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Oleuropein exhibits anticancer effects by inducing apoptosis and inhibiting cell motility in MCF7 and MDA-MB231 breast cancer cells

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ABSTRACT

Background: Globally, breast cancer stands as one of the primary causes of cancer-related deaths, with chemotherapy as the primary treatment modality. However, drug resistance and the absence of effective treatment options for aggressive subtypes, particularly triple-negative breast cancer (TNBC), necessitate the exploration of novel therapeutic agents derived from natural sources. Oleuropein, a bioactive polyphenolic compound found in olive fruits and leaves, has demonstrated potential anticancer properties.

Objective: This research is aimed to examine the effects of oleuropein in its isolated compound form (oleuropein compound, OLC) and extract form (olive leaf extract, OLE), on two distinct breast cancer cell lines: estrogen, CF7 and progesterone receptor-positive (HR+), TNBC MDA-MB231, cells. The focus of this research was to assess cytotoxicity, apoptosis induction, inhibition of cell motility and spreading at low concentrations of oleuropein (1, 5, and 10 µM).

Methods: Oleuropein effects on the breast cancer cell lines were explored using flow cytometry, utilizing 7-Aminoactinomycin D and Annexin V staining to measure cytotoxicity and apoptosis. Functional experiments were performed to evaluate the effects of oleuropein on cell motility and spreading. Using IC_{50} values that were determined for both cell lines, the compound's antiproliferative effects were quantified

Results: The IC_{50} values for oleuropein were 16.99 ± 3.4 µM for MCF7 and 27.62 ± 2.38 µM for MDA-MB231 cells, indicating greater sensitivity in HR+ cells compared to TNBC cells. Using oleuropein significantly reduced cell growth and induced apoptosis in both cell lines. Functional assays revealed that oleuropein impaired cell motility and reduced spreading, suggesting its potential to suppress metastatic behavior.

Conclusion: This research offers new insights into the anticancer potential of oleuropein, a bioactive compound from olive leaves and fruits. It demonstrates oleuropein's ability to induce apoptosis and inhibit cell motility in both hormone receptor-positive (MCF7) and triple-negative (MDA- MB231) breast cancer cells. Unlike prior studies focusing solely on oleuropein's cytotoxicity, this work emphasizes its dual role in reducing metastatic behavior and promoting cell death at low concentrations, suggesting its promise as a functional food-derived therapeutic agent for breast cancer management.

Keywords: Oleuropein; breast cancer; MCF7; MDA-MB231; apoptosis; functional food; natural bioactive compound

Graphical Abstract: Oleuropein exhibits anticancer effects by inducing apoptosis and inhibiting cell motility in MCF7 and MDA-MB231 breast cancer cells

INTRODUCTION

Breast cancer remains one of the leading causes of cancer-related deaths worldwide, posing a major public health challenge despite advancements in diagnostic tools and therapeutic strategies [1, 2]. This heterogeneous disease comprises distinct molecular subtypes characterized by unique biological behaviors and treatment responses. Among the most studied breast cancer cell lines, MCF7 and MDA-MB231 are critical models for investigating cancer progression and evaluating potential therapeutic interventions. MCF7 cells, representing the luminal A subtype, are defined by the presence of estrogen receptor positivity (ER+), progesterone receptor positivity (PR+), and the absence of HER2 (HER2-), exhibiting an epithelial-like morphology and a low-invasive phenotype. In contrast, MDA-MB231 cells represent triple-negative breast cancer (TNBC), lacking the expression of ER, PR, and HER2. This subtype is highly aggressive and mesenchymal-like, displaying enhanced migratory and invasive properties. The stark differences between these cell lines underscore their importance in exploring the molecular mechanisms underlying breast cancer and assessing the efficacy of potential therapeutic agents [3, 4].

A major challenge in breast cancer treatment lies in its clinical heterogeneity, largely driven by tumor-initiating cells (TICs) and the tumor microenvironment (TME). These factors contribute significantly to therapeutic resistance, recurrence, and metastasis [5, 6]. Breast cancer is categorized into three main molecular subtypes: hormone receptor-positive (HR+), HER2-positive (HER2+), and TNBC. While early-stage, non-metastatic breast cancer has a relatively high cure rate, chemoresistance and metastatic progression remain major barriers to effective and successful treatment [5, 7–9].

Cell adhesion, spreading, and migration on extracellular matrix (ECM) substrates play pivotal roles in cancer progression, particularly in invasion and metastasis. Spreading assays, which evaluate cell adhesion and morphological changes over time, are widely used to study these processes. MCF7 cells exhibit slow spreading with a rounded morphology, while MDA-MB231 cells spread rapidly, forming lamellipodia and filopodia to enhance adhesion and migration [10–13]. The dynamic reorganization of the cytoskeleton, including actin filaments and microtubules, orchestrates these processes, regulated by key proteins involved in actin dynamics and Rho-family GTPases. Recent studies highlight the intricate coupling of microtubule-associated processes with actin polymerization as a key mechanism driving cell migration [14].

In recent years, natural compounds derived from foods with evidence of clinically proven health benefits beyond basic nutrition— have gained significant attention for their potential in cancer prevention and therapy. These compounds are valued for their low toxicity and diverse biological activities [3, 15–21]. Among them, oleuropein, a bioactive polyphenolic compound naturally present in olive leaves and oil, has shown promising anticancer properties. Oleuropein influences physiological processes and promotes health outcomes through multiple mechanisms. Its anticancer effects are mediated by inducing apoptosis, disrupting mitochondrial function, modulating key signaling pathways such as Wnt/β-Catenin, PI3K/Akt, and NF-κB, inhibiting cell proliferation, suppressing angiogenesis, and preventing metastasis [3, 22–25].

The growing interest in natural compounds as complementary or alternative cancer treatments emphasizes the need for further exploration of oleuropein's potential in breast cancer management. While its general anticancer properties have been widely studied, the specific effects of oleuropein on breast cancer cell motility, spreading, and apoptosis remain underexplored. This study aims to bridge this gap by investigating the cytotoxic and apoptotic effects of oleuropein, both in its compound form (oleuropein

compound, OLC) and extract form (oleuropein extract, OLE), on MCF7 and MDA-MB231 breast cancer cells. By evaluating oleuropein's ability to inhibit cell motility, reduce spreading, and induce apoptosis, this research seeks to elucidate its potential as a natural therapeutic agent for overcoming the limitations of current breast cancer treatments. Furthermore, the results may contribute to the development of functional food-based strategies for managing breast cancer, focusing on the health-promoting effects of natural products.

MATERIALS AND METHODS

Plant Material and Phenolic Compounds Extraction: This study utilized olive leaves from *Olea europaea* L., a Tunisian species. The extraction process followed previously established methods [26]. The purity of the oleuropein (OLE) fraction was assessed using high-performance liquid chromatography (HPLC). The purified OLE was subsequently used to treat MCF7 and MDA-MB231 cell cultures.

HPLC Analysis of Phenolic Compounds: Phenolic compounds in the aqueous extract of *Olea europaea* L. were analyzed using the method described by Goupy et al. [27]. The analysis used an HPLC system (Agilent 1100, Germany) equipped with a variable wavelength detector, autosampler, quaternary pump, degasser, and column compartment. A reverse-phase stainless-steel column C18 (BDS 5 µm, Labio, Czech Republic) measuring 4 × 250 mm was utilized for compound separation. Peak identification was conducted by comparing retention times and UV spectra with reference standards. After purification using a silica gel column, oleuropein (OLE) yield was determined per gram of dry leaf weight.

Cells Lines and Culture: The MCF7 (ATCC® HTB-22™) and MDA-MB231 (ATCC® HTB-26™) cancer cell lines used in this study were obtained from the American Type Culture Collection (ATCC). Both cancer cell lines were maintained in DMEM supplemented with 4.5 g/L D-glucose, 10% fetal bovine serum (FBS), 2 mM L-glutamine, and 1% penicillin/streptomycin. Cells were incubated at 37 °C in a humidified atmosphere with 5% CO_2 and passaged every 2-3 days upon reaching 80-90% confluency. For passaging, cells were trypsinized, diluted, and replated. The OLC and OLE extracts were prepared as stock solutions in DMSO and diluted in culture medium to the desired concentrations prior to treatment. Control cells were treated with medium containing 0.1% DMSO [28, 29].

Cell Proliferation Assay: The inhibitory effect of oleuropein extract (OLE) was assessed using the MTT (methylthiazolyldiphenyl-tetrazolium bromide) assay, with oleuropein compound (OLC) used as the positive control. MCF7 and MDA-MB231 cells were seeded at a density of 5×10^3 cells per well in a 96-well plate and allowed to adhere for 24 hours. Following attachment, the cells were exposed to different concentrations of OLC and OLE and incubated for an additional 24 hours. After treatment, 20 µL of MTT solution (5 mg/mL) was added to each well, and the plates were incubated for 4 hours to allow the formation of purple formazan crystals. The crystals were then dissolved by adding 100 µL of dimethyl sulfoxide (DMSO) to each well, and the absorbance was measured at 570 nm using a multimode microplate reader (Biotek Synergy H1, BioTek Instruments, Agilent, Winooski, VT, USA). The IC_{50} values, representing the concentrations required to inhibit cell growth by 50%,, were determined from the MTT viability curve.

Cell Protrusion Quantification: MCF7 and MDA-MB231 cells were cultured, fixed, and stained following the standard protocols described previously. The number of protrusions per cell was manually counted for 100 cells in each condition. Protrusions were defined as observable membrane projections.

Scratch Wound Migration Assay: A total of 5×10^5 MCF7 and MDA-MB231 cells per well were plated in 6-well plates and subjected to serum starvation for 24 hours. A

single scratch wound was created in each well using a P10 pipette tip, and wound closure was monitored over 24 hours using an inverted microscope (Leica Microsystems, Germany). Imaging was captured with a Hamamatsu CCD camera (Hamamatsu Corporation, Bridgewater, NJ, USA) attached to the inverted microscope (Leica Microsystems, Germany) with a 10× objective lens.

The wound area was analyzed using Image J Analysis Software (Version 1.54g, NIH, USA), measuring the total wound area across three fields per condition at time points 0 and 24 hours. Mean wound areas were calculated by averaging measurements from each location. The percentage of wound closure at each time point was determined by subtracting the remaining wound area from 100% of the original wound area,

Assessment of Apoptosis Using Flow Cytometry: MCF7 and MDA-MB231 cells were seeded at 5×10^5 cells per well in 6-well plates and allowed to adhere overnight. The cells were treated with oleuropein compound (OLC) and oleuropein extract (OLE) at concentrations of 0, 1, and 5 µM. After 48 hours of treatment, the cells were harvested, washed twice with DPBS, and resuspended in 500 µL of 1X Annexin V binding buffer. To distinguish between live, apoptotic, and necrotic cells, the samples were stained with 5 µL each of PE-Annexin V (Phycoerythrin-Annexin V) and 7-AAD (7-Aminoactinomycin D) (BD Pharmingen™, Catalog number 559763, BD Biosciences, USA). The stained cells were analyzed within 10 minutes using a FACSCalibur flow cytometer (BD Biosciences, San Jose, CA, USA). Control cells were incubated with medium supplemented with 0.1% DMSO.

Statistical Analysis: Data was collected from a minimum of three independent experiments. Results are presented as mean ± standard error of the mean (SEM). Statistical analyses were performed using GraphPad Prism 9 software, employing Student's t-test and two-way ANOVA. Significance levels are denoted as follows: *$p < 0.05$, **$p < 0.01$, ***$p < 0.001$, and ****$p < 0.0001$ compared to the DMSO-treated control, with "ns" indicating no significant difference.

RESULTS

Chemical Characterization of Oleuropein Leaf Extract: Oleuropein constitutes the primary phenolic compound in olive leaf extract (OLE), with a concentration of 20% ± 0.1 in the dry extract [30]. Additionally, the OLE detected a minor hydroxytyrosol peak (Figure 1A). The molecular structure of oleuropein consists of elagic acid, 3,4-dihydroxyphenylethanol (hydroxytyrosol), and a glycosidic bond attached to an ester group (Figure 1A). Hydroxytyrosol's smaller size and higher bioavailability allow it to penetrate cell membranes more effectively, contributing to its therapeutic potential.

Antiproliferative and Cytotoxic Effects of Oleuropein in MCF7 and MDA-MB231 Cells: To explore the anticancer properties of oleuropein, we assessed its cytotoxic and antiproliferative effects on two breast cancer cell lines: MCF7 and MDA-MB-231. Both oleuropein extract (OLE) and oleuropein compound (OLC) were tested at concentrations ranging from 0 to 20 µM over a 48-hour treatment period.

Using the MTT assay, we confirmed the antiproliferative effects of OLE and OLC over 48 hours. At 20 µM, OLC reduced live cell percentages to 28.95% ± 1.87 in MCF7 and 54.5% ± 3.5 in MDA-MB231, while OLE resulted in 43.67% ± 2.38 live cells in MCF7 and 72.87% ± 11.8 in MDA-MB231 (Figures 1B, 1E). This dose-dependent inhibition highlights the higher sensitivity of MCF7 cells to oleuropein compared to MDA-MB231, suggesting subtype-specific mechanisms of action.

The dose-response curves revealed that MCF7 cells were approximately twice as sensitive to OLE and OLC compared to MDA-MB-231 cells. The IC50 values for MDA-MB-231 were 22.85 ± 1.86 µM (OLC) and 27.62 ± 2.38 µM (OLE), while for MCF7, they were 14.17 ± 3.5 µM (OLC) and 16.99 ± 3.4 µM (OLE) (Figures 1C-D, 1F-G). Significant cytotoxicity ($p < 0.0001$) was observed at 20

µM in both cell lines, with a notable reduction in MCF7 cell viability even at 10 µM ($p < 0.01$). These findings indicate that oleuropein effectively inhibits cell proliferation, particularly in MCF7 cells.

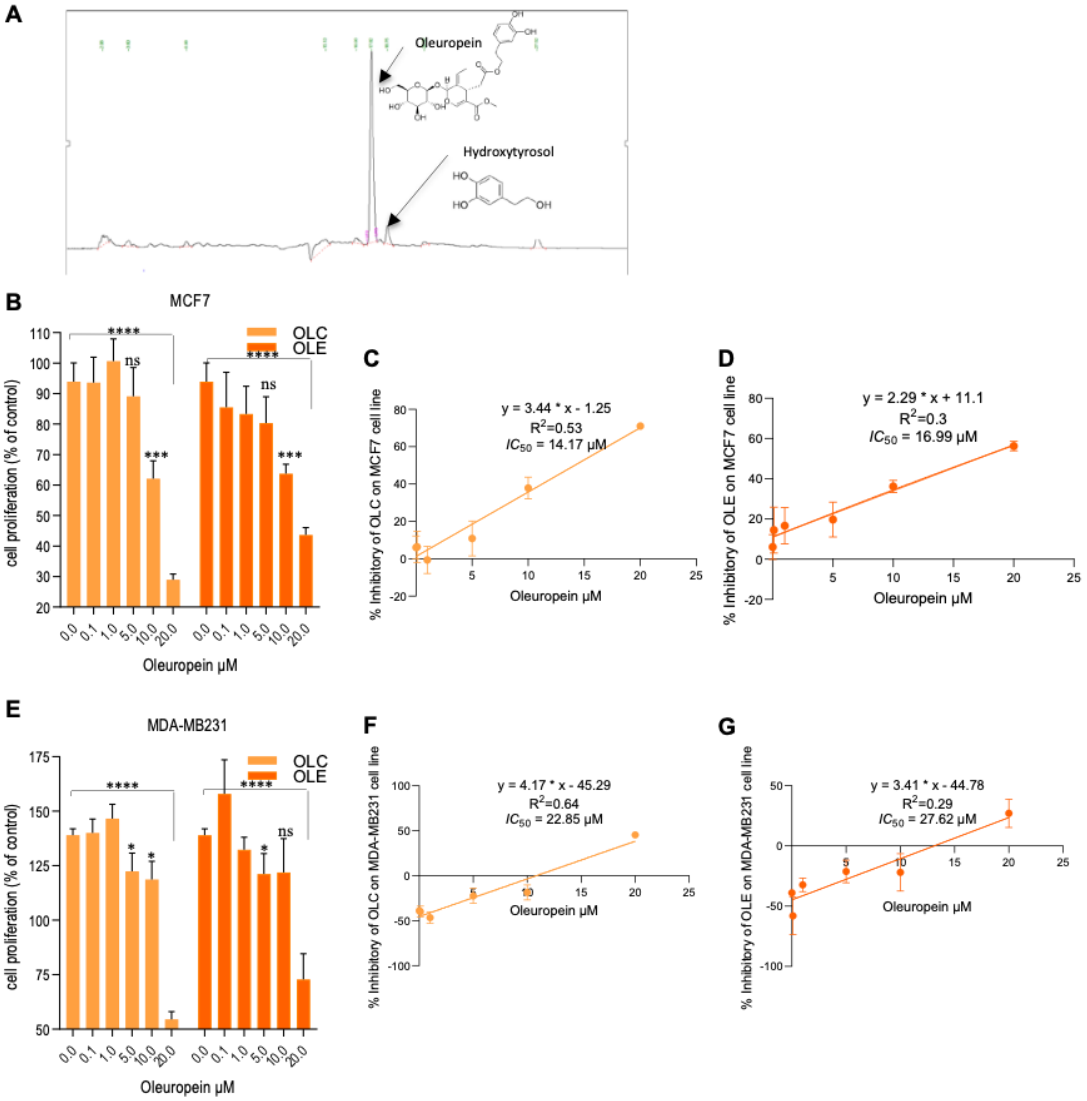

Figure 1. Proliferation and cytotoxicity assay of oleuropein in (B-D) The dose-response inhibitory cytoxicity curve for (C) MCF7 shows IC50 values 14.17 ± 3.5 µM and 16.99 ± 3.4 µM for OLC and OLE, respectively (D) MDA-MB-231 shows IC50 values of 22.85 ± 1.86 µM and 27.62 ± 2.38 µM for OLC and OLE, respectively. The data points represent the mean ± standard error of the mean (SEM) from three independent experiments, with n=12. Two-way analysis of variance (ANOVA) was applied to determine the significance of differences between the DMSO-treated control and experimental cells. Significance was indicated as ** $p < 0.01$ and **** $p < 0.0001$, while ns denotes nonsignificant differences. MCF7 and (E-G) MDA-MB-231 Cells. (A) HPLC profile of the olive leaves extract. (B) The MCF7 and (E) MDA-MB-231 triple-negative breast cancer (TNBC) cell lines were plated in 96-well plates and treated for 48 hours with concentrations of oleuropein ranging from 0, 0.1, 1, 5, 10 and 20 µM. Cell viability was expressed as a percentage of survival compared to the DMSO-treated control cells.

Preferential Sensitivity of Breast Cancer Cells to Oleuropein: To investigate the preferential sensitivity of breast cancer cells to oleuropein (OLC and OLE), we analyzed the effects of treatment on cell remodeling dynamics in MCF7 and MDA-MB231 cells. Microscopy was used to monitor 100 cells per condition over 24 hours following exposure to 1, 5, 10, and 20 µM concentrations of OLC and OLE. Each experiment was repeated twice for reproducibility (Figure 2A). Protrusion formation assays revealed dose-dependent reductions in protrusion

formation in both cell lines after treatment (Figures 2A', 2B). Notably, at 20 µM, OLC and OLE significantly impaired protrusion formation and reduced adherence to well surfaces in all analyzed cells.

Cell spreading was assessed at 60, 120, and 180 minutes post-seeding. Untreated cells exhibited progressive spreading with the development of lamellipodia and filopodia by 120 minutes, while treated cells showed delayed spreading and incomplete protrusions throughout the observation period (Figures 2A', 2C). By 180 minutes, untreated cells demonstrated extensive cytoskeletal organization, whereas treated cells maintained rounded shapes with reduced adhesion and lacked robust lamellipodia and filopodia formation.

Cells treated with oleuropein displayed typical apoptotic characteristics, such as reduced cell size, rounded mitotic morphology, and multilobed nuclei, suggesting impaired cytokinesis. (Figure 2A'). While some cells progressed through growth and division, they failed to complete cytokinesis, leading to enlarged nuclei. These disruptions were observed across all treated cells, resulting in decreased adhesion, increased floating, and eventual apoptosis. Both MCF7 and MDA-MB231 cells transitioned to rounded shapes by 180 minutes post-seeding, highlighting the compound's ability to inhibit cell spreading and induce apoptosis.

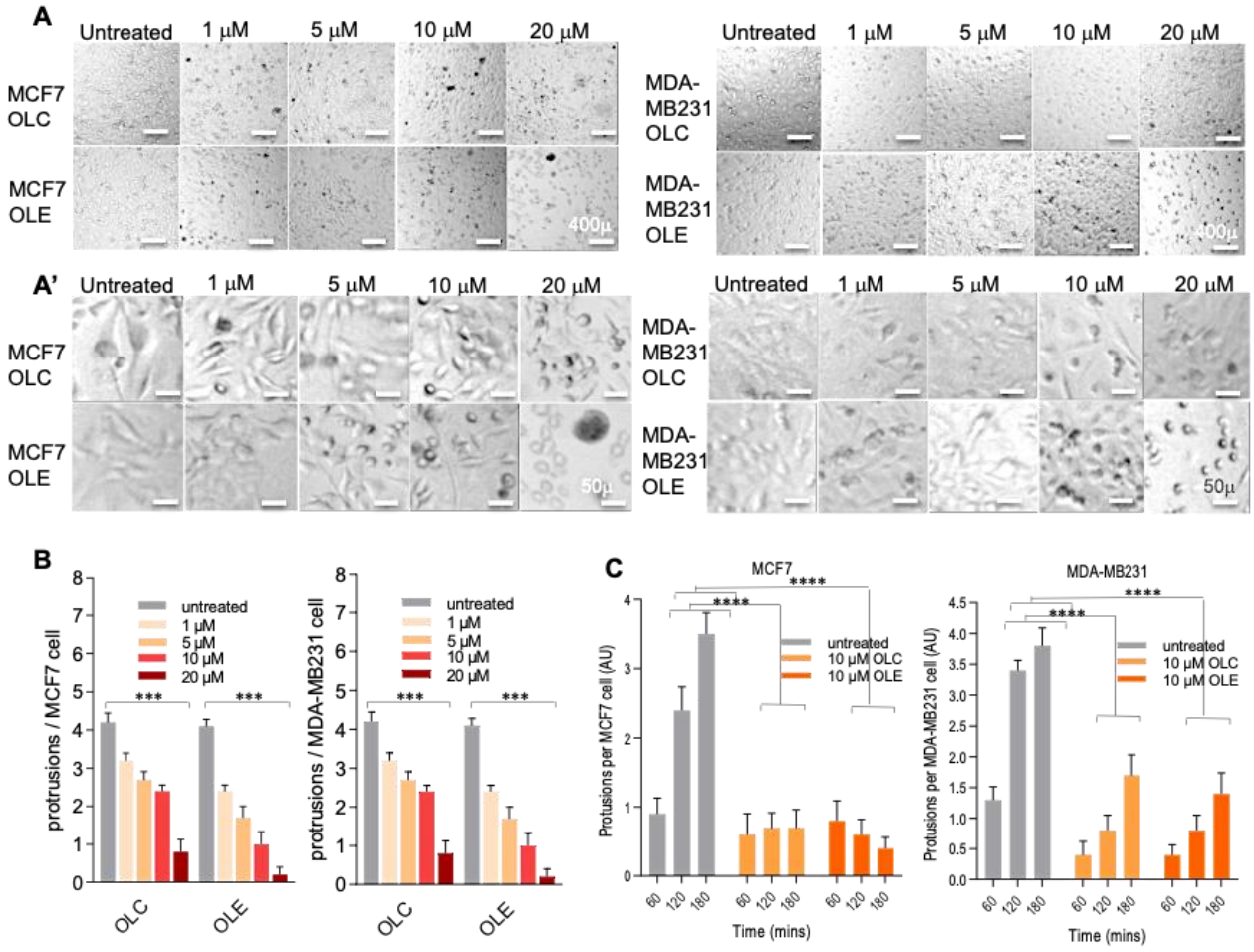

Figure 2. Oleuropein inhibits cell polarization and spreading in MCF7 and MDA-MB-231 Cells. (A-A') Spreading cells were analyzed using the microscopy to measure cell shape at different time points after 24 hours post-seeding for following treatment with 1 µM, 5 µM, 10 µM, and 20 µM concentrations of OLC and OLE, respectively with each experiment repeated twice per condition. Scales bars 50 and 400 µm (B) Quantitative analysis revealed differences in cell spreading between MCF7 and MDA-MB-231 cells. Values were taken for untreated and OLC and OLE treatments of MCF7 cells at 24 hours for each experiment (mean ± s.e.m., n = 2 experiments, *p = 0.05, ***p = 0.0001 compared to DMSO-treated control). (C) Protrusion formation was observed at 60, 120, and 180 minutes post-seeding for 100 cells per condition seeding (scale bar: 15 µm). Quantification of protrusion numbers per cell during spreading showed significant differences between conditions (mean ± s.e.m., n = 100 cells, ****p = 0.0001 vs DMSO-treated control).

Selective Inhibition of Breast Cancer Cell Motility by Oleuropein: To evaluate the impact of oleuropein (OLC and OLE) on breast cancer cell motility, we analyzed the migratory dynamics of MCF7 and MDA-MB231 cells using a scratch assay over 24 hours. Cells were treated with 1 µM and 5 µM concentrations of OLC and OLE, and their responses were assessed via microscopy (Figure 3A-B). Both compounds significantly impaired wound healing in both cell lines, with a more pronounced delay in wound closure observed at higher concentrations. Notably, MDA-MB231 cells, which are highly motile, showed a substantial reduction in migration, with fewer cells invading the scratched area compared to untreated controls (0.1% DMSO) (Figure 3C-D). This effect is attributed to disruptions in cytoskeletal reorganization and adhesion mechanisms. While MCF7 cells also exhibited reduced migration, the effects were less dramatic, consistent with their lower baseline invasiveness. Morphological changes, including cell shrinkage and rounding, were evident in treated cells, impairing their ability to adhere and migrate effectively. Increased apoptosis was further indicated by the detachment and floating of treated cells in the culture medium (Figure 3A-B). These findings demonstrate the preferential sensitivity of MDA-MB231 cells to oleuropein, underscoring its potential as a functional food-derived therapeutic agent targeting aggressive breast cancer subtypes.

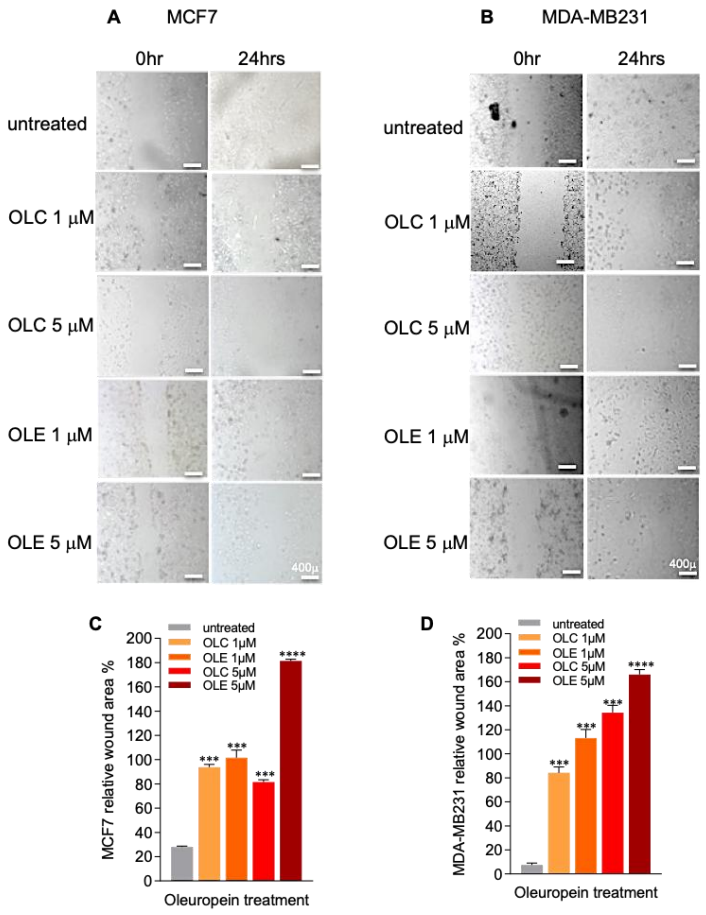

Figure 3. Oleuropein Inhibits Cell Migration in MCF7 and MDA-MB-231 Cells. The inhibitory effects of oleuropein on cell migration were evaluated using a scratch wound assay in MCF7 and MDA-MB-231 breast cancer cells. (A, B) The assay revealed distinct wound healing rates between the two cell lines, with scale bars set at 400 µm. (C, D) The percentage of wound closure relative to the initial wound area was measured at specific time points for both MCF7 and MDA-MB-231 cells. Data are presented as mean ± standard error of the mean (s.e.m.), with n=2 replicates per cell line and four independent experiments. Statistical significance was denoted as *$p < 0.05$ compared to the DMSO-treated control and **$p < 0.05$ between different treatment conditions.

Oleuropein-Induced Apoptosis in MCF7 and MDA-MB231 Cells: To investigate the mechanisms underlying the antiproliferative and pro-apoptotic potentials of OLC and OLE on breast cancer cells, flow cytometry was used to analyze apoptotic responses in MCF7 and MDA-MB231 cell lines treated with 1 µM and 5 µM concentrations of OLC and OLE for 48 hours. Using the PE Annexin V apoptosis detection kit with 7AAD, early apoptotic cells were identified by PE Annexin V positivity and 7-AAD negativity, while late apoptotic and dead cells were characterized by dual positivity. Results showed a dose-dependent increase in apoptosis for both cell lines ($p < 0.05 – p < 0.0001$), with MCF7 cells exhibiting greater sensitivity to OLE compared to OLC (Figures 4A-B, 4H-I). Specifically, at 5 µM, OLE induced 68.5% ± 4.7 late apoptotic MCF7 cells versus 46.4% ± 12.1 for OLC, highlighting OLE's superior efficacy. In MDA-MB231 cells, OLE also outperformed OLC, inducing 56.06% ± 5.7 late apoptotic cells compared to 49.03% ± 6.8 (Figures 4A-B, 4H-I).

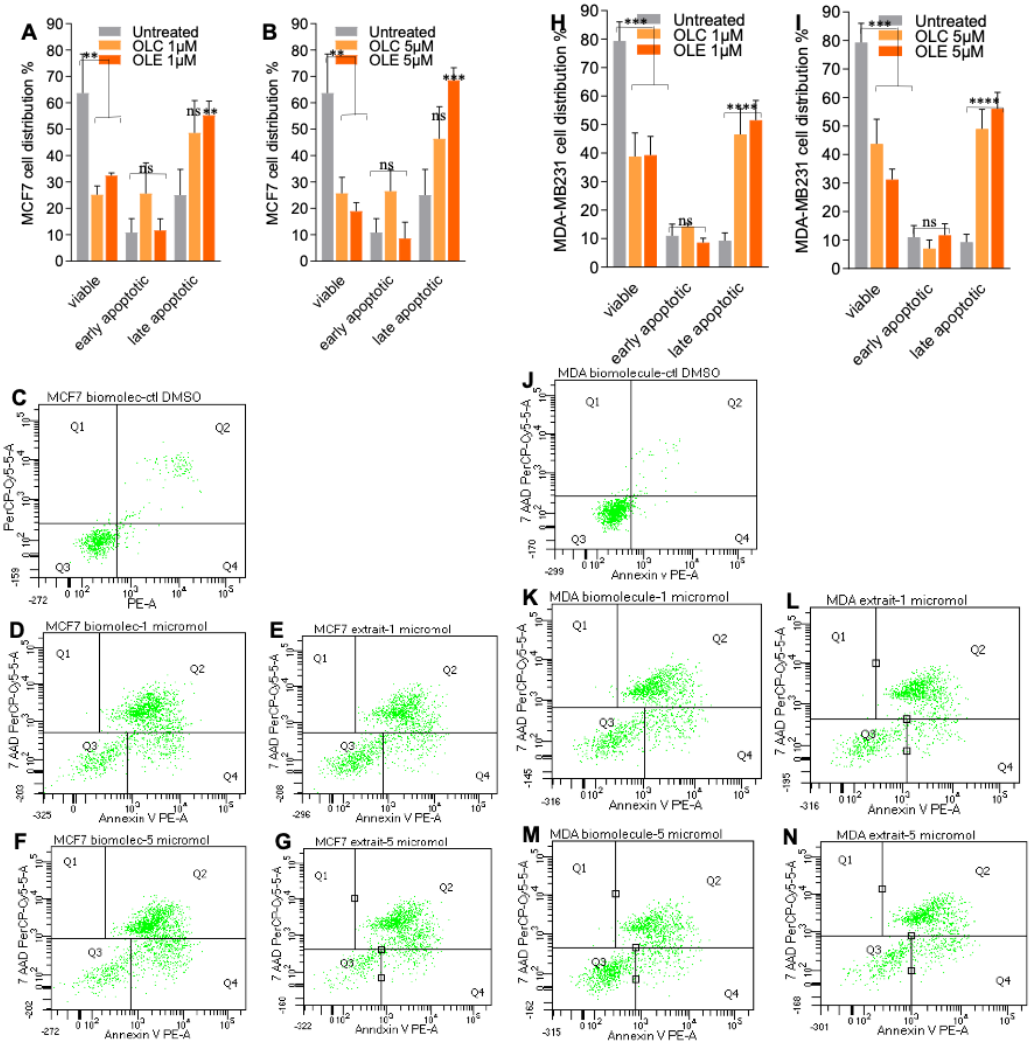

Figure 4. Apoptotic Effects of OLC and OLE in MCF7 and MDA-MB231 Cells. The apoptotic effects of OLC and OLE were evaluated in (A–G) MCF7 and (H–N) MDA-MB231 cells. Cells were treated with OLC and OLE at concentrations of 1 and 5 µM for 48 hours, while untreated control cells (0.1% DMSO) were incubated with experimental media alone. After treatment, cells were stained using an Annexin V-FITC apoptosis detection kit combined with 7-AAD (7-aminoactinomycin D). The percentages of apoptotic and necrotic cells were quantified using CellQuest software. The bar graphs represent the mean ± SEM (standard error of the mean) of the combined early and late apoptotic cell populations, derived from three independent experiments (n = 3 per experiment). Statistical analysis was performed using two-way ANOVA to compare treated groups with DMSO-treated controls. Significance levels were denoted as *$p < 0.05$, **$p < 0.01$, ***$p < 0.001$, and ****$p < 0.0001$, while "ns" indicates nonsignificant differences. PE-A refers to PE Annexin V, and PerCP-Cy5-5A corresponds to 7-AAD.

Flow cytometric analysis further quantified total cell death (Figures 5A, 5G). In MCF7 cells, OLE demonstrated enhanced cytotoxicity over OLC, with dead cell percentages increasing from 59.13% ± 5.01 at 1 µM to 71.23% ± 3.59 at 5 µM. For MDA-MB231 cells, OLE again surpassed OLC, inducing 51.83% ± 7.59 and 59.00% ± 8.33 dead cells at 1 µM and 5 µM, respectively. Overall, MCF7 cells exhibited higher sensitivity to both OLC and OLE, particularly at 5 µM, while MDA-MB231 cells displayed a more moderate response (Figures 5A, 5G).

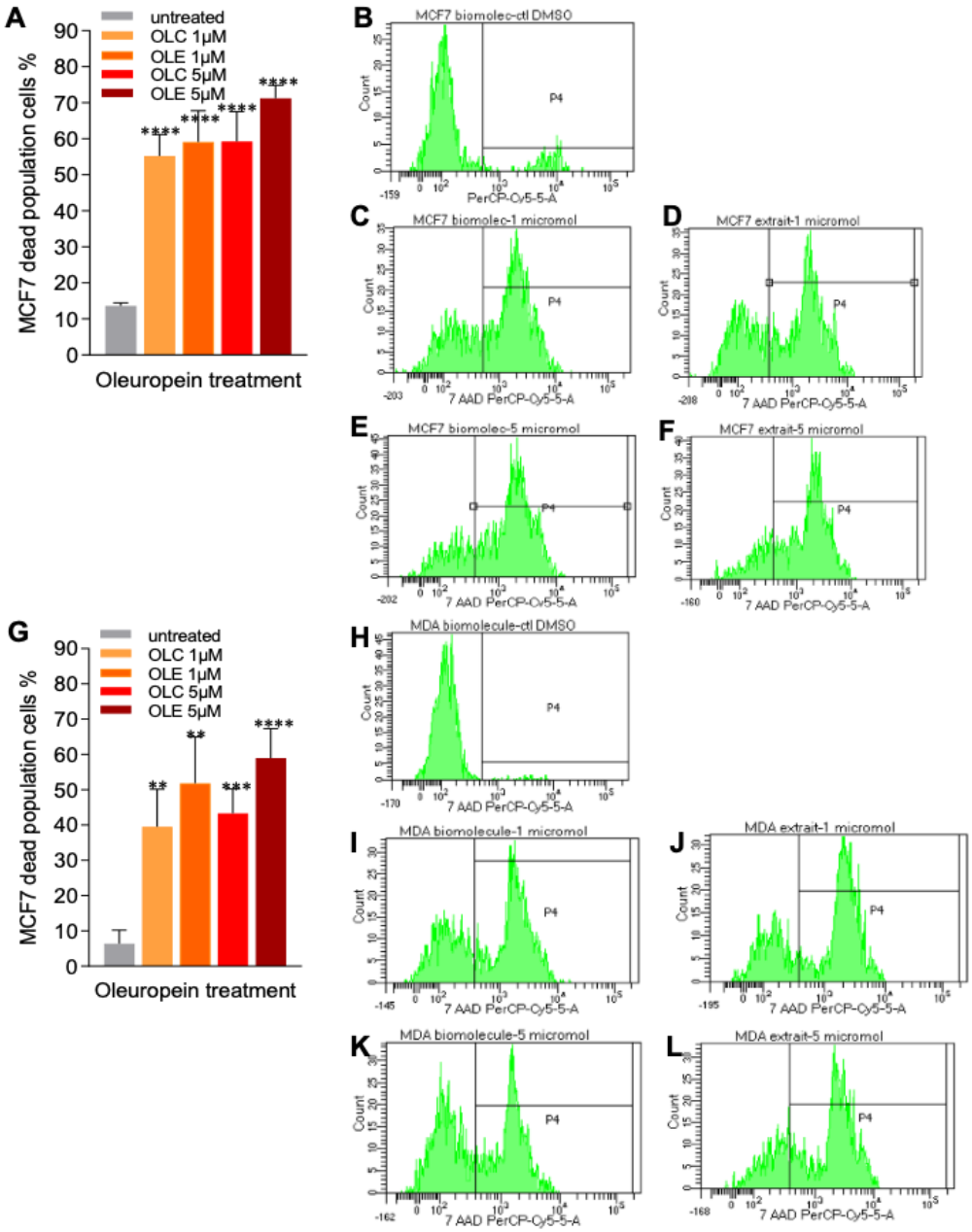

Figure 5. Oleuropein induces cell death in (A-F) MCF7 and (G-L) MDA-MB231 cells. MCF7 and MDA-MB231 cells were treated with 1 µM and 5 µM concentrations of OLC and OLE for 48 hours, while untreated control (0.1% DMSO) cells were incubated with experimental media only. Dead population cells were identified using a PE Annexin V and 7-AAD apoptotic markers. Flow cytometric analysis quantified the percentages of late apoptotic cells (PE Annexin V-positive and 7-AAD-positive) and dead population cells across treatments. Each bar in the graphs represents the mean percentage ± SEM of late apoptotic and dead cells derived from three independent experiments, each conducted in triplicate (n = 3). Statistical differences between DMSO-treated control and treatment groups were assessed using two-way ANOVA. Significance was marked as *p < 0.05, **p < 0.01, ***p < 0.001, ****p < 0.0001. ns indicates no significant difference.

These findings indicate that apoptosis is the primary mode of cell death in both cell lines, with minimal necrosis observed. The stronger apoptotic response in MCF7 cells may stem from differences in receptor status and intrinsic susceptibility to oleuropein-induced signaling pathways. Conversely, the high sensitivity of MDA-MB231 cells could be attributed to their altered genomic profiles, which predispose them to apoptosis upon treatment.

DISCUSSION

Oleuropein, a bioactive polyphenolic compound predominantly found in olive oil, has gained significant attention for its potential role in cancer therapy [3, 31]. Our study highlights its ability to induce apoptosis, inhibit cell migration, and disrupt cellular spreading in breast cancer cells, particularly in MCF7 (ER+/PR+) and MDA-MB231 (TNBC) subtypes. These findings underscore oleuropein's potential as a multi-targeted therapeutic agent, addressing the heterogeneity of breast cancer and the limitations of current treatments [32–34].

Our study demonstrates oleuropein's effectiveness at low concentrations (1–10 µM), revealing its potential as a safer therapeutic agent compared to high-dose studies (>100 µM) [35, 36]. This is significant, as lower doses typically offer better safety profiles while maintaining efficacy. Treatment resulted in dose-dependent reductions in cell viability, with IC_{50} values of 16.99 µM and 27.62 µM for MCF7 and MDA-MB231 cells, respectively (Figures 1D, 1G). MCF7 cells exhibited greater sensitivity, likely due to differences in receptor status and intrinsic signaling pathways [37]. Despite MDA-MB231 resistance to conventional therapies, oleuropein significantly impaired the spreading and migratory capacities of MDA-MB231 cells, reducing wound closure by over 65% (Figures 2B-C, 3D). This highlights its potential to prevent metastasis in aggressive subtypes.

The induction of apoptosis by oleuropein was confirmed through flow cytometry, with late-apoptotic populations increasing by 35% in MCF7 cells and 50% in MDA-MB231 cells after 48 hours of treatment (Figures 4A-B, 4H-I). This suggests that oleuropein activates both intrinsic and extrinsic apoptotic pathways, which could explain its effectiveness against cancer cells that are resistant to conventional therapies. Additionally, oleuropein's ability to modulate cyclin-dependent kinases (CDKs) and inhibit cell cycle progression further supports its role as a potent antiproliferative agent [34,38–40].

Another notable finding is oleuropein's impact on cytoskeletal dynamics. In MCF7 cells, treatment with 10 µM oleuropein reduced cellular protrusions and spreading by 70%, indicating its ability to disrupt structures essential for cell motility and invasion (Figure 2C). While we did not directly analyze actin polymerization or microtubule organization, the observed inhibition of migration suggests that oleuropein interferes with cytoskeletal remodeling [41,42]. In MDA-MB231 cells, the suppression of migration and adherence points to its potential to target proteins involved in actin polymerization, such as guanine exchange factors (GEFs) and RhoA pathway components. [43]. These effects may also extend to the inhibition of matrix metalloproteinase activity, further limiting cancer progression [44].

The implications of our findings are significant, particularly in the context of functional foods and natural therapeutics. Oleuropein's dual potential of action—inducing apoptosis and disrupting cytoskeletal dynamics—positions it as a promising candidate for complementary or alternative cancer therapies. Its natural origin and efficacy at low concentrations make it an attractive option for integration into dietary strategies aimed at cancer prevention and management.

Our study demonstrates that oleuropein effectively inhibits breast cancer cell proliferation, migration, and spreading while inducing apoptosis through multiple mechanisms. These findings highlight its potential as a selective anticancer agent, particularly for aggressive subtypes like TNBC. By integrating oleuropein into

functional foods or developing it as a therapeutic agent, we may offer new avenues for breast cancer prevention and treatment, addressing the limitations of current therapies and improving patient outcomes.

CONCLUSIONS

This study demonstrates that oleuropein, a natural bioactive compound from olive oil, effectively inhibits breast cancer cell growth, induces apoptosis, and reduces migration, even at low concentrations. It showed stronger effects on aggressive MDA-MB231 cells, highlighting its potential for treating resistant subtypes. With its low toxicity and multi-targeted action profile, oleuropein is a promising candidate for complementary cancer therapy.

Future research should explore its molecular mechanisms, *in vivo* efficacy, and potential synergies with existing treatments. Additionally, its presence in olive oil suggests a role in cancer prevention through dietary strategies. In summary, oleuropein offers significant potential for breast cancer therapy and prevention, warranting further investigation for clinical and functional food applications.

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Conflicts of Interest: The authors declare no conflicts of interest.

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