

Cytotoxicity of gypsogenic acid isolated from *Gypsophila trichotoma*

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Submitted: 18-12-2012

Revised: 17-01-2013

Published: 28-05-2014

ABSTRACT

Background: *Gypsophila trichotoma* Wend. (Caryophyllaceae) is a medicinal plant which is protected in Bulgaria by the Biodiversity Law. Previous studies have showed the presence of triterpene saponins, sterols, flavonoids, triterpens, etc. **Objective:** Gypsogenic acid, isolated from *Gypsophila trichotoma* roots, was evaluated for cytotoxic activity. **Materials and Methods:** The structure of the compound was elucidated by spectral methods. The cell survival fraction was determined by the MTT dye reduction assay, performed with some modifications. **Results:** Gypsogenic acid was tested in a panel of human tumor cell lines and was found to inhibit the proliferation of malignant cells. It was active against leukemic cells with lymphoid (SKW-3 and BV-173) or myeloid phenotype (HL-60, K-562, and LAMA-84), as well as against the EJ bladder carcinoma cell line. Bcr-Abl expressing myeloid cells (LAMA-84 and especially K-562) displayed lower sensitivity. HL-60/Dox cells were less sensitive to gypsogenic acid than the parent cell line, which shows that gypsogenic acid is probably a substrate of MRP-1.

Key words: Cytotoxic activity, gypsogenic acid, *Gypsophila trichotoma*

Access this article online

Website:
www.phcog.com

DOI:
10.4103/0973-1296.133299

Quick Response Code:



INTRODUCTION

Triterpenes comprise one of the most interesting groups of natural products due to their high potential as pharmacological agents. They have been described as anti-inflammatory, antiangiogenic, antiviral, antioxidant, antibacterial, anticancer agents, as well as being immunomodulator compounds.^[1] In the literature available, there are insufficient data about the pharmacological properties of gypsogenic acid. Lee *et al.*,^[2] isolated this compound from *Aceriphyllum rossii* and evaluated it for *in vitro* cytotoxicity against the K-562 and HL-60 cell lines.^[2]

Gypsophila species have been found to accumulate many saponins including both monodesmosidic and bidesmosidic triterpene saponins of gypsogenin, oleanolic acid, quillaic acid, etc.^[3] Some of them have and gypsogenic acid as sapogenin.^[3-8] Triterpenes can be found rarely in the genus and they occur usually as saponins. Only a few have been isolated in native form from the plants of *Gypsophila*.^[9,10] The presence of a gypsogenic acid has been reported for

G. oldhamiana.^[10] In our previous investigation we isolated from *G. trichotoma* three triterpenes, including gypsogenic acid.^[11]

Gypsophila trichotoma is a perennial herbaceous plant located in Southeastern Europe, Southwestern Asia, Kazakhstan, and Turkmenistan. The plant is found along the Black Sea coast in Bulgaria. Our previous studies of *G. trichotoma* resulted in the isolation of saponins, flavonoids, sterols, triterpenes, etc.^[11-13] The hepatoprotective activity of the flavon glycoside saponarin was studied too.^[14] In continuation of our investigations of *G. trichotoma*, in this paper we describe the cytotoxic activity of gypsogenic acid, isolated from the roots of the species, against six human tumor cell lines.

MATERIALS AND METHODS

General experimental procedure

¹H Nuclear Magnetic Resonance (NMR) (400 MHz) and ¹³C NMR (100.6 MHz) spectra were recorded on Bruker DPX-400 using transcranial magnetic stimulation (TMS) as internal standard. All spectra were recorded in CD₃OD. High-resolution electron spray ionization mass spectra (HR-ESIMS) was carried out on Agilent 6210 ESI-TOF (Agilent Technology) mass spectrometer. Thin

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layer chromatography (TLC) study was carried out on silica gel plates (Kieselgel 60 F₂₅₄, Merck) using solvent systems n-BuOH/AcOH/H₂O (4:1:1) and CHCl₃/MeOH (9:1). The spots were visualized by spraying anisaldehyde/conc. H₂SO₄ reagent, followed by heating at 110°C. Chromatography (CC) was carried out with Diaion HP-20 (Supelco) and silica gel 60 (40-60 µm, Merck).

Plant material

The roots of *G. trichotoma* were collected in August 2008 at the Black Sea coast, Bulgaria. A voucher specimen (SO 103887) was deposited at the Herbarium of the Faculty of Biology, Sofia University.

Extraction and isolation

Air-dried powdered plant material of *G. trichotoma* (740 g) was exhaustively extracted with 80% methanol. After partial evaporation the aqueous solutions were extracted with CH₂Cl₂, EtOAc, and n-BuOH successively. The residue from the n-BuOH layer was separated by CC on a Diaion HP-20 column, using H₂O/MeOH (100:0 → 0:100) and further purified by flash chromatography over silica gel with CH₂Cl₂/MeOH/H₂O (18:11:1) to yield gypsogenic acid (50 mg) [Figure 1]. The structure of the compound was determined based on the spectral evidence (HRESI-MS, ¹H and ¹³C NMR, correlation spectroscopy (COSY), heteronuclear single-quantum correlation (HSQC), heteronuclear multiple bond correlation (HMBC) experiments).^[11]

Cell lines and culture conditions

The following human cell lines were used: EJ (urinary bladder carcinoma), SKW-3 (T-cell leukemia), BV-173, K-562 and LAMA-84 (chronic myeloid leukemia), and HL-60 (acute myeloid leukemia) and its resistant variant HL-60/Dox which is characterized by the expression of the multi-drug resistance-associated protein MRP-1. All leukemic cell lines were obtained from DSMZ (Braunschweig, Germany) and EJ cells were obtained from the American Type Culture Collection (Rockville, MD, USA). All cells were grown in RPMI-1640 medium supplemented with 10% fetal calf serum (FCS) and 2 mM L-glutamine (all from Lonza, Belgium) under standard conditions (37°C in an incubator with humidified atmosphere containing 5% CO₂). The cell cultures were supplemented with fresh medium two or three times per week to maintain them in log phase. HL-60/Dox cells were maintained in medium containing 0.2 µM doxorubicin in order to sustain their multidrug-resistance (MDR) phenotype. One week prior to cytotoxicity determination however, they were kept in drug-free medium in order to avoid synergistic interaction between doxorubicin and the tested compound.

MTT assay for cell survival and proliferation

Exponentially growing cells were seeded into 96-well microplates (100 µl/well at a density of

2×10^5 cells/ml for leukemic cells or 5×10^4 for EJ cells) and exposed to various concentrations of gypsogenic acid for 72 h. The cell survival fraction was determined by the MTT (3-[4,5-dimethylthiazol-2-yl]-2,5-diphenyltetrazoliumbromide) dye reduction assay,^[15] performed with some modifications.^[16] Briefly, after incubation with the test compound, MTT solution (10 mg/ml in PBS) was added (10 µl/well). Plates were further incubated for 3 h at 37°C and the formazan crystals formed were dissolved by addition of 110 µl solvent (5% formic acid in 2-propanol) per well and mixing. Absorption was measured by an automated microtiter plate spectrophotometer (Labexim LMR-1, Lengau, Austria) at 550 nm. For each concentration at least four wells were used. Complete medium (100 µl), MTT solution (10 µl) and 5% formic acid in 2-propanol (110 µl) were used as blank solution.

Statistics and evaluation of cytotoxic effects

Cell survival fractions were calculated as percentages of respective untreated controls, taken for 100%. Using the GraphPad Prism 5.01 program (GraphPad Software, San Diego, California, USA), concentration-effect curves was fitted, which were then used to interpolate respective IC₅₀ values and their 95% confidence intervals (CI).

RESULTS AND DISCUSSION

Fractionation of methanolic extract, obtained from the roots of *G. trichotoma*, by a combination of CC over Diaion HP-20 and silica gel resulted in the isolation of gypsogenic acid.^[11]

Gypsogenic acid has previously been shown to possess antibacterial and trypanocidal activity. It inhibited the growth of six cariogenic gram-positive bacterial strains^[17] and was active against blood trypomastigote forms of

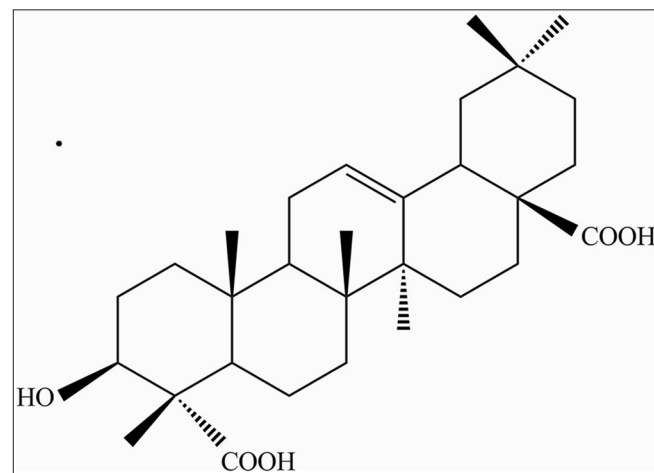


Figure 1: Chemical structure of gypsogenic acid

Trypanosoma cruzi.^[18] Gypsogenic acid was also found to have antihepatotoxic activity in a CCl₄-based model of liver injury.^[19] In the present study, gypsogenic acid was evaluated for cytotoxic activity against a panel of human

tumor cell lines and concentration-dependent cytotoxic effects were observed [Figures 2 and 3]. BV-173 cells were most sensitive (IC₅₀ = 41.4 μM; 95% CI: 38.6-44.3 μM), HL-60 cells ranked second (IC₅₀ = 61.1 μM; 95%

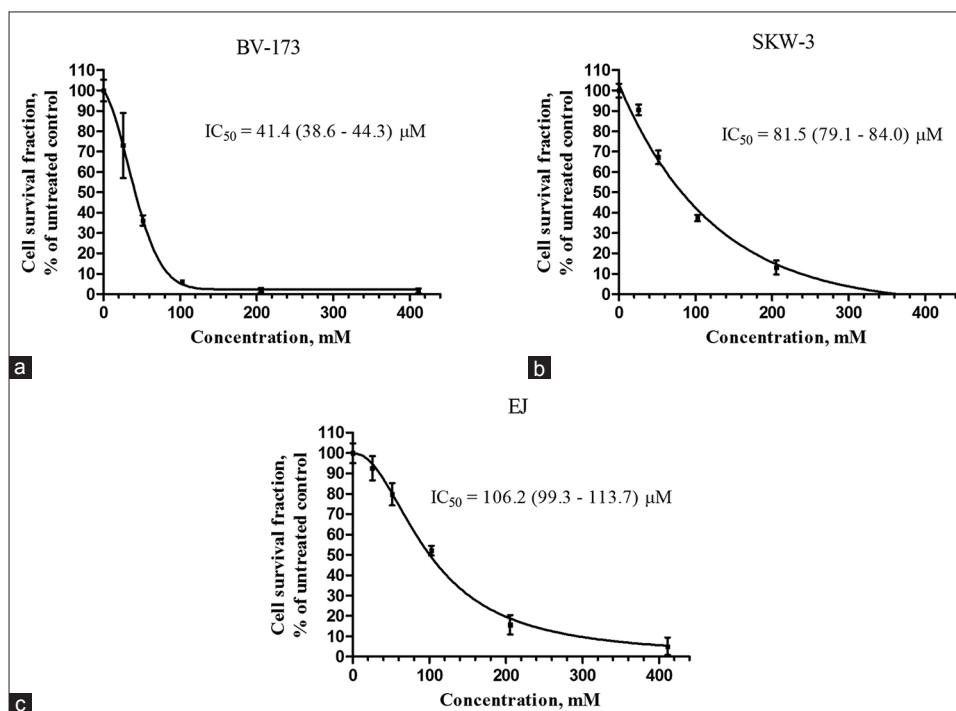


Figure 2: Survival of (a) BV-173, (b) SKW-3, and (c) EJ, tumor cells after exposure to gypsogenic acid for 72 h. Cell survival fractions were measured using the MTT dye reduction assay and are given as percentages of the respective untreated controls. Bars denote standard deviation

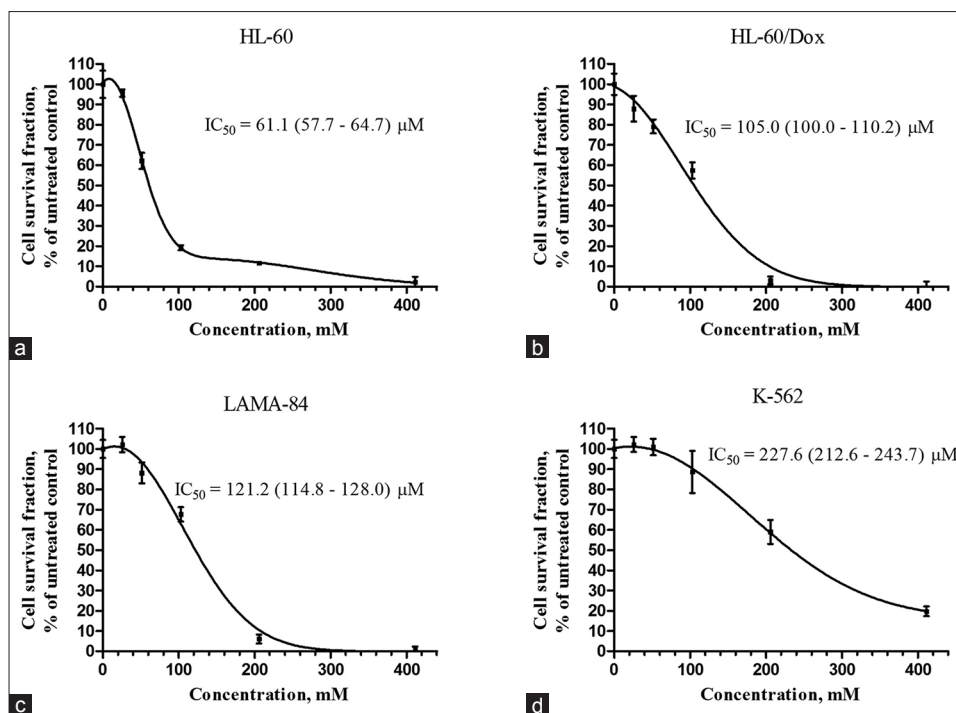


Figure 3: Survival of (a) HL-60, (b) HL-60/Dox, (c) LAMA-84, and (d) K-562, tumor cells after exposure to gypsogenic acid for 72 h. Cell survival fractions were measured using the MTT dye reduction assay and are given as percentages of the respective untreated controls. Bars denote standard deviation

CI: 57.7-64.7 μM), and SKW-3 third ($\text{IC}_{50} = 81.5 \mu\text{M}$; 95% CI: 79.1-84.0 μM). Gypsogenic acid had similar efficacy against HL-60/Dox, LAMA-84, and EJ cells with IC_{50} values ranging between 100 and 125 μM . K-562 cells were the least sensitive to gypsogenic acid ($\text{IC}_{50} = 227.6 \mu\text{M}$; 95% CI: 212.6-243.7 μM). Our data for the K-562 cell line are in accordance with those obtained by Lee *et al.*,^[2] who studied gypsogenic acid isolated from *A. rossii*, while HL-60 cells were more sensitive in our experiment.

CONCLUSION

In summary, gypsogenic acid isolated from *G. trichotoma* exhibited moderate cytotoxic activity against leukemic cells with lymphoid (SKW-3 and BV-173) or myeloid phenotype (HL-60, K-562, and LAMA-84), as well as against the EJ bladder carcinoma cell line. Bcr-Abl expressing myeloid cells (LAMA-84 and especially K-562) displayed lower sensitivity. HL-60/Dox cells were less sensitive to gypsogenic acid than the parent cell line, which shows that the compound is probably a substrate of MRP-1.

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Cite this article as: Krasteva I, Yotova M, Yosifov D, Benbassat N, Jenett-Siems K, Konstantinov S. Cytotoxicity of gypsogenic acid isolated from *Gypsophila trichotoma*. *Phcog Mag* 2014;10:430-3.

Source of Support: Nil, **Conflict of Interest:** None declared.