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Journal Menu

 Contents

PDF/EPUB

... More

## Abstract

### Aim/Background

This study aimed to investigate the antioxidant capacity, hemolytic effect, and cytotoxic potential of the alkaloid fraction isolated from *Fumaria agraria*, with an emphasis on its possible use as a therapeutic agent against cancer cells.

### Materials and Methods

The antioxidant activity of the alkaloidal fractions was evaluated using the 2,2-diphenyl-1-picrylhydrazyl (DPPH), reducing power, and phosphomolybdate assays. Hemolytic activity was assessed to determine potential erythrocyte toxicity. Cytotoxicity was tested on Michigan Cancer Foundation-7 (MCF-7), Michigan Cancer Foundation-231 (MDA-MB-231), and Michigan Cancer Foundation-10A (MCF-10A) breast cell lines. Cell cycle distribution and cyclin expression were analyzed by flow cytometry, and apoptosis was detected by dual staining with propidium iodide (PI) and Annexin V.

### Results

The basic fraction (BF) showed strong antioxidant activity (half-maximal inhibitory concentration [IC<sub>50</sub>] = 50.34 µg/mL in the DPPH assay) and low hemolytic toxicity (4.1% at 2 mg/mL), lower than boldine (5.8%). It exhibited notable cytotoxicity against MCF-7 cells and moderate effects on MDA-MB-231 cells, while normal MCF-10A cells were less sensitive. Flow

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cytometry revealed G<sub>0</sub>/G<sub>1</sub> arrest in MCF-7 cells and G<sub>2</sub>/M accumulation in MDA-MB-231 cells. In MCF-7 cells, cyclin D1, cyclin E, and MYC proto-oncogene, bHLH transcription factor (MYC) expression decreased, whereas cyclin-dependent kinase 1 (CDK1)/cyclin B1 activity increased in MDA-MB-231 cells. Apoptosis occurred in MCF-7 cells but not in MDA-MB-231 cells.

## Conclusion

The alkaloid fraction from *F. agraria* displayed potent antioxidant and selective cytotoxic effects of BF, particularly against MCF-7 breast cancer cells, with minimal toxicity toward normal cells. These findings highlight its potential as a promising source of bioactive compounds for therapeutic development.

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## Introduction

Breast cancer is one of the most prevalent forms of cancer affecting women and can result from genetic, environmental, or ageing factors ([Russo & Russo, 1998](#)). It can originate in the cells of the lobules (milk-producing glands) or ducts (passages that drain milk from the lobules to the nipple) ([Ali & Coombes, 2002](#)). Breast cancer stands as the leading cause of cancer-related death in Western countries ([Russo & Russo, 1998](#)). Conventional cancer treatments have played a crucial role as a therapeutic approach, typically combining surgery, multi-therapeutic agents, and anti-cancer drugs ([Butler, 2004](#); [Paterson & Anderson, 2005](#)). These anti-cancer agents induce cell cycle arrest or death through apoptotic or non-apoptotic mechanisms ([Brown & Attardi, 2005](#); [Okada & Mak, 2004](#)).

While drugs like doxorubicin, gemcitabine, mitomycin, vinorelbine, and paclitaxel are employed to treat breast cancer, they come with significant side effects, including anemia, diarrhea, fatigue, hair loss, mouth and throat sores, vomiting, and weight changes ([Kurebayashi, 2003](#)). A crucial issue lies in the fact that these drugs may become ineffective at advanced stages of cancer. Therefore, there is an urgent need for new agents capable of treating breast cancer at all stages with few or no side effects. Moreover, phytochemistry represents an alternative source for the exploration of anti-cancer compounds such as alkaloids.

*Fumaria agraria* is one of these plants of pharmaceutical interest. It belongs to the family of Fumariaceae, which includes about 40 species ([Suau et al., 2004](#)). They are annual flowering plants with a wide distribution in the Mediterranean region ([Suau et al., 2005](#)). These plants

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have been used in the indigenous system for the treatment of various diseases ([Goetz et al., 2009](#)) and are still used in traditional medicine, in cases of hepatobiliary dysfunction, gastrointestinal disorders, and antioxidant activity ([Maiza-Benabdesselam et al., 2007](#); [Singh et al., 2010](#)). The biological activity of *Fumaria* is mostly associated with the presence of isoquinoline alkaloids ([Suau et al., 2005](#)).

Despite its traditional use for presumed health benefits, the anti-cancer potential of *F. agraria* remains unexplored. This study aimed to evaluate the antioxidant capacity and selective cytotoxicity of its alkaloid fraction against human breast cancer cell lines, representing the first report of its kind and highlighting its potential as a novel therapeutic source.

## Materials and Methods

### Plant Material

Aerial parts of *F. agraria* Lag. were collected in March 2022, at the flowering and fruit setting stages, in Bejaia city, North East Algeria (36°36'28.80"; 4°41'2.40") and referenced under code NCY013571. A dried voucher specimen was archived in the Herbarium of the Conservatoire and Botanical Gardens of Nancy, France.

The plant material was washed, oven-dried at 40 °C for 5 days, ground to a 63 µm powder using an electric mill, and stored in the dark at 20 °C until use.

### Cell Culture

Michigan Cancer Foundation-7 (MCF-7) and Michigan Cancer Foundation-231 (MDA-MB-231) breast cancer cell lines, obtained from the American Type Culture Collection (ATCC, Manassas, VA), were cultivated in Eagle's Minimum Essential Medium supplemented with 5% fetal calf serum (Lonza, Levallois-Perret, France), 2 mM L-glutamine, and 0.06% 4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid (HEPES). The immortalized human mammary epithelial cell line Michigan Cancer Foundation-10A (MCF-10A) was cultured in a complete growth medium composed of Dulbecco's modified Eagle's medium/nutrient mixture (DMEM/F12) supplemented with 5% fetal calf serum, 20 ng/mL epidermal growth factor, 10 ng/mL insulin, and 0.5 mg/mL hydrocortisone. All cell lines were incubated in a 5% CO<sub>2</sub>-humidified environment at 37 °C.

The alkaloid fractionation and extraction of *F. agraria* followed [Bougoffa-Sadaoui et al. \(2016\)](#). Dried aerial parts (15 g) were extracted with 150 mL ethanol in a Soxhlet apparatus. The concentrated ethanolic extract was suspended in water, defatted with petroleum ether, and fractionated by chloroform extraction. The neutral fraction (NF) was obtained from the initial chloroform extraction. After acidification to pH 2.5, the acidic fraction (AF) was extracted, and finally, after basification to pH 8.5, the basic fraction (BF) was collected.

A preliminary phytochemical screening was performed to confirm the presence of alkaloids in the extract fractions of *F. agraria*.

## Determination of Antioxidant Activity

### DPPH• Radical Scavenging Activity

The scavenging activity of the alkaloid fractions from the aerial part of *F. agraria* against the 2,2-diphenyl-1-picrylhydrazyl (DPPH•) radical was assessed using the method described by [Shirwaikar et al. \(2006\)](#). Briefly, 1.5 mL of each fraction was mixed with 1.5 mL of an ethanolic DPPH• solution (0.1 mM). After 20 min of incubation, the absorbance of the reaction mixture was measured at 517 nm, and the percentage of inhibition was calculated using Equation (1).

$$\text{DPPH}\cdot \text{ Radical Scavenger \%} = \frac{A_c - A_s}{A_c} \times 100 \quad (1)$$

$A_c$ : The control absorbance

$A_s$ : The sample absorbance

### Measurement of Reducing Power

The reducing power was determined according to the method recommended by [Amarowicz et al. \(2004\)](#). A volume of 1 mL of each fraction or standard was mixed with 1 mL of phosphate buffer (0.2 M, pH 6.6) and 1 mL of  $K_3Fe(CN)_6$  (1%). After incubation at 50 min, 1 mL of 10% trichloroacetic acid (TCA), followed by 1.5 mL of  $H_2O$  and 0.3 mL of (0.1%), was added. The absorbance was measured at 700 nm.

### Total Antioxidant Capacity

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The total antioxidant activity was measured by the molybdate phosphate test according to the protocol of [Prieto et al. \(1999\)](#). 0.1 mL of each fraction at different concentrations, as well as the standard, was mixed with 1 mL of reagent (0.6 M sulfuric acid, 28 mM sodium phosphate, and 4 mM ammonium molybdate). Then the mixture was incubated at 95 °C for 90 min, and the absorbance was read at 695 nm.

### Cytotoxicity Test

According to the method of [Okoko and Ere \(2012\)](#), with some modifications, 500 µL of erythrocytes (10%) (prepared as described previously) were added to 500 µL of the alkaloid fraction (AF, NF, and BF) at various concentrations. The mixture was incubated for 30 min at 37 °C, then centrifuged at 3,000 rpm for 10 min. The absorbance of the supernatant was measured at 540 nm. The increase in absorbance is proportional to the rate of hemolysis (Equation (1)).

### Study of Apoptosis Induction and Cell Cycle Arrest

The effect of the BF on the cell cycle and apoptosis was assessed by flow cytometry (fluorescence-activated cell sorting [FACS]). After 48 h of adhesion, MCF-7, MDA-MB-231, and MCF-10A cells were treated for 72 h with the BF at 50, 100, and 150 µg/mL, respectively. The cells were then collected, fixed in cold ethanol, and prepared for analysis. Cell cycle distribution was examined after ribonuclease (RNase) treatment and propidium iodide (PI) staining, while apoptosis was evaluated using double staining with Annexin V conjugated with fluorescein isothiocyanate (Annexin V-FITC/PI). Samples were analyzed by flow cytometry, and the data were processed with Cyflogic software to determine the distribution of cell cycle phases and apoptotic populations.

### Western Blotting Analysis

Cell extracts were prepared in radioimmunoprecipitation assay (RIPA) buffer containing protease inhibitors, and the soluble protein fraction was collected after centrifugation. Protein concentration was determined using a bovine serum albumin (BSA) standard curve. A total of 25 µg of denatured proteins were subjected to sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE), transferred onto a nitrocellulose membrane, and incubated overnight at 4 °C with specific primary antibodies. Membranes were then incubated with horseradish peroxidase (HRP)-conjugated secondary antibodies, detected by chemiluminescence, and quantified by densitometric analysis. The antibodies used included cyclin-dependent kinase 1 (CDK1), cyclin-dependent kinase 2 (CDK2), cyclin-dependent kinase

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4 (CDK4), cyclin D1, cyclin E, cyclin B1, cyclin-dependent kinase inhibitor (p27<sup>kip1</sup>), B-cell lymphoma 2 protein (Bcl-2), Bcl-2-associated X protein (Bax), and  $\beta$ -actin as an internal control.

Statistical Analysis

Results were expressed as means  $\pm$  standard error of the mean (SEM). Statistical comparisons were performed using two-tailed unpaired *t*-tests (two groups) or one-way analysis of variance (ANOVA) (three or more groups), followed by comparison with the control group. A *p* value < .05 was considered statistically significant.

Results

Antioxidant Activity

The anti-radical activity of the alkaloid fractions NF, AF, and BF, as well as that of boldine, showed a dose-dependent relationship between concentration (0–200  $\mu$ g/mL) and DPPH• radical inhibition. At 200  $\mu$ g/mL, the inhibition percentages were 70% for BF, 63% for NF, and 59% for AF, compared with 88% for boldine. The corresponding half-maximal inhibitory concentration (IC<sub>50</sub>) values were 136.12  $\pm$  2.5  $\mu$ g/mL (NF), 106.78  $\pm$  3.4  $\mu$ g/mL (AF), 50.34  $\pm$  4.8  $\mu$ g/mL (BF), and 38.75  $\pm$  2.85  $\mu$ g/mL for boldine ([Table 1](#)), indicating a stronger antioxidant activity for the BF.

**Table 1.** The IC<sub>50</sub> Values of the Different Fractions and Boldine Against the DPPH• Radical.

| Fraction | IC <sub>50</sub> ( $\mu$ g/mL) |
|----------|--------------------------------|
| NF       | 106.78 $\pm$ 3.4               |
| AF       | 136.12 $\pm$ 2.5               |
| BF       | 50.34 $\pm$ 4.8                |
| Boldine  | 38.75 $\pm$ 2.85               |

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**Note:** AF: Acidic fraction; BF: Basic fraction; DPPH: 2,2-diphenyl-1-picrylhydrazyl; NF:

Neutral fraction; IC<sub>50</sub>: Half-maximal inhibitory concentration.

The reducing power and total antioxidant activity assays revealed a marked reducing potential (Table 2). The BF exhibited the highest absorbance for reducing power ( $0.89 \pm 0.03$  at 200 µg/mL), exceeding that of boldine ( $0.75 \pm 0.01$ ), while the NF ( $0.40 \pm 0.01$ ) and AF ( $0.35 \pm 0.01$ ) were less active. Total antioxidant activity showed significant reducing capacities for all fractions, with absorbances of  $0.56 \pm 0.01$  (NF),  $0.33 \pm 0.004$  (AF), and  $0.76 \pm 0.01$  (BF) at 100 µg/mL; however, BF remained less active than boldine ( $1.13 \pm 0.04$ ).

**Table 2.** The Reducing Power and Total Antioxidant Activity of the Different Fractions and Boldine.

| Test                                       | AF               | NF              | BF              | Boldine         |
|--------------------------------------------|------------------|-----------------|-----------------|-----------------|
| Reducing power (200 µg/mL)                 | $0.35 \pm 0.01$  | $0.40 \pm 0.01$ | $0.89 \pm 0.03$ | $0.75 \pm 0.01$ |
| The total antioxidant activity (100 µg/mL) | $0.33 \pm 0.004$ | $0.56 \pm 0.01$ | $0.76 \pm 0.01$ | $1.13 \pm 0.04$ |

**Note:** AF: Acidic fraction; BF: Basic fraction; NF: Neutral fraction.

Study of the Cytotoxicity of Alkaloid Fractions of *Fumaria agraria*

The hemolytic effect of the alkaloid fractions of *F. agraria* was evaluated at concentrations of 500, 1,000, and 2,000 µg/mL (Table 3). All fractions showed a dose-dependent response. At 500 µg/mL, the BF and boldine exhibited hemolysis rates of 1.21% and 1.89%, respectively, values comparable to the control (2%), indicating no significant cytotoxicity. The AF and NF showed higher hemolysis rates (2.5% and 4.4%).

**Table 3.** Hemolysis Rate of Erythrocytes Treated with the Different Fractions of *Fumaria agraria* and the Standard.

| Extract (µg/mL) | 500   | 1,000 | 2,000 |
|-----------------|-------|-------|-------|
|                 | 1.21% | 1.8%  | 4.1%  |

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|         |       |       |        |
|---------|-------|-------|--------|
| AF      | 2.5%  | 3.01% | 5.45%  |
| NF      | 4.4%  | 8.5%  | 12.83% |
| Boldine | 1.89% | 3.83% | 5.8%   |
| Control | 2%    |       |        |

**Note:** AF: Acidic fraction; BF: Basic fraction; NF: Neutral fraction.

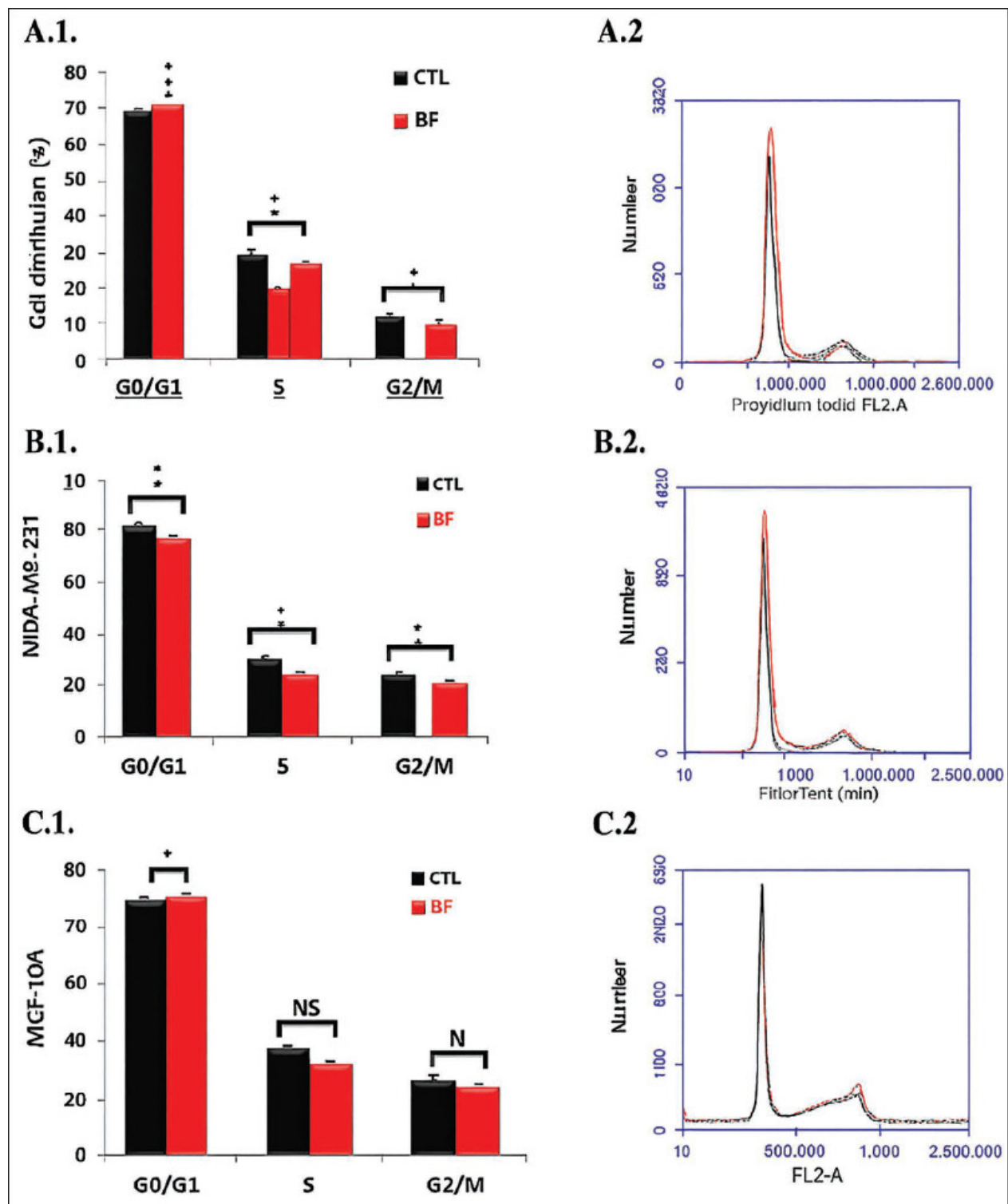
At 1,000 µg/mL, a slight increase in hemolysis was observed for BF (1.8%) and AF (3.01%), while boldine reached 3.83%. The NF showed the strongest effect (8.5%).

At 2,000 µg/mL, all fractions exceeded the control, with BF showing a moderate effect (4.1%). The AF (5.45%) and boldine (5.8%) presented similar values, while NF showed the highest hemolytic activity (12.83%).

### Basic Fraction of Fumaria agraria Induces Cell Cycle Arrest

This study assessed the impact of the BF on cell-cycle progression and its anti-proliferative effects on breast cancer cells (estrogen receptor [ER<sup>+</sup>] MCF-7, ER<sup>-</sup> MDA-MB-231) and normal mammary epithelial cells (MCF-10A) ([Figure 1](#)). After 72 h of treatment with 150 µg/mL, MCF-10A cells showed overall stable cell-cycle distributions, indicating minimal cytotoxicity.





**Figure 1.** Cell-cycle Arrest Triggered by the Basic Fraction (BF).

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In MCF-7 cells, treatment with 50  $\mu\text{g/mL}$  of the BF led to significant  $G_1$ -phase accumulation ( $74\% \pm 0.91\%$ ) compared to controls ( $60.65\% \pm 1.18\%$ ), indicating  $G_1$  cell-cycle arrest. This was associated with marked downregulation of cyclins D1 and E, key regulators of the  $G_1$ -to-S

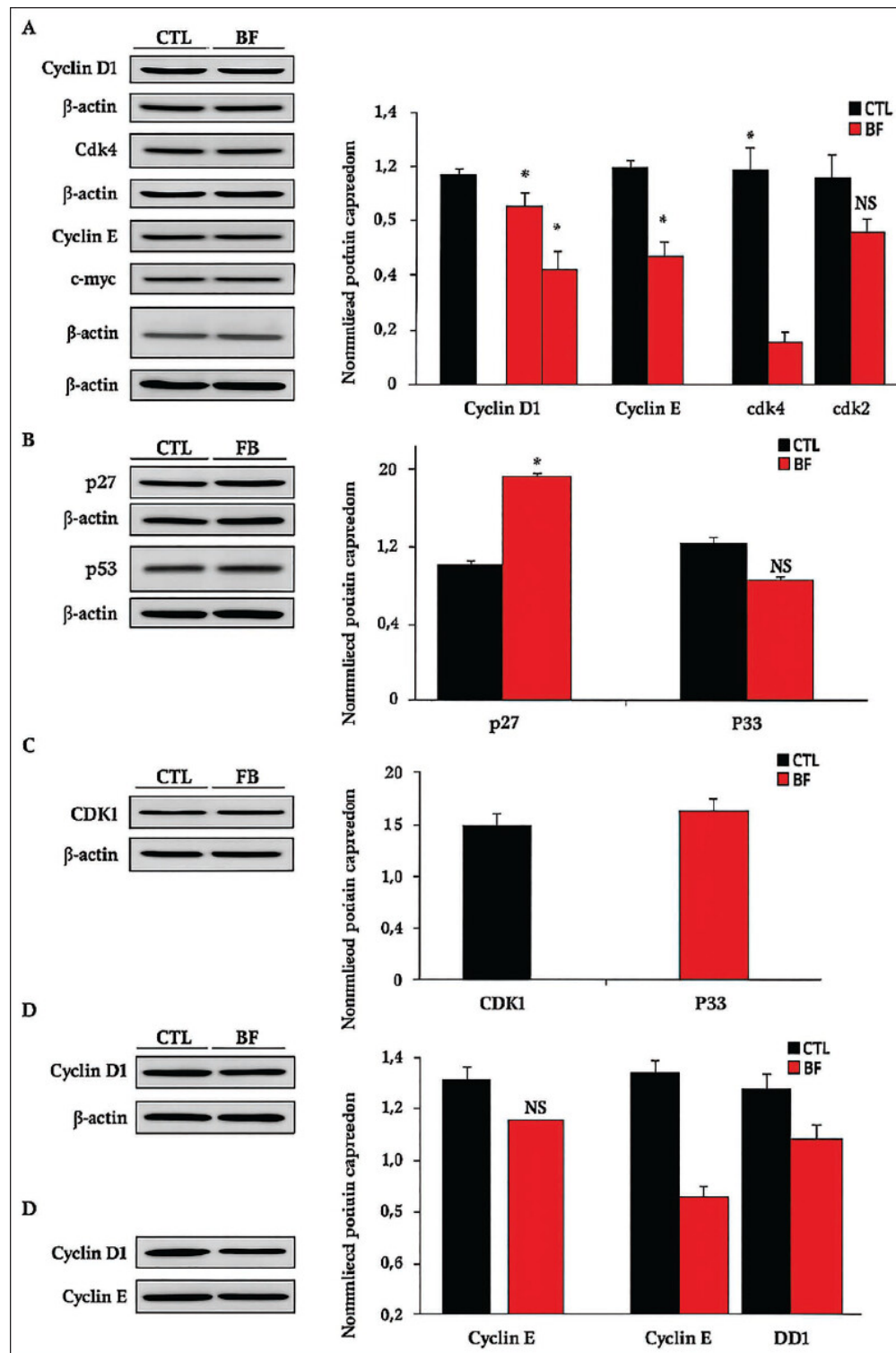
transition, while CDK2 and CDK4 expression remained unchanged. MYC proto-oncogene, bHLH transcription factor (c-MYC) protein levels were also reduced.

In MDA-MB-231 cells treated with 100 µg/mL of the BF, a pronounced G<sub>2</sub>-M phase accumulation (20.3% ± 1.63% vs 14% ± 1.17% in controls) was observed, accompanied by reduced CDK1 expression, whereas cyclin B1 levels remained stable.

Overall, the BF induced cell-cycle arrest in a cell-type-specific manner: G<sub>1</sub> arrest in ER<sup>+</sup> MCF-7 cells and G<sub>2</sub>-M accumulation in triple-negative MDA-MB-231 cells, with minimal impact on normal MCF-10A cells.

### Basic Fraction of *Fumaria agraria* Affects Apoptosis in Cells

Next, we explored the impact of the essential alkaloid fraction extract of *F. agraria* on cell death. Cells were collected after 72 h of treatment, double-stained with PI and Annexin V-FITC, and analyzed by FACS; only Annexin V<sup>+</sup> cells were considered apoptotic ([Figure 2](#)).



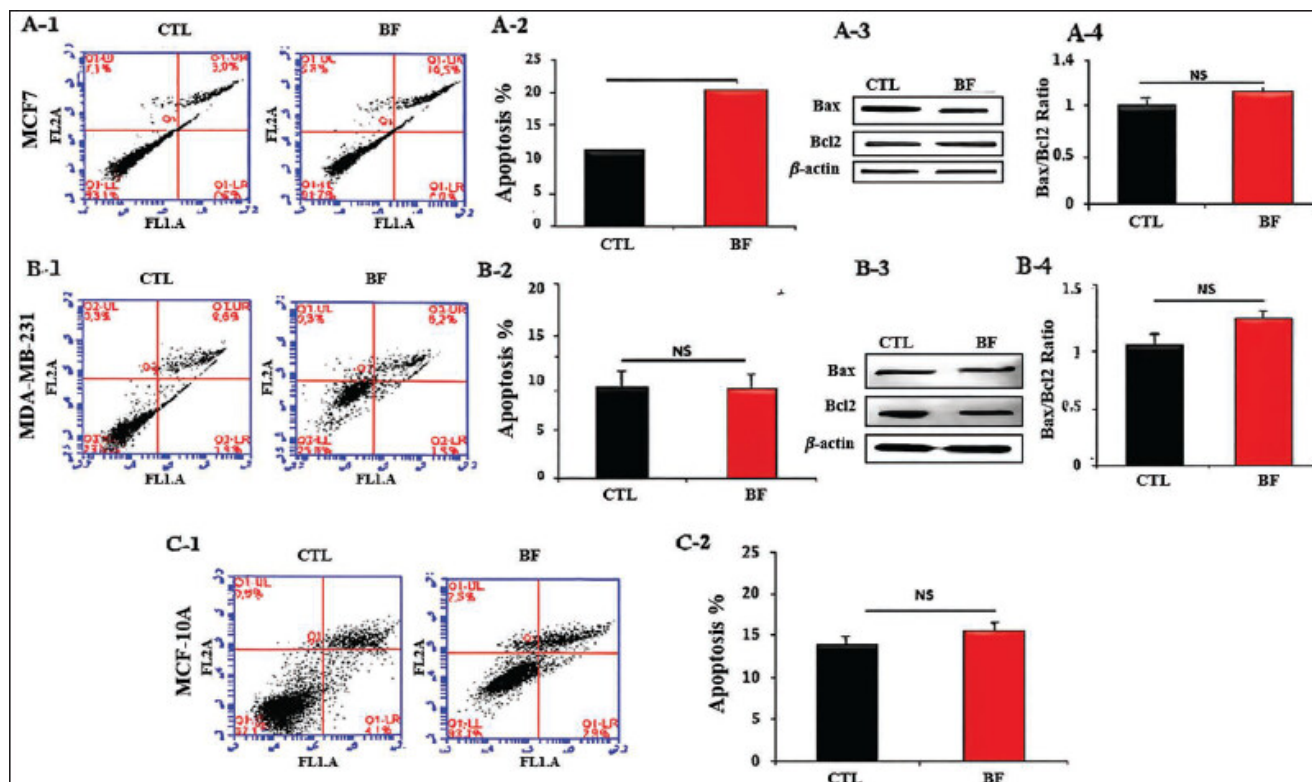
**Figure 2.** Differential Cell-cycle Regulation by the Basic Fraction (BF) from *Fumaria agraria* in Cancer and Normal Cells.

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A disparity in behavior was observed between the breast cancer cell lines. In MCF-7 cells, a apoptotic subpopulation (PI<sup>+</sup>/Annexin V<sup>+</sup>) was detected (16.5%  $\pm$  0.05% vs 5.8%  $\pm$  0.04%

for the BF and control, respectively) ([Figure 3A-1 and 3A-2](#)). In MDA-MB-231 cells, the population shifted directly to the PI<sup>+</sup> quadrant ( $6.5\% \pm 0.009\%$  vs  $0.3\% \pm 0.003\%$  for the BF and control, respectively) without passing through the PI<sup>-</sup>/Annexin V<sup>+</sup> stage ([Figure 3B-1 and 3B-2](#)).



**Figure 3.** Apoptosis Test of the Basic Fraction (BF) Performed by Annexin V Labeling.

To better understand the mode of cell death in MCF-7 cells, we examined the expression of the anti-apoptotic protein Bcl-2 and the pro-apoptotic protein Bax by immunoblot ([Figure 3A-3 and 3A-4](#)). No changes were observed in the Bax/Bcl-2 ratio compared to control.

The apoptotic effect of the BF on MCF-10A cells was negligible ( $0.1\% \pm 0.03\%$  vs  $0.3\% \pm 0.03\%$  for the BF and control) ([Figure 3C](#)).

## Discussion

The stronger DPPH• radical scavenging activity of the BF is likely due to its higher purity, whereas NF and AF may be affected by antagonistic effects or steric hindrance ([Bougoffa-Bououi et al., 2016](#)). Modifications at C6 and C7 in alkaloids can diminish activity, explaining

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the differences between fractions. These results align with previous studies reporting strong DPPH• scavenging by isoquinoline alkaloids such as *N*-methyl-tetrahydropapaveroline, fumarizine, and berberine, which can outperform standard antioxidants like α-tocopherol and ascorbic acid ([Iwasa et al., 2001](#); [Racková et al., 2004](#)).

The high reducing activity of the BF may be related to its chemical composition, including protopine, which facilitates electron transfer. Hydroxyl- and nitrogen-rich molecules in BF enhance electron transfer, explaining its superior activity compared to NF and AF ([Bougoffa-Sadaoui et al., 2016](#); [Hinneburg et al., 2006](#); [Soobratte et al., 2008](#)). Literature comparisons, such as berberine's IC<sub>50</sub> of 42.7 µg/mL versus ascorbic acid's 62.4 µg/mL ([Shirwaikar et al., 2006](#)), support that quaternary isoquinoline alkaloids can be potent radical scavengers.

Assessing the hemolytic effect provides a simple method to evaluate cytotoxicity. The alkaloids of *F. agraria*, belonging to the isoquinoline alkaloid family, are known for their anti-cancer properties. Compared to classical chemotherapeutic agents, their cytotoxicity appears relatively low, suggesting that *F. agraria* alkaloids could be considered low-cytotoxicity agents in the context of conventional chemotherapy ([Mameri et al., 2021](#)).

The differing responses of MCF-7 and MDA-MB-231 cells reflect their distinct molecular profiles. MCF-7 cells (ER<sup>+</sup>, wild-type p53) efficiently activate p53-dependent pathways, leading to G<sub>1</sub>-phase arrest and apoptosis, whereas MDA-MB-231 cells (triple-negative, mutant p53) show limited apoptotic response due to impaired p53 function and absence of hormonal receptors ([Zhang et al., 2024](#)).

G<sub>1</sub> arrest in MCF-7 cells is associated with reduced cyclin D1 and E, preventing Rb phosphorylation, inactivating E2F, and blocking transcription of S-phase genes. The observed decrease in c-MYC reinforces the anti-proliferative effect ([Amendola et al., 2009](#)). In contrast, G<sub>2</sub>-M accumulation in MDA-MB-231 cells is linked to reduced CDK1 levels, indicating interference with mitotic machinery without disrupting normal cell-cycle regulation ([Wang et al., 2024](#)).

The anti-proliferative effects may be partly attributed to protopine, the main compound in the BF (69%), which modulates cyclins, CDKs, and mitochondrial apoptotic pathways ([Bougoffa-Sadaoui et al., 2016](#)).

The BF also induces apoptosis-related effects in breast cancer cells. In MCF-7 cells, apoptosis is observed, whereas in MDA-MB-231 cells, the unchanged Bax/Bcl-2 ratio suggests resistance, possibly involving alternative mechanisms such as autophagy. Supporting this, treatment of PC-3 cells with the BF induced Bcl-2 hyperphosphorylation and reduced

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myeloid cell leukemia 1 (Mcl-1) expression. Finally, the BF exhibits low sensitivity to P-gp, a broad-spectrum adenosine triphosphate (ATP)-dependent efflux pump, suggesting potential for overcoming multidrug resistance and developing therapeutic strategies against cancers such as prostate cancer ([Chen et al., 2012](#); [Levine & Kroemer, 2008](#)).

## Conclusion

In the present study, the alkaloid fractions isolated from *F. agraria* exhibited significant biological activity. Among them, the BF proved to be the most potent, showing remarkable antioxidant activity and selective cytotoxicity against MCF-7 breast cancer cells, while exhibiting minimal hemolytic and cytotoxic effects on normal MCF-10A cells. These findings suggest that the bioactive alkaloids present in this fraction, particularly protopine, may contribute to its antioxidant and anti-cancer properties. In conclusion, *F. agraria* represents a promising natural source of alkaloid compounds with potential therapeutic applications in the prevention and treatment of breast cancer.

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The authors would like to express their sincere gratitude to Professor Bedjou, Director of the Research Laboratory, as well as to the engineers and all laboratory members for their valuable assistance and support throughout this study.

## Ethical Approval

The study was approved by the Institutional Ethics Committee (Approval No. EA4667-LPCM/2023/015) for *in vitro* research on human cell lines.

## Informed Consent

Not applicable. This study involved *in vitro* experiments using established human cell lines and did not involve human participants.

## Declaration of Conflict of Interests

The authors declared no potential conflicts of interest with respect to the research, authorship, and/or publication of this article.

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## Abbreviations

AF: Acidic fraction; Annexin V FITC: Annexin V conjugated with fluorescein isothiocyanate; Bax: Bcl-2-associated X protein; Bcl-2: B-cell lymphoma 2 protein; BF: Basic fraction; CDK1: Cyclin-dependent kinase 1; CDK2: Cyclin-dependent kinase 2; CDK4: Cyclin-dependent kinase 4; CDK6: Cyclin-dependent kinase 6; DPPH: 2,2-Diphenyl-1-picrylhydrazyl; EDTA: Ethylenediamine-tetraacetic acid; ER: Estrogen receptor; HRP: Horseradish peroxidase; MCF-10A: Michigan Cancer Foundation-10A; MCF-7: Michigan Cancer Foundation-7; Mcl-1: Myeloid cell leukemia 1; MDA-MB-231: Michigan Cancer Foundation-231; MYC: MYC proto-oncogene, bHLH transcription factor; NF: Neutral fraction; p27<sup>kip1</sup>: Cyclin-dependent kinase inhibitor; PI: Propidium iodide; RIPA buffer: Radioimmunoprecipitation assay buffer; RNase: Ribonuclease; SDS-PAGE: Sodium dodecyl sulfate-polyacrylamide gel electrophoresis; TAC: Total Antioxidant Capacity; TCA: Trichloroacetic acid.

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