



Efficacy of medicinal plants from Algeria on cell proliferation: Involvement of caspases 3/7 and cell cycle disorder

Original Study

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Abstract

Introduction. Cancer is the main cause of mortality worldwide, and finding effective treatments with fewer side effects is crucial. Medicinal plants offer significant potential in this area. They contain bioactive compounds that may have antitumor effects. The aim of this study is to assess the cytotoxicity of various medicinal plants towards several cancer cell lines.

Materials and Methods. Antiproliferative activity of extracts from *Crataegus monogyna*, *Rhamnus alaternus*, *Lavandula dentata*, *Aristolochia baetica*, *Erica arborea*, *Cistus lanifedus* on cell lines: A-498 (kidney carcinoma), HepG2 (hepatocellular carcinoma), PLC/PRF/5 (hepatoma), MDA-MB-231 (breast adenocarcinoma), MV-4-11 (biphenotypic B myelomonocytic leukemia), and one non-tumorigenic murine fibroblast BALB/3T3 by colorimetric methods using Sulforhodamine B (SRB) and tetrazolium salt (MTT), enzyme caspases 3/7 activity, and cell cycle analysis by flow cytometry. Statistical analysis was performed by the Shapiro–Wilk and Brown–Forsythe tests ensured normality and variance equality, respectively, followed by one-way ANOVA (Dunnett's and Tukey's tests) for caspase 3/7 and two-way ANOVA (Dunnett's test) for cell cycle analysis.

Results. *Aristolochia baetica* (Aristolochiaceae) and *Lavandula dentata* (Lamiaceae) extracts have significant antiproliferative properties with caspase 3/7-independent activity which implies that the cytotoxicity of this extracts induces cell death through non-apoptotic mechanisms. Extracts from lavender and aristolochia exhibit antiproliferative effects by disrupting cell cycle progression, leading to the accumulation of dead cells in the sub-G1 phase.

Conclusion. These two species show promising therapeutic potential as antiproliferative agents, operating through mechanisms distinct from those of conventional chemotherapeutic agents.

Keywords

Aristolochia baetica • *Lavandula dentata*, caspases 3/7 • antiproliferative activity • cell cycle

1. Introduction

In 2022, there were 20 million new cases of cancer and 9.7 million deaths. Nearly 53.5 million people survived their diagnosis within five years. The data show that one in five people would develop cancer during their lifetime; including that one in nine men and one in twelve women will be affected. The number of new cases of cancer is estimated to reach 35 million by 2050, an increase of 77% compared to 2022. Aging, population growth, and changes in exposure to risk factors related to socioeconomic development are responsible for this increase. Other factors also incriminated in the progression of this disease include smoking, alcohol consumption, obesity, and air pollution [1]. Cancer, which is the leading cause of death and morbidity worldwide, is the result of disruption of essential cellular mechanisms such as growth signaling, anti-apoptotic signaling, immune response, genetic stability, and regulation of the stromal microenvironment [2].

Although often effective, conventional cancer treatments such as chemotherapy, radiation therapy and chemical drugs can lead to side effects, toxicity and reduced quality of life for patients.

These challenges, such as recurrence, multidrug resistance, fever, and fatigue, prompt the search for alternative solutions. Some anticancer drugs can cause adverse effects on tissues, mutations and tumors, disrupting antibody production and the cellular immune response [3,4,5,6]. Cancer treatment has been improved by antibody therapies and immunotherapy, which have allowed more precise targeting of cancer cells. However, their effectiveness is not universal and they have limitations such as limited reach, high cost, disease recurrence, and treatment resistance. Combination therapies and novel approaches are being explored to find more effective and less toxic anticancer molecules [2].

Secondary metabolites and semi-synthetic derivatives from plants offer a range of promising anticancer treatments which have demonstrated their efficacy against different tumors. Phytochemicals present in fruits, vegetables, spices and cereals have anticancer properties, and regular consumption can reduce the risk of cancer incidence. They act by selective inhibition of cancer cells, often associated with conventional

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chemotherapeutic treatments in order to improve the efficacy of therapy and reduce side effects [4]. According to Mizanur Rahaman et al. [7], phytochemicals can prevent carcinogenesis due to their significant potential for chemoprevention and chemotherapy. Their cytotoxic properties are attributed to DNA damage, inhibition of topoisomerases I and II, induction of apoptosis, and inhibition of tumor growth. In combination with chemotherapy drugs, they selectively kill tumor cells without affecting healthy cells such as lymphocytes and fibroblasts. Phenolics exhibit inhibitory properties on NF- κ B and AP-1 signaling pathways, enhance the immune system to recognize and destroy cancer cells, and inhibit angiogenesis, which is a key factor in tumor growth. In addition, they decrease the adhesion and invasion of cancer cells, thereby reducing their ability to metastasize [8].

The originality of this study lies in evaluating the anticancer activity of various medicinal plants "*Crataegus monogyna* (Rosaceae), *Rhamnus alaternus* (Rhamnaceae), *Lavandula dentata* (Lamiaceae), *Aristolochia baetica* (Aristolochiaceae), *Erica arborea* (Ericaceae), *Cistus lanifedus* (Cistaceae)" collected from the Bissa forest, a region renowned for its rich biodiversity in aromatic and medicinal plants. Most of these plants have never been or have been scarcely investigated in scientific research, making this an innovative exploration of potentially valuable natural resources for cancer treatment. By focusing on these specific species, the study contributes to filling a significant gap in the scientific literature and opens new perspectives for research on natural-origin anticancer treatments.

2. Materials and methods

To evaluate the activity of plant extracts four human cancer cell lines: A-498 (kidney carcinoma), HepG2 (hepatocellular carcinoma), PLC/PRF/5 (hepatoma), MDA-MB-231 (breast adenocarcinoma), and one non-tumorigenic murine fibroblast BALB/3T3 clone A31 were chosen. A series of antiproliferative activity tests were performed (3- 4 test on each cell line). The tested extracts were weighed on an analytical scale XP26/M Mettler Toledo with the accuracy 0.00001g and serial dilutions were freshly prepared for each test.

Based on the results of antiproliferative tests, two plant extracts: *Aristolochia baetica* and *Lavandula dentata* were selected for further analysis. Enzymatic caspase-3/7 activity assay was performed in HepG2 (hepatocellular carcinoma) cell line and cell cycle analysis was performed in MV-4-11 (biphenotypic B myelomonocytic leukemia) cell line.

2.1. Plant extracts

The plants, which were the subject of this study, were collected in the region of Beni Houa (Algeria) and identified by the botanists Dr Belhacine Fatima, and Dr Nadji Omar, teacher researcher at the University of Chlef. The methanol extracts were prepared from

10 g of dried powder macerated in 100 ml of methanol (leaves and fruits of *Crataegus monogyna*, leaves of *Rhamnus alaternus*, and flowers of *Lavandula dentata*) for 24 hours. The aqueous extracts (leaves of *Aristolochia baetica*, flowers of *Erica arborea*, leaves of *Cistus lanifedus*) were prepared by infusing 20 g of dried powder in 100 ml of hot water for 15 minutes. After filtration, all macerates were evaporated using a rotary evaporator to remove excess solvent. The obtained extracts were dried for 72 hours until a dry residue at 39°C was obtained, weighed, and stored refrigerated at 4°C.

2.2. Cell lines

All cell lines were obtained from American Type Culture Collection (ATCC, Rockville, Maryland, USA) and are being maintained at the Hirsfeld Institute of Immunology and Experimental Therapy, Polish Academy of Sciences (Wroclaw, Poland).

2.3. Culture media

The A-498 was cultured in a mixture (1:1) of two media: RPMI 1640 with GlutaMAX and Opti-MEM (both from Gibco, Scotland, UK/ Life Technologies Poland) containing 10% fetal bovine serum (HyClone, Life Technologies Poland) and 1.0 mM sodium pyruvate (Sigma-Aldrich Chemie GmbH, Steinheim, Germany).

The HepG2 and PLC/PRF/5 cell lines were cultured in Eagle's medium (ILET PAS, Wroclaw, Poland) containing 10% fetal bovine serum (HyClone, Life Technologies Poland) and 2 mM L-glutamine (Sigma-Aldrich Chemie GmbH, Steinheim, Germany).

The MDA-MB-231 cell line was cultured in RPMI 1640 with stable glutamine medium (Biowest, Nuaille France) containing 10% fetal bovine serum (Sigma-Aldrich Chemie GmbH, Steinheim, Germany).

The BALB/3T3 clone A31 cell line was maintained in Dulbecco Modified Eagle Medium (DMEM) (Gibco, Scotland, UK/Life Technologies Poland) medium supplemented with 10% fetal bovine serum (HyClone, Life Technologies Poland) and 2 mM L-glutamine (from Sigma-Aldrich Chemie GmbH, Steinheim, Germany).

The MV-4-11 cell line was cultured in RPMI 1640 with stable glutamine medium (Biowest, Nuaille France) containing 10% fetal bovine serum and 1.0 mM sodium pyruvate (both Sigma-Aldrich Chemie GmbH, Steinheim, Germany).

The medium used as a solvent for compounds was a mixture (1:1) of RPMI 1640 and Opti-MEM (Gibco, Scotland, UK/ Life Technologies Poland) media supplemented with 5% fetal bovine serum (HyClone, Life Technologies Poland) and 2 mM L-glutamine (Sigma-Aldrich Chemie GmbH, Steinheim, Germany).

All culture media were supplemented with 100 U/mL penicillin (Polfa Tarchomin S.A., Warsaw, Poland) and 100 μ g/mL streptomycin (Sigma-Aldrich Chemie GmbH, Steinheim, Germany). The cells were grown at 37°C in a humid atmosphere saturated with 5% CO₂.

2.4. Compounds

For the SRB assay, there were 7 plant extracts. Cisplatin, the active cytostatic drug, was used as a control for the antiproliferative assay. Solutions of the extracts were prepared freshly before being added to cells. Prior to usage, the extracts were weighed and dissolved in culture medium to the concentration of 10 mg/ml and subsequently diluted in culture medium to reach the required concentrations, ranging from 1 to 1000 µg/ml. The cytostatic compound, cisplatin (CDDP, Cisplatin 1 mg/ml Concentrate for Solution for Infusion, Accord Healthcare Poland) was diluted in culture medium to reach the required concentrations (ranging from 0.01 to 10 µg/ml).

For other assays, two plant extracts: *Aristolochia baetica* and *Lavendula dentata* were used. Cisplatin, the active cytostatic drug, was used as a control. Samples of the extracts were prepared as seen above. Camptothecin (Pol-Aura, Poland) and doxorubicin (Doxorubicinum Accord 2mg/ml, Accord Healthcare Poland) were used as controls for the caspase-3/7 activity assay. Camptothecin was dissolved in DMSO. All anticancer drugs were diluted in culture medium to reach the required concentrations depending on the type of test.

2.5. The SRB anti-proliferative assay

The antiproliferative tests were performed on all five cell lines: A-498, HepG2, PLC/PRF/5, MDA-MB-231, and BALB/3T3 as described previously by Wietrzyk et al. [9]. Briefly, 24 h prior to the addition of the tested extracts, HepG2, PLC/PRF/5, MDA-MB-231, and BALB/3T3 cells were plated in 96-well plates (Sarstedt, Germany) at a density of 1.0×10^5 /mL and A-498 cells at a density of 0.5×10^5 /mL. To determine the *in vitro* antiproliferative activity of the tested extracts, the assays were performed after 72 h exposure of the cultured cells to the varying concentrations of tested extracts (total plate incubation time: 96 h) using the sulforhodamine B (SRB) assay. All cell lines were exposed to each tested extracts at four different concentrations in the range of 1–1000 µg/ml.

After incubation, the cells were fixed with cold 50% (w/v) trichloroacetic acid (Avantor Performance Materials, Gliwice, Poland) at 4 °C for 1 h, rinsed with tap water and stained with 0.4% (w/v) solution of sulforhodamine B (Sigma-Aldrich, Germany) dissolved in 1% (v/v) acetic acid (Avantor Performance Materials, Gliwice, Poland) for 30 min. The plates with stained cells were rinsed with 1% (v/v) acetic acid and air dried at room temperature. The protein-bound dye was extracted from the stained cells with 10 mM TRIS base (Avantor Performance Materials, Gliwice, Poland) solution, and the absorbance at 540 nm was measured using a Biotek Hybrid H4 reader (BioTek Instruments, Inc., Winooski, Vermont, USA).

2.6. MTT antiproliferative assay

MTT assay was used for cells growing in suspension. 24 h prior to the addition of the tested extracts, MV4-11 cells were plated

in 96-well plates (Sarstedt, Germany) at a density of 1.0×10^5 /mL. Cells were exposed to each tested extracts at four different concentrations in the range of 1–1000 µg/ml and to cisplatin at four different concentrations in the range of 0.01 to 10 µg/ml.

After 72 h compound treatment, 20 µL of 5 mg/mL 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide (Sigma-Aldrich, Steinheim, Germany) solution in PBS (phosphate buffered saline, IIET PAS, Wrocław, Poland) was added to each well and plates were further incubated at 37°C. After 4 h, plates were centrifuged and media from each well without disturbing cells were carefully removed. Dissolution of cell-produced formazan from thiazolyl blue tetrazolium bromide was performed by adding 200 µl/well of dimethyl sulfoxide DMSO (Avantor Performance Materials, Gliwice, Poland). After 10 min, absorbance was read using a Synergy H4 Hybrid reader (BioTek Instruments, Inc., Winooski, Vermont, USA) at 570 nm wavelength. Four antiproliferative activity tests were performed as described previously by Wietrzyk et al. [9].

The activity of the tested agents was compared to the activity of cisplatin (for both SRB and MTT assays). The results were calculated as IC_{50} value (inhibitory concentration 50%)—the concentration (µg/mL) of tested agent which inhibits proliferation of 50% of the cancer cell population. IC_{50} values were calculated in Prolab-3 system based on Cheburator 0.4, Dmitry Nevozhay software for each experiment [10]. Each compound in each concentration was tested in triplicate in a single experiment, which was repeated at least four times.

2.7. Enzymatic caspase-3/7 activity assay

Cells were seeded on 24-well plates (Greiner Bio-One, Austria) at a density of 10×10^4 /well, cultured overnight and treated with extracts at various concentrations for 72 h. Cells were lysed using ice-cold lysis buffer (50 mM HEPES, 10% (w/v) sucrose, 150 mM NaCl, 2 mM EDTA, 1% (v/v) Triton X-100, pH 7.3) (IIET, Wrocław, Poland) at 4°C for 20 min. Next, 40 µL of each sample was transferred to a white, 96-well plate (Corning, NY, USA) containing 160 µL of reaction buffer (20 mM HEPES, 10% (w/v) sucrose, 100 mM NaCl, 1 mM EDTA, 10 mM DTT, 0.02% (v/v) Triton X-100, pH 7.3) (IIET PAS, Wrocław, Poland) with 10 mM Ac-DEVD-ACC ($\lambda_{ex} = 360$ nm, $\lambda_{em} = 460$ nm) as a fluorogenic substrate. Fluorescence increase correlated with caspase-3/7 level was continuously recorded at 37°C for 2 h using a Synergy H4 Hybrid reader (BioTek Instruments, Inc., Winooski, Vermont, USA).

In parallel, the SRB anti-proliferative assay was performed. Extracts at each concentration were tested in duplicate in a single experiment and each experiment was repeated at least three times. Results of caspase-3/7 activity assays were normalized to the protein content determined using the SRB method and are reported as mean relative caspase-3/7 activity in comparison to the untreated control $\pm$ SD.

Table 1. The antiproliferative activity of tested extracts presented as the mean IC₅₀ value $\pm$ standard deviation (SD). The extracts were dissolved in culture medium

	A-498	HepG2	PLC/PRF/5	MDA-MB-231	MV4-11	BALB/3T3
	IC ₅₀ (mean $\pm$ SD) [μ g/mL]					
<i>Aristolochia baetica</i>	430.20 $\pm$ 138.23	340.94 $\pm$ 143.0	722.25 $\pm$ 103.98	241.21 $\pm$ 73.38	277.08 $\pm$ 87.26	387.69 $\pm$ 142.75
<i>Cistus lanifedus</i>	528.65 $\pm$ 277.15	759.24 $\pm$ 200.31	n.a.	403.77 $\pm$ 157.14	n.t.	604.93 $\pm$ 315.09
<i>Crataegus Monogyna</i> (Leaves)	n.a.	n.a.	n.a.	598.15 $\pm$ 45.96	n.t.	n.a.
<i>Crataegus Monogyna</i> (Fruit)	n.a.	n.a.	n.a.	84.99 $\pm$ 11.11*	n.t.	n.a.
<i>Erica arborea</i>	347.27 $\pm$ 106.93	481.40 $\pm$ 148.33	n.a.	450.48 $\pm$ 158.56	n.a.	288.68 $\pm$ 11.00
<i>Rhamnus alaternus</i>	n.a.	729.25 $\pm$ 205.32	n.a.	n.a.	n.t.	n.a.
<i>Lavandula dentata</i>	375.60 $\pm$ 87.05	317.38 $\pm$ 25.75	354.15 $\pm$ 13.99	277.91 $\pm$ 24.73	684.34 $\pm$ 192.55	226.85 $\pm$ 25.63
Cisplatin	2.64 $\pm$ 0.78	1.32 $\pm$ 0.94	1.64 $\pm$ 0.29	3.11 $\pm$ 0.48	0.39 $\pm$ 0.047	0.50 $\pm$ 0.32

n.a. - not active in the range of concentrations used; n.t. - not tested.

Each compound in each concentration was tested in triplicate in a single experiment, which was repeated at least four times.

2.8. Cell cycle analysis

Cells were seeded on 24-well plates (Sarstedt, Germany) at a density of 10×10^4 /well, cultured overnight and treated with extracts at concentrations of 100 or 1000 μ g/mL and CDDP at 0.4 μ g/mL for 72 h. Then, cells were fixed for at least 24 h in 70% (v/v) ethanol, washed with PBS and incubated for 1 h at 37°C with 500 mL of 8 mg/mL RNase (Thermo Scientific, USA). Next, 25 μ L of 0.5 mg/mL propidium iodide solution (Sigma-Aldrich, Germany) was added to each sample and after 30 min incubation in darkness, samples were analyzed by flow cytometry using a BD LSR Fortessa cytometer (BD Bioscience, San Jose, USA). Extracts at each concentration were tested at least three times. Obtained results were analyzed using Flowing Software 2.5.1 (Perttu Terho, Turku Centre for Biotechnology, Finland).

2.9. Statistical analysis

The Shapiro-Wilk normality test and Brown-Forsythe test for the equality of group variances were used prior further data analysis. One-way ANOVA tests with Dunnett's multiple comparisons test and Tukey post-hoc test were used for caspase 3/7 assay. Two-way ANOVA and Dunnett's multiple comparisons test was used for cell cycle analysis. Statistical analysis was performed in GraphPad Prism 7 (GraphPad Software Inc., USA). *P*-values lower than 0.05 were considered statistically significant.

3. Results

3.1. Anti-proliferative activity of tested extracts

The obtained results from the antiproliferative assays are presented in Table 1. The antiproliferative effect of the 7 plant extracts was evaluated on human cancer cell lines: A-498 (kidney carcinoma), HepG2 (hepatocellular carcinoma), PLC/PRF/5 (hepatoma), MDA-MB-231 (breast adenocarcinoma), MV4-11 (leukemia), and a BALB/3T3 non-tumorigenic murine fibroblast

clone A31. All extracts inhibited the proliferation of cancer cells with IC₅₀ > 241.21 $\pm$ 73.38 μ g/mL with a notable effect on non-tumorigenic BALB/3T3 cells. Cisplatin, synthetic anticancer drug, expressed greater effectiveness than the extracts, tested with IC₅₀, which oscillate between 1.32 $\pm$ 0.94 and 3.11 $\pm$ 0.48 μ g/mL with side effects affecting healthy BALB/3T3 cells. Only extracts of *Lavandula dentata* and *Aristolochia baetica* were effective on PLC/PRF/5 (hepatoma).

However, the leaves and fruits extracts of *Crataegus monogyna* are inactive towards all types of cell lines except for MDA-MB-231 (breast adenocarcinoma), these extracts inhibit the proliferation of this cell line type and even more effective for fruits, which have an IC₅₀ of 84.99 $\pm$ 11.11 μ g/mL, compared to the leaves extracts whose IC₅₀ was 598.15 $\pm$ 45.96 84.99 $\pm$ 11.11 μ g/mL. Note that these two extracts were not active on control BALB/3T3 cells. The same findings for the extract of *Rhamnus alaternus*, which inhibited only HepG2 cancer cells (hepatocellular carcinoma) with an IC₅₀ of the order of 729.25 $\pm$ 205.32 μ g/mL.

The antiproliferative activity of *Lavandula dentata* and *Aristolochia baetica* extracts was also tested on MV-4-11 (biphenotypic B myelomonocytic leukemia cell line) in comparison with cisplatin, a cytostatic antineoplastic drug. The extracts expressed an inhibitory activity with an IC₅₀ of 277.08 $\pm$ 87.26 and 684.34 $\pm$ 192.55 μ g/mL.

The antiproliferative activity of *Lavandula dentata* and *Aristolochia baetica* extracts was observed against all cancer cell lines tested, therefore these extracts were chosen for further studies.

3.2. Enzymatic caspase-3/7 activity assay

Enzymatic activity of caspase 3/7 was tested at 200 μ g/mL (based on the SRB results) of extracts from *Lavandula dentata* and *Aristolochia baetica*. The tested extracts did not increase activity of caspase 3/7 at concentrations used, whereas we observed

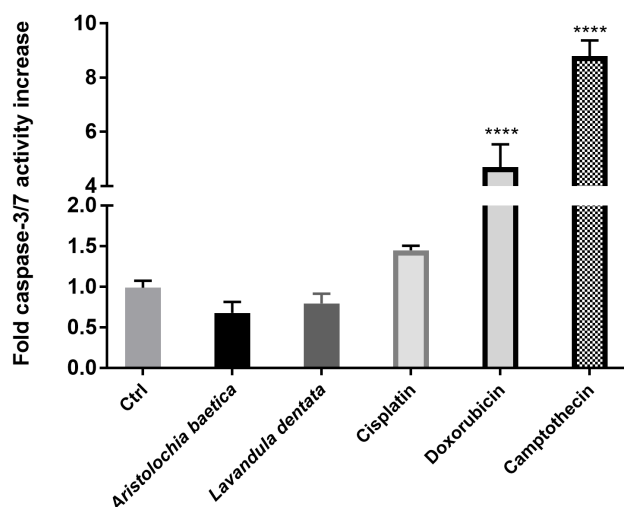


Figure 1. Caspase-3/7 activity after 72 h of HepG2 cells treatment with extracts at 200 µg/ml concentration. Concentrations of cytostatics: Cisplatin – 1 µg/ml, Doxorubicin – 10 µM, and Camptothecin – 0,5 µg/ml. Doxorubicin and Camptothecin were added 24 h before Caspase-3/7 assay. The normality of the data distribution was performed using the Shapiro-Wilk test. One-way ANOVA tests with Dunnett's multiple comparisons test were performed. **** - $p < 0,0001$ vs. ctrl. The number of observations for ctrl and *Aristolochia baetica* – 7, *Lavandula dentata* – 6, Cisplatin – 5, Doxorubicin, and Camptothecin – 3.

significant increase of its activity caused by positive control anticancer drugs (Figure 1 and Table 2).

3.3. Cell cycle analysis

Figures 2 and 3 show the impact on cell cycle progression of extracts from *Lavandula dentata* and *Aristolochia baetica* at two different concentrations (1000 and 100 µg/mL).

In a concentration of 1000 µg/mL *Lavandula dentata* and *Aristolochia baetica* extracts significantly reduced the percentage of MV4-11 cells in the G0/G1 (both extracts) and G2/M (only *Aristolochia baetica*) cell cycle phase, however, this effect is due to the high cell death rate, as evidenced by the significant increase in percentage of cells in the subG1. The effect of extracts on cell cycle progression in a concentration of 100 µg/ml are not statistically significant.

4. Discussion

Lavandula dentata L. (*Lamiaceae*) is a shrub of the *Lamiaceae* family, commonly known as "Djaïda," used in herbal medicine including for sedative, antibacterial, antifungal, antidepressant, antioxidant, and anti-inflammatory properties [11]. *Cistus lanifedus* (*Cistaceae*) or *Ouerd* in the Algerian dialect is renowned for its medicinal, ecological, and aromatic properties [12]. *Cistus* has antioxidant, immunomodulatory, bacteriostatic, and antifungal activities. In addition, it treats colds and the flu [13]. *Aristolochia baetica* (*Aristolochiaceae*) is among the plants listed in Algeria known as *Beli litha*, and its traditional uses are as an antidote, an antipyretic, an anti-inflammatory, an analgesic and

Table 2. Fold mean relative caspase-3/7 activity in comparison to the untreated control ± SD in HepG2 cells

	Mean relative caspase-3/7 activity ± SD
<i>Aristolochia baetica</i> (200 µg/ml)	0.680±0.134
<i>Lavandula dentata</i> (200 µg/ml)	0.795±0.120
Cisplatin (1 µg/ml)	1.425±0.067
Doxorubicin (10 µM)	4.890±1.096
Camptothecin (0,5 µg/ml)	8.975±0.028

an antirheumatic [14]. *Crataegus monogyna* (*Rosaceae*) *Bou mekheri* by the indigenous population is a shrub present in many Mediterranean and European countries including Algeria except on the highlands. In traditional Algerian medicine, this species is used as an antispasmodic, tonicardiac, or anti-diarrheal. In times of famine, the fruit is a source of food. *Rhamnus alaternus* (*Rhamnaceae*) or locally known by *el Quaced*, *elkheir Aouid* and *M'liles*, is widely distributed in North Africa, the Middle East, and southern Europe. In Algeria, *Rhamnus alaternus* grows in scrubland and limestone hillsides exposed to the sun. The species is purgative so it is used as a gargle against sore throats. *Erica arborea*'s (*Ericaceae*) popular name in Algeria is *Ariga* and *Bouhaddad*. It is a species native to the Mediterranean basin, although it is also found in the Canary Islands and the mountains of Central Africa. In Algeria, it is common in the Tell and is present in forests, scrubland, and on acidic soils. It is astringent, antiseptic, and diuretic [15].

Plants offer a considerable potential and reservoir of natural chemical products that could have a chemoprotective effect. Some of these compounds act as anticancer agents through various biological responses, such as controlling the action of proteins and enzymes, inducing apoptosis, inhibiting cyclin-dependent kinases and the nuclear factor-kappa B cascade. Others are anti-metastatic and hinder the invasion of cancer cells. The leaves and roots of the *Aristolochiaceae* family have anticancer effects on several types of human cancer cell lines, including A-549, HCT-116, PC-3, and THP-1 [5].

Lavender and aristolochia extracts were the only extracts active against all the tested cancer cell lines and the only ones active against the PLC/PRF/5 line. Akindele et al. [16], reported anticancer activity of aristolochia root extract on A549 (lung), HCT-116 (colon), PC3 (prostate), A431 (skin), HeLa (cervix), and THP-1 (leukemia) cell lines, as detected by the sulforhodamine B (SRB) *in vitro* cytotoxicity assay. In an ethnobotanical survey about anticancer medicinal plants used in Morocco, Alami Merrouni and Elachouri [17] cited the use of a decoction of *Aristolochia baetica* in the treatment of cancer.

In a literature review, Bouyahya et al. [18] reported several biological activities related to *Lavandula dentata*, including its anticancer activity. Even at low concentrations, its extracts

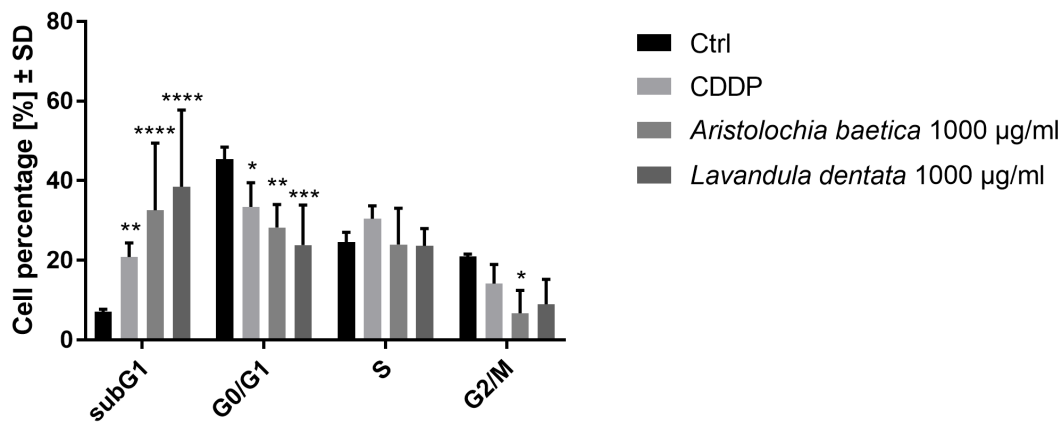


Figure 2. The cell cycle analysis for extracts at 1000 µg/ml after 72 hours of treatment of MV4-11 cells. Concentrations of cisplatin – 0.4 µg/ml. The number of observations for ctrl – 5, *Aristolochia baetica* and *Lavandula dentata* – 4, Cisplatin – 6. Two-way ANOVA and Dunnett's multiple comparisons test was used for cell cycle analysis. *– $p < 0.05$, **– $p < 0.01$, ***– $p < 0.001$, ****– $p < 0.0001$ vs. ctrl

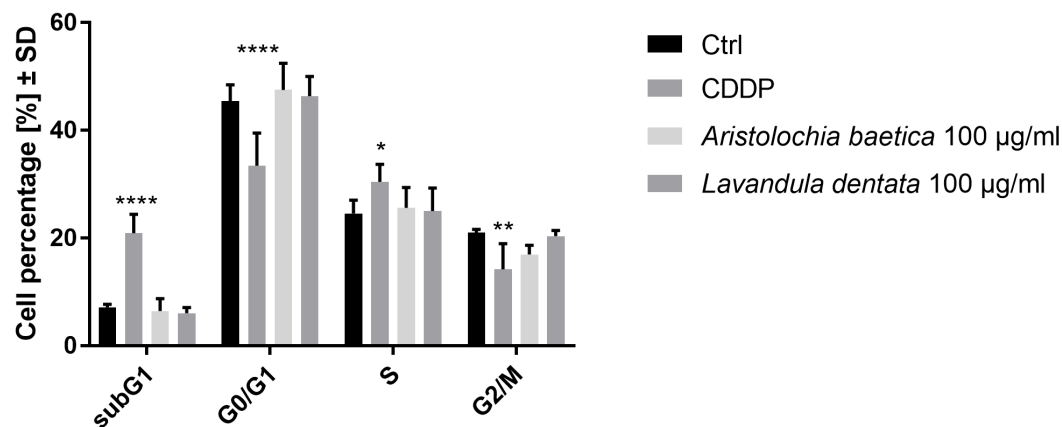


Figure 3. The cell cycle analysis for extracts at 100 µg/ml after 72 hours of treatment of MV4-11 cells. Concentrations of cisplatin – 0.4 µg/ml. The number of observations for ctrl – 5, *Aristolochia baetica* and *Lavandula dentata* – 4, Cisplatin – 6. Two-way ANOVA and Dunnett's multiple comparisons test was used for cell cycle analysis. *– $p < 0.05$, **– $p < 0.01$, ****– $p < 0.0001$ vs. ctrl.

specifically inhibit enzymes, membrane or intracellular receptors, proteins, or signaling pathways involved in carcinogenesis. Indeed, various plants have demonstrated promising properties for preventing liver cancer.

The aqueous extracts of the fruits and leaves of *Crataegus monogyna* were effective only against the MDA-MB-231 cell line. The hawthorn fruit extract exhibited significant activity against the A549 lung cancer cell line [19]. The extracts obtained from various parts of hawthorn inhibited the growth of four human tumor cell lines: MCF-7, NCI-H460, HeLa, and HepG2 [20].

The extract of *Rhamnus alaternus* was effective only on the HepG2 cell line. The experimental investigations of Chatti et al. [21], on murine B16-F10 melanoma cells, showed that *Rhamnus alaternus* extract inhibited the proliferation of cancer cells, leading to the accumulation of cells in sub-G1 and S phase in a dose-dependent manner. Additionally, a pronounced

antiproliferative effect was observed on human leukemia K562 cells [22] and induction of apoptosis in this same cancer cell line [23]. On the other hand, the aqueous extracts of *Cistus lanifedus* and *Erica arborea* exhibited antiproliferative activity on all tested cancer cell lines except the PLC/PRF/5 line. The aqueous extract from the leaves of *C. lanifedus* demonstrated a strong ability to inhibit the proliferation of various human cancer cells as M220 pancreatic cancer cells, MCF7/HER2, and JIMT-1 breast cancer cells [24]. The inhibitory power of cell proliferation by the species *C. ladanifer* tested by Bouothmany et al. [25], using MTT assay. This medicinal plant exhibited promising antiproliferative activity against the HepG2, 22Rv1, and MDA-MB-231 cancer cell lines as revealed by the MTT assay.

The extracts of *Lavandula dentata* and *Aristolochia baetica* have shown antiproliferative effects by disrupting cell cycle progression. These tested extracts had cytotoxic effects on the

proliferation of MV4-11 cells and induced cell death of cancer cells, especially in the G2/M phase. The fact that cytotoxic effects were observed in the G2/M phase but not in other phases suggests the selectivity of these extracts for cell division. Extracts from both species (and even more so in the case of *Aristolochia baetica*) clearly induced cell death. This extract has a pronounced inhibitory effect on cell cycle progression in the G2/M phase (a very strong inhibitory effect on mitosis) and inhibits cell growth, possibly through DNA damage or activation of cell cycle checkpoints, suggesting that it prevents progression to and through mitosis. Elshnoudy et al. [26] previously reported these findings. Indeed, many medicinal plants play a crucial role as checkpoint inhibitors in HepG2 cancer cells. *Ammi majus* induces of G2/M cell cycle arrest and apoptosis that lead to inhibition the proliferation of these cells. The essential oil of *Phlomis* causes apoptosis in HepG2 cells by increasing cell accumulation in the subG1 and G2/M phases of the cell cycle, decreasing the S and G0/G1 phases through the activation of caspases-3 and -9, and inhibiting cyclin-dependent kinase.

The disruption of the cell cycle by lavender and aristolochia extracts was caspase 3/7-independent, as these two extracts did not activate caspase 3/7 enzymes, which are indicators of apoptosis. This suggests that our extracts induce cell death through non-apoptotic mechanisms. The accumulation of cells in the sub-G1 phase is often associated without repair DNA damage, another potential predictor of a caspase-independent cell death pathway. Indeed, according to Kroemer et al. [27], cell death can be triggered through alternative pathways such as autophagy or necroptosis. Autophagy (type II or macroautophagic cell death) is a complex degradation process leading to sequestration the sequestration of cytoplasm and organelles within vesicles for subsequent degradation by lysosomes to maintain homeostasis or in response to various stimuli. This process allows the cell to 'self-cannibalize' [28].

5. Limitations of the study

The chemical composition of medicinal plants varies depending on geographic origin, cultivation conditions, harvesting time, and post-harvest processing, and leading to potential inconsistencies in results. Additionally, many bioactive compounds have low bioavailability and stability, limiting their *in vivo* efficacy despite promising *in vitro* outcomes. Finally, high concentrations of certain extracts may cause toxicity to normal cells, highlighting the need for dose-response studies and targeted delivery strategies to optimize therapeutic applications.

6. Conclusion

The plant extracts that were the subject of this study expressed promising therapeutic potential as antiproliferative agents, acting by mechanisms distinct from those of conventional chemotherapeutic agents. Indeed, *Aristolochia baetica* and

Ladandula dentata demonstrated antiproliferative activity on several types of cancer cell lines marked by decreased cell viability, cell cycle disorder visualized by flow cytometry and absence of activation of caspases 3/7 (apoptotic markers), suggesting that plant extracts induce cell death by a non-apoptotic mechanism. These results indicate that plant extracts may act by an alternative mechanism of cell death induction, paving the way for future research to elucidate and understand the underlying mechanisms.

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Authors' contributions

Conceptualization, L.G.; methodology, L.G., D.K.; software, L.G., D.K.; validation, D.K.; formal analysis, D.K.; investigation, L.G., D.K.; resources, L.G., D.K.; data curation, D.K.; writing—original draft preparation, L.G.; writing—review and editing, J.W., D.K.; visualization, L.G., D.K.; supervision, J.W.; project administration, L.G.; funding acquisition, L.G., J.W. All authors who contributed to the article approved the submitted version.

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Conflict of interest

The authors have no conflicts of interest to declare.

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