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Antioxidant, anti-inflammatory, and antiproliferative activities of aqueous extract from *Calamintha nepeta* L. leaves

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ABSTRACT

Calamintha nepeta L. (Lamiaceae) is traditionally used in Morocco for its medicinal properties such as inflammation disorders. The current study aims to investigate the antioxidant, anti-inflammatory, and antiproliferative activities of aqueous extract from *Calamintha nepeta* L. leaves. Antioxidant properties were assessed *in vitro* through the scavenging activities of DPPH and ABTS radicals, along with ferric-reducing antioxidant power assays. Anti-inflammatory potential was examined on BV2 microglial cells, by determining the efficacy of the extract in reducing nitric oxide (NO) production in lipopolysaccharide (LPS)-activated microglial cells. In addition, anti-proliferative properties were tested on the A431 skin cancer cell line. HPLC analysis revealed the presence of compounds with potent anti-cancer properties, including cinnamic acid (1.2 %), rosmarinic acid (4.8 %), quercetin (21.1 %), and caffeic acid (8.3 %). The aqueous extract showed potent antioxidant activity with an IC_{50} of 28.1 ± 0.5 µg/mL for DPPH, an IC_{50} of 10.0 ± 0.1 µg/mL for ABTS, and 108.5 ± 2.4 mmol TE/100 g dw for FRAP. Significant anti-inflammatory activity was also demonstrated, inducing significant inhibition of NO production at the concentration of 1 mg/mL without influencing cell viability. It also exerted a significant antiproliferative action on A431 cancer cells at concentrations of 250 µg/mL and above. The cytotoxic effect was dose-dependent, with an IC_{50} of 360.7 ± 14.4 µg/mL.

In conclusion, the aqueous extract of *Calamintha nepeta* L. leaves displays significant antioxidant properties, anti-inflammatory activity, and a promising cytotoxic effect against A431 cancer cells.

Introduction

Cancer is the leading cause of mortality worldwide. Skin cancers are one of the most frequently diagnosed groups of cancers in the world, with over 1.5 million new cases estimated in 2022 (WHO, 2022), which is mainly linked to the increase in environmental pollutants, such as chemical hazards and radiation [1,2]. Medical and surgical approaches, including topical and systemic drug treatments, are used to treat the disease [1].

Recent studies have revealed that various herbal extracts display anticancer properties with minimal side effects when used against skin cancer. The biological properties of plant extracts used in traditional medicine are mainly associated with their phenolic chemical

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content. These constitute a large group of secondary metabolism products of plants, known to exhibit strong antioxidant activities [3]. In fact, phenolic acids, flavonoids, and non-flavonoids constitute the three major categories of phenolic components [4]. The redox properties and the chemical structure of these compounds, which neutralize free radicals, chelate metals, and decompose peroxides, are responsible for their antioxidant properties [5]. Given that free radicals are a principal factor in DNA damage, which can trigger cancer development, inhibiting these reactive molecules may significantly contribute to preventing the onset of cancer [6].

Calamintha nepeta (Lamiaceae) is known by its local vernacular name "manta' [7]. It is commonly used to treat a variety of complaints, including muscular pains, fever, and arthritis [8]. It is also used for respiratory diseases, gastroenteric diseases, and infectious diseases [9,10]. Several studies have reported that *Calamintha nepeta* L. displays many biological properties, including antioxidant, antibacterial, and antiulcer activity [11–13]. Analgesic and anti-inflammatory properties were also reported [14,15]. Although extensive scientific research has focused on essential oils derived from *Calamintha nepeta* L. (Lamiaceae), aqueous extracts are the type of extract most widely used in traditional medicine. The present study aimed to investigate the anti-inflammatory, antioxidant, and antiproliferative properties of *Calamintha nepeta* L. aqueous extract on microglial and squamous cell carcinoma.

Methods

Plant material

Calamintha nepeta L. was collected in March 2021 in the region of Ksar El Kebir (Northern Morocco) at an altitude of 134 m (34°06′03, 11′'N 03°56′35, 94′'W). A specimen of *Calamintha nepeta* L. was carefully prepared after identification and deposited in the Herbarium of the Institut Scientifique in Rabat (Morocco) under voucher number RAB114271. The plant was dried and the leaves were grounded into a fine powder.

Preparation of plant extract

The decoction method was used to prepare the aqueous extract of *Calamintha nepeta* L. leaves. Thirty grams of plant leaf powder were boiled for 15 min in 300 mL of distilled water. The extract was filtered through Whatman paper (no. 1), evaporated in a rotary evaporator, and stored at 4 °C.

Total polyphenolic and flavonoid contents

Total polyphenol content (TPC)

The amount of TPC was measured using the Folin-Ciocalteu reagent [16]. 500 μL of Folin-Ciocalteu reagent (1/10) was added to 100 μL of aqueous extract. Next, 400 μL of a water solution of sodium carbonate (7.5 % w/v) was added to the mixture. The mixture was left at room temperature for 60 min. Absorbance was measured at 765 nm. The calibration curve was developed using gallic acid as a standard, and TPC was expressed as milligrams of gallic acid equivalents per gram of dry residue (mg GAE/g dr).

Total flavonoid content (TFC)

The $AlCl_3$ technique was utilized to evaluate the TFC of the aqueous extract [17]. In short, 500 μL of sample extract was mixed with 1.5 mL of methanol, 100 μL of aluminum chloride (10 %), and 100 μL of potassium acetate (1 M). A total volume of 5 mL was achieved by adding 2.8 mL of deionized water. After 30 min of incubation, the absorbance at 415 nm of the mixture was determined. The results were given in milligrams of quercetin equivalents per gram of dry residue (mgQE/g dr).

High-performance liquid chromatography analysis

The phytochemical composition of the plant extract was identified by high-performance liquid chromatography (UV-HPLC) coupled with visible UV detection. Peaks were identified by comparing retention times and UV spectra with reference standards. Analyses are carried out on a PRP-C18, 4.6 cm x 250 mm internal diameter, with a particle size of 5 μm. The injection volume is 20 μL, and the mobile phase is water/acetic acid (98: 2) (solvent A) and acetonitrile /acetic acid (98: 2) (solvent B). The flow rate is 1.2 mL/min. Chromatograms are extracted at 280 nm.

Antioxidant activity

DPPH radical scavenging activity

The free radical scavenging capacity of the extract was assessed by the DPPH assay [17]. Different concentrations of decoction extract were prepared, and 0.5 mL of each sample extract was mixed with 3 mL of a freshly DPPH (0.1 mM) methanolic solution. The result mixtures were kept at room temperature under darkness for 20 min. Then the absorbance was measured at 517 nm. The DPPH radical scavenging efficiency of each sample was then estimated as a percentage of inhibition:

$$\text{PI}\ (\%) = \frac{A\ (control)\ -\ A\ (sample)}{A(control)} \times\ 100$$

A (sample) represents the absorbance of the extract or standard, and A (control) represents the absorbance of the control. Under

identical test circumstances, ascorbic acid was employed as a positive control. By plotting inhibition percentages versus extract concentrations, the concentrations providing 50 % radical inhibition (IC_{50}) were determined.

ABTS radical scavenging activity

This assay was carried out as previously described [18]. The ABTS radical cations (ABTS•+) were produced by reacting 2.45 mM of potassium persulphate aqueous solution with 7 mM of an ABTS aqueous solution. Prior to use, the resulting solution was kept at room temperature for 12 to 16 h in the dark, an absorbance of 0.700 ± 0.005 at 734 nm was then established after dilution with deionized water. The extract was prepared at different concentrations, 10 µL of each concentration was added to 990 µL of ABTS•+ solution which was then left to sit for 6 min at room temperature. At 734 nm, the absorbance was subsequently determined. The following formula was used to determine the percentage of ABTS•+ radical inhibition:

$$\text{PI } (\%) = \frac{A\ (control)\ -\ A\ (sample)}{A(control)} \times\ 100$$

A (sample) represents the absorbance of the extract or standard (Ascorbic acid), and A (control) represents the absorbance of the control. The results were presented as IC_{50}, which is the concentration that scavenges 50 % of ABTS•+ radicals.

Ferric reducing antioxidant power assay

This assay measures the capacity of an antioxidant to reduce ferric tripyridyl-triazine (Fe^{3+}-TPTZ) to ferrous (Fe^{2+}-TPTZ) at pH=3.6 [19]. Briefly, a 0.3 M acetate buffer (pH=3.6), a 10 mM solution of TPTZ (2,4,6-tripyridyl-s-triazine) solubilized in 40 mM hydrochloric acid, and a 20 mM ferric chloride ($FeCl_3$) solution were used to prepare the FRAP reagent with volumes 10:1:1 respectively. Before each experiment, a fresh solution was prepared. 10 µL of the extract was mixed with 2 mL of FRAP reagent and incubated for 10 min at the temperature of the laboratory. The absorbance was then measured at 593 nm. The standard curve was constructed using Trolox. The results were expressed as mmol Trolox equivalent / 100 g dry weight.

Anti-inflammatory activity

The effect of aqueous extract on nitric oxide (NO) production was assessed on BV2 microglial cells. Nitric oxide formation can be studied by measuring nitrite (NO2-), the main stable, non-volatile degradation product of NO The test is based on a diazotization reaction first described by Griess (1879).

BV2 cells were seeded in a 96-well plate at 250,000/well/500 µL culture medium, giving a concentration of 500,000 cells/mL. Four hours after seeding, extracts were added at four concentrations: 125, 250, 500, and 1000 µg/mL (5µL/well). Cells were then incubated for 24 h at 37 °C. A solution of lipopolysaccharide (LPS) was then added to the wells at a concentration of 200 ng/mL, and the cells were then incubated for a further 24 h at 37 °C. A solution of 1 % sulfanilamide (solubilized in 5 % phosphoric acid) and 0.1 % N-1-naphthyl ethylenediamine dihydrochloride (NED) were mixed into equivalent volumes. 100 µL of the mixture was added to 100 µL of cell culture supernatant, then incubated for 10 min at room temperature, in the dark. Absorbance was measured at 550 nm. The concentration of nitrite in the supernatant was determined using a standard curve prepared with sodium nitrite.

Antiproliferative activity against skin cancer cell lines

Before starting the experiment, a mixture of Alamar Blue and culture medium was prepared at a dilution ratio of 1:10. In a 96-well plate, cells were placed and treated with the extract for 24 h, then 100 µL of the culture medium was removed and replaced by the mixture already prepared. The microplate was then placed in an incubator for 4 h (37 °C, 5 % CO_2). A microplate reader was used to measure fluorescence levels, 560 nm being the excitation wavelength and 590 nm being the emission wavelength. The ratio between the fluorescence intensity of treated cells and that of control cells was used to determine cell viability.

Apoptosis induction

Cell line A431, treated with different concentrations of the aqueous extract (250 and 500 µg/mL) using Annexin V/FITC/FVD eFluor 780, was tested for apoptosis induction and measured by flow cytometry (FACS Fortessa, BD, Erembodegem, Belgium). In a plate of 24 wells, A431 cells were seeded at a density of 5×10^5 cells per well and incubated for 48 h at 37 °C with 5 % CO_2. Then, cells were collected and washed with PBS before being reconstituted in a binding buffer (prepared by diluting a stock solution 1:10 with distilled water). Subsequently, cells were suspended in PBS and stained with 1 µL of eFluor 780 FVD per condition, followed by a 30-min incubation at 2–8 °C in darkness. After two washes with flow cytometry buffer or PBS and one wash with binding buffer, cells were resuspended in 100 µL of binding buffer and incubated with 5 µL of Annexin V for 10–15 min at room temperature. Once the supernatant had been removed, 2 mL of binding buffer was added and the cell suspension was centrifuged at 1500 rpm for 5 min. Finally, for flow cytometric analysis, cells were resuspended in 500 µL of binding buffer after removal of the supernatant.

Data analysis

GraphPad Prism was used for statistical analysis. Data were statistically evaluated by one-way analysis of variance (ANOVA), or

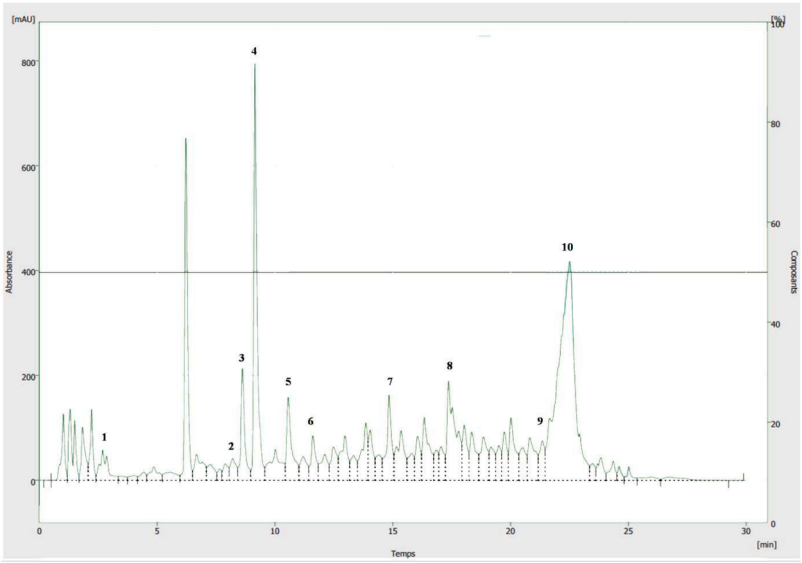

Fig. 1. HPLC analysis of phenolic compounds in the aqueous extract of *Calamintha nepeta* L. Peaks identified: (1) gallic acid, (2) catechin, (3) chlorogenic acid, (4) caffeic acid, (5) vanillin, (6) coumaric acid, (7) rutin, (8) rosmarinic acid, (9) cinnamic acid, (10) quercetin. cinnamic acid, (10) quercetin.

using the non-parametric Kruskal–Wallis test followed by Dunn's multiple comparison tests. Data were represented as mean±SEM or mean±SD of three separate trials. $P < 0.05$ was considered statistically significant.

Results and discussion

Phenolic content of Calamintha nepeta L. aqueous extract

The extraction yield obtained from *Calamintha nepeta* L. leaves was 10 %, and this value is low compared to other studies. Indeed, the aqueous extract of *Calamintha nepeta* L. gave a yield of 22.19 % in the study conducted by Bougandoura & Bendimerad [20], while the methanolic extract yielded 23.1 %, as reported by Amira et al. [21], and 34.4 % as reported by Hayani et al. [22]. Generally, the extraction process is affected by various factors, such as temperature, plant particle size, solvent nature, pH, and solid-liquid ratio. Variations in any of these parameters may explain the differences in yield observed among studies [23,24].

Results of total phenolic content showed that the extracts contained 163.03±3.18 mg GAE/g dr, and flavonoids were 67.91±0.84 mg QE/g dr. The higher amount of phenolic compounds obtained is probably due to the methodology used. Indeed, as other researchers have also shown, high temperatures weaken cell walls, promoting fluid diffusion through the cells and improving solute solubility and diffusion coefficients, which in turn improves extraction. In addition, bound phenolic compounds are released by hydrolysis reactions of esters or ether bonds, promoting the high solubility of phenolic compounds [24]. Confirming our findings, several similar studies have reported that decoction is the best technique for extracting phenolic compounds, whether phenolic acids or flavonoids [25].

The chemical profile of *Calamintha nepeta* L. decoction extract was studied using HPLC (Fig. 1). 10 compounds were identified, among them caffeic acid (8.3 %), quercetin (21.1 %), catechin (0.7 %), cinnamic acid (1.2 %), coumaric acid (1.2 %), gallic acid (1.4 %), rosmarinic acid (4.8 %), rutin (2.5 %), vanillin (2.5 %), and vanillic acid (0.5 %). Quercetin and caffeic acid were the major components of this extract. In line with the results obtained, caffeic acid and its derivatives were the main constituents of the hydroalcoholic extract of *Calamintha nepeta* L., with acetin, quercetin, kaempferol, rosmarinic acid, and quinic acid in varying amounts [26]. Caffeic acid has been shown in prior research to possess antioxidant capacity, anti-inflammatory, immunomodulatory, antimicrobial, neuroprotective, and antiproliferative properties [27–30]. As well as their anticancer capacity against various types of cancer [31–34]. Numerous studies have also reported that quercetin demonstrates varied pharmacological properties, which include anti-histaminic, anti-cancer, and anti-inflammatory properties. These beneficial effects are mainly attributed to its antioxidant properties, suggesting potential application in various medical and nutritional contexts [33,34].

Antioxidant activity

To provide a better assessment of antioxidant capacity, the extract was tested by three different but complementary *in vitro* model systems, namely DPPH, ABTS, and FRAP.

Table 1
Antioxidant activity of *Calamintha nepeta* L. aqueous extract.

	DPPH IC_{50} (µg/mL)	ABTS IC_{50} (µg/mL)	FRAP mmol TE/100 g dw
Calamintha nepeta L.	28.1 ± 0.5	9.96±0.09	108.49±2.42
Standard (Ascorbic acid)	3.30±0.46 *	2.15±0.08*	

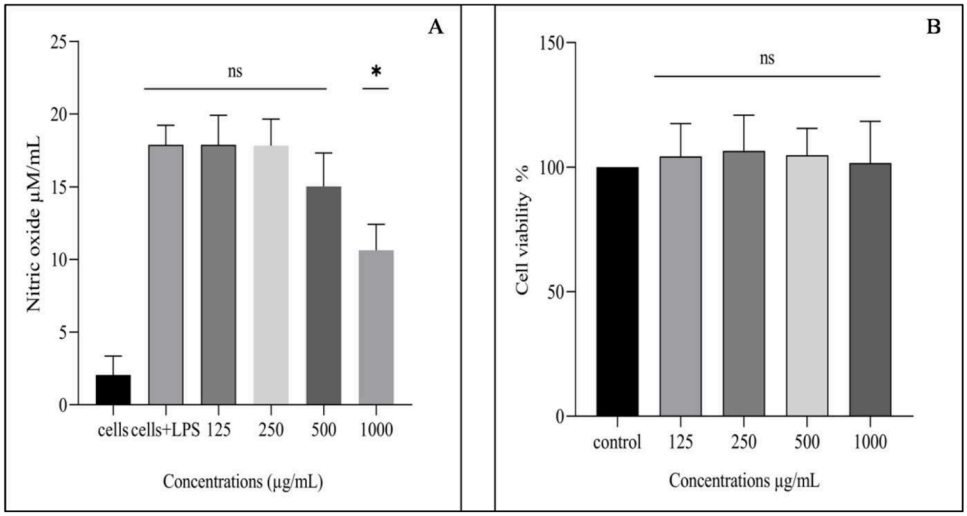

Fig. 2. Effects of *Calamintha nepeta* L. aqueous extract on NO production by BV2 cells treated with LPS. Cells are pretreated with different concentrations of *Calamintha nepeta* L. extract for 24 h, followed by LPS treatment. (A) NO levels in culture media. (B) Cell viability assessed by Alamar Blue assay. The values are expressed as means ± standard deviation of three independent experiments ($n = 3$). * $p < 0.05$ vs. control.

DPPH, and ABTS radical scavenging activity

The DPPH test measures the electron donation ability of the antioxidant, thus neutralizing the DPPH radical. Similarly, the ABTS test assesses the antioxidant capacity to donate electrons or hydrogen atoms, thus deactivating this radical species [35]. As indicated in Table 1, the decoction extract was an effective radical scavenger against DPPH (IC_{50}= 28.1 ± 0.5 µg/mL), and ABTS (IC_{50}= 9.96 ± 0.09 µg/mL). These findings were superior to those obtained by Khodja et al. [36] where the IC_{50} value was 766 ± 58 µg/mL by decoction extract. As antioxidant activity is closely linked to phenolic compound content, and the nature of the phenolic compounds extracted depends on extraction conditions, this may explain the differences in results observed. In addition, ecological, genetic, and harvesting factors may also play an important role in the variation of polyphenol composition [36].

Ferric reducing antioxidant power assay

The FRAP (Ferric Reducing Antioxidant Power) test is a practical and cost-efficient test [35]. It is an electron transfer mechanism rather than a hydrogen atom transfer mechanism, and therefore measures the ability of phenolic compounds to reduce Fe^{3+} to Fe^{2+} [37]. The aqueous extract of *Calamintha nepeta* L. showed a powerful capacity to reduce ferric ions to ferrous ions (108.49 ± 2.42 mmol TE/100 g dw). The powerful antioxidant activity recorded in aqueous extracts is strictly linked to the phytochemical composition of the plant, in particular to polyphenols and flavonoids, due to their chemical structure characterized by one or more hydroxyl groups linked to one or more aromatic rings [38]. ROS and free radicals can damage biomolecules like proteins, lipids, and DNA, resulting in a range of chronic diseases, notably cancer, inflammation, neurodegenerative diseases, and premature aging [39–41]. Antioxidants can prevent or eradicate these oxidative stress-related diseases by reducing the damaging effect of ROS and preserving cellular functions [42]. Polyphenols can easily scavenge free radicals through hydrogen or electron donation, and mitigate the harmful effects of metal redox-active ions such as Fe(II) or Cu(II). As antioxidants, some flavonoids inhibit enzymes that generate free radicals such as NO synthase and xanthine oxidase, or combine free-radical scavenging and enzymatic functions [43].

Anti-inflammatory activity

Inflammation is a pathophysiological response involving a series of defensive reactions against various agents, including physical injury, infection, toxic chemicals, or tumor growth, to re-establish tissue homeostasis [44]. As they are involved in both innate and adaptive immunity, macrophages have a key role in inflammatory processes. Activated macrophages in response to bacterial lipopolysaccharides (PLS) initiate defensive responses and release pro-inflammatory cytokines, including interleukin 6 (IL-6), TNF-α, and interleukin 1b (IL-1β), as well as inflammatory mediators like NO and PGE2. Numerous inflammatory diseases can be mediated by an excess of NO, which can be dangerous [45].

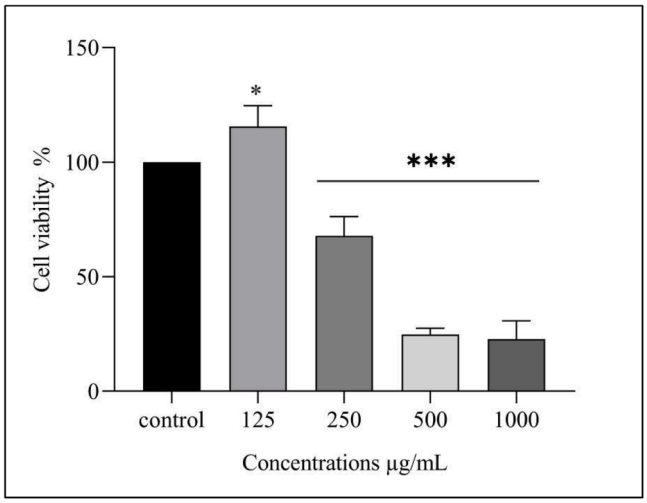

Fig. 3. The effect of *Calamintha nepeta* L. extract on A431 cell viability. Results expressed as Mean ± Standard Deviation from three independent experiments ($n = 3$). * $P < 0.05$, ** <0.01, *** <0.001 compared with untreated cells.

Calamintha nepeta L. leaf decoction was evaluated for its anti-inflammatory properties by examining its capacity to reduce nitric oxide (NO) production, a crucial signaling molecule implicated in inflammation. This evaluation was carried out using BV2 cells (Fig. 2). The *Calamintha nepeta* L. extract induced a significant decrease in NO production by LPS-activated BV2 cells at the concentration of 1000 µg/mL ($p < 0.05$) without inducing cell death in all tested concentrations. Our results were in line with those of Conforti et al. [46] who reported that the methanolic, ethanolic, dichloromethane, and petroleum ether fractions of *Calamintha nepeta* L. inhibits NO production in LPS-stimulated RAW 264.7 cells with IC_{50} values of 286 ± 3.6, 142 ± 1.8, 219 ± 2.6 and 167 ± 2.1 µg/mL respectively [46].

The recorded anti-inflammatory property of this extract may be attributable to its content of phenolic compounds. Rosmarinic acid, a compound present in this extract, has been demonstrated to produce an anti-inflammatory effect in experimental models in vitro and in vivo, by modulating several mechanisms [47]. It inhibits iNOS activity, resulting in lower levels of NO release [48]. Quercetin has also been shown to have powerful anti-inflammatory effects by reducing prostaglandin E2 and NO synthesis [49]. In addition, caffeic acid, the most abundant component of the present extract, can act on multiple signaling pathways associated with inflammation. It inhibits the inflammatory response by inhibiting the activation of NF-κB, p38 MAPK, and c-Jun NH2-terminal kinase. Caffeic acid may also effectively inhibit the expression of IL-8, IL-1β, IL-6, and TNF-α [50]. Indeed, the aqueous extract is comprised of multiple, complex substances, and its biological properties appear to be the result of a synergy between these compounds. Our results highlight the safety profile of *Calamintha nepeta* L. aqueous extract, which significantly inhibited LPS-induced NO production in the BV2 cell line, underlining its strong anti-inflammatory potential without affecting cell viability at concentrations up to 1000µg/mL. The safety profile of *Calamintha nepeta* L. justifies and reinforces its suitability as a condiment and food flavoring.

Cytotoxicity of Calamintha nepeta L. aqueous extract on A341 cells

Cancer is a complex of disorders characterized mainly by abnormal and unregulated cell proliferation, occurring in various parts of the body. Carcinomas, which account for the majority of cancer cases (80–90 %), are malignant tumors derived from the epithelial cells that line the surface of organs, glands, or other body structures. Melanoma, basal cell carcinoma, and epidermal cancer are the best-known skin cancers [41]. A431 cells were found to be more suitable for skin cancer research [1]. *Calamintha nepeta* L. leaf extract showed very high cytotoxicity against A431 cells in a dose-dependent manner. Increasing the extract concentration from 250 µg/mL to 1000 µg/mL induced a marked reduction in cell viability, with percentages decreasing from 67 % to 22 %, respectively. The cytotoxic effect was dose-dependent with IC_{50} values of 360.7 ± 14.42 µg/mL. These results demonstrated an interesting anti-proliferative action of the extract, suggesting that this plant can be used as a potentially active component in developing pharmaceutical formulations with anti-cancer properties.

The antiproliferative activity of *Calamintha nepeta* L. extract was investigated on the human breast cancer cell line (MCF-7) employing the MTT assay, and significant antiproliferative activity was recorded [51]. Likewise, the hydromethanolic extract was tested on the breast cancer cell line (MDA-MB-231) for its cytotoxic effect, showing significant inhibition of cell viability at a dose of 100 µg/mL [26].

Two doses of *Calamintha nepeta* L. leaf extract (250 and 500 µg/mL) demonstrated strong antiproliferative activity against the A431 cancer cell line, were evaluated for apoptosis induction, and flow cytometry results are shown in Fig. 4. According to this assay, the majority of cells remained alive after extract treatment (Q3). Thus, the anti-cancer effect of the plant extract does not appear to be mediated by the induction of apoptosis. This suggests that other mechanisms may be involved, such as cell cycle arrest, inhibition of cell proliferation, or alterations in signaling pathways.

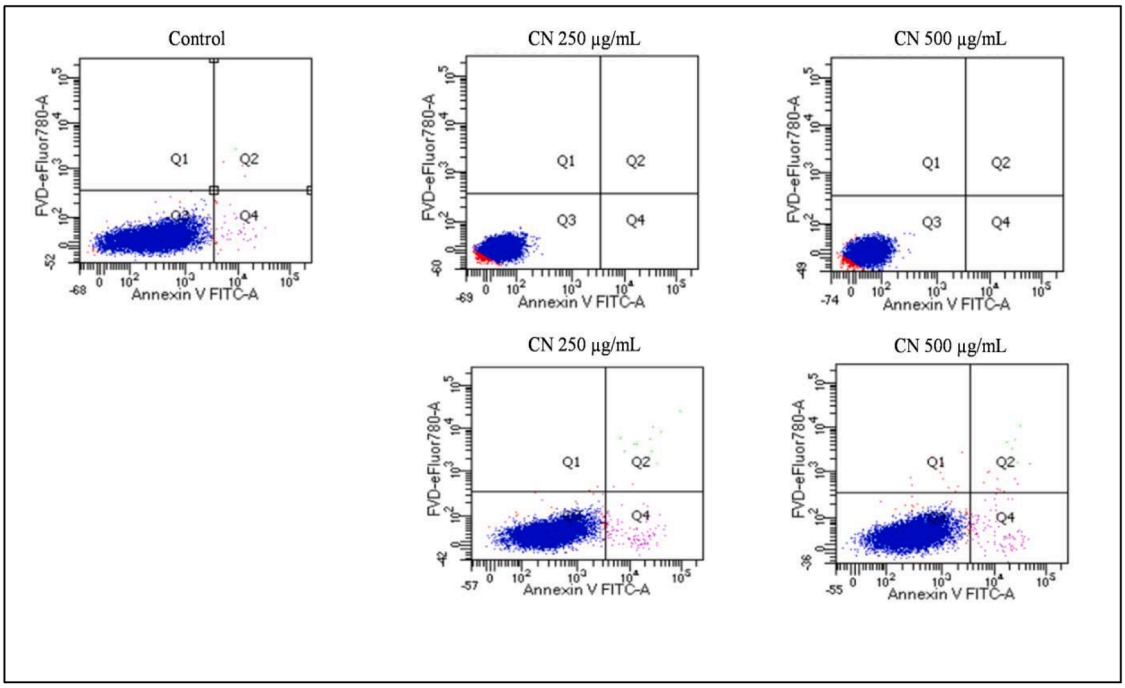

Fig. 4. Induction of apoptosis in A431 cells by *Calamintha nepeta* L. aqueous extract at 250 µg/mL and 500 µg/mL using flow cytometry analysis.

Coumaric acid, a compound detected in *Calamintha nepeta* L. extract, was studied in combination with naringin to assess their antiproliferative activity on A431 squamous cell carcinoma cells. The results indicated that combined treatment resulted in cell cycle arrest, activated apoptosis, and altered ROS levels and mitochondrial membrane potential in A431 cells [52]. Similarly, caffeic acid derivatives induced G0/G1-phase cell cycle arrest in colorectal cancer cells and inhibited cell proliferation via the PI3K/Akt signaling pathway [53]. The anticancer actions of this substance are mediated in part by the control of many cell signaling pathways, including apoptosis, PI3K/Akt, Wnt/β-catenin, MAPK, JAK/STAT, NF-Kb, and p53-independent pathway. Additionally, rutin regulates DNA damage [54]. In-depth investigations of other phenolic compounds, notably rosmarinic acid, have also been carried out. These studies have highlighted the diversity of mechanisms of action of rosmarinic acid, including prevention of tumor formation, reduction of lipid peroxidation, modulation of pro-apoptotic protein expression, decreasing levels of IL-6, COX-2, and TNF-α, regulating p65 expression, as well as stimulating apoptosis by altering the expression of multiple genes, making it effective in the treatment of cancer [55,56].

Conclusion

The present study revealed the richness of the traditional aqueous extract of *Calamintha nepeta* L. in phenolic compounds, particularly caffeic acid, quercetin, and rosmarinic acid, giving the extract significant antioxidant activity demonstrated by several tests. The richness of phenolic compounds and the strong antioxidant activity of the extract may be responsible for the antiproliferative activity against squamous cell carcinoma and the anti-inflammatory activity observed. The results justify the safe traditional use of the decoction extract, underline the potent anti-inflammatory and anti-cancer potential of the extract studied, and pave the way for future research aimed at elucidating the bioactive components involved in these effects. Fig 3

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Declaration of competing interest

The authors have no competing interest to declare.

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