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Short Communication

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In vitro cytotoxicity of sesquiterpene lactones isolated from *Centaurea pullata* L. native to Algeria

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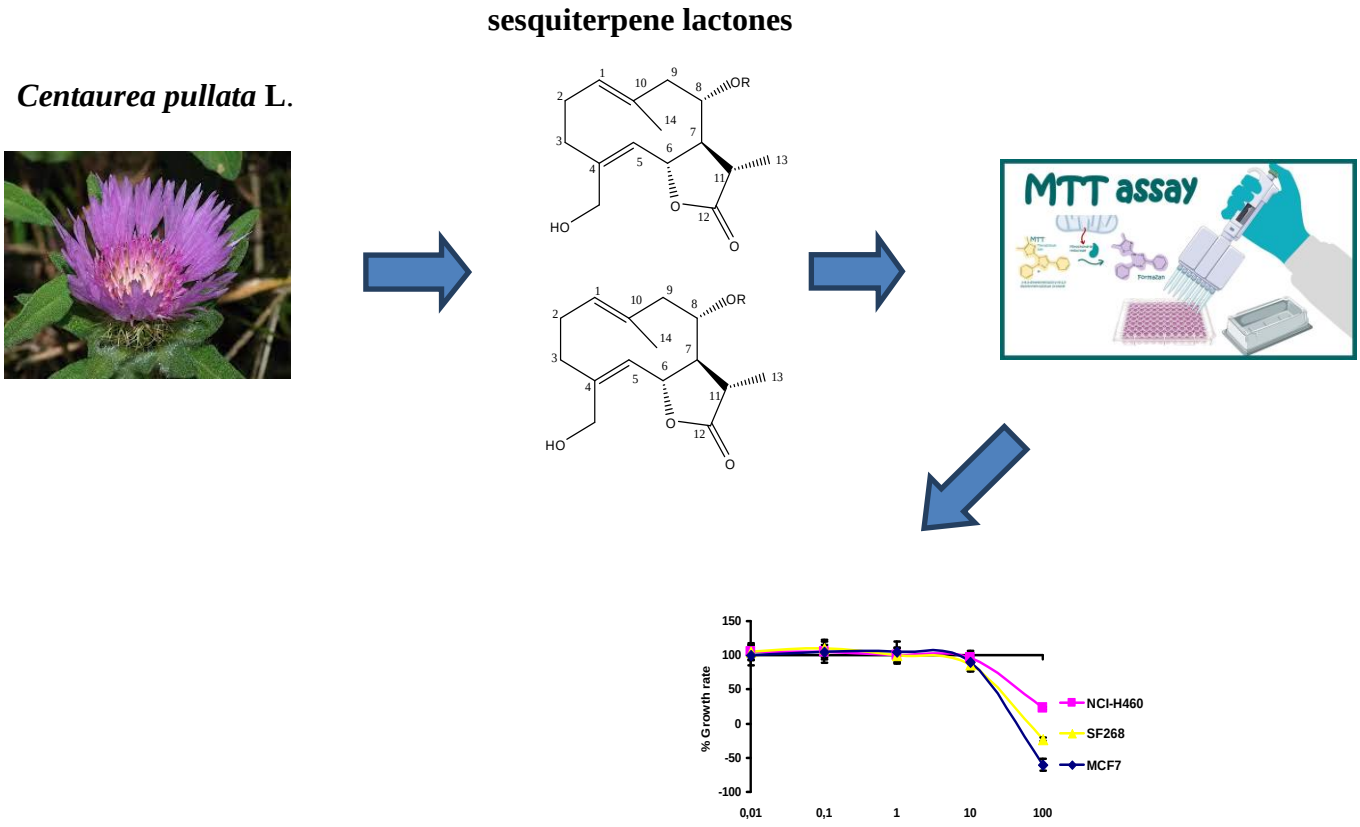
Graphical Abstract



Abstract
The phytochemical investigation of the aerial parts of *Centaurea pullata* L. from Algeria led to the isolation and identification of ten sesquiterpene lactones. The *in vitro* cytotoxic and antiproliferative potential of the isolated sesquiterpene lactones was tested against fives human cell lines (i.e., DLD1, SF268, MCF7, NCI-H460 and OVCAR3) using the sulforhodamine B (SRB) assay. In total ten compounds were tested, among which the elemanolide melitensin (5) proved the most active demonstrating a growth inhibitory activity below 100 μM against three cell lines NCI-H460, MCF7 and SF268, from the five tested.

Keywords: *Centaurea pullata* L., Asteraceae, Sesquiterpene lactone, Melitensin, Cytotoxic activity.

1. Introduction

Centaurea pullata L. (Asteraceae) is a biennial plant belonging to the section Melanoloma, distributed from Spain to France and North Africa (Khammar and Djeddi, 2012).
It is well known that sesquiterpene lactones constitute the more distributed natural products in the genus *Centaurea* L. which display a wide spectrum of biological activity such as antimicrobial, antifeedant, cytotoxic and antifungal (Louaar et al., 2011). We report here on the cytotoxic potential of sesquiterpene lactones possessing an α-methyl γ-lactone moiety isolated from *C. pullata* L. (Djeddi et al., 2007; Djeddi et al., 2008) using the sulforhodamine B (SRB) assay.

2. Materials and Methods

2. 1. Plant material

Aerial parts of *C. pullata* L. were collected from Chrea mountain (36°25′37″N 2°52′35″E) in Blida (North Algeria) in April 2006 and authenticated by Mr. Beloued abd El Kader (Agronomic National Institute, Algiers). A voucher specimen has been deposited in the Herbarium of the Department of Biology, Environmental Laboratory, University of Annaba, under the code: Ann-BV 2006/0010.

2. 2. Extraction and isolation

As previously described, the fresh plant material (1.2 kg) was ground finely and extracted at room temperature with cyclohexane–Et_2O–MeOH (1:1:1) and MeOH–H_2O (5:1), successively. The first extract was washed with brine, with the aqueous layer re-extracted with EtOAc, and the organic layer dried with Na_2SO_4 and concentrated under reduced pressure. The latter residue (12.7 g) was prefractionated by VLC on silica gel, using cyclohexane–EtOAc– Me_2CO mixtures of increasing polarity as eluents .
Lipophilic extract was subjected to silica gel column chromatography, and than all fractions were elucidated by spectroscopic methods (Djeddi et al., 2007; Djeddi et al., 2008).

2. 3. *In vitro* cytotoxic activity

The human cell lines: DLD1 (colon), SF268 (CNS), MCF7 (breast), NCI-H460 (non-small cell lung cancer), and OVCAR3 (ovarian) were obtained from the National Cancer Institute, NIH (Bethesda, MD, USA). The cultures were grown in a humidified 37°C-incubator with 5% CO_2 atmosphere.

Cell viability was assessed at the beginning of each experiment by the trypan blue dye exclusion method, and was always greater than 95%. Cells were seeded into 96-well culture microplates in 100 µL of medium at densities ranging from 5000 to 15000 cells per well, and subsequently, the plates were incubated at standard conditions for 24 h to allow the cells to resume exponential growth. Then, in order to measure the cell population, cells in one plate were fixed and further stained with sulforhodamine B (SRB) assay as described elsewhere (Mahaira et al., 2011)

To determine the sesquiterpene lactones (SLs) activity, the compounds dissolved in DMSO were added at 10-fold dilutions (from 100 to 0.01 µM) and incubation continued for an additional period of 48 h. The assay was terminated by the addition of cold TCA followed by SRB staining and absorbance measurement at 530 nm, in a microplate ELISA reader (EL-311 BIOTEK, Winooski, VT, USA). The data represent the mean of three experiments run in triplicates and were analyzed using a two-tailed Student's t-test.

The following parameters were determined through our own customized software: GI_{50}, TGI, and LC_{50} (Mashadesh et al., 2000). Briefly, GI_{50} is the concentration where 100 x (T-T0)/(C-T0) = 50 and measures the growth-inhibitory potency of the tested compound. TGI is the concentration of the test compound where 100 x (T-T0)/(C-T0) = 0 and measures the cytostatic effect of the compound. LC_{50} is the concentration of the test compound, where (T/T-T0) x100 = -50 and represents the cytotoxic activity of the compound. T is the optical density of the test well after a 48 h period of exposure to the test compound; T0 is the optical density of the cell population at time zero (when the compound is added), and C is the optical density of the control well, where cells were incubated for 48 h.

3. Results and discussion

The lipophilic extract afforded the following sesquiterpene lactones: 11β, 13 dihydrosalonitenolide **1** (1.1 mg), 11*β*,13-dihydrocnicin **2** (3.4 mg), 11*β*,13-dihydro 19-desoxycnicin **3** (20.0 mg), 8*α*-O-(4-acetoxy-5-hydroxy-angeloyl)-11*β*,13-dihydrocnicin **4** (1.0 mg), melitensin **5** (15.2 mg), 8*α*-O-(4-hydroxy-2-methylenebutanoyloxy) melitensine **6** (2.0 mg), 8*α* -hydroxy-11β, 13-dihydro-4-epi sonchucarpolide **7** (1.1 mg), 8*α*-O-(4-hydroxy-2-methylene-butanoyloxy)-11*β*,13-dihydro-4-epi-sonchucarpolide **8** (5.5 mg), 8*α*-O-(4-hydroxy-2-methylene-butanoyloxy)-11*β*,13-dihydro-sonchucarpolide **9** (1.7 mg) and 8*α*-hydroxy-11β, 13-dihydro-onopordaldehyde **10** (2.2 mg).

All compounds were elucidated by spectroscopic methods (Djeddi et al., 2007; Djeddi et al., 2008) (Figure 1).

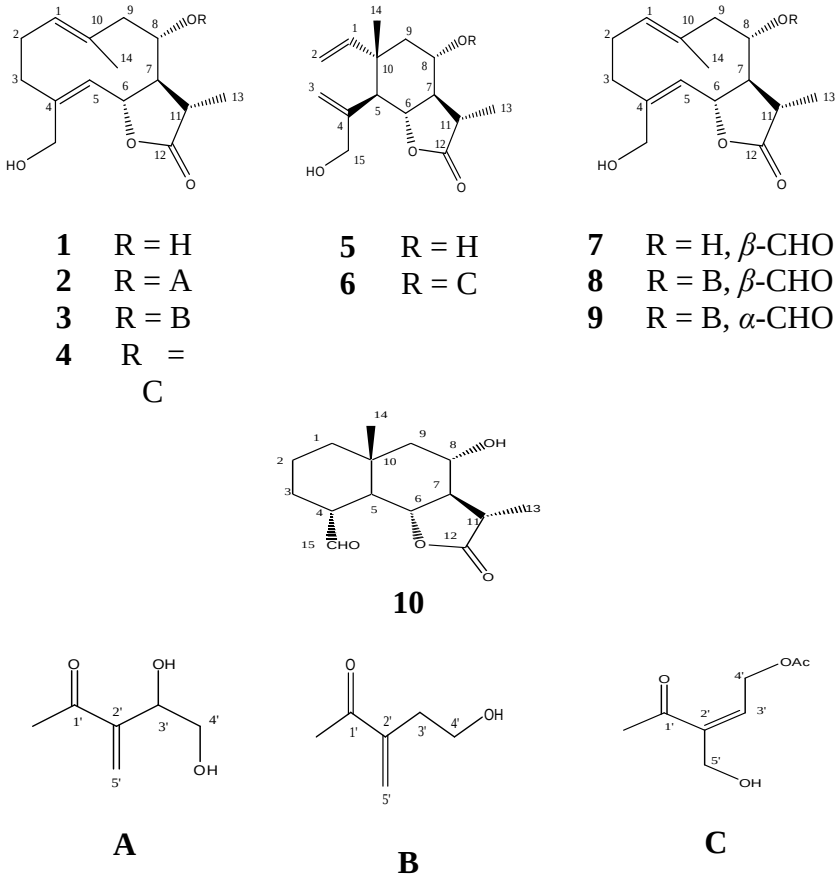


Figure 1. Chemical structure of the sesquiterpene lactones **1-10.**

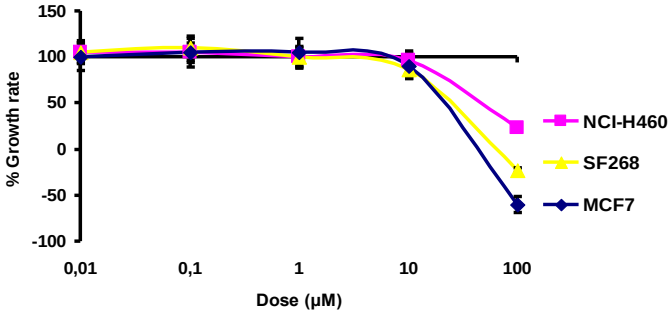
The obtained results revealed that the germacranolide 11*β*, 13-dihydrocnicine (**2**) showed a moderate growth inhibitory activity (GI_{50}) at dose of 51.8 µM against MCF7 (Table 1). In the other hand, the elemanolide melitensin (**5**) showed a better growth ihnibitory activity (GI_{50}) at doses 66.9 µM, 33.9 µM and 40.3 µM against NCI-H460, MCF7 and SF268, respectively. Its cytostatic effect (TGI) was proved to be moderate against MCF7 (63.8 µM) and SF268 (81.1 µM), while the cytotoxic effect (LG_{50}) was found to be very low at all cell lines tested as the LC_{50} was less than 100µM only against MCF7 (93.8 µM) (Table 1, Figure 2).

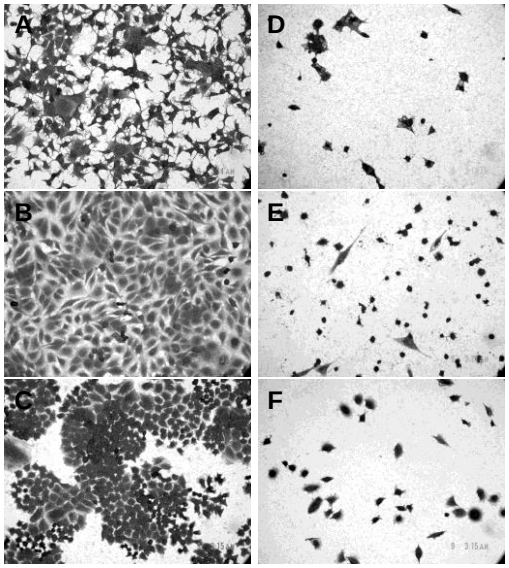
From the literature survey, cytotoxic properties of crude extracts obtained from various *Centaurea* species were reported, namely *C. musimomum* chloroform extract showed a cytotoxic activity with growth inhibition of 89 % at 10 µg/ml and 26 % at 1 µg/ml against the cell line KB (human carcinoma of the nasopharynx) (Medjroubi et al., 2005), while the ethanol extract of *C. ainetensis* an endemic plant from Lebanon, inhibit the poliferation of the human colon carcinoma cells HCT-116 and HT-29 (El-Najjar et al., 2008).

Table 1. GI_{50}, TGI, and LC_{50} Data (μM) of Compounds **2**, **5** and **5-FU.**

	2			**5**			**5-FU**		
NCI-H460	**GI_{50}**	**TGI**	**LC_{50}**	**GI_{50}**	**TGI**	**LC_{50}**	**GI_{50}**	**TGI**	**LC_{50}**
MCF7	*b*	*b*	*b*	66.9	*b*	*b*	1.0	50.0	*b*
SF268	51.8	*b*	*b*	33.9	63.8	93.8	2.0	31.0	*b*
DLD1	*b*	*b*	*b*	40.3	81.1	*b*	50.0	*b*	*b*
OVCAR3	*b*	*b*	*b*	*b*	*b*	*b*	*c*	*c*	*c*

b: >100μM, **c:** not tested





A-C: Cells untreated.

D-F: Cells treated with compound 5 in 100μM stained with SRB 48h at the end of the incubation.

Figure 2. Response of MCF7 cells (A and D), SF268 (B and E) and NCI-H460 (C and F) treated with compound **5**.

Some sesquiterpene lactones isolated from *Centaurea* species were also reported to be active. *C. ainetensis* afforded the guaianolide salograviolide A, which induced apoptosis and inhibition of the growth of colon cancer cell lines (El-Najjar et al., 2008); onopordopicrin and cnicin, isolated from several *Centaurea* sp. showed high cytotoxicity against human-derived macrophages (Bach et al., 2011). In addition, the cytotoxic effect of cnicin, isolated from *C.*

calolepis, an endemic species of Anatolia, was also studied toward pig kidney epithelial (LLC-PK11), human malignant melanoma (SK-MEL) and human ductal carcinoma (BT-549) cells with IC_{50} values of 23.3, 14.0 and 18.3 µM, respectively (Erel et al., 2011). Furthermore, cnicin showed inhibition of nuclear factor (NF-κB) and inhibition of inducible nitric oxide synthase (iNOS) activity with IC_{50} values of 1.8 and 6.5 µM, respectively (Erel et al., 2011). Aguerin B, isolated from the aerial parts of *C. deflexa* was assessed against human pancreatic and colonic cancer cells and the results revealed that this compound was efficient to induce apoptotic cell death (Chicca et al., 2011).

In comparison with the data on the cytostatic/cytotoxic activities of sesquiterpene lactones isolated from *Centaurea* sp., it appears that the majority of the sesquiterpene lactones studied possess an α-methylene γ-lactone moiety (Saroglou et al., 2005; El-Najjar et al., 2008), while no previous study reported on sesquiterpene lactones possessing an α-methyl γ-lactone ring, present in the Algerian *Centaurea* sp. (Djeddi et al., 2008; Djeddi et al., 2007). The present results corroborate with previously obtained, which gave evidence that among the studied sub-groups of SLs, those of elemanolides seems to be the most active (Koukoulitsa et al., 2002; Saroglou et al., 2005; Chadwick et al., 2013) and the presence of either α-methylene or α-methyl γ-lactone moiety doesn't affect the potential of cytotoxicity.

Author Contribution Statement

Dimas Konstantinos carried out the cytotoxic assays; Skaltsa Helen supervised the chemical analysis; All authors have given approval to the final version of the manuscript.

Conflicts of interest

The authors declare there are no conflicts of interest.

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