

Multifaceted Biological Activities of Linalool: Antimicrobial, Antibiofilm, Cytotoxic, Antiproliferative and Pro-Apoptotic Effects

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Abstract

Linalool is a natural bioactive monoterpene with antimicrobial, antibiofilm, and anticancer potential. Its multifaceted biological activity was investigated using microbial and mammalian cell-based models, addressing the limited integrated characterisation of these effects. In this study, the antimicrobial and antibiofilm efficacy of linalool was evaluated against selected pathogens, including *Staphylococcus aureus*, *Escherichia coli*, *Pseudomonas aeruginosa*, methicillin-resistant *Staphylococcus aureus* (MRSA), *Klebsiella pneumoniae*, and *Candida albicans*, and its cytotoxic, antiproliferative, morphological, and apoptotic effects were delineated in the C6 glioma cell line at concentrations of 25, 50, 100, 200, 400, and 800 μM . Linalool exhibited broad-spectrum antimicrobial activity against all tested microorganisms and significantly suppressed biofilm formation. Among the tested strains, *S. aureus* was the most susceptible pathogen, showing the lowest minimum inhibitory concentration (MIC) (418.8 μM) and the highest biofilm inhibition (60%). MTT analysis showed that linalool exerted dose-dependent cytotoxicity in C6 glioma cells after 24 h, with an IC_{50} of 563.3 μM (95% CI: 370.3–871.7 μM). Longitudinal trypan blue exclusion counting revealed that 400 μM linalool produced the strongest antiproliferative effect over 4 days, resulting in a 1.6-fold reduction in viable C6 cell numbers compared with the untreated control, accompanied by distinct morphological alterations. In addition, treatment with 400 μM linalool increased executioner caspase-3/7 activity by up to 2.07-fold, supporting the involvement of apoptosis in linalool-induced cytotoxicity. These findings demonstrate the multifaceted biological activity of linalool, encompassing antimicrobial, antibiofilm, cytotoxic, antiproliferative, and pro-apoptotic effects, and support its potential as a multifunctional natural bioactive compound for further investigation.

1. INTRODUCTION

Linalool ($\text{C}_{10}\text{H}_{18}\text{O}$) is a naturally occurring monoterpene alcohol that is gaining increasing attention due to its wide range of significant biological effects, especially antimicrobial and anticancer (An et al, 2021; Singh and Mishra, 2024). Previous studies have reported that linalool has antimicrobial activity against Gram-positive and Gram-negative bacteria, as well as fungal pathogens, and inhibits biofilm formation (Guo et al., 2021; Herman et al, 2015; Aslan and Alim, 2025). In addition, linalool has been found to have anticancer effects against various types of cancer, including prostate, lung, and breast cancer in vitro and in vivo models (Elbe et al., 2022; Zhang and Chen, 2024; Zhao et al., 2020; Rodenak-Kladniew et al., 2020). Beyond its antimicrobial and anticancer properties, linalool also exhibits antioxidant, anti-inflammatory, and anticancer activities, further supporting its potential as a multifunctional bioactive compound (An et al., 2021).

Antimicrobial resistance (AMR) is one of the major global public health challenges, leading to treatment failure, prolonged illness, and an increasing healthcare burden worldwide (WHO, 2025; Murray et al., 2022). The continued emergence of resistant microorganisms highlights the urgent need for novel antimicrobial agents with alternative mechanisms of action.

One of the most important factors contributing to antimicrobial resistance is biofilm formation. Biofilms are structured microbial communities in which microorganisms attach to surfaces and are embedded in a self-produced extracellular polymeric matrix composed mainly of polysaccharides, proteins, and extracellular DNA (Flemming et al., 2016). Microorganisms within biofilms exhibit significantly greater tolerance to antimicrobial agents than planktonic cells. This tolerance is associated with limited drug penetration, reduced metabolic activity, and the activation of specific resistance mechanisms (Hall and Mah, 2017). Furthermore, biofilms enable pathogens to evade host immune responses, thereby enhancing microbial persistence and virulence (Montanari et al., 2025). Therefore, preventing biofilm formation and inhibiting microbial attachment are considered key strategies in the fight against antimicrobial resistance. In this context, the identification of natural compounds, such as linalool, that exhibit both antimicrobial and antibiofilm activities has gained considerable importance (Herman et al., 2015; Aslan and Alim, 2025).

Brain and central nervous system (CNS) cancers, despite their relatively low incidence, represent a major public health challenge because of their high mortality and profound impact on patients' quality of life (Ilic & Ilic, 2023). CNS malignancies account for approximately 2.5% of all cancer-related deaths, with gliomas representing the most common and aggressive primary brain tumors characterised by poor prognosis and limited therapeutic success (Filho et al., 2025; Kim et al., 2025; Rezanejad-Asl et al., 2025). Although current therapeutic strategies have improved patient management, conventional chemotherapeutic agents often lack tumor specificity and may induce severe toxicity in normal brain tissues (Was et al., 2022). Consequently, naturally derived bioactive compounds have attracted increasing attention as potential alternative or complementary therapeutic agents because of their diverse biological activities and favourable safety profiles (Ravizza et al., 2008; Han et al., 2016; Zhao et al., 2020). Previous studies have demonstrated that linalool exerts anticancer effects in several tumor models, including breast, prostate, colon, and human glioblastoma cells, by suppressing cell proliferation and promoting apoptosis-related pathways (Elbe et al., 2022; Zhao et al., 2020; Rodenak-Kladniew et al., 2020; Cheng et al., 2017; Zhang & Chen, 2024). Although linalool has been reported to exhibit diverse biological activities, its effects in the C6 rat glioma model remain insufficiently characterised. Therefore, a comprehensive evaluation of its cytotoxic, antiproliferative, morphological, and pro-apoptotic effects, together with its antimicrobial and antibiofilm activities, may provide a broader understanding of the biological potential of this natural monoterpene.

Accordingly, the present study investigated the antimicrobial and antibiofilm activities of linalool against *Staphylococcus aureus*, *Escherichia coli*, *Pseudomonas aeruginosa*, methicillin-resistant *Staphylococcus aureus* (MRSA), *Klebsiella pneumoniae*, and *Candida albicans*, and evaluated its cytotoxic, antiproliferative, morphological, and pro-apoptotic effects in the C6 rat glioma cell line. By examining linalool across both microbial and mammalian cell-based models, this study provides a broader characterization of its biological activity and highlights its potential as a multifunctional natural bioactive compound.

2. MATERIALS AND METHODS

2.1. Antimicrobial activity of linalool against bacterial and fungal strains

The antimicrobial activity of linalool was evaluated using the broth microdilution (BMD) method in accordance with the guidelines of the Clinical and Laboratory Standards Institute (CLSI) and the European Committee on Antimicrobial Susceptibility Testing (EUCAST), with minor modifications based on previous studies (CLSI, 2023; EUCAST, 2024). Standard reference strains were used in all antimicrobial assays. The tested microorganisms included *E. coli* ATCC 25922, *S. aureus* ATCC 25923, Methicillin-Resistant *S. aureus* (MRSA) ATCC 43300, *P. aeruginosa* ATCC 27853, *K. pneumoniae* ATCC 700603, and *C. albicans* ATCC 10231. Antimicrobial assays were performed in cation-adjusted Mueller-Hinton Broth (CAMHB) for all microbial strains. The assays were carried out in 96-well U-bottom microtiter plates. Linalool stock solutions were prepared in dimethyl sulfoxide (DMSO) and diluted with appropriate culture medium. Two-fold serial dilutions were prepared to obtain a range of test concentrations (2048 µg/mL to 4 µg/mL). The final DMSO concentration in all test wells did not exceed 1% (v/v). Microbial inocula were prepared from fresh overnight cultures and adjusted to a final concentration of approximately 5×10^5 CFU/mL for bacterial strains and $0.5\text{--}2.5 \times 10^3$ CFU/mL for yeast. Each well was inoculated with the standardised microbial suspension and the corresponding concentration of linalool. Reference antimicrobial agents were tested in parallel under identical experimental conditions to obtain comparative MIC values. Wells containing medium and inoculum without linalool or antibiotics served as growth controls, while wells containing medium alone were used as sterility controls. Solvent control wells containing the highest DMSO concentration were also included. The microplates were incubated at 37 °C for 24 hours under aerobic conditions. After incubation, microbial growth was evaluated by visual inspection. The minimum inhibitory concentration (MIC) was defined as the lowest concentration of linalool that prevented visible microbial growth compared with the growth control wells (Aslan and Alim, 2025).

2.2. Inhibition of biofilm formation by linalool

The effect of linalool on biofilm formation was evaluated using the crystal violet (CV) microtiter plate (MtP) method (Stepanović et al., 2000). Biofilm formation was assessed at a single sub-inhibitory concentration (MIC/2) to determine the percentage of biofilm inhibition compared with untreated control wells. Biofilm assays were performed in 96-well, U-bottom microplates. Tryptic Soy Broth (TSB) medium supplemented with 1% glucose was used to support biofilm formation. Microbial suspensions were prepared from fresh overnight cultures and adjusted to approximately 1×10^6 CFU/mL. Linalool was added to the wells at sub-inhibitory concentrations (MIC/2). Wells containing medium and microorganisms, without linalool, served as positive biofilm controls, while wells containing medium alone served as negative controls. The plates were incubated at 37 °C under static conditions for 48 hours. After incubation, planktonic cells were removed, and the wells were washed three times with sterile phosphate-buffered saline (PBS) to remove non-adherent cells. The remaining attached biofilms were fixed with methanol for 15 minutes and then stained with 0.1% CV for 15 minutes at room

temperature. Excess stain was removed by washing with distilled water. The bound CV was dissolved using 95% ethanol. Biofilm formation was quantified by measuring absorbance at 570 nm using a microplate reader. The percentage of biofilm inhibition was calculated by comparing the absorbance values of linalool-treated wells with those of the untreated control wells using the following formula:

Biofilm Inhibition (%) = $100 - [(OD_{\text{sample}} / OD_{\text{control}}) \times 100]$ (Aslan and Alim, 2025).

2.3. Cell culture

Glioma cell line (C6) cells were cultured in DMEM (SERANA EUROPE - MCL-004-500ML) supplemented with 10% FBS (Capricorn, FBS-HI-11A), 100 Units/mL penicillin, and 100 µg/mL streptomycin. Cells were incubated with 5% CO₂ at 37°C.

2.4. Cytotoxic effects of linalool on C6 glioma cells

C6 cells were plated at 7500 cells/well in a 96-well plate in DMEM and allowed to adhere for 24 hours. After incubation, the medium was removed, and linalool in different concentrations (25, 50, 100, 200, 400 µM) was applied to the cells. After 24 h, the growth medium was removed, and 5 mg/mL MTT (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide) reagent was added to the wells. After 4 h incubation, the medium containing the MTT solution was removed, and 100 µL DMSO was added to each well to dissolve the formazan crystals. Their absorbance was measured at 540 nm using a microplate spectrophotometer (Thermo Multiskan Go), and the number of living cells was calculated using the equation described by Subasi et al. (2020). The IC₅₀ (half-maximal inhibitory concentration) value for each compound was determined individually by plotting concentration versus the number of viable cells. The experiments were carried out at three different times.

2.5. Morphological alterations induced by linalool in C6 cells

20x10⁴ C6 cells were plated in a 24-well plate and allowed to adhere for 24 hours. After incubation, the medium was removed, and linalool at 100, 200, and 400 µM was added to the cells. In the control group, cells were incubated in DMEM. After 24 hours of incubation, changes in cell morphology were examined and photographed in bright-field mode at 20X, and 40X using a fluorescence microscope (Zeiss Axio) workstation under transmitted light, without the application of any exogenous fluorescent stains (Atalay et al., 2023).

2.6. Antiproliferative effects of linalool on C6 glioma cells

20x10⁴ C6 cells were plated in a 24-well plate and allowed to adhere for 24 hours. After incubation, the medium was removed, and linalool (100, 200, and 400 µM) was applied to the cells. In the control group, cells were incubated in DMEM. After 48, 72, and 96 hours, cells in a 24-well plate were harvested and mixed with 0.4% (w/v) Trypan Blue at a 1:1 ratio to exclude non-viable cells. The remaining viable cells were quantified using a Thoma slide to determine the cellular proliferation rate under a light microscope (Atalay et al., 2023).

2.7. Linalool induces caspase-3/7 activity in C6 glioma cells

Caspase-3/7 activity was determined using a colorimetric Caspase-3/7 Activity Assay Kit (Elabscience, Cat. No. E-CK-A383, USA) according to the manufacturer's instructions (Cakmak et al., 2026). C6 cells were treated with linalool in 100, 200, and 400 μM concentrations, while untreated cells served as the control group. Following linalool treatment, cells were collected, washed with PBS, and counted. 1.5×10^6 cells were lysed using the supplied cell lysis buffer on ice for 30 min. The lysates were centrifuged at $11,000 \times g$ for 10 min at 4°C , and the supernatants were used for enzymatic analysis. For each reaction, the reaction mixture contained $2\times$ reaction buffer and Ac-DEVD-pNA substrate in a final volume of 100 μL . The reaction mixtures were incubated at 37°C for 2 h, and the absorbance was measured at 405 nm using a microplate reader.

Relative caspase-3/7 activity was calculated after blank correction and normalization to total protein concentration using the following formula:

Relative caspase-3/7 activity = $[(A_{405} \text{ treated} - A_{405} \text{ blank}) / \text{protein concentration of treated sample}] \div [(A_{405} \text{ control} - A_{405} \text{ blank}) / \text{protein concentration of untreated control}]$

2.8. Protein extraction

C6 glioma cells were treated with linalool at concentrations of 100, 200, and 400 μM , while untreated cells served as the control group. Following 24 h of treatment, cells were harvested, washed twice with phosphate-buffered saline (PBS), and counted. A total of 1.5×10^6 cells were lysed in ice-cold RIPA lysis buffer (SERVA, Germany) and sonicated for 30 s to ensure complete cell disruption. The lysates were incubated on ice for 10 min and subsequently centrifuged at 10,000 rpm for 15 min at 4°C . The resulting supernatants containing total cellular proteins were collected for further analysis.

Total protein concentrations were quantified using the Bradford assay (SERVA, Germany) according to the manufacturer's instructions. A bovine serum albumin (BSA) standard curve (500–4000 $\mu\text{g/mL}$) was generated for protein quantification. Briefly, 10 μL of each protein sample or BSA standard was mixed with 190 μL of freshly prepared BCA working reagent in a 96-well microplate and incubated at room temperature for 5 min. Absorbance was measured at 595 nm using a microplate reader (Thermo Scientific Multiskan GO). All measurements were performed in triplicate.

2.9. Statistics

All experiments were performed using at least three independent biological replicates, each with technical replicates as indicated. Statistical analyses were conducted using GraphPad Prism version 8.0 (GraphPad Software, CA, USA). Data are presented as mean $\pm$ standard error of the mean (SEM). Differences were considered statistically significant at $p < 0.05$.

3. RESULTS

3.1. Antimicrobial Activity

The antimicrobial activity of linalool against the tested standard reference strains was assessed using the broth microdilution method, and the results are summarised in Table 1 and Fig. 1. Linalool showed antimicrobial activity against all tested microorganisms, with MIC values ranging from 414.8 to 1659.4 μ M. Among the tested strains, *S. aureus* exhibited the lowest MIC value (414.8 μ M), followed by *E. coli* and *C. albicans* (829.7 μ M). Higher MIC values were recorded for MRSA, *P. aeruginosa*, and *K. pneumoniae* (1659.4 μ M). MIC values were defined as the lowest concentrations that showed no visible microbial growth and are displayed on a logarithmic scale in Fig. 1.

Table 1
Antimicrobial and antibiofilm activity of linalool against standard reference strains

Microorganisms	MIC (μ M)	Reference Drug	MIC of Reference Drug	Biofilm Inhibition (%)
E. coli	829.7	Ampicillin	11.45	43.211
S. aureus	414.8	Ampicillin	2.86	64.767
MRSA	1659.4	Vancomycin	1.35	37.755
P. aeruginosa	1659.4	Ciprofloxacin	2.72	33.126
K. pneumoniae	1659.4	Ciprofloxacin	2.72	31.394
C. albicans	829.7	Fluconazole	26.12	51.482
MIC values were determined by the microdilution method and defined as the lowest concentration showing no visible growth. The MIC values of the reference antimicrobial agents shown in this table were experimentally determined under the same test conditions and presented for comparative purposes. Biofilm inhibition (%) was calculated relative to untreated control wells using the crystal violet assay.				

The antibiofilm activity of linalool was assessed using the CV assay, and biofilm formation inhibition was expressed as percentage values relative to untreated control wells. As shown in Table 1, linalool inhibited biofilm formation in all tested microorganisms. Biofilm inhibition at MIC/2 ranged from 31.39% to 64.77%. The highest biofilm inhibition was observed against *S. aureus* (64.77%), followed by *C. albicans* (51.48%) and *E. coli* (43.21%). Moderate levels of biofilm inhibition were detected for MRSA (37.76%), *P. aeruginosa* (33.13%), and *K. pneumoniae* (31.39%) (Table 1, Fig. 2).

3.2. Anticancer activities

The cytotoxic effects of linalool at different concentrations (25–400 μ M) have been investigated by a colourimetric MTT assay. The results showed that linalool displayed cytotoxic activity in a dose-dependent manner (Fig. 3). Non-linear regression analysis performed via GraphPad Prism software

revealed that the exact IC_{50} value of linalool on C6 cells was $563.3 \mu M$ (95% Confidence Interval: $370.3-871.7 \mu M$) after 24 hours of exposure (Fig. 3).

The changes in cell morphology were observed using fluorescence microscopy in bright-field mode. The C6 cells exhibit an epithelial-like morphology; these large cells often contain multiple nucleoli. In the control group, the cells maintain their regular shape and membrane integrity (Fig. 4a). However, after 24 hours of linalool treatment (100, 200, and $400 \mu M$), noticeable morphological changes occurred. The axonal segments of linalool-treated cells have disappeared, and the cells have become separated from each other (Fig. 4b-d). Some irregular cells are indicated with a red arrow in the Fig. at 40X magnification. It is hypothesised that linalool treatment induces molecular and phenotypic changes, including morphological alterations such as changes in cell shape and appearance.

To assess the effect of linalool on C6 cell proliferation, cell numbers were counted over four days following treatment with 100, 200, and $400 \mu M$ linalool. As shown in Fig. 5, linalool treatment leads to a gradual decrease in cell count over this period. The cell numbers did not significantly differ from the control at the end of the first and second days for all concentrations (Fig. 5). By the end of the fourth day, cell numbers decreased by 1.2-, 1.4-, and 1.6-fold in the 100, 200, and $400 \mu M$ linalool-treated groups, respectively, compared to the control (Fig. 5). Overall, the results indicate that linalool significantly inhibits cell proliferation after four days.

To determine whether the cytotoxic and antiproliferative effects of linalool were mediated through apoptosis, caspase-3/7 activity was assessed in C6 glioma cells following exposure to linalool. Caspase-3/7 activity was significantly increased compared with the untreated control at all tested concentrations. After 2 h of substrate incubation, enzyme activity remained elevated, reaching 1.70-, 1.92-, and 2.07-fold of the control (Fig. 6). Among the tested concentrations, 200 and $400 \mu M$ linalool produced the highest caspase-3/7 activation throughout the assay, suggesting enhanced activation of apoptotic pathways in C6 glioma cells (Fig. 6).

4. DISCUSSION

Linalool is an acyclic monoterpene alcohol found in the essential oils of more than 200 plant species and exhibits a broad biogeographical distribution (Kamatou and Viljoen, 2008; Elsharif et al., 2015; Pereira et al., 2018). Because of its antioxidant, anti-inflammatory, antimicrobial, and anticancer properties, linalool has been widely used in diverse sectors, including food, household products, and healthcare. In this study, we investigated the potential application of linalool in healthcare by evaluating its antimicrobial, antibiofilm, and anticancer activities. In this study, linalool exhibited broad-spectrum antimicrobial activity, significantly inhibited biofilm formation, and exerted cytotoxic, antiproliferative, and pro-apoptotic effects in C6 glioma cells. These findings support the multifaceted biological activity of linalool and extend previous evidence by characterizing its antimicrobial, antibiofilm, and cellular effects within an integrated experimental framework, including its activity in the relatively less explored C6 glioma model.

Among the tested microorganisms, *S. aureus* showed the strongest antimicrobial activity and the highest biofilm inhibition by linalool (Figs. 1 and 2). This finding is consistent with previous studies reporting that *Staphylococcus* species are among the most susceptible microorganisms to linalool. Silva et al. (2015) demonstrated that *S. aureus* was more susceptible to linalool than Gram-negative bacteria, while Herman et al. (2016) also reported greater antimicrobial activity against Gram-positive bacteria. This higher susceptibility has been attributed to structural differences in the bacterial cell wall, which may facilitate interactions between lipophilic compounds, such as linalool, and the cell membrane (Burt, 2004; Nazzaro et al., 2013). Together, these findings support our results and suggest that *S. aureus* is a particularly suitable model for investigating the antimicrobial and antibiofilm potential of linalool.

In the present study, biofilm formation was evaluated after 48 h of incubation, allowing the development of a stable biofilm before assessing the inhibitory effect of linalool. Similar incubation periods have been used in CV-based biofilm assays to ensure sufficient biofilm establishment before treatment or quantitative evaluation (Merritt et al., 2005; O'toole, 2011). Biofilm formation is especially problematic in healthcare environments, where it often develops on medical devices such as catheters, prosthetic implants, endotracheal tubes, and dental materials. Biofilm-related infections are known to contribute to prolonged hospitalisation, reduced therapeutic efficacy, increased morbidity and mortality, and higher healthcare costs (Jamal et al., 2018). An additional important finding from this study is that antibiofilm activity does not always align with planktonic antimicrobial susceptibility. Similar discrepancies have been reported in previous studies, where linalool and linalool-rich preparations have been shown to disrupt biofilm formation even when their effects on planktonic growth are moderate. These findings suggest that antibiofilm activity may involve mechanisms distinct from growth inhibition, including interfering with initial adhesion or extracellular matrix formation (Herman et al., 2015; Mączka et al., 2022). Moreover, it has been demonstrated that linalool can inhibit biofilm formation and decrease biofilm development by disrupting microbial adhesion and extracellular matrix production (Aslan and Alim, 2025; Wang et al., 2025). Together, these findings highlight the importance of assessing both planktonic and biofilm-associated responses when evaluating the biological potential of natural compounds such as linalool.

Several bacterial and fungal pathogens have been classified as priority organisms due to their high capacity to develop antimicrobial resistance, including *S. aureus*, *Acinetobacter baumannii*, *E. coli*, *Enterococcus spp.*, *P. aeruginosa*, *K. pneumoniae*, and *C. albicans* (WHO, 2024). These pathogens are among the leading causes of hospital- and community-acquired infections and are often associated with severe clinical outcomes, particularly in hospitalised and immunocompromised patients (Murray, 2022). In our study, the antimicrobial activity of linalool was evaluated against these microorganisms, yielding promising results (Fig. 1 and Fig. 2). Our data showed that linalool is effective against all target microorganisms (Table 1), further supporting its potential as a broad-spectrum antimicrobial agent. The antimicrobial activity of linalool against these organisms, as observed in the present study, further supports its potential as a broad-spectrum antimicrobial agent. The antimicrobial activity observed in the present study is consistent with previous reports suggesting that linalool primarily exerts its effects

through membrane disruption, increased membrane permeability, and leakage of intracellular components (Herman et al., 2015; Aslan and Alim, 2025).

Brain cancer remains a significant global health burden, with approximately 322,000 new cases and 249,000 deaths reported worldwide in 2022, highlighting the urgent need for more effective therapeutic approaches (Filho et al., 2025; Rezanejad-Asl et al., 2025). Despite global efforts, including the WHO and United Nations Sustainable Development Goals aimed at reducing premature cancer-related mortality by 2030, current trends indicate that achieving these targets may be challenging, particularly in developing regions (Ilic & Ilic, 2023). Consequently, attention has increased on naturally derived multifunctional compounds that may complement conventional cancer therapies because of their diverse biological activities and favourable safety profiles (Newman & Cragg, 2020; Ali et al., 2025). Among these compounds, linalool has emerged as a promising candidate owing to its reported anti-proliferative, pro-apoptotic, and anti-metastatic activities in several experimental cancer models (Elbe et al., 2022; Zhao et al., 2020; Rodenak-Kladniew et al., 2020). Furthermore, a previous study demonstrated that linalool induced apoptosis in human glioblastoma cells by activating the SIRT3–SOD2–ROS signalling pathway, supporting its potential as a therapeutic agent against glioma (Cheng et al., 2017).

Previous studies have demonstrated that the cytotoxic activity of linalool varies with cancer type, treatment duration, and concentration used. Linalool exhibits potent cytotoxicity at relatively low concentrations in lymphoma and cervical cancer cells, whereas higher concentrations are required to achieve comparable effects in breast, colon, and prostate cancer models (Chang et al., 2015; Elbe et al., 2022; Zhao et al., 2020; Rodenak-Kladniew et al., 2020). Treatment with 200 and 400 μM linalool significantly reduced C6 glioma cell viability after 24 h, with the strongest cytotoxic effect observed at 400 μM (Fig. 3). The IC_{50} value obtained in the present study (563.3 μM) is consistent with the concentration range reported by Cheng et al. (2017), who observed an approximately 50% reduction in the viability of U87-MG glioblastoma cells at linalool concentrations between 400 and 800 μM . Although the underlying molecular mechanisms were not investigated in the present study, the observed reduction in cell viability suggests that linalool may affect conserved cellular pathways involved in glioma cell survival, warranting further mechanistic investigations.

To date, studies on the anticancer activity of linalool have mainly focused on its antiproliferative, pro-apoptotic, and anti-invasive properties (Chang et al., 2015; Elbe et al., 2022; Zhao et al., 2020; Rodenak-Kladniew et al., 2020). In addition to these key hallmarks, morphological evaluation provides valuable insight into treatment-induced phenotypic alterations associated with cancer progression (Hanahan and Weinberg, 2011). In the present study, treatment with 100, 200, and 400 μM linalool induced marked morphological changes in C6 glioma cells (Fig. 4). Such changes are commonly associated with cytoskeletal reorganisation and apoptosis-related processes (Elbe et al., 2022; Rodenak-Kladniew et al., 2020). Furthermore, 24 h of linalool treatment increased caspase-3/7 activity at all tested concentrations (Fig. 6). As executioner caspases, caspase-3 and -7 play a central role in the final stage of apoptosis (Elmore, 2007; Galluzzi et al., 2018). Our findings are consistent with those of Cheng et al. (2017), who demonstrated that linalool induced apoptosis in human glioblastoma cells through activation of the

SIRT3-SOD2-ROS pathway. Similar pro-apoptotic effects of linalool have also been reported in other cancer models (Elbe et al., 2022; Zhao et al., 2020). Taken together with the observed reduction in cell viability and proliferation, the increased caspase-3/7 activity supports apoptosis as a major mechanism underlying the anticancer effects of linalool in C6 glioma cells.

Uncontrolled cell proliferation is a defining hallmark of cancer and represents a major target in anticancer therapy (Huang et al., 2025). Consistent with this concept, linalool showed significant antiproliferative effects at 200 and 400 μ M throughout the four-day treatment period, with the strongest inhibition observed on the fourth day. (Fig. 5). These results are in agreement with previous studies conducted on breast and prostate cancer cell lines (Elbe et al., 2022; Zhao et al., 2020). Our findings extend these observations to the C6 glioma cells and indicate that linalool consistently suppresses tumor cell proliferation across different cancer types. The sustained anti-proliferative effect observed over time further supports the potential of linalool to interfere with long-term cancer cell growth.

Despite the promising findings, this study has several limitations. First, the antimicrobial and antibiofilm activities of linalool were evaluated under in vitro conditions using standard reference strains, which may not fully recapitulate the complexity of clinical infections. Therefore, future studies should incorporate clinical isolates and mixed-species biofilm models to more accurately assess the therapeutic potential of linalool. Second, the present study was primarily designed as a phenotypic evaluation of linalool's biological activity; therefore, the molecular mechanisms underlying its cytotoxic and anti-proliferative effects were not investigated. Nevertheless, the observed cytotoxicity, inhibition of cell proliferation, and pronounced morphological alterations collectively suggest the possible involvement of regulated cell death pathways (Elmore, 2007; Galluzzi et al., 2018). Third, the anticancer effects of linalool were examined using a single glioma cell line (C6). Further studies using additional human glioma cell lines, normal brain cells, and mechanistic approaches, including analyses of apoptosis and the cell cycle, will be essential to validate and extend these findings. Finally, in vivo studies are necessary to evaluate the pharmacokinetics, bioavailability, safety, and therapeutic efficacy of linalool before any potential clinical application.

In conclusion, linalool demonstrated broad-spectrum antimicrobial activity and significant antibiofilm effects against the tested microorganisms, with *S. aureus* showing the highest susceptibility. In C6 rat glioma cells, linalool exerted cytotoxic and antiproliferative effects accompanied by marked morphological alterations and increased caspase-3/7 activity, supporting the involvement of apoptosis in linalool-induced cytotoxicity. Together, these findings provide an integrated characterization of the antimicrobial, antibiofilm, cytotoxic, antiproliferative, and pro-apoptotic activities of linalool and further support its multifaceted biological activity. Overall, this study highlights linalool as a natural bioactive compound with potential for further investigation in antimicrobial and cancer-related research.

Declarations

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Competing Interests

The authors have no relevant financial or non-financial interests to disclose.

Author Contributions

All authors contributed to the study conception and design. Conceptualisation, investigation, data curation, methodology, and writing-review and editing were performed by E.B.A. Conceptualization, data curation, methodology, and writing of the original draft were performed by R.A. All authors read and approved the final version of the manuscript.

Data Availability

The data supporting the findings of this study are available from the corresponding author upon reasonable request.

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Figures

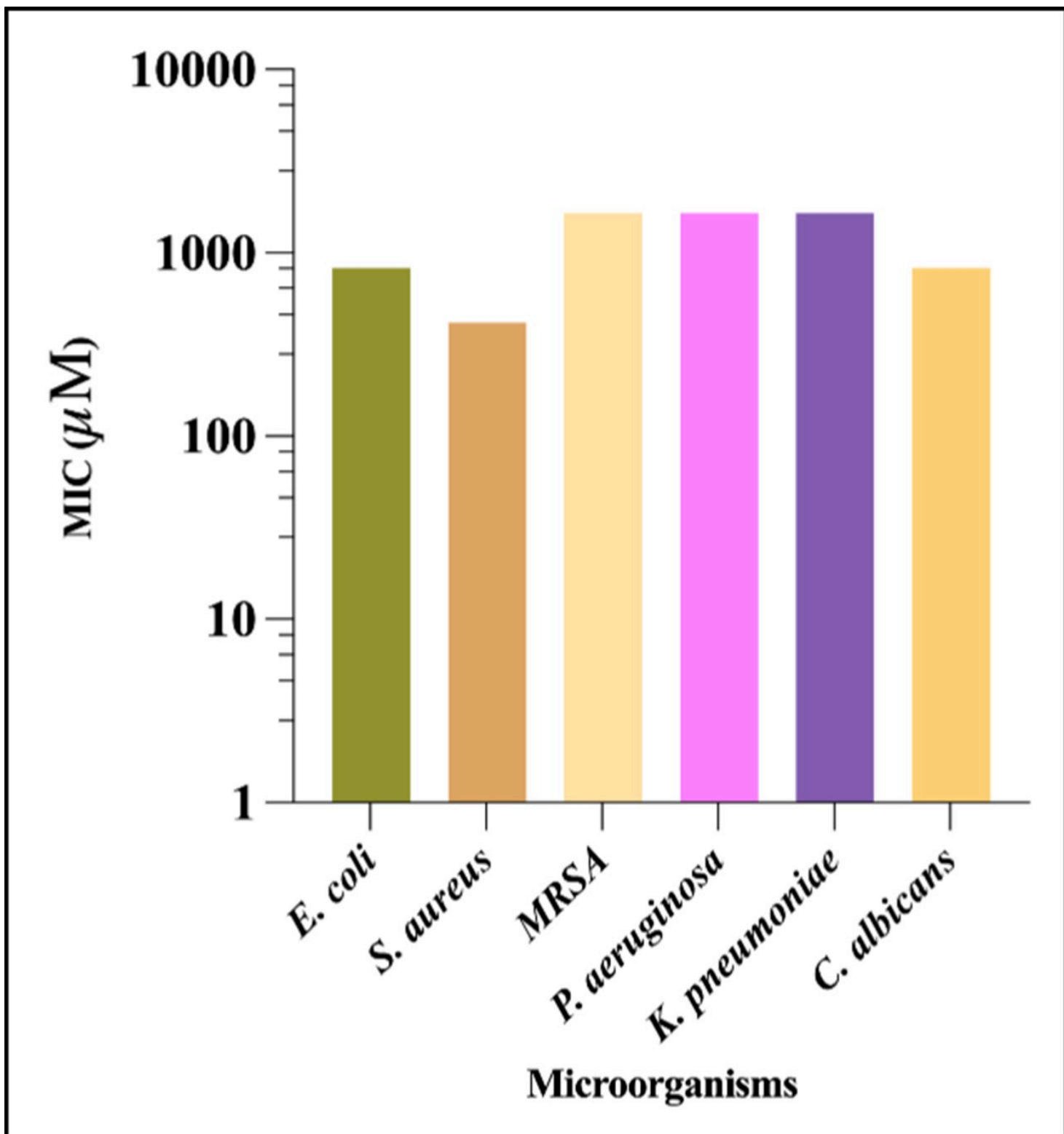


Figure 1

MIC values of linalool against standard reference strains. MIC was determined by the broth microdilution method and defined as the lowest concentration showing no visible microbial growth; results are presented on a logarithmic scale.

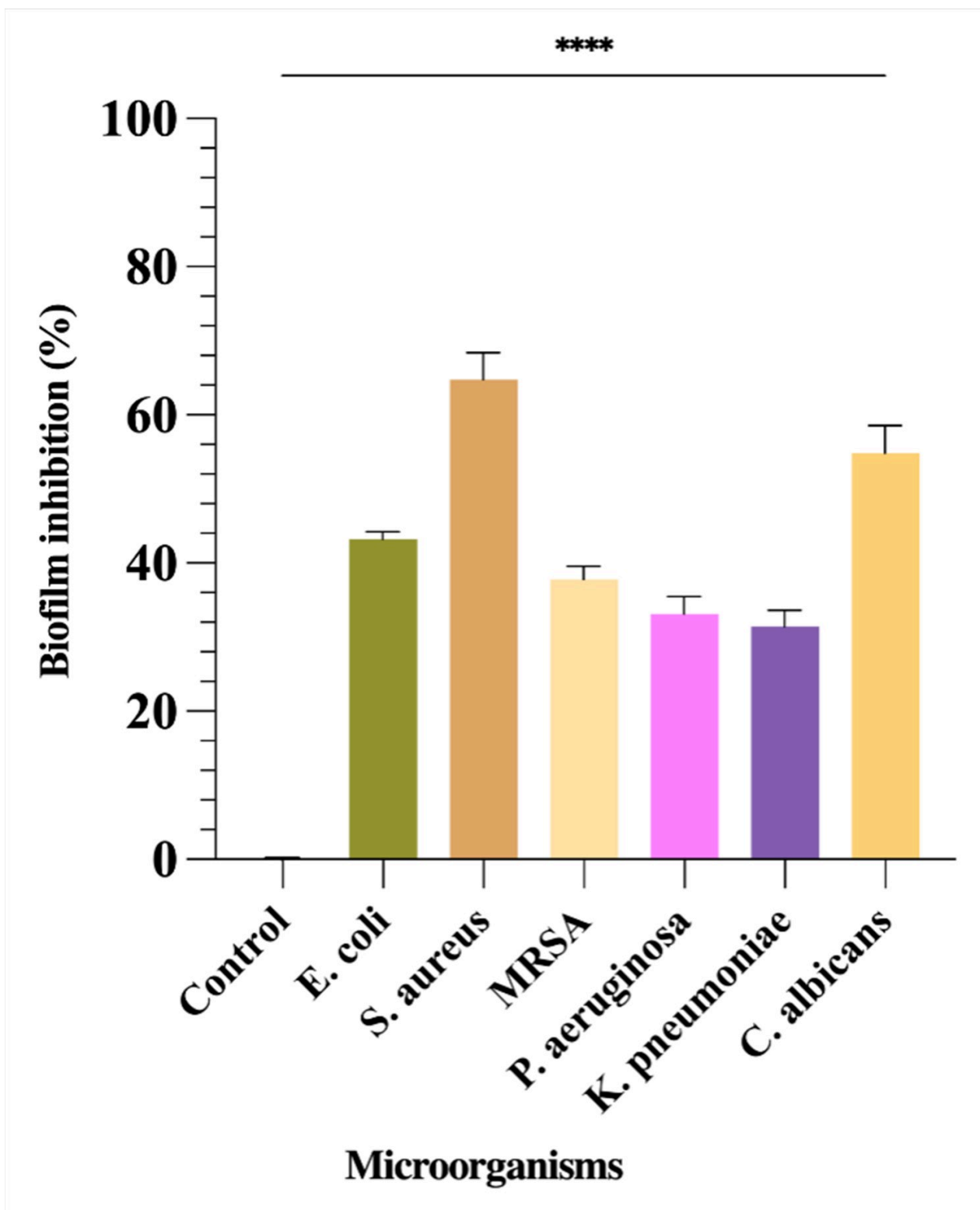


Figure 2

Biofilm formation inhibition (%) by linalool at MIC/2 against standard reference strains. Statistical analysis was performed using one-way ANOVA followed by Dunnett's multiple comparison test. All treated groups were compared with the control group. **** $p < 0.0001$.

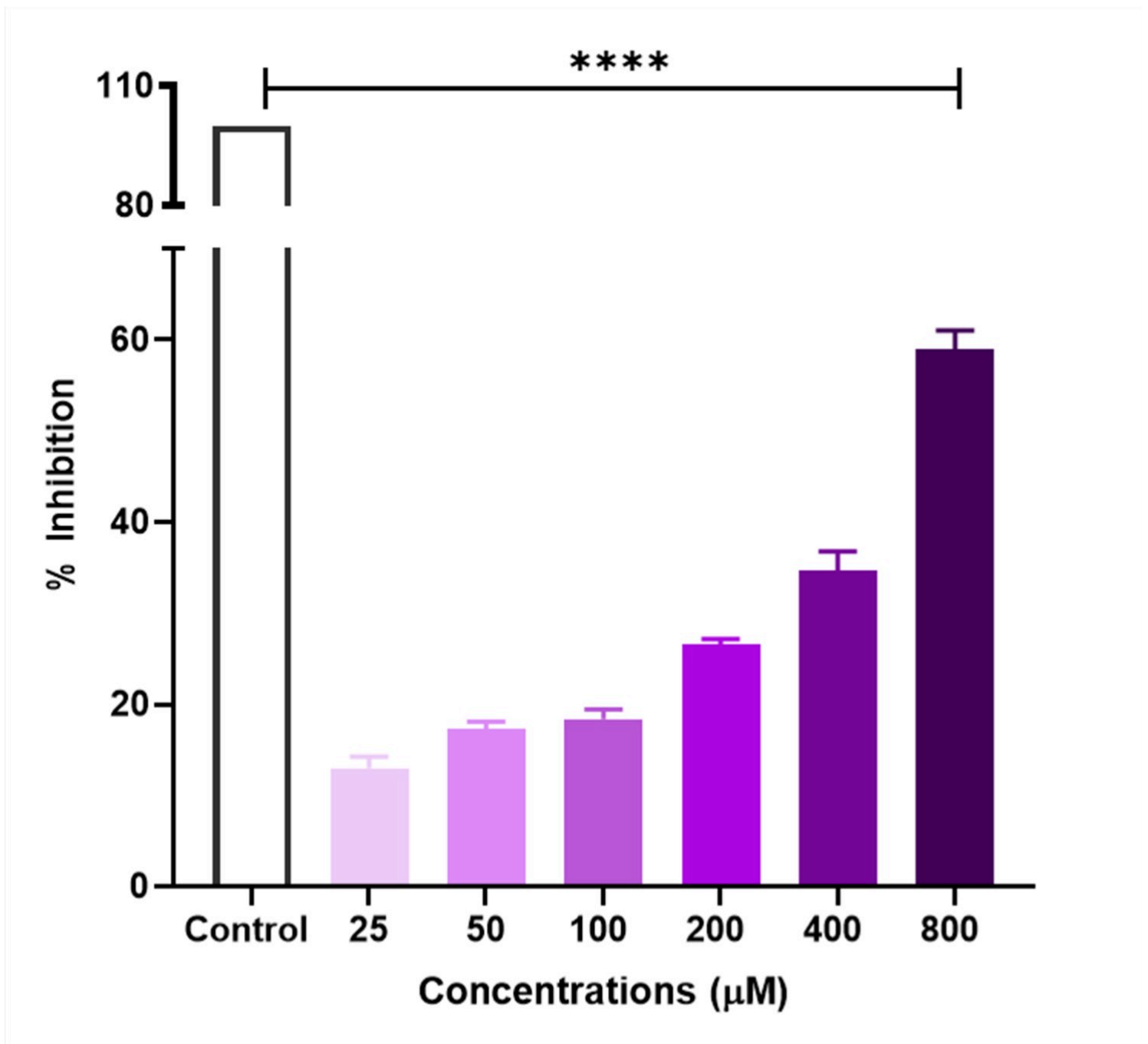


Figure 3

The cytotoxic effect of linalool on glioma cells. The effect of 25, 50, 100, 200, 400, and 800 μM linalool on C6 cell cytotoxicity was investigated using an MTT assay over 24 hours. Statistical analysis was performed using one-way ANOVA followed by Dunnett's multiple comparison test. In the control, cells were not treated. All treated groups were compared with the control group. The corresponding IC_{50} value (563.3 μM, 95% CI: 370.3-871.7 μM) is indicated on the graph. **** $p < 0.0001$.

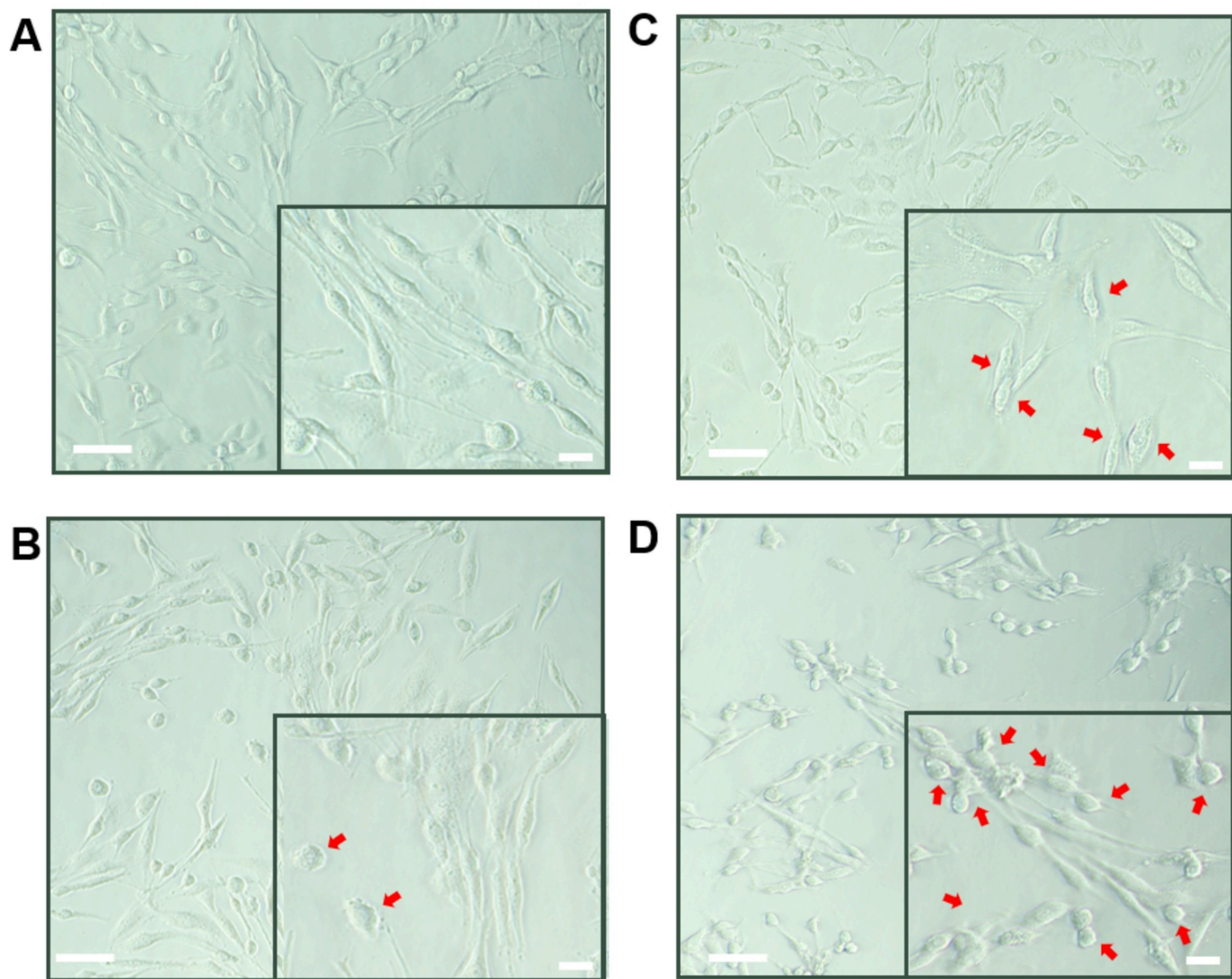


Figure 4

Morphological changes in C6 glioma cells following linalool treatment. Representative

images of untreated control cells (A) and cells treated with 100 μ M (B), 200 μ M (C), or 400 μ M (D) linalool for 24 h. Images were acquired at $\times 20$, and $\times 40$ magnifications. The scale bar represents 200 μ m.

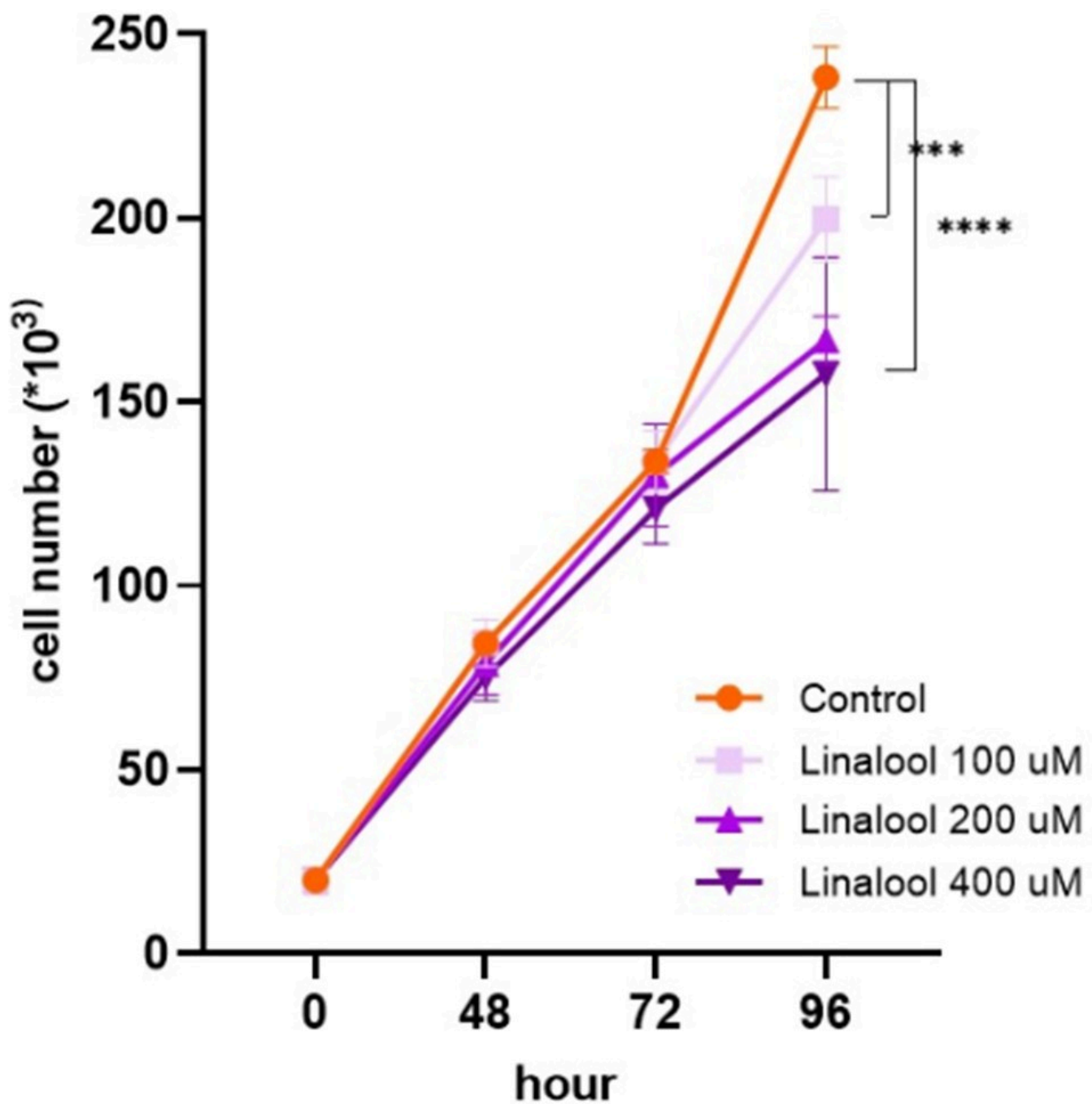


Figure 5

Anti-proliferative effects of linalool on C6 glioma cells. Cell proliferation was evaluated over four days following treatment with 100, 200, or 400 µM linalool. Data were analysed using two-way ANOVA followed by Dunnett's multiple comparisons test, with each treatment group compared with the untreated control. *** $p < 0.001$. **** $p < 0.0001$

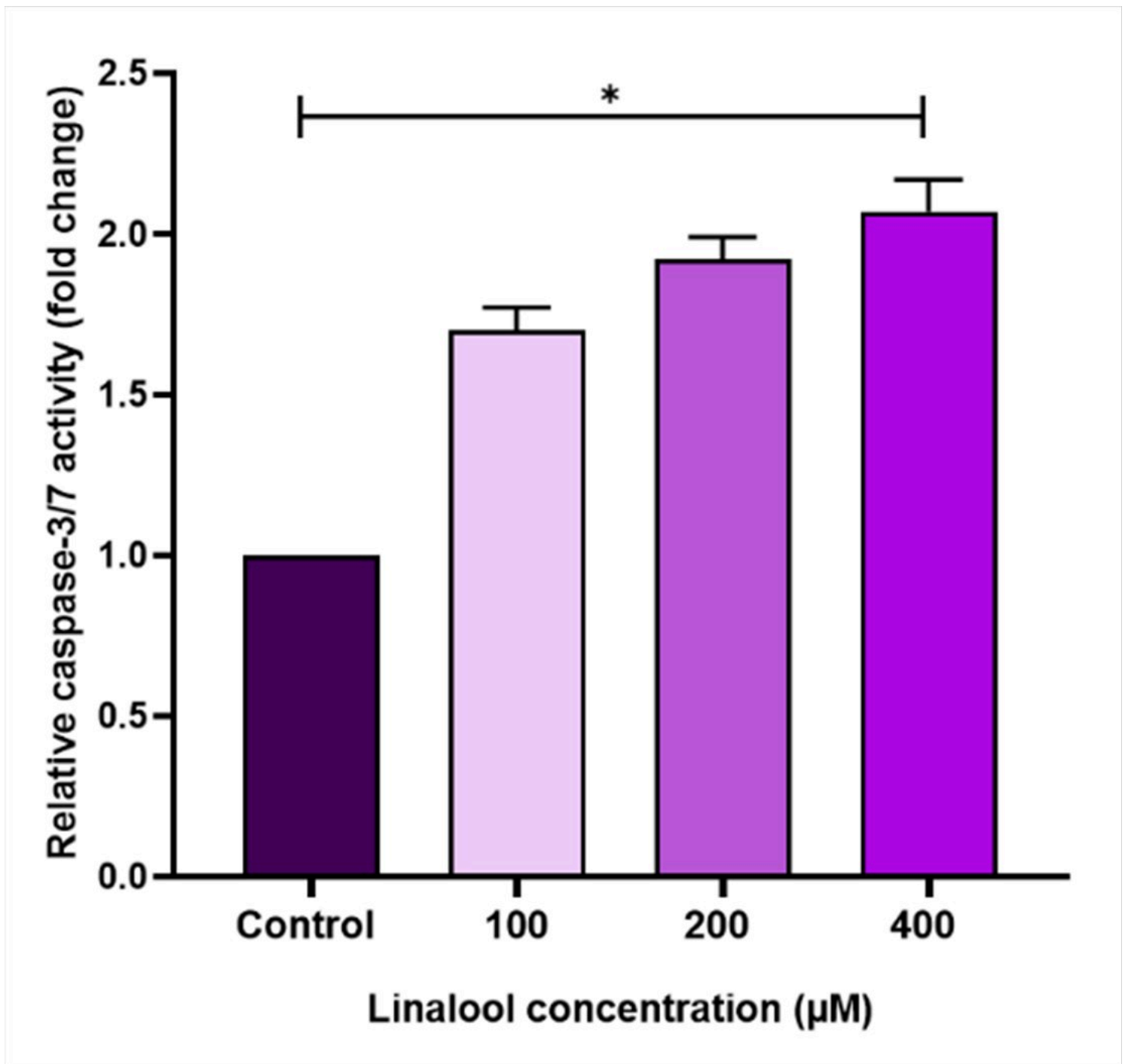


Figure 6

Effect of linalool on caspase-3/7 activity in C6 glioma cells. Cells were treated with 100, 200, and 400 µM linalool for 24 h. Caspase-3/7 activity was determined after 2 h of incubation with the Ac-DEVD-pNA substrate. Data were analysed using two-way ANOVA followed by Dunnett's multiple comparisons test, with each treatment group compared with the untreated control. * $p < 0.05$.