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# Triterpene glycosides from *Silene odontopetala*

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ABSTRACT

*Silene* species are known for their use in traditional medicine in treating several diseases. To the authors' knowledge there is no report on the chemical composition of *S. odontopetala*. Therefore, the phytochemical investigation of the methanol extract of *S. odontopetala* was carried out, leading to the isolation of six undescribed oleanane-type glycosides along with the known saponin azukisaponin IV. Their structures were elucidated by the analysis of 1D and 2D-NMR experiments, along with mass spectrometry analysis. The cytotoxic activity of oleanane-type saponins was evaluated against a small panel of cancer cell lines, including PC-3 (prostate carcinoma cells), MCF-7 (breast cancer cells), A549 (alveolar basal carcinoma cells), and HeLa (cervical carcinoma cells). Furthermore, the activity of isolated compounds against a normal cell line HEK-293, used for assessing their cytotoxicity, was evaluated.

## 1. Introduction

The Caryophyllaceae family comprises 104 genera and more than 2000 species and is represented in the Flora of Central, Eastern and South-Eastern Anatolia by 32 genera and about 500 species (Horo et al., 2015; Kilinc et al., 2019). Caryophyllaceae are named 'pink family' for the colour of the flowers and plants belonging to this family are generally perennial. *Silene* is a large genus with more than 700 species growing in various temperate regions of the world (Golea et al., 2017). This genus is represented by 150 taxa in the Flora of Turkey, of which 67 endemic. Several phytochemical studies have been carried out on *Silene* species (Kilinc et al., 2019), highlighting the occurrence of ecdysteroids (Shakhmurova et al., 2012). A number of *Silene* species are used in Traditional Medicine to treat inflammation, bronchitis, cold, and infections or as a diuretic, antipyretic, analgesic, and emetic (Mamadalieva et al., 2014). Furthermore fungicidal activity has been reported for some *Silene* species (Mamadalieva et al., 2014). To the authors' knowledge there is no report on the chemical composition of *Silene odontopetala* Fenzl. Thus on the basis of the biological activities reported for *Silene* species, the phytochemical investigation of *S. odontopetala* was carried out. Herein, we report the isolation and structural characterization by NMR experiments of six undescribed oleanane-type saponins (**1–6**), in addition to the known azukisaponin IV.

Moreover, the cytotoxic activity of oleanane-type saponins (**1–7**) has been evaluated against a small panel of cancer cell lines, including PC-3 (prostate carcinoma cells), MCF-7 (breast cancer cells), A549 (alveolar basal carcinoma cells), HeLa (cervical carcinoma cells) along with a normal cell line HEK-293, used for assessing the selective cytotoxicity of the compounds.

## 2. Results and discussion

The MeOH extract of *S. odontopetala* was dissolved in $H_2O$ and successively partitioned with *n*-Hexane, $CH_2Cl_2$ and *n*-BuOH; the n-BuOH extract was chromatographed by MPLC on silica gel. The obtained fractions were submitted to further chromatographic steps to afford six underscribed triterpene saponins (Fig. 1), along with azukisaponin IV. Their structures were elucidated by extensive spectroscopic methods including 1D- ($^1$H and $^{13}$C) and 2D-NMR (DQF-COSY, HSQC, HMBC, and TOCSY) experiments as well as ESIMS analysis. The aglycones of the isolated compounds were identified as oleanane-type triterpenes by $^1$H NMR and $^{13}$C NMR analysis (Table 1) (Gulcemal et al., 2013; Koz et al., 2010).

The HR-ESI-MS spectrum of **1** (*m/z* 793.4006 $[M-H]^-$, calcd for $C_{41}H_{61}O_{15}$, 793.4010) supported a molecular formula of $C_{41}H_{62}O_{15}$. The MS/MS spectrum of this ion showed fragment ions at *m/z* 643.3461 $[M-H-132-18]^-$, due to the loss of a pentosyl unit with a $H_2O$ molecule

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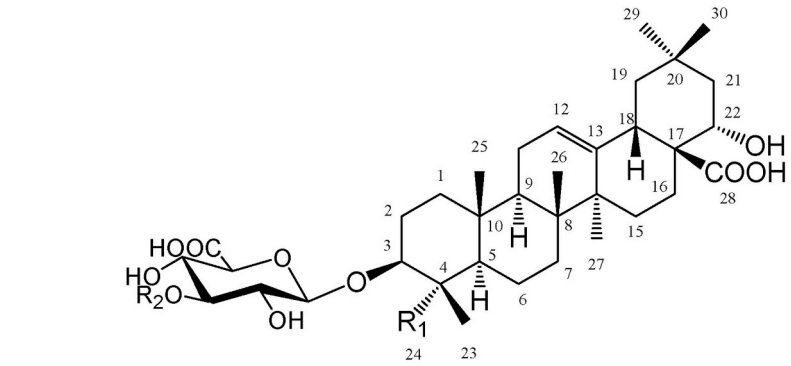
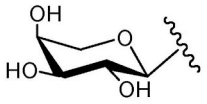
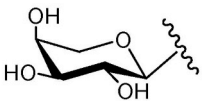



| | $R_1$ | $R_2$ |
|---|---|---|
| **1** | CHO |  |
| **2** | CHO | H |
| **3** | $CH_3$ |  |
| **4** | CHO | |
| **5** | $CH_3$ | |

**6**

**Fig. 1.** Triterpene saponins from *Silene odontopetala*.

**Table 1**
$^{13}C$ and $^{1}H$ NMR data (*J* in Hz) of the aglycone moieties of compounds **1–6** (600 MHz, δ ppm, in $CD_3OD$).

| | 1 | | 2 | | 3 | | 4 | | 5 | | 6 | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | $\delta_C$ | $\delta_H$ (*J* in Hz) | $\delta_C$ | $\delta_H$ (*J* in Hz) | $\delta_C$ | $\delta_H$ (*J* in Hz) | $\delta_C$ | $\delta_H$ (*J* in Hz) | $\delta_C$ | $\delta_H$ (*J* in Hz) | $\delta_C$ | $\delta_H$ (*J* in Hz) |
| 1 | 38.7 | 1.73, 1.15, *m* | 38.7 | 1.73, 1.15, *m* | 39.3 | 1.64, 1.02, *m* | 39.8 | 1.69, 1.10, *m* | 39.6 | 1.60, 1.02, *m* | 39.0 | 1.74, 1.17, *m* |
| 2 | 25.1 | 2.05, 1.82, *m* | 25.1 | 2.08, 1.80, *m* | 26.4 | 2.00, 1.72, *m* | 25.4 | 2.03, 1.80, *m* | 26.4 | 1.98, 1.75, *m* | 25.4 | 2.05, 1.62, *m* |
| 3 | 82.2 | 3.98, *dd* (11.5, 4.6) | 82.3 | 3.98, *dd* (11.5, 4.6) | 90.5 | 3.22, *dd* (11.6, 4.4) | 85.5 | 3.88, *dd* (11.5, 4.5) | 91.8 | 3.21, *dd* (11.6, 4.4) | 72.6 | 3.80, *dd* (11.6, 4.4) |
| 4 | 56.0 | – | 56.1 | – | 40.0 | – | 56.4 | – | 40.0 | – | 56.7 | – |
| 5 | 48.6 | 1.37, *m* | 48.7 | 1.37, *m* | 56.7 | 0.82 *brd* (11.5) | 48.6 | 1.34, *m* | 56.9 | 0.78 *brd* (11.5) | 48.6 | 1.35, *m* |
| 6 | 21.0 | 1.60, 0.95, *m* | 21.0 | 1.60, 0.95, *m* | 19.3 | 1.53, 1.47, *m* | 21.3 | 1.51, 0.92, *m* | 19.4 | 1.56, 1.48, *m* | 21.7 | 1.55, 0.92, *m* |
| 7 | 33.0 | 1.56, 1.28, *m* | 33.2 | 1.58, 1.28, *m* | 33.9 | 1.53, 1.35, *m* | 33.3 | 1.52, 1.24, *m* | 33.7 | 1.52, 1.33, *m* | 33.1 | 1.55, 1.28, *m* |
| 8 | 40.4 | – | 40.0 | – | 40.3 | – | 40.8 | – | 40.9 | – | 40.8 | – |
| 9 | 48.9 | 1.75, *m* | 48.7 | 1.75, *m* | 48.7 | 1.63, *m* | 48.8 | 1.69, *m* | 48.8 | 1.58, *m* | 48.8 | 1.74, *m* |
| 10 | 36.6 | – | 36.9 | – | 37.9 | – | 36.8 | – | 37.8 | – | 36.8 | – |
| 11 | 24.0 | 1.97, 1.90, *m* | 24.0 | 1.95, 1.90, *m* | 24.2 | 1.96, 1.90, *m* | 24.7 | 1.94, 1.90, *m* | 24.5 | 1.96, 1.90, *m* | 24.8 | 1.94, 1.89, *m* |
| 12 | 121.8 | 5.26, *t* (3.5) | 121.6 | 5.25, *t* (3.5) | 122.2 | 5.25, *t* (3.5) | 122.4 | 5.26, *t* (3.5) | 122.3 | 5.26, *t* (3.5) | 122.4 | 5.27, *t* (3.5) |
| 13 | 145.2 | – | 145.2 | – | 145.0 | – | 145.4 | – | 145.4 | – | 145.4 | – |
| 14 | 43.6 | – | 43.6 | – | 43.4 | – | 42.8 | – | 43.0 | – | 42.8 | – |
| 15 | 28.5 | 1.70, 1.09, *m* | 28.5 | 1.70, 1.09, *m* | 28.5 | 1.71, 1.12, *m* | 29.2 | 1.87, *m* | 28.9 | 1.69, 1.12, *m* | 29.0 | 1.87, *m* |
| 16 | 18.1 | 1.94, 1.69, *m* | 18.1 | 1.94, 1.69, *m* | 18.2 | 1.94, 1.70, *m* | 24.9 | 1.85, 1.79, *m* | 24.9 | 1.85, 1.79, *m* | 24.9 | 1.85, 1.78, *m* |
| 17 | 53.0 | – | 53.1 | – | 53.0 | – | 50.8 | – | 50.9 | – | 50.8 | – |
| 18 | 43.1 | 2.84, *dd* (13.8, 3.7) | 43.1 | 2.83, *dd* (13.8, 3.7) | 42.9 | 2.83, *dd* (13.8, 3.7) | 42.5 | 2.97, *dd* (13.8, 3.7) | 42.4 | 2.98, *dd* (13.8, 3.7) | 42.4 | 2.98, *dd* (13.8, 3.7) |
| 19 | 47.7 | 1.76, 1.15, *m* | 47.7 | 1.75, 1.13, *m* | 47.5 | 1.76, 1.13, *m* | 48.9 | 1.80, 1.19, *m* | 49.0 | 1.82, 1.19, *m* | 49.0 | 1.80, 1.18, *m* |
| 20 | 31.8 | – | 31.8 | – | 31.9 | – | 37.5 | – | 37.0 | – | 37.5 | – |
| 21 | 41.8 | 1.41, 1.36, *m* | 41.8 | 1.39, 1.34, *m* | 41.7 | 1.41, 1.35, *m* | 85.0 | 3.48, *m* | 85.1 | 3.50, *m* | 85.0 | 3.50, *m* |
| 22 | 72.0 | 3.75, *dd* (11.6, 5.2) | 72.0 | 3.75, *dd* (11.6, 5.2) | 72.0 | 3.75, *dd* (11.6, 5.2) | 41.8 | 1.93, 1.72, *m* | 41.0 | 1.94, 1.75, *m* | 41.4 | 1.95, 1.74, *m* |
| 23 | 208.5 | 9.44, *s* | 208.7 | 9.44, *s* | 27.9 | 1.08, *s* | 210.4 | 9.46, *s* | 28.1 | 1.10, *s* | 208.4 | 9.32, *s* |
| 24 | 9.8 | 1.13, *s* | 9.8 | 1.13, *s* | 16.5 | 0.87, *s* | 10.4 | 1.17, *s* | 16.8 | 0.90, *s* | 9.2 | 1.04, *s* |
| 25 | 15.7 | 1.03, *s* | 15.7 | 1.02, *s* | 15.7 | 0.98, *s* | 14.8 | 1.01, *s* | 15.7 | 0.96, *s* | 15.8 | 1.00, *s* |
| 26 | 17.7 | 0.94, *s* | 17.9 | 0.92, *s* | 17.7 | 0.93, *s* | 17.9 | 0.89, *s* | 17.8 | 0.89, *s* | 18.0 | 0.90, *s* |
| 27 | 26.0 | 1.21, *s* | 26.3 | 1.20, *s* | 25.9 | 1.19, *s* | 25.3 | 1.18, *s* | 26.1 | 1.15, *s* | 26.2 | 1.19, *s* |
| 28 | 183.8 | – | 183.9 | – | 181.8 | – | 184.1 | – | 180.5 | – | 180.8 | – |
| 29 | 33.4 | 0.95, *s* | 33.5 | 0.94, *s* | 33.6 | 0.95, *s* | 29.6 | 1.04, *s* | 29.6 | 1.04, *s* | 29.6 | 1.05, *s* |
| 30 | 24.9 | 1.00, *s* | 25.0 | 1.00, *s* | 24.6 | 1.00, *s* | 18.3 | 1.00, *s* | 18.4 | 1.01, *s* | 18.3 | 1.01, *s* |

and at *m/z* 485.3253 $[M-H-132-176]^-$ attributable to the aglycone moiety.

The $^{13}C$ NMR spectrum showed 41 carbon signals, of which 30 were assigned to the aglycone moiety and 11 to a sugar portion made up of two sugar units (Tables 1 and 2). The $^{1}H$ NMR spectrum displayed signals for an aldehyde proton at $\delta_H$ 9.44, six tertiary methyl groups at $\delta_H$ 0.94, 0.95, 1.00, 1.03, 1.13, and 1.21, an olefinic proton at $\delta_H$ 5.26 (t, $J$ = 3.5 Hz), and two oxygen-bearing methine protons at $\delta_H$ 3.98 (dd, $J$ = 11.5, 4.6 Hz, H-3) and 3.75 (dd, $J$ = 11.6, 5.2 Hz, H-22) (Table 1). These signals along with the carbon resonances for an aldehyde function at $\delta_C$ 208.5, a carboxyl group at $\delta_C$ 183.8, six methyl groups at $\delta_C$ 9.8, 15.7, 17.7, 24.9, 26.0, and 33.4, two olefinic carbons at $\delta_C$ 121.8, 145.2 in the $^{13}C$ NMR spectrum, suggested that the aglycone of compound **1** was a 3-hydroxyolean-12-en-28-oic acid with an aldehyde function and an additional secondary alcoholic function (Masullo et al., 2014). The HMBC correlations between the proton signal at $\delta_H$ 9.44 and the carbon resonances at $\delta_C$ 82.2 (C-3), 56.0 (C-4), 48.6 (C-5) and 9.8 (C-24), along with the ROESY correlations between the proton signal at $\delta_H$ 9.44 and the proton signals at $\delta_H$ 3.98 (H-3) and 1.37 (H-5) allowed to locate the aldehyde group at C-23. The additional secondary alcoholic function was placed at C-22 on the basis of the HMBC correlations between the proton signals at $\delta_H$ 1.01 (Me-30) and 0.95 (Me-29) with the carbon resonance at $\delta_C$ 41.8 (C-21) and on the basis of the COSY correlations of $H_2$-21 at $\delta_H$ 1.41 and 1.36 with the proton signal at $\delta_H$ 3.75 (H-22). In case of an oleanane-type triterpene the configuration of C-18 can be assigned on the basis of the chemical shift of C-18, which appears 7–8 ppm upfield shifted in the H-18α series if compared with the H-18β series, and of the basis of the chemical shift of C-28, which is reported to resonate at higher field of about 10–11 ppm in the H-18α series (Mahato and Kundu, 1994). The $^{13}C$ NMR resonances of C-18 ($\delta_C$ 43.1) and C-28 ($\delta_C$ 182.0) were in agreement with a β orientation of H-18. Thus the α-orientation of the alcoholic function at C-22 was deduced from the ROESY correlations between H-18 ($\delta_H$ 2.84), H-22 ($\delta_H$ 3.75) and Me-30 ($\delta_C$ 1.00), in agreement with literature data on triterpene derivatives with alcoholic functions on the E-ring (De Freitas et al., 2017) (Fig. 2). Thus, the aglycone of **1** was identified as 3β,22α-dihydroxyolean-12-en-23-al-28-oic acid. To the best of our knowledge, this is the first report of this aglycone in literature. The downfield shift of C-3 ($\delta_C$ 82.2) suggested that this carbon was a glycosidation site. The $^{1}H$ NMR spectrum displayed in the sugar region signals corresponding to two anomeric protons at $\delta_H$ 4.60 (d, $J$ = 7.0 Hz) and 4.26 (d, $J$ = 7.5 Hz). On the basis of 1D-TOCSY, DQF-COSY and HSQC analysis, the presence of a β-glucuronopyranosyl unit ($\delta_H$ 4.26) and an α-arabinopyranosyl unit ($\delta_H$ 4.60) was deduced. The configurations of glucuronic acid and arabinose units were established as D and L, respectively, after hydrolysis of **1** with 1 N HCl, trimethylsilation and determination of retention time by GC (Polat et al., 2010). An unambiguous determination of the sequence and linkage sites was obtained from the HMBC spectrum which showed key correlation peaks between the proton signal at $\delta_H$ 4.26 (H-1glcA) and the carbon resonance at $\delta_C$ 82.2 (C-3), the proton signal at $\delta_H$ 4.60 ($H\text{-}1_{ara}$) and the carbon resonance at $\delta_C$ 85.5 ($C\text{-}3_{glcA}$). On the basis of the above evidences, the structure of compound **1** was established as 3β,22α-dihydroxyolean-12-ene-23-al-28-oic acid 3-*O*-α-L-arabinopyranosyl-(1 → 3)-β-D-glucuronopyranoside.

The HR-ESI-MS spectrum of **2** (*m/z* 661.3574 $[M-H]^-$, calcd for $C_{36}H_{53}O_{11}$, 661.3588) supported a molecular formula $C_{36}H_{54}O_{11}$. The MS/MS spectrum of this ion showed a fragment ion at *m/z* 485.3254 $[M-H-176]^-$, due to the loss of a hexosyluronic acid and corresponding to the aglycone.

The $^{1}H$ and $^{13}C$ NMR chemical shifts of the aglycone moiety of **2** were superimposable on those of **1** (Table 1). In the sugar region, the $^{1}H$

**Table 2**
$^{13}C$ and $^{1}H$ NMR data (*J* in Hz) of the sugar portions of compounds **1–6** (600 MHz, δ ppm, in $CD_3OD$).

| | 1 | | 2 | | 4 | |
|---|---|---|---|---|---|---|
| | $\delta_C$ | $\delta_H$ (*J* in z) | $\delta_C$ | $\delta_H$ (*J* in Hz) | $\delta_C$ | $\delta_H$ (*J* in Hz) |
| | β-D-GlcA (at C-3) | | β-D-GlcA (at C-3) | | β-D-GlcA (at C-3) | |
| 1 | 103.7 | 4.26, *d* (7.5) | 103.7 | 4.20, *d* (7.5) | 103.9 | 4.37, *d* (7.0) |
| 2 | 74.5 | 3.32, *dd* (7.5, 9.0) | 75.4 | 3.12, *dd* (7.5, 9.0) | 78.5 | 3.70, *dd* (7.5, 9.0) |
| 3 | 85.2 | 3.57, *dd* (9.0, 9.0) | 77.5 | 3.32, *dd* (9.0, 9.0) | 85.8 | 3.68, *dd* (9.0, 9.0) |
| 4 | 71.0 | 3.53, *dd* (9.0, 9.0) | 73.8 | 3.43, *dd* (9.0, 9.0) | 71.8 | 3.57, *dd* (9.0, 9.0) |
| 5 | 76.0 | 3.58, *d* (9.0) | 76.3 | 3.52, *d* (9.0) | 77.4 | 3.59, *d* (9.0) |
| 6 | 176.0 | – | 176.0 | – | 176.1 | – |
| | α-L-Ara (at C-$3_{glcA}$) ( | | | | β-D-Gal (at C-$2_{glcA}$) | |
| 1 | 104.8 | 4.60, *d* (7.0) | | | 103.3 | 4.81, *d* (8.0) |
| 2 | 72.2 | 3.64, *dd* (8.0, 7.0) | | | 73.3 | 3.48, *dd* (8.5, 8.0) |
| 3 | 73.8 | 3.58, *dd* (8.0, 3.0) | | | 75.0 | 3.45, *dd* (8.5, 3.0) |
| 4 | 69.3 | 3.86, *m* | | | 69.5 | 3.81, *dd* (3.0, 1.2) |
| 5 | 66.7 | 3.96, *dd* (12.5, 3.0) | | | 76.3 | 3.51, *m* |
| | | 3.62, *dd* (12.5, 2.6) | | | | |
| | | | | | 61.8 | 3.77, *dd* (12.0, 2.5) |
| | | | | | | 3.74, *dd* (12.0, 4.5) |
| 1 | | | | | α-L-Ara (at C-$3_{glcA}$) | |
| 2 | | | | | 104.4 | 4.64, *d* (7.0) |
| 3 | | | | | 72.4 | 3.60, *dd* (8.0, 7.0) |
| 4 | | | | | 74.3 | 3.52, *dd* (8.0, 3.0) |
| 5 | | | | | 70.1 | 3.81, *m* |
| 6 | | | | | 67.0 | 3.91, *dd* (12.5, 3.0) |
| | | | | | | 3.57, *dd* (12.5, 2.6) |
| | | | | | β-D-Xyl (at C-21) | |
| 1 | | | | | 106.2 | 4.32, *d* (7.5) |
| 2 | | | | | 74.4 | 3.33, *dd* (7.5, 9.2) |
| 3 | | | | | 87.4 | 3.42, *t* (9.2) |
| 4 | | | | | 69.5 | 3.52, *m* |
| 5 | | | | | 65.6 | 3.85, *dd* (5.2, 11.7) |
| | | | | | | 3.23, *t* (11.7) |
| | | | | | β-D-Qui (at C-$3_{xyl}$) | |
| 1 | | | | | 104.9 | 4.51, *d* (8.2) |
| 2 | | | | | 74.1 | 3.30, *dd* (8.2, 9.5) |
| 3 | | | | | 77.3 | 3.36, *dd* (9.5, 9.5) |
| 4 | | | | | 76.0 | 3.06, *dd* (9.5, 9.5) |
| 5 | | | | | 73.4 | 3.40, *m* |
| 6 | | | | | 17.0 | 1.31, *d* (6.5) |

a The chemical shift values of the sugar moieties of **3** were superimposable to those of **1**.
The chemical shift values of the sugar moieties of **5** were superimposable to those of **4**.
The chemical shift values of the sugar chain at C-21 of **6** were superimposable to those of the sugar chain at C-21 of **4**.

NMR spectrum displayed a unique anomeric proton signal at $\delta_H$ 4.20 (d, $J$ = 7.5 Hz), assigned on the basis of HSQC, HMBC, COSY and 1D-TOCSY experiments to a β-glucuronopyranosyl unit. The configuration of the glucuronic acid unit was established as D after hydrolysis of **2** with 1 N HCl, trimethylsilation and determination of retention time by GC (Horo et al., 2012). The linkage of the sugar unit to C-3 was established by the HMBC correlation between the proton signal at $\delta_H$ 4.20 with the carbon resonance at $\delta_C$ 82.2. Therefore, the structure of compound **2** was elucidated as 3β,22α-dihydroxyolean-12-en-23-al-28-oic acid 3-*O*-β-D-glucuronopyranoside.

The HR-ESI-MS spectrum of **3** (*m/z* 779.4189 [M-H]$^-$, calcd for $C_{41}H_{63}O_{14}$, 779.4218) supported the molecular formula $C_{41}H_{64}O_{14}$. The MS/MS spectrum of this ion showed a fragment ion at *m/z* 471.3454 [M-H-176-132]$^-$, corresponding to the loss of a sugar chain made up of a hexosyluronic acid and a pentosyl unit.

The $^{1}H$ NMR spectrum displayed signals for seven tertiary methyl groups at $\delta_H$ 0.87, 0.93, 0.95, 0.98, 1.00, 1.08 and 1.19, an olefinic proton at $\delta_H$ 5.25 (t, $J$ = 3.5 Hz), two oxygen-bearing methine protons at $\delta_H$ 3.22 (dd, $J$ = 11.6, 4.4 Hz, H-3), and 3.75 (dd, $J$ = 11.6, 5.2 Hz, H-22) (Table 1). A detailed analysis of NMR data revealed that the aglycone of compound **3** differed from the aglycone of **1** for the replacement of a 23-CHO with a methyl function (Table 1). The $^{1}H$ NMR spectrum displayed in the sugar region signals corresponding to two anomeric protons at $\delta_H$ 4.26 (d, $J$ = 7.5 Hz), and 4.60 (d, $J$ = 7.0 Hz). A detailed comparison of NMR data of the sugar portion of compounds **3** and **1** demonstrated that the two compounds possessed the same sugar chain. Consequently, the structure of compound **3** was established as 3β,22α-dihydroxyolean-12-en-28-oic acid 3-*O*-α-L-arabinopyranosyl-(1 → 3)-β-D-glucuronopyranoside.

The HR-ESI-MS spectrum of **4** (*m/z* 1233.5518 [M-H]$^-$, calcd for $C_{58}H_{89}O_{28}$, 1233.5540) supported the molecular formula $C_{58}H_{90}O_{28}$. The MS/MS spectrum of this ion showed fragment ions at *m/z* 1083.4966 [M-H-132-18]$^-$, corresponding to the loss of a pentosyl unit with a $H_2O$ molecule and at *m/z* 763.4247 [M-H-132-162-176]$^-$, corresponding to the further loss of a hexosyl unit and a hexosyluronic acid.

The $^{13}C$ NMR spectrum showed 58 carbon signals, of which 30 were assigned to the aglycone moiety and 28 to a sugar portion made up of five sugar units (Table 2). The $^{1}H$ NMR spectrum showed signals for an aldehyde proton at $\delta_H$ 9.46, six tertiary methyl groups at $\delta_H$ 0.89, 1.00, 1.01, 1.04, 1.17, and 1.18, an olefinic proton at $\delta_H$ 5.26 (t, $J$ = 3.5 Hz), and two oxygen-bearing methine protons at $\delta_H$ 3.48 (m, H-21) and 3.88 (dd, $J$ = 11.5, 4.5 Hz, H-3) (Table 1). These signals along with the carbon resonances in the $^{13}C$ NMR spectrum suggested that the aglycone of compound **4** showed the same chemical features of compound **1**, except for the difference observed in the chemical shift of an oxygen-bearing methine proton ($\delta_H$ 3.48 in **4**), instead of the signal at $\delta_H$ 3.75 attributed to H-22 in **1**. This secondary alcoholic function was located

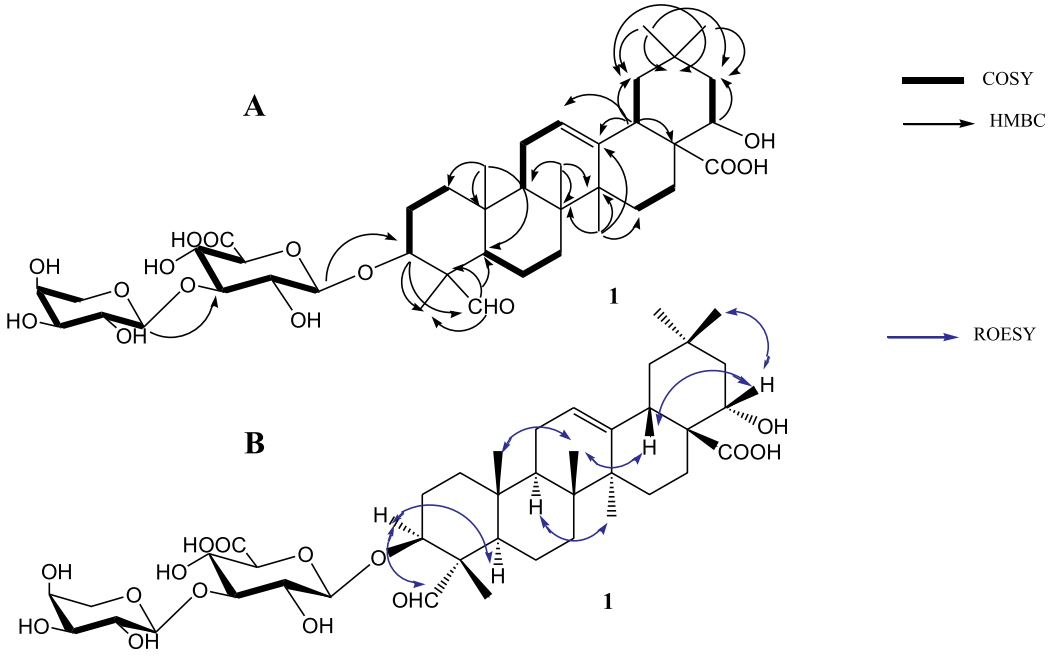



**Fig. 2.** (**A**) $^{1}$H–$^{1}$H COSY and key HMBC correlations for **1**; (**B**) Selected ROESY correlations for **1**.

at C-21 on the basis of the HMBC correlations between the proton signals at $\delta_H$ 1.00 (Me-30) and 1.04 (Me-29) with the carbon resonance at $\delta_C$ 85.0 (C-21), and the COSY correlations between the proton signal at $\delta_H$ 3.48 and the proton signals at $\delta_H$ 1.93 and 1.72 ($H_2$-22). The β-orientation of the alcoholic function was deduced from the correlations observed in the ROESY spectrum between H-21 ($\delta_H$ 3.48) and Me-29 ($\delta_H$ 1.04) and between H-18 ($\delta_H$ 2.97) and Me-30 ($\delta_H$ 1.00). Thus, the aglycone of **4** was identified as 3β,21β-dihydroxyolean-12-en-23-al-28-oic acid. The downfield shifts of C-3 ($\delta_C$ 85.5) and C-21 ($\delta_C$ 85.0) of the aglycone suggested that compound **4** was a bidesmosidic glycoside. The $^{1}$H NMR spectrum displayed in the sugar region signals corresponding to five anomeric protons at $\delta_H$ 4.32 (d, $J$ = 7.5 Hz), 4.37 (d, $J$ = 7.0 Hz), 4.64 (d, $J$ = 7.0 Hz), 4.51 (d, $J$ = 8.2 Hz), and 4.81 (d, $J$ = 8.0 Hz). On the basis of 1D-TOCSY, DQF-COSY and HSQC analysis, the presence of a β-xylopyranosyl unit ($\delta_H$ 4.32), a β-glucuronopyranosyl unit ($\delta_H$ 4.37), a β-quinovopyranosyl unit ($\delta_H$ 4.51), a α-arabinopyranosyl unit ($\delta_H$ 4.64), and a β-galactopyranosyl unit ($\delta_H$ 4.81) was deduced. The configuration of glucuronic acid, galactose, xylose and quinovose was established as D, while the configuration of arabinose was established as L, after hydrolysis of **4** with 1 N HCl, trimethylsilation and determination of retention time by GC (De Marino et al., 2003; Horo et al., 2012; Montoro et al., 2013). An unambiguous determination of the sequence and linkage sites was obtained from the HMBC spectrum, which showed key correlation peaks between the proton signal at $\delta_H$ 4.81 (H-$1_{gal}$) and the carbon resonance at $\delta_C$ 78.5 (C-$2_{glcA}$), the proton signal at $\delta_H$ 4.64 (H-$1_{ara}$) and the carbon resonance at $\delta_C$ 85.8 (C-$3_{glcA}$), the proton signal at $\delta_H$ 4.37 (H-$1_{glcA}$) and the carbon resonance at $\delta_C$ 85.5 (C-3); further correlations were observed between the proton signal at $\delta_H$ 4.51 (H-$1_{qui}$) and the carbon resonance at $\delta_C$ 87.4 (C-$3_{xyl}$), the proton signal at $\delta_H$ 4.32 (H-$1_{xyl}$) and the carbon resonance at $\delta_C$ 85.0 (C-21). On the basis of these evidences, the structure of compound **4** was established as 21-*O*-β-D-quinovopyranosyl-(1 → 3)-β-D-xylopyranosyl-3β,21β-dihydroxyolean-12-en-23-al-28-oic acid 3-*O*-α-L-arabinopyranosyl-(1 → 3)-[β-D-galactopyranosyl-(1 → 2)]-β-D-glucuronopyranoside.

The HR-ESI-MS spectrum of **5** (*m/z* 1219.5714 [M-H]$^-$, calcd for $C_{58}H_{91}O_{27}$, 1219.5748) supported a molecular formula of $C_{58}H_{92}O_{27}$. The MS/MS spectrum of this ion showed a peak at *m/z* 749.4228 [M-H-132-162-176]$^-$, corresponding to the loss of a sugar chain made up of pentosyl and hexosyl units and hexosyluronic acid.

The $^{1}$H NMR spectrum displayed signals for seven tertiary methyl groups at $\delta_H$ 0.89, 0.90, 0.96, 1.01, 1.04, 1.10 and 1.15, an olefinic proton at $\delta_H$ 5.26 (t, $J$ = 3.5 Hz), and two oxygen-bearing methine protons at $\delta_H$ 3.50 (m, H-21) and 3.21 (dd, $J$ = 11.6, 4.4 Hz, H-3) (Table 1). The NMR data of the aglycones of compounds **4** and **5** were superimposable except for an additional methyl signal in **5**, replacing the 23-CHO group of **4** (Table 1). A detailed comparison of NMR spectroscopic data of the sugar region of compounds **5** and **4** demonstrated that they possessed the same sugar chain. Therefore, the structure of compound **5** was established as 21-*O*-β-D-quinovopyranosyl-(1 → 3)-β-D-xylopyranosyl-3β,21β-dihydroxyolean-12-en-28-oic acid 3-*O*-α-L-arabinopyranosyl-(1 → 3)-[β-D-galactopyranosyl-(1 → 2)]-β-D-glucuronopyranoside.

The HR-ESI-MS spectrum of **6** (*m/z* 763.4247 [M-H]$^-$, calcd for $C_{41}H_{63}O_{13}$, 763.4269) supported a molecular formula of $C_{41}H_{64}O_{13}$. The MS/MS spectrum of this ion showed a fragment ion at *m/z* 485.3253 [M-H-146-132]$^-$, corresponding to the loss of pentosyl and hexosyl units, attributable to the aglycone moiety.

A detailed analysis of NMR spectroscopic data of compound **6** showed that compounds **6** and **4** share the same aglycone moiety, namely 3β,21β-dihydroxyolean-12-en-23-al-28-oic acid. The downfield shift exhibited only by C-21 ($\delta_C$ 85.4) of the aglycone suggested that compound **6** was a monodesmosidic glycoside. The comparison of NMR spectroscopic data of the sugar region of compounds **6** and **4** demonstrated that they possessed the same sugar chain linked to C-21. Therefore, compound **6** was determined as 21-*O*-β-D-quinovopyranosyl-(1 → 3)-β-D-xylopyranosyl-3β,21β-dihydroxyolean-12-ene-23-al-28-oic acid.

Additionally, azukisaponin IV, for the first time described in *Vigna angularis*, was isolated and determined by comparison of its spectroscopic data with those present in literature (Kitagawa et al., 1983).

The abundance and widespread occurrence of triterpene saponins is a typical feature of the family Caryophyllaceae (Mamadalieva et al., 2014). Several oleanane saponins isolated from *Silene* genus show as characteristic features an aldehyde or carboxyl group at C-23, a carboxyl group at C-28 and secondary alcoholic functions at C-16, and rarely at C-11 (Mamadalieva et al., 2014; Takahashi et al., 2016). In agreement with saponins previously reported in *Silene* spp., compounds

**Table 3**
$IC_{50}$ (μM) values of compounds **1–6** and doxorubicin on treated cell lines.[a].

| Compound | A549 | HEK293 | Hela | MCF7 | PC3 |
|---|---|---|---|---|---|
| **1** | > 50 | > 50 | 45.59 ± 2.32 | > 50 | 0.52 ± 0.16 |
| **2** | > 50 | > 50 | > 50 | > 50 | > 50 |
| **3** | 0.21 ± 0.02 | > 50 | 42.92 ± 1.77 | > 50 | > 50 |
| **4** | 0.12 ± 0.01 | > 50 | 10.23 ± 0.43 | > 50 | > 50 |
| **5** | 3.25 ± 0.09 | > 50 | 23.58 ± 3.15 | > 50 | 8.12 ± 1.51 |
| **6** | 8.57 ± 1.10 | > 50 | 45.68 ± 4.37 | > 50 | 2.34 + 0.61 |
| azukisaponin IV | 0.09 ± 0.01 | > 50 | 19.68 ± 1.69 | > 50 | > 50 |
| Doxorubicin | 0.17 ± 0.02 | 0.24 ± 0.05 | 1.73 ± 0.08 | 6.43 ± 0.38 | 14.88 ± 0.23 |

[a] Data are expressed as the mean values ± SD of three experiments.

**1**, **2**, **4** and **6** are characterized by the presence of an aldehyde group and a carboxyl group at C-23 and C-28, respectively. Their peculiarity is in the occurrence of further secondary alcoholic functions at positions 21 or 22.

Naturally occurring oleanane saponins have been reported to show anticancer effect through various pathways, such as antimetastasis, immunostimulation and chemoprevention (De et al., 2018; Martucciello et al., 2018; Masullo et al., 2017b). In order to evaluate the cytotoxic activity of isolated compounds against four cancer cell lines, including PC-3 (prostate carcinoma cells), MCF-7 (breast cancer cells), A549 (alveolar basal carcinoma cells), HeLa (cervical carcinoma cells), an MTT assay, detecting the activity of mitochondrial reductase of viable cells, was performed. The achieved results showed that compounds **1–6** isolated from *S. odontopetala* inhibited cell viability in a dose-dependent manner with $IC_{50}$ values in a range of 0.09–45.68 μM. The $IC_{50}$ values for all affected cell lines are shown in Table 3. Azukisaponin IV and compound **4** showed significant activity as compared to positive control, doxorubicin, against A549 cell line. All isolated compounds did not exhibit any activity at the tested concentrations on MCF7 cells and showed moderate cytotoxic activity against HeLa cells. Compounds **1**, **5** and **6** showed more than twenty-eight fold, twice-fold and six fold activity than doxorubicin against PC3 cell line, respectively. In addition, the cytotoxic activity of compounds **1–6** against a normal cell line HEK-293 was tested. None of the tested compounds exhibited activity against this cell line.

## 3. Materials and methods

### 3.1. General procedures

Optical rotations were measured on a Autopol IV (Rudolph Research Analytical) polarimeter. IR measurements were obtained with a Bruker IFS-48 spectrometer. NMR experiments were acquired in methanol-$d_4$ (99.95%, Sigma-Aldrich) with a Bruker DRX-600 spectrometer (Bruker BioSpin GmBH, Rheinstetten, Germany) equipped with a Bruker 5 mm TCI CryoProbe at 300 K. Data processing were carried out with Topspin 3.2 software. The ROESY spectra were acquired with $t_{mix}$ = 400 ms. GC analyses were performed on a Termo Finnigan Trace GC apparatus using a l-Chirasil-Val column (0.32 mm × 25 m). HRESIMS data were acquired on an LTQ Orbitrap XL mass spectrometer (Thermo Fisher Scientific, San Jose, CA, USA) operating in negative ion mode.

### 3.2. Plant material

Whole plants of *Silene odontopetala* Fenzl. (Caryophyllaceae) were collected from Malatya Darende Ağılbaşı, 1600 m, Turkey (GPS coordinates 38°32′52.0"N; 37°50′49.3"E) in May 2010, and identified by Serdar G. Şenol (Department of Biology, Faculty of Science, Ege University, İzmir, Turkey). In the Herbarium of Ege University (EGE-HERB 42722) a voucher specimen has been deposited.

### 3.3. Extraction and isolation procedure

The air-dried and powdered plant material (*S. odontopetala*; whole plant, 500 g) was first extracted with *n*-Hexane (2 × 3 L), $CH_2Cl_2$ (2 × 3 L) and MeOH (3 × 4 L) at room temperature. After carrying out filtration and evaporation procedures, *n*-Hexane (5.8 g), $CH_2Cl_2$ (8.4 g) and MeOH (71.0 g) extracts were obtained, respectively. The MeOH extract (71.0 g) was dissolved in $H_2O$ (500 mL) and successively partitioned with *n*-Hexane (2 × 200 mL), $CH_2Cl_2$ (2 × 200 mL) and *n*-BuOH saturated with $H_2O$ (3 × 300 mL). The *n*-BuOH phase (15.0 g) was subjected to a MPLC using reverse-phase material (Lichroprep. RP-18, 25–40 mm, 300.0 g) eluting with $H_2O$:MeOH (100:0–0:100) to give 5 main fraction (1–5).

Fraction 1 (3.0 g) was subjected to silica gel (Merck. 7734, 250.0 g) column chromatography with the solvent system $CH_2Cl_2$:MeOH:$H_2O$ (90:10:1) to give 7 sub-fractions (1a-1g). The sub-fraction 1 b (30.0 mg) was subjected to a MPLC using reverse-phase material (RP-18, 50.0 g) eluting with $H_2O$:MeOH (1:1) to give compound **6** (1.7 mg). Fraction 1e (95.8 mg) was submitted to silica gel (Merck. 7734, 250 g) column chromatography with the following solvent system $CH_2Cl_2$:MeOH:$H_2O$ (80:20:2–70:30:3) to give sub-fraction 1e1 and 1e1 (37.0 mg) was subjected to MPLC (RP-18, 5.0 g) eluting with $H_2O$:MeOH (1:1) to give azukisaponin IV (2.5 mg). Fraction 1 d (339.0 mg) was applied to silica gel (Merck. 7734, 250.0 g) column chromatography with the solvent system $CH_2Cl_2$:MeOH:$H_2O$ (80:20:2) to give sub-fraction 1d1 and 1d1 (27.0 mg) was subjected to MPLC (RP-18, 5 g) eluting with $H_2O$:MeOH (3:2) to give compounds **1** (5.9 mg) and **3** (1.4 mg). Fraction 1f (43.7 mg) was applied to silica gel (Merck. 7734, 90.0 g) column chromatography with the solvent system $CH_2Cl_2$:MeOH:$H_2O$ (70:30:3) to give sub-fraction 1f2 and 1f2 (27.0 mg) was subjected to MPLC (RP-18, 5.0 g) eluting with $H_2O$:MeOH (7:3) to give compounds **4** (2.3 mg) and **5** (3.5 mg).

Fraction 3 (590.0 mg) was subjected to silica gel (Merck. 7734, 30.0 g) column chromatography with the solvent system $CH_2Cl_2$:MeOH:$H_2O$ (90:10:1–80:20:2) to give 4 sub-fractions (3a-3d). The sub-fraction 3 d (21.0 mg) was subjected to a Sephadex (LH-20 Amersham Biosciences) column with MeOH to give compound **2** (1.0 mg).

The extraction and isolation procedure has been repeated to afford the required amount of each compound for further chemical and biological analyses.

### 3.4. 3β,22α-dihydroxyolean-12-en-23-al-28-oic acid 3-O-α-L-arabinopyranosyl-(1 → 3)-β-D-glucuronopyranoside (1)

Amorphous white solid; $C_{41}H_{62}O_{15}$; $[\alpha]_D^{25}$ +8.7 (*c* 0.1 MeOH); IR $\nu_{max}^{KBr}$ $cm^{-1}$: 3430, 2925, 1650, 1615; for $^1H$ and $^{13}C$ NMR (methanol-$d_4$, 600 MHz and 150 MHz) data, see Tables 1 and 2; HR-MS $[M-H]^- m/z$ 793.4006 ($C_{41}H_{61}O_{15}$, 793.4010).

### 3.5. 3β,22α-dihydroxyolean-12-en-23-al-28-oic acid 3-O-β-D-glucuronopyranoside (2)

Amorphous white solid; $C_{36}H_{54}O_{11}$; $[\alpha]_D^{25}$ +27.3 (c 0.1, MeOH); IR νKBrmax $cm^{-1}$: 3420, 2935, 1715, 1650; for $^1H$ and $^{13}C$ NMR (methanol-$d_4$, 600 MHz and 150 MHz) data, see Tables 1 and 2; HR-MS *m/z* 661.3574 $[M-H]^-$ (calcd for $C_{36}H_{53}O_{11}$, 661.3588).

### 3.6. 3β,22α-dihydroxyolean-12-en-28-oic acid 3-O-α-L-arabinopyranosyl-(1 → 3)-β-D-glucuronopyranoside (3)

Amorphous white solid; $C_{41}H_{64}O_{14}$; $[\alpha]_D^{25}$ +9.6 (c 0.1, MeOH); IR νKBrmax $cm^{-1}$: 3430, 2945, 1725, 1650; for $^1H$ and $^{13}C$ NMR (methanol-$d_4$, 600 MHz and 150 MHz) data, see Tables 1 and 2; HR-MS *m/z* 779.4189 $[M-H]^-$ (calcd for $C_{41}H_{63}O_{14}$, 779.4218).

### 3.7. 21-O-β-D-quinovopyranosyl-(1→3)-β-D-xylopyranosyl-3β,21β-dihydroxyolean-12-en-23-al-28-oic acid 3-O-α-L-arabinopyranosyl-(1 → 3)-[β-D-galactopyranosyl-(1 → 2)]-β-D-glucuronopyranoside (4)

Amorphous white solid; $C_{58}H_{90}O_{28}$; $[\alpha]_D^{25}$ +16.4 (c 0.1, MeOH); IR νKBrmax $cm^{-1}$: 3425, 2935, 1725, 1650; for $^1H$ NMR and $^{13}C$ NMR (methanol-$d_4$, 600 MHz and 150 MHz) data see Tables 1 and 2; HR-MS *m/z* 1233.5518 $[M-H]^-$ (calcd for $C_{58}H_{89}O_{28}$, 1233.5540).

### 3.8. 21-O-β-D-quinovopyranosyl-(1→3)-β-D-xylopyranosyl-3β,21β-dihydroxyolean-12-en-28-oic acid 3-O-α-L-arabinopyranosyl-(1 → 3)-[β-D-galactopyranosyl-(1 → 2)]-β-D-glucuronopyranoside (5)

Amorphous white solid; $C_{58}H_{92}O_{27}$; $[\alpha]_D^{25}$ +14.3 (c 0.1, MeOH); IR νKBrmax $cm^{-1}$: 3430, 2940, 1725, 1650; for $^1H$ and $^{13}C$ NMR (methanol-$d_4$, 600 MHz and 150 MHz) data, see Tables 1 and 2; HR-MS *m/z* 1219.5714 $[M-H]^-$ (calcd for $C_{58}H_{91}O_{27}$, 1219.5748).

### 3.9. 21-O-β-D-quinovopyranosyl-(1 → 3)-β-D-xylopyranosyl-3β,21β-dihydroxyolean-12-en-23-al-28-oic acid (6)

Amorphous white solid; $C_{41}H_{64}O_{13}$; $[\alpha]_D^{25}$ +14.6 (c 0.1, MeOH); IR νKBrmax $cm^{-1}$: 3415, 2935, 1720, 1645; for $^1H$ and $^{13}C$ NMR (methanol-$d_4$, 600 MHz and 150 MHz) data, see Tables 1 and 2; HR-MS *m/z* 763.4274 $[M-H]^-$ (calcd for $C_{41}H_{63}O_{13}$, 763.4269).

### 3.10. Acid hydrolysis

The configuration of sugar units was established by hydrolysis of compounds **1**–**6** with 1 N HCl, trimethylsilation and determination of the retention times by GC operating in the experimental conditions previously reported (Altunkeyik et al., 2012). The peaks of D-glucuronic acid (15.84 min) was detected in the hydrolysate of compounds **1**–**5**. The peaks of L-arabinose (8.94 and 9.80 min) were detected in the hydrolysate of **1**, **3**–**6**. The peaks of D-xylose (10.98 and 12.01 min), and D-quinovose (11.67, 12.72) were detected in the hydrolysate of **4**–**6**. The peak of D-galactose (13.99 min) was detected in the hydrolysate of **4** and **5**. Retention times for authentic samples after being treated in the same manner with 1-(trimethylsilyl)-imidazole in pyridine were detected at 15.81 min (D-glucuronic acid), 13.99 (D-galactose), D-quinovose (11.65, 12.70), 10.98 and 12.0 (D-xylose), 8.90 and 9.78 (L-arabinose) (De Marino et al., 2003; Horo et al., 2012; Montoro et al., 2013; Santos et al., 2017; Savran et al., 2012).

### 3.11. Cancer cell lines

PC-3 (prostate), MCF-7 (breast), A549 (lung), HeLa (human epitheloid cervix carcinoma) and a normal cell line HEK-293 were used for assessing cytotoxicity of the compounds. All the cell lines were purchased from the American Type Culture Collection (ATCC, Manassas, VA, U.S.A.). The cell lines were maintained in Dulbecco's modified Eagle's medium F12 (DMEM/F12), (10% FBS, 1% L-glutamin, 1% gentamicin, and 1 mM HEPES). The cells were incubated at 37 °C in a humidified atmosphere of 5% $CO_2$. During the experiments, exponential growth phase were used and these cells were sub-cultured twice a week.

### 3.12. Analysis of cell viability

The cytotoxicity of pure compounds was determined using a modified MTT assay, which detects the activity of mitochondrial reductase of viable cells (Hamed et al., 2006; Masullo et al., 2017a; Mosmann, 1983). The assay principle is based on the cleavage of MTT that forms formazan crystals by cellular succinate-dehydrogenases in viable cells. Briefly, all the cell lines were cultivated for 24 h in 96-well microplates with an initial cell number of $1 \times 10^5$ cells/mL in a humidified atmosphere with 5% $CO_2$ at 37 °C. Hence, the cultured cells were treated with different concentrations of compounds (0.05, 0.1, 0.5, 5, 50 μM), followed by incubation for 48 h at 37 °C. Doxorubicin (Sigma, St. Louis, MO, U.S.A.) was used as a positive control. After 48 h, MTT (2.5 mg/mL) stock solution was added as 25 μL at the end of each incubation period and incubated for 4 h at 37 °C. Dissolved formazan crystals in 150 μL DMSO were measured at 570 nm (reference filter 620 nm) with a UV visible spectrophotometer. The viability (%) was determined using the following formula:

$$\%\text{Viable cells} = \frac{\text{Absorbance of treated cells} - \text{Absorbance of blank}}{\text{Absorbance of control} - \text{Absorbance of blank}} \times 100$$

The mean $IC_{50}$ represents the concentration of an agent that reduces cell growth by 50% under the experimental conditions, and it is the average calculated from at least three independent measurements that would be reproducible and statistically significant. The $IC_{50}$ values were reported with ± 95% confidence intervals ( ± 95% CIs). This analysis was performed using Graph Pad Prism 5 (San Diego, CA, U.S.A.).

## Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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## Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.phytochem.2020.112404.

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