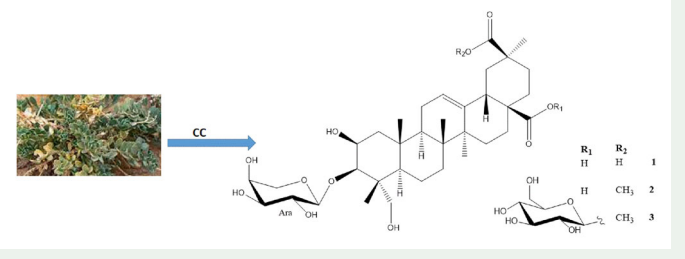
ABSTRACT

The polar fraction of the MeOH extract of the roots of *Corrigiola litoralis* subsp. *telephiifolia* (Pourr.) Briq. (Caryophyllaceae) was investigated for its constituents and two previously unreported monodesmosides triterpene saponins, telephiifoliosides A and B (**1** and **2**), along with the known bonushenricoside A (**3**) were isolated. Their structures were elucidated by combined spectroscopic and spectrometric techniques (^{1}H NMR, ^{13}C NMR, HSQC, ^{1}H-^{1}H COSY, HMBC, TOCSY, NOESY, HRESIMS) and chemical methods. The structures of the new saponins were established as; 3-*O*-α-L-arabinopyranosyljaligonic acid (**1**), and 3-*O*-α-L-arabinopyranosyl-phytolaccagenin ester (**2**). Upon evaluation of the antiproliferative activity on human malignant epithelial (HeLa) cells, none of the isolated compounds was efficient at the concentration of 33 μM.



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Caryophyllaceae; *Corrigiola litoralis*; triterpenoid saponins; antiproliferative activity

HIGHLIGHTS

- This is the first phytochemical study on *Corrigiola litoralis* subsp. *telephiifolia*.
- Two new saponins were isolated from the roots of *Corrigiola litoralis* subsp. *telephiifolia*.
- The isolated compounds were tested for their antiproliferative activity.

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1. Introduction

The Caryophyllaceae family (Kingdom: Plantae, Subkingdom: Tracheobionta, Superdivision: Spermatophyta, Division: Magnoliophyta, Class: Magnoliopsida, Subclass: Caryophyllidae, Order: Caryophyllales) includes 86 genera and about 2200 species; distributed mainly in temperate regions of the Northern hemisphere (Bittrich 1993). *Corrigiola litoralis* subsp. *telephiifolia* (Pourr.) Briq. (Synonym: *Corrigiolla telephiifolia* Pourr.) (Pourret 1788) is an herbaceous Caryophyllaceae specie which has been recognised by Moroccan legislation as a medicinal plant and is locally called "*Sarghina*". In Morocco, it grows in cultivated beds on rocky and sandy soils. It is an herb, widely branched, with slender prostrate branches and tiny compact inflorescences. When burned, its roots release an aromatic fume (Lakmichi et al. 2011). They are also used to treat flu, dermatological diseases, inflammation, cough and jaundice (Faïz et al. 2006-2007). *C. litoralis* root's consumed with honey, or simply sprinkled on food is part of a traditional remedy given to pregnant women (Bellakhdar 1997; Lakmichi et al. 2011). Although *C. litoralis* is used for the treatment of several diseases, very few imformation is reported concerning its chemical composition (Rimbau et al. 1999; Lakmichi et al. 2011).

Triterpene saponins are naturally occurring sugar conjugates of triterpenes. They are surface-active compounds which gave stable foams in water (Arslan 2014) and have been shown to possess a broad spectrum of biological and pharmacological activities (Dinda et al., 2010). In a continuation of our collaborative program of valorisation of African medicinal plants potentially containing saponins (Fouedjou et al. 2014; Tapondjou et al. 2013, 2015), we have examined the saponins containing fraction of the MeOH extract from *Corrigiola litoralis*. The present work describes the isolation and the structural elucidation of two new triterpene saponins: telephiifoliosides **A** (**1**) and B (**2**), possessing jaligonenic and phytolaccagenic acids as aglycones, respectively together with one known compound (**3**) (Figure 1). Furthermore, the isolated compounds were tested for their antiproliferative activity on human malignant epithelial cells (HeLa).

2. Results and discussion

2.1. Isolation and structure elucidation

The air dried and pulverised roots of *C. litoralis* was extracted at room temperature with MeOH. Repeated column chromatography of the MeOH extract over silica gel and Sephadex LH-20 afforded three natural compounds (**1-3)** including two new saponins (**1** and **2**).

Compound **1** was obtained as a white amorphous powder. Its molecular formula $C_{35}H_{54}O_{11,}$ was obtained from the positive-mode HR-ESI-MS which showed the pseudo-molecular ion peak at *m/z* 673.3391 $[M+Na]^+$ (calcd for $C_{35}H_{54}O_{11}Na$, 673.3564) and, 1323.6861 $[2M+Na]^+$. On the basis of the ^1H and ^{13}C NMR spectral data, compound **1** was identified as an olean-12-ene type pentacyclic triterpene saponin, and this was further confirmed by comparison of its NMR data with those of known olean-12-ene derivatives (Mahato and Kundu 1994). The aglycone part of **1**

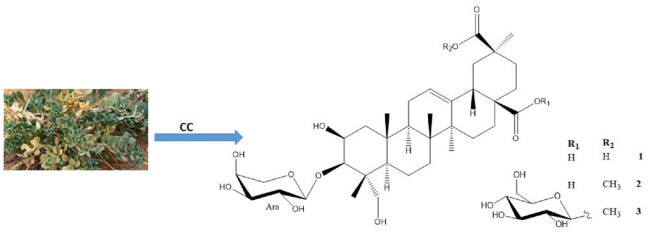

Figure 1. Structures of compounds **1-3.**

was identified as jaligonic acid and all the ^{1}H and ^{13}C NMR spectral data were in good agreement with literature values of closely related compounds (Wang et al. 2008). The signal of one anomeric proton at δ_H 5.05 (d, $J = 7.3$, H-1′), giving HSQC correlation with the anomeric carbon at δ_C 106.2 (C-1′) was also observed. The identification of protons belonging to the sugar unit was achieved *via* TOCSY experiment and their assignment to the respective carbon atoms was deduced from ^{1}H-^{1}H COSY and HSQC experiments starting from anomeric protons. The analysis of the chemical shifts of the sugar part allowed the identification of one arabinopyranosyl unit. The α anomeric configuration of the arabinopyranosyl units was deduced from the coupling constant ($J = 7.3$ Hz). Extensive survey of pentacyclic triterpenoid saponins from Caryophyllaceae species showed that, the sugar chains are preferably attached at C-3 and C-28 (Böttger and Melzig 2011; Arslan 2014). In the case of triterpenoid saponins having jaligonic acid or its C-30 O-methylester (Phytolaccagenin) as aglycone, C-3 of the aglycone resonates at about 82 ppm (Wang et al. 2008). The downfield shift observed for C-3 (δ 82.6) suggested the linkage site of the sugar at C-3. Furthermore, the HMBC correlations observed between the anomeric protons at δ_H 5.05 (H-1′) and the carbon at δ_C 82.6 (C-3) the linkage positions of the sugar. The *L* configuration of the sugar unit was determined by acid hydrolysis followed by GC analysis of its cysteine and thiazolidine derivatives (see experimental). On the basis of these data, the structure of **1** was determined as jaligonic acid 3-*O*-α-L-rabinopyranoside, a new secondary metabolite to which we gave the trivial name telephiifolioside A.

Compound **2** was also isolated as a white amorphous powder. Its positive ion mode HR-ESI-MS showed the pseudo-molecular ion peak at *m/z* 687.3564 $[M + Na]^+$ (calcd for $C_{36}H_{56}O_{11}Na$, 687.3754), corresponding to the molecular formula $C_{36}H_{56}O_{11}Na$. This indicated an increment of 14 amu as compared to compound **1,** suggesting the presence of one additional methyl group. An ion fragment was also observed at *m/z* 515.3222 $[M + H\text{-}132\text{-}18]^+$. Both ^{1}H and ^{13}C NMR of **2** were very similar to those of **1.** However, a signal of an *O*-methyl group was observed at δ_H 3.70 (OCH_3), as the only difference detected between **1** and **2**. The presence of the *O*-methyl group was further supported on the ^{13}C NMR spectrum where an additional signal was observed at δ_C 51.0 (OCH_3). Compound **2** was then a methyl ester derivative of **1**. The additional *O*-methyl group was attached to C-30 of the aglycone due to the HMBC cross peak depicted between protons of at δ_H 3.70 and C-30 of aglycone at δ_C 177.2. Based on the above findings, the structure of compound **2** was elucidated as 3-*O*-α-L-arabinopyranosyl phytolaccagenin, a new triterpene saponin to which we gave the trivial name telephiifolioside B.

In order to verify if compounds **2** and **3** are natural products and not artifacts formed during extraction, comparative TLC was performed and revealed their presence in an aliquot of the EtOH extract of this plant. C-30 *O*-methyl ester triterpenoid derivatives were previously reported from Chenopodiaceae (Kokanova-Nedialkova et al. 2019), Phytolaccaceae (Wang et al. 2008; Ma et al. 2019) and Molluginaceae (Zhang et al. 2019) plant species as naturally occurring compounds.

The known metabolite **3** was identified by comparison of its NMR data with those reported in the literature as 3-O-α-*L*-arabinopyranosylphytolaccagenin-28-O-β-*D*-glucopyranosyl ester (bonushenricoside A) previously isolated from the roots of *Chenopodium bonushenricus* L. (Kokanova-Nedialkova et al. 2019).

2.2. Antiproliferative activity

Due to the fact that, cancer is one of the most prominent human diseases and because several triterpene saponins were reported to be cytotoxic against a large panel of cancer cells (Lacaille-Dubois 2005), the three isolated saponins were tested for their antiproliferative activity against Human malignant epithelial cells (HeLa). All the compounds were not efficient at the concentration of 33 mM.

3. Experimental

3.1. General experimental procedures and instrumentation

Optical rotations were measured on a JASCO DIP-370 Digital Polarimeter. High-Resolution ESI-TOF-MS were measured on a Bruker Micro TOF II LC/MS Spectrometer (Column oven: 50 °C, Flow rate: 0.4 mL/min, Run time: 0.8 min for **1** and 0.1 min for **2**). The 1 D and 2 D NMR spectra (^{1}H NMR, ^{13}C NMR, HSQC, ^{1}H-^{1}H COSY, HMBC, TOCSY, NOESY*)* were performed in C_5D_5N-D_2O (20:1) using a Varian INOVA-600 NMR spectrometer (600 MHz for ^{1}H and 150 MHz for ^{13}C spectra). All chemical shift (δ) values are given in ppm units with reference to tetramethylsilane (TMS) as the internal standard, and the coupling constants (*J*) are in Hz. Silica gel (63-200 μm Merck 64271) and Sephadex LH-20 (Sigma 9041-37-6) were used for column chromatography. TLC was carried out on precoated Kieselgel 60 F_{254} (0.25 mm, Merck) plates developed with EtOAc-MeOH and EtOAc-MeOH-H_2O mixtures and/or on Kieselgel 60 RP-18 $F_{254}S$ (0.25 mm, Merck KGaA 64271 Darmstadt, Germany) developed with MeOH-H_2O mixtures. Spots on TLC plates were visualised by spraying with 20% H_2SO_4 and heating for 5 min at 70 °C. GC-MS was performed with GC-MS QP2010SE (Shimadzu, Japan) with Inert Cap 5MS/Sil i.d. 0.25 × 30 m (GL Sciences Inc., Japan) [Column temperature: 100-280 °C, rate of temperature increase: 10 °C/min]. The following sugar samples were commercially obtained and used for GC-MS analysis; D-glucose, L-glucose (Aldrich Chem. Co., Japan), D-arabinose, L-arabinose (Kishida Chemical Co., Ltd., Japan), L-cysteine methyl ester hydrochloride (Kanto Chemical Co., Inc., Japan), *N*-trimethylsilylimidazole (TMS-imidazole) (Tokyo Kasei Kogyo Co., Ltd., Japan). Fetal bovine serum (FBS) was purchased from Nichirei Bioscience Inc. (Tokyo, Japan) and heat-inactivated at 56 °C for 30 min for cell culture.

3.2. Plant material

The dried roots of *Corrigiola litoralis* were provided from the local market in the west of Morroco in June 2016. Professor A. Ouyahya, a taxonomist from the Scientific National Institute (Rabat) where a voucher specimen (N ° RAB65892) was deposited in the Botany Department, identified the plant.

3.3. Extraction and isolation

The dried and ground roots of *C. litoralis* (2 Kg) were extracted three times (each time for 24 h) with MeOH (4 L) at room temperature. The filtrate obtained was concentrated until dryness under reduced pressure to yield a dark crude extract (290 g) using a rotary evaporator (yield 14.5%). Part of this extract (270 g) was subjected to column chromatography on silica gel, using a gradient of EtOAc in hexane then, MeOH in EtOAc to give seven fractions (CT1-CT7).

Fraction CT3 was subjected to Sephadex LH-20 column chromatography eluted with MeOH to give four main subfractions ($CT3_{1\text{-}4}$). One of the above subfractions ($CT3_4$; 157 mg) was submitted to silica gel column chromatography eluted with EtOAc-MeOH (98:2) to afford **1** (2.16 mg). Sephadex LH-20 filtration of fraction CT5 using MeOH as elution solvent afforded five subfractions ($CT5_{1\text{-}5}$). Further silica gel column chromatography with isocratic elution (EtOAc-MeOH-H_2O; 97:2:1) of subfraction $CT5_3$ (57 mg) gave two subfractions $CT5_{31}$ and $CT5_{32}$. $CT5_{31}$ (23 mg) was further purified over silica gel column chromatography with isocratic elution (EtOAc-MeOH-H_2O; 97:2:1) to give **2** (1.73 mg). Subfraction CT6 was purified over silica gel column chromatography eluted with EtOAc-MeOH-H_2O (95:5:2) to afford **3** (4.33 mg).

3.3.1. Telephiifolioside A (1)

White amorphous powder (MeOH); $[a]_D^{22} = +\ 40.00^{\circ}$ ($C = 0.0009$ g/mL, MeOH); ^{1}H-NMR (600 MHz, C_5D_5N:D_2O, 20:1): δ_H (ppm) **Aglycone**: 5.71 (brs, H-12), 4.76 (d, $J = 3.7$ Hz, H-2), 4.30 (o, H_1-23), 4.29 (o, H-3), 3.65 (d, $J = 11.2$ Hz, H_2-23), 3.59 (brd, $J = 9.7$ Hz, H-18), 2.48 (brd, $J = 11.2$ Hz, H_1-19), 2.39 (o, H_1-7), 2.39 (H_1-21), 2.32 (dd, $J = 14.2$ and 3.2 Hz, H_1-1), 2.18 (o, H-16), 2.17 (o, H-15), 2.05 (o, H-11),2.02 (o, H_2-7), 1.90 (H_2-19), 1.84 (H-5), 1.80 (H-9), 1.78 (H-6), 1.75 (H_1-22), 1.54 (H_2-21), 1.52 (s, H-25), 1.39 (s, H-29), 1.34 (s, H-27), 1.33 (H_2-22), 1.28 (o, H_1-1), 1.28 (s, H-24), 1.06 (s, H-26); **Arabinose**: 5.05 (d, $J = 7.3$ Hz, H-1′), 4.51 (dd, $J = 7.3$ and 9.1, H-2′), 4.30 (o, H-4′), 4.28 (o, H_1-5′), 4.15 (dd, $J = 3.3$ and 9.1, H-3′), 3.75 (H_2-5′); and ^{13}C-NMR (150 MHz, C_5D_5N:D_2O; 20:1): δ_C (ppm) **Aglycone**: 180.0 (C-28), 179.6 (C-30), 144.6 (C-13), 123.1 (C-12), 82.6 (C-3), 70.4 (C-2), 64.7 (C-23), 48.3 (C-9), 47.3 (C-5), 46.2 (C-17), 43.8 (C-1), 43.2 (C-18 and C-20), 42.9 (C-19), 42.6 (C-4), 42.0 (C-14), 39.6 (C-8), 36.7 (C-10), 34.2 (C-7), 32.7 (C-22), 30.9 (C-21), 28.9 (C-29), 28.1 (C-15), 26.0 (C-27), 23.7 (C-11 and C-16), 17.7 (C-6), 17.3 (C-26), 17.0 (C-25), 14.8 (C-24); **Arabinose**: 106.2 (C-1′), 74.2 (C-3′), 72.5 (C-2′), 69.2 (C-4′), 66.8 (C-5′); HR-ESI-MS *m/z* 673.3391 $[M + Na]^+$ (calcd for 673.3564, $C_{35}H_{54}O_{11}Na$).

3.3.2. Telephiifolioside B (2)

White amorphous powder (MeOH); $[a]_D^{22} = +\ 60.00^{\circ}$ ($C = 0.0010$ g/mL, MeOH); δ_H (ppm) **Aglycone**: 5.62 (brs, H-12), 4.76 (d, $J = 3.2$ Hz, H-2), 4.30 (o, H_1-23), 4.28 (o, H-3),

3.65 (d, $J = 11.2$ Hz, H_2-23), 3.28 (brd, $J = 9.7$ Hz, H-18), 2.33 (dd, $J = 14.2$ and 3.6 Hz, H_1-1), 2.25 (H-19), 2.16 (H_1-21), 2.09 (o, H-16), 2.16 (o, H-15), 2.07 (H_1-22), 2.02 (o, H-11), 1.96 (H_2-22), 1.82 (H-5), 1.81 (H_2-19), 1.79 (H-9), 1.78 (H-6), 1.66 (o, H-7), 1.53 (s, H-25), 1.45 (H_2-21), 1.29 (s, H-27), 1.28 (o, H_2-1), 1.28 (s, H-24), 1.22 (s, H-29), 1.05 (s, H-26), 3.70 (s, OCH_3); **Arabinose**: 5.06 (d, $J = 7.4$ Hz, H-1′), 4.52 (dd, $J = 7.4$ and 9.0, H-2′), 4.29 (o, H-4′), 4.27 (o, H_1-5′), 4.15 (dd, $J = 3.4$ and 9.1, H-3′), 3.76 (H_2-5′); and ^{13}C-NMR (150 MHz, C_5D_5N:D_2O; 20:1): δ_C (ppm) **Aglycone**: 181.8 (C-28), 177.2 (C-30), 144.4 (C-13), 123.3 (C-12), 82.5 (C-3), 70.4 (C-2), 64.6 (C-23), 48.3 (C-9), 47.3 (C-5), 43.9 (C-17), 43.8 (C-1), 43.2 (C-18), 42.6 (C-4), 42.5 (C-14, C-19 and C-20), 39.6 (C-8), 36.6 (C-10), 34.3 (C-22), 32.6 (C-7), 30.5 (C-21), 28.2 (C-15), 28.0 (C-29), 26.0 (C-27), 23.7 (C-11), 23.6 (C-16), 17.7 (C-6), 17.3 (C-26), 17.0 (C-25), 14.7 (C-24), 51.0 (OCH_3); **Arabinose**: 106.2 (C-1′), 74.1 (C-3′), 72.5 (C-2′), 69.2 (C-4′), 66.8 (C-5′); HR-ESI-MS *m/z* 687.3564 $[M + Na]^+$ (calcd for 687.3754, $C_{36}H_{56}O_{11}Na$).

3.4. Acid hydrolysis of saponins and GC-MS analysis

Each saponin (0.3 mg) was heated in 1 M HCl (0.1 mL) at 90 °C for 3 h. The reaction mixture was dried *in vacuo* and dissolved in pyridine (0.2 mL). TMS-imidazole (50 μL) was added to the part of solution (0.1 mL) then heated at 50 °C for 30 min. The reaction mixture was diluted with H_2O (0.2 mL) and extracted with hexane (0.1 mL) then analysed by GC-MS by comparison with standard samples derivatised in the same conditions. *L*-Cysteine methyl ester hydrochloride (*ca.* 1.0 mg) was added to the remaining pyridine solution (0.1 mL) and heated at 60 °C for 1 h then the TMS derivative was also prepared as described for each isolated compound and analysed by GC-MS. Only the derivatives of monosaccharide *L*-arabinose (R_t 17.75) was characterised for compounds **1** and **2** while *D*-glucose (R_t 19.51 min) and *L*-arabinose (R_t 17.75) were characterised for compound **3** by comparison of their retention times with those of authentic samples treated in the same way as described above.

Standard TMS-Cys-Sugars (R_t, min)

TMS-Cys-D-glucose, R_t =19.51; TMS-Cys-L-glucose, R_t = 19.64;

TMS-Cys-L-arabinose, R_t = 17.67; TMS-Cys-D-arabinose, R_t = 17.90

3.5. Antiproliferative assay

Human malignant epithelial cells (HeLa) were cultured in Eagle's minimum essential medium (EMEM) supplemented with 10% fetal bovine serum (FBS) kept in an incubator at 37 °C in a humidified air containing 5% CO_2. FBS was purchased from Nichirei Bioscience Inc. (Tokyo, Japan). Cell viability was determined by a Cell-Titer 96 Aqueous Non-Radioactive Cell Proliferation (MTS) Assay (Promega, WI, USA) according to the manufacturer's protocol. HeLa cells (1×10^4 cells/well) were seeded in 96 well plates and incubated for 24 h, subsequently grown with compounds for additional 48 h, and then cell proliferation assay was performed (Teponno et al. 2016). Cells were counted after 24 h then, after 48 h. Cisplatin was used as a reference drug.

4. Conclusion

Two new triterpene saponins namely telephiifoliosides A and B were isolated from the commercial roots of *C. litoralis*, a Moroccan medicinal plant. Their structures were elucidated on the basis of extensive NMR, mass spectroscopic data, chemical reactions, and in comparison of their NMR data with those reported in the literature. The isolated compounds showed no antiproliferative activity against the human cancer cell lines HeLa at the concentration of 33 μM. The isolation of saponins from *C. litoralis* is in perfect agreement with the result reported by Daoudi et al. (2017), which revealed the presence of saponins in the aqueous extract of a sample of this plant collected in the Atlas Region-Morocco. The Caryophyllaceae family is well known to be a rich source of triterpene saponins having an oleanane-type skeleton as aglycone (Böttger and Melzig 2011). This work corroborated the previously reported results and let us to suggest that the oleanane-type skeleton might represent a chemiotaxonomic marker of the Caryophyllaceae family.

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Disclosure statement

The authors confirm that this article content has no conflict of interests.

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