SUPPLEMENTARY MATERIAL

Perisomalien A, a new cytotoxic scalarane sesterterpene from the fruits of Periploca somaliensis

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Abstract

The CHCl₃ fraction of MeOH extract of *Periploca somaliensis* (family Asclepiadaceae) fruits afforded a new scalarane sesterterpene, namely perisomalien A (1), along with lupeol acetate (2), β-amyrin (3), cycloart-23Z-ene-3β,25-diol (4), and β-sitosterol-3-O-β-D-glucopyranoside (5). Their chemical structures were established by various spectroscopic analyses, in addition to comparison with the formerly reported data. Moreover, the cytotoxic activity of these metabolites was assessed towards MCF-7, HepG2, and HCT-116 tumour cell lines using sulphorhodamine B (SRB) assay. Compound 4 showed the most potent cytotoxic profile with IC₅₀ 9.0 µM towards MCF-7, compared to doxorubicin (IC₅₀ 0.18 µM). Also, 1 and 4 possessed the most potent effect towards HepG2 with IC₅₀s 26.7 and 25.9 µM, respectively. In addition, all tested compounds showed cytotoxic effects with IC₅₀ values ranging from 19.9 to 39.3 µM against HCT-116.

**Keywords:** *Periploca somaliensis*; Asclepiadaceae; scalarane sesterterpene; perisomalien A; cytotoxic
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**Figure S1.** $^1$H NMR (A) and expanded $^1$H NMR (B) spectra of compound 1 (850 MHz, CDCl$_3$).

**Figure S2.** Expanded $^1$H NMR spectrum of compound 1 (850 MHz, CDCl$_3$).

**Figure S3.** $^{13}$C NMR spectrum of compound 1 (214 MHz, CDCl$_3$).

**Figure S4.** $^1$H-$^1$H COSY spectrum of compound 1.

**Figure S5.** HSQC spectrum of compound 1.

**Figure S6.** HMBC spectrum of compound 1.

**Figure S7.** Some Key COSY and HMBC correlations of 1.

**Figure S8.** NOESY spectrum of compound 1.

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**Figure S11.** The dose response curve of different chemicals compounds on the cytotoxicity in MCF-7, HepG2, and HCT-116 cell lines. Cells were exposed to compounds, 2 (A), 3 (B) and 4 (C) with different dilutions of compounds for 72h. Cell viability was determined by SRB stain.

**Figure S12.** The dose response curve of different chemicals compounds on the cytotoxicity in MCF-7, HepG2, and HCT-116 cell lines. Cells were exposed to compounds 1 (A) and 5 (B) with different dilutions of compounds for 72h. Cell viability was determined by SRB stain.

**Table S1.** IC$_{50}$ (µM) of the isolated compounds against different tumor cell lines.
Experimental

General experimental procedures

UV spectra were recorded in MeOH on a Shimadzu 1601 UV/VIS spectrophotometer (Shimadzu, Kyoto, Japan. Optical rotation was measured on a JASCO DIP-370 digital polarimeter (Jasco Co., Tokyo, Japan) at 25 °C at the sodium D line (589 nm). The IR spectrum was measured on a Shimadzu Infrared-400 spectrophotometer (Shimadzu, Kyoto, Japan). EIMS were recorded on JEOL JMS-SX/SX 102A mass spectrometer. HRESIMS was carried out by LTQ-Orbitrap spectrometer (ThermoFinnigan, Bremen, Germany). 1D and 2D NMR spectra (chemical shifts in ppm, coupling constants in Hz) were recorded on Bruker Avance DRX 850 MHz spectrometers (Bruker BioSpin, Billerica, MA, USA). Sephadex LH-20 (0.25-0.1 mm, Pharmacia Fine Chemical Co. Ltd, Piscataway, NJ), RP-18 (0.04-0.063 mm), and silica gel 60 (0.04-0.063 mm, Merck, Darmstadt, Germany) were used for column chromatography. Pre-coated silica gel plates Kieselgel 60 F_{254} (0.25 mm, Merck, Darmstadt, Germany) were used for thin-layer chromatographic (TLC) analysis. The compounds were detected by UV absorption at \( \lambda_{max} \) 255 and 366 nm followed by spraying with a \( p \)-anisaldehyde:H\(_2\)SO\(_4\) spray reagent, then heating at 110 °C for 1–2 min.

Plant material

*P. somaliensis* fruits were collected in April 2014 from Al-Taif city, Saudi Arabia. The plant was kindly identified by a taxonomist at the Department of Natural products and Alternative Medicine, King Abdulaziz University, Saudi Arabia, in addition to its morphological features and the library database (Collenette, 1999). It was confirmed by Dr. Emad Al-Sharif, Associate Professor of Plant Ecology, Dept. of Biology, Faculty of Science & Arts, Khulais, King Abdulaziz University, Saudi Arabia. A voucher specimen (PS-1033) was archived at the Department of Natural Products and Alternative Medicine herbarium, King Abdulaziz University, Saudi Arabia.

Extraction and isolation
The air-dried powdered fruits of *P. somaliensis* (370 g) were extracted with MeOH/H₂O (70:30, v/v) (6 × 3 L) at room temperature (Kwape et al. 2016). The MeOH extract was evaporated and concentrated under reduced pressure to afford a dark brown residue (42.5 g). The latter was suspended in distilled H₂O (250 mL) and successively partitioned between CHCl₃ and EtOAc to yield CHCl₃ (10 g), EtOAc (4.0 g), and aqueous (19.7 g) fractions, respectively. From total, CHCl₃ and EtOAc fractions, 5.0, 2.0, and 1.0 g, respectively were stored for biological studies. The CHCl₃ fraction (7.7 g) was chromatographed over a SiO₂ CC (400 g × 100 × 3 cm) using *n*-hexane:EtOAc gradient to obtain eight sub-fractions: PSC-1 to PSC-8. Sub-fraction PSC-5 (698 mg) was subjected to a SiO₂ CC (50 g × 50 × 2 cm) using *n*-hexane:EtOAc gradient to get impure 1. Its purification was achieved on RP-18 CC (50 g × 50 × 2 cm) eluting with MeOH:H₂O (80:20) to yield 1 (10.4 mg). Sub-fraction PSC-3 (97:3, 980 mg) was chromatographed on SiO₂ CC (70 g × 50 × 2 cm) and eluted with *n*-hexane:EtOAc (99:1 to 97:3) to give impure 2, that was purified on RP-18 CC (50 g × 50 × 2 cm) eluting with MeOH:H₂O gradient to yield 2 (28.2 mg). SiO₂ CC (150 g × 50 × 3 cm) of sub-fraction PSC-4 (2.7 g) using *n*-hexane:EtOAc (96:4 to 90:10) gave two major spots. Separation of the two major spots was achieved on RP-18 column eluting with MeOH:H₂O gradient to get 3 (18.6 mg) and 4 (17.4 mg). Sub-fraction PSC-7 (850 mg) was chromatographed over a sephadex LH-20 CC (170 g × 50 × 2 cm) using MeOH:CHCl₃ (90:10) to obtain impure compound 5, which was purified on a SiO₂ CC (50 g × 50 × 2 cm) using CHCl₃:MeOH gradient to yield 5 (13.4 mg).

**In vitro cytotoxic activity**

**Cell culture**

Human hepatocellular carcinoma (HepG-2), colorectal adenocarcinoma cell line (HCT-116), and breast adenocarcinoma (MCF-7) cell lines were obtained from the American type culture collection (ATCC). Cells were maintained in RPMI-1640 supplemented with (100 μg/mL); penicillin (100 units/mL) and heat-inactivated fetal bovine serum (10% v/v) in a humidified, 5% (v/v) CO₂ atmosphere at 37 °C (Mahmoud et al. 2012). Doxorubicin was used as a positive control.

**Cytotoxicity assessment**
The cytotoxicity of the isolated compounds was tested against various human tumor cells using Sulphorhodamine B assay (SRB) (Skehan et al. 1990; Mohamed et al. 2017). Healthy growing cells were cultured in a 96 well tissue culture plate (3000 cells/well) for 24 hrs before treatment with the tested compounds to allow attachment of the cells to the plate. Cells were exposed to the five different concentrations of each compound (0.01, 0.1, 1, 10, and 100 μM); untreated cells (control) was added. Triplicate wells were incubated with the different concentrations for 72 hrs and subsequently fixed with TCA (10% w/v) for 1 hr at 4 °C. After several washings, cells were stained by 0.4% (w/v) SRB solution for 10 min in dark place. Excess stain was washed with 1% (v/v) glacial acetic acid. After drying overnight, the SRB-stained cells were dissolved with tris-HCl and the color intensity was measured in microplate reader at 540 nm. The linear relation between viability percentage of each tumor cell line and compounds concentrations was analyze to get the IC_{50} (dose of the drug which reduces survival to 50%) using SigmaPlot 12.0 software (Alahdal et al. 2018).

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Table S1: IC\textsubscript{50} (µM) of the isolated compounds against different tumor cell lines.

| Compd. No. | MCF-7       | HepG2       | HCT-116     |
|------------|-------------|-------------|-------------|
| 1          | 19.2 ± 0.9  | 26.7 ± 2.3  | 25.4 ± 3.8  |
| 2          | 24.0 ± 0.2  | 29.9 ± 0.6  | 31.9 ± 0.5  |
| 3          | 21.1 ± 0.8  | 31.7 ± 0.84 | 28.3 ± 0.6  |
| 4          | 9.0 ± 0.5   | 25.9 ± 0.6  | 19.9 ± 2.7  |
| 5          | 19.2 ± 0.6  | 33.6 ± 0.5  | 39.3 ± 3.9  |
| Doxorubicin| 0.18 ± 0.005| 0.6 ± 0.05  | 0.2 ± 0.008 |