Published Online: 2026 February 18

Research Article

Evaluation of Cytotoxic Effects and Selectivity of *Nigella sativa* Extracts on MCF-7 and HDF Cell Lines

Roghayeh Daneshyar [1], Mohammad Zandi [2, *], Fariba Sharifnia [1], Mohammad Reza Sanjabi [2], Annahita Ghaedrahmati [3]

[1] Department of Biology, North Tehran Branch, Islamic Azad University, Tehran, Iran
[2] Department of Agriculture, Iranian Research Organization for Science and Technology (IROST), Tehran, Iran
[3] Department of Animal Science, Agricultural Sciences and Natural Resources University of Khuzestan, Khuzestan, Iran

[*] ***Corresponding Author:*** Department of Agriculture, Iranian Research Organization for Science and Technology (IROST), Tehran, Iran. P. O. Box 33535111, Email: mz1075@yahoo.com

Received: 21 December, 2025; **Revised:** 1 February, 2026; **Accepted:** 7 February, 2026

Abstract

Background: Breast cancer, one of the most common and lethal cancers in women, faces multiple therapeutic challenges, including side effects and the lack of selectivity of conventional drugs. In this regard, *Nigella sativa*, due to its numerous bioactive compounds and proven therapeutic properties, has emerged as a promising candidate for cancer treatment.

Objectives: This study aimed to investigate the anticancer effects and toxicity of aqueous and hydroalcoholic extracts of *N. sativa* on human breast cancer (MCF-7) cells and normal human dermal fibroblasts (HDF).

Methods: Aqueous and hydroalcoholic extracts of *N. sativa* were prepared, and their compound profiles were determined using high-performance liquid chromatography (HPLC). Cytotoxicity was evaluated using the MTT assay across a concentration range of 6.25 to 6400 µg/mL after 24 hours of treatment. Statistical analysis was performed using ANOVA with a significance level of $P < 0.05$.

Results: The results demonstrated that the effects of the extracts were dependent on their type, concentration, and the nature of the target cells. The aqueous extract significantly reduced the viability of HDF cells at concentrations of 100 µg/mL and above, while its significant anticancer effect on MCF-7 cells began only at 400 µg/mL and above. The hydroalcoholic extract exhibited a dual effect: At low concentrations (12.5 - 50 µg/mL), it increased the viability of normal cells, whereas at high concentrations, it decreased it. This extract also showed a dual effect on cancer cells, increasing their viability at very low concentrations (6.25 and 12.5 µg/mL) and significantly reducing it only at a high concentration (800 µg/mL) ($P < 0.05$).

Conclusions: The findings indicate that although *N. sativa* extracts possess concentration-dependent anti-proliferative potential, they lack the desired therapeutic selectivity, as concentrations effective against cancer cells also impose significant toxicity on normal cells. These results underscore the pharmacological complexity of the response and highlight the need for future studies to optimize dosage and formulation for the safe and effective exploitation of this potential.

Keywords: *Nigella sativa*, Cytotoxicity, MCF-7 Cells, HDF Cells

1. Background

Breast cancer remains one of the most prevalent and deadly malignancies in women, constituting a major global public health concern (1). With an approximate annual incidence of 2.3 million new cases, it is the second leading cause of cancer-related mortality in the female population (2). The complexity of this cancer stems from its significant molecular and phenotypic heterogeneity, influenced by genetic, epigenetic, and transcriptomic alterations, which profoundly impact diagnosis strategies, treatment response, and patient prognosis (3).

Current standard treatments include surgery, radiotherapy, chemotherapy, and hormone therapy. However, despite significant advancements, the efficacy

Copyright © 2026, Daneshyar et al. This open-access article is available under the Creative Commons Attribution 4.0 (CC BY 4.0) International License (https://creativecommons.org/licenses/by/4.0/), which allows for unrestricted use, distribution, and reproduction in any medium, provided that the original work is properly cited.

How to Cite: Daneshyar R, Zandi M, Sharifnia F, Sanjabi MR, Ghaedrahmati A. Evaluation of Cytotoxic Effects and Selectivity of Nigella sativa Extracts on MCF-7 and HDF Cell Lines. Gene Cell Tissue. 2026;13(1):e169056. doi: https://doi.org/10.5812/gct-169056

of these approaches is compromised by major limitations, including considerable side effects, the non-selective targeting of many anticancer drugs, systemic toxicity, the development of drug resistance, and inadequate bioavailability (4, 5). This highlights the necessity for the search for novel and safer therapeutic agents. In this context, the MCF-7 cell line is widely employed as a standard and validated in vitro laboratory model for the initial assessment of cytotoxicity and antiproliferative effects (4).

A broad spectrum of anticancer drugs has been derived from natural sources (6, 7). Among promising medicinal plants, *Nigella sativa* has attracted modern research interest due to its rich historical background in traditional medicine and a wide range of bioactive compounds (8). Pharmacological studies have confirmed its anti-inflammatory, antioxidant, and antimicrobial properties. Notably, its significant anticancer potential against various neoplasms, including breast cancer, has been extensively reported (9-11).

The most important and abundant bioactive compound of *N. sativa* is thymoquinone (TQ), which constitutes 30–50% of its essential oil content (12, 13). Other important compounds include monoterpenes such as p-cymene, thymol, 4-terpineol, and carvacrol. Furthermore, *N. sativa* contains valuable amounts of non-volatile compounds, including alkaloids, saponins, flavonoids, and unsaturated fatty acids, which may exert synergistic effects. Extracts of *N. sativa* and its key compounds, particularly thymoquinone, have demonstrated potent antiproliferative effects in various in vitro models of breast cancer, including the MCF-7 cell line (11, 14).

2. Objectives

Therefore, considering the aforementioned background and the importance of discovering more selective and safer therapeutic agents, the primary objective of this study is the preliminary evaluation of the cytotoxic effects of aqueous and hydroalcoholic extracts of *N. sativa* on the MCF-7 breast cancer cell line, as well as the assessment of their selective toxicity on normal human dermal fibroblasts. It does not investigate molecular mechanisms but aims to provide essential baseline data for more in-depth future research.

3. Methods

3.1. Chemicals

All chemicals were purchased from Sigma-Aldrich (St. Louis, MO, USA), and all plasticware was obtained from Corning (USA).

3.2. Initial Preparation

Raw *N. sativa* seeds were purchased and assessed for purity. After washing with distilled water, they were immersed in a 20% ethanol solution for surface sterilization. The seeds were thoroughly dried in the shade at room temperature. Subsequently, the samples were placed in an oven at 50°C for 24 h to achieve complete dryness. To increase the surface area and facilitate better extraction, the dried seeds were ground into a fine powder using a mill. The powder was stored in an airtight container, protected from light and moisture, until further use.

3.3. Preparation of Hydroalcoholic Extract

For the hydroalcoholic extraction, 40 g of the seed powder was mixed with 400 mL of a 50% ethanol solution (v/v, diluted with distilled water). This mixture was stirred on a shaker at 130 revolutions per minute (RPM) for 24 hours at room temperature. Upon completion of the extraction, the contents of the container were vacuum-filtered using filter paper to obtain a clear extract.

3.4. Preparation of Aqueous Extract

The aqueous extract was obtained using a Soxhlet apparatus. For this purpose, 40 g of the seed powder was placed into filter paper thimbles, and the soluble components were extracted with distilled water. The resulting dilute extracts were then transferred to a rotary evaporator for concentration and removal of excess solvents, whereby the water and alcohol were evaporated off.

3.5. Extract Concentration and Dry Matter Determination

The obtained dilute extracts (both types) were placed in a rotary evaporator under vacuum to remove the solvent and achieve concentration, allowing the excess water and ethanol to be gently evaporated off, resulting in a concentrated extract. To determine the dry matter content of the final extracts, a precise quantity of each

concentrated extract (approximately 2 milliliters) was poured into pre-weighed Petri dishes. These dishes were then dried in an oven at 50°C until a constant weight was achieved. The dry matter of each extract was calculated by subtracting the weight of the empty Petri dish from the weight of the dish containing the dried extract and was reported in grams per gram (g/g).

3.6. Qualitative High-performance Liquid Chromatography Profiling

The analysis of phenolic compounds in the *N. sativa* seed extract was performed using high-performance liquid chromatography (HPLC). This analysis was conducted solely for qualitative identification. The analyses were carried out using a Knauer (Germany) HPLC system, consisting of a K-2700 binary pump, a K-3700 diode array detector, and an RP-C18 column (model Perfectsil Target ODS-3, dimensions: 4.6 × 250 mm, particle size 5 µm). The mobile phase used was a mixture of acetonitrile and distilled water (containing 0.1% acetic acid) in a volume ratio of 60:40, delivered isocratically at a flow rate of 1 mL/min. The injection volume was 20 µL, and the detector wavelength was set to 360 nm for the measurement of phenolic compounds.

3.7. Cell Lines

The cancerous and normal cell lines used in this study were obtained from the Iranian Biological Resource Center (IBRC). The cell lines used were human dermal fibroblasts (HDF; IBRC code: C10506) and breast cancer cells (MCF-7; IBRC code: C10682). DMEM culture medium containing penicillin, streptomycin, and 10% FBS was used for cell culture. The cells were cultured in an incubator at 37°C, with 5% CO_2 and saturated humidity.

3.8. Evaluation of Cell Viability Using the MTT Assay

3-Methyl-2,5-diphenyltetrazolium bromide (MTT), a yellow, water-soluble tetrazolium salt, is reduced by the mitochondrial enzyme succinate dehydrogenase in living cells. The reduction of this compound leads to the cleavage of the tetrazolium ring and the formation of insoluble purple formazan crystals. These crystals are dissolved using the organic solvent dimethyl sulfoxide (DMSO), and the resulting color intensity is measured at a wavelength of 570 nm. The amount of formazan produced is directly proportional to the number of cells with active metabolism. To perform the assay, 100 µL of cells were seeded per well in a 96-well plate. Cells were treated with concentrations of 6.25, 12.5, 25, 50, 100, 200, 400, 800, 1600, 3200, and 6400 µg/mL of both *N. sativa* seed extract types (aqueous and hydroalcoholic) for 24 h. After the treatment period, MTT solution was added to the wells, and the plate was incubated for 4 hours in a 5% CO_2 incubator at 37°C. Subsequently, the supernatant containing MTT was removed, and 100 µL of DMSO was added to each well to dissolve the formazan crystals. The plate was shaken in an incubator shaker at 250 rpm for 20 min, and finally, the optical density at 570 nm was measured using an ELISA reader.

3.9. Statistical Analysis

Data analysis was performed using SPSS (version 16, IBM Corporation, USA). Data are presented as Mean ± SEM from three independent replicates (n = 3). After confirming normality (Shapiro-Wilk test) and homogeneity of variances (Levene's test), a one-way ANOVA followed by Duncan's post hoc test was used for group comparisons. Differences were considered significant at $P < 0.05$.

4. Results

4.1. Analysis of the Active Compound Profile in Nigella sativa

Based on HPLC analysis, the predominant peak in the aqueous extract of *N. sativa* corresponded to p-coumaric acid, followed by peaks for ferulic acid, quercetin, and hydroxybenzoic acid, respectively (Figure 1).

The HPLC profile of the hydroalcoholic extract of *N. sativa* also indicated that hydroxybenzoic acid, syringic acid, and ferulic acid showed the highest peak areas among the identified active compounds, respectively (Figure 2).

4.2. Evaluation of the Cytotoxic Effect of the Aqueous Extract of Nigella sativa on the Viability of Human Dermal Fibroblasts Cells

The evaluation of the cytotoxic effects of different concentrations of aqueous and hydroalcoholic extracts of *N. sativa* seeds on the viability of HDF cells and MCF-7 cells demonstrated that in this study, concentration ranges of 6.25, 12.5, 25, 50, 100, 200, 400, 800, 1600, 3200, and 6400 µg/mL from both extract types were assessed, and the data obtained from cell treatment with the aqueous extract are presented in Figures 3. and 4.

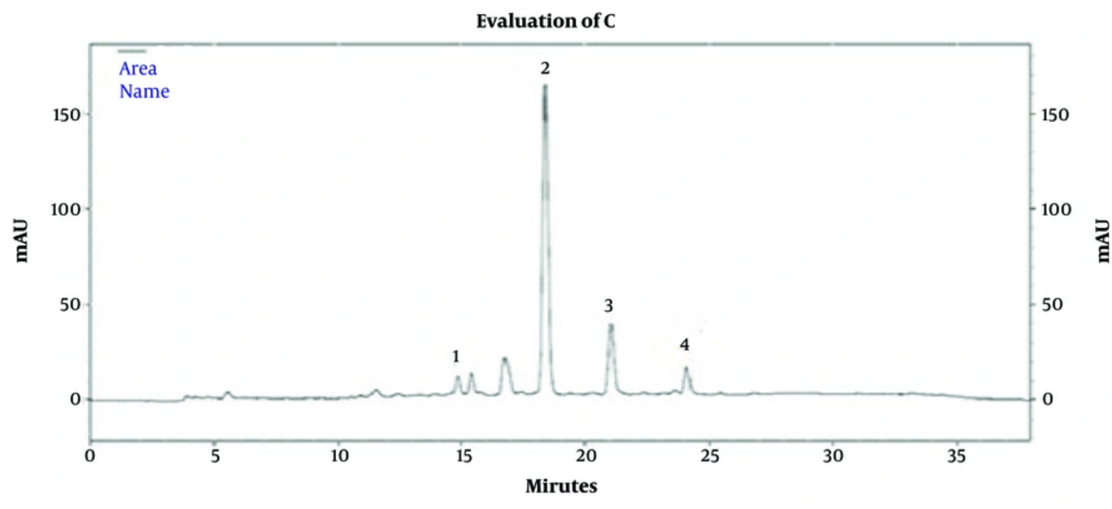

Figure 1. High-performance liquid chromatography (HPLC) chromatogram of compounds in the aqueous extract of *Nigella sativa*; the identified peaks correspond to the following: (1) Hydroxybenzoic acid; (2) P-Coumaric acid; (3) Ferulic acid; (4) Quercetin

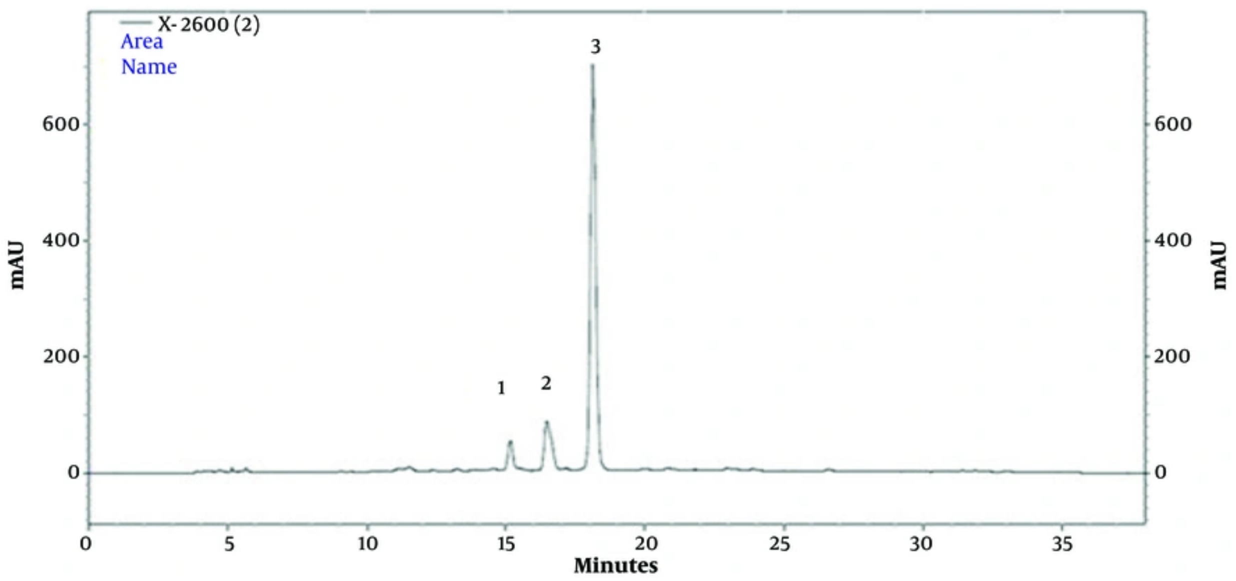

Figure 2. High-performance liquid chromatography (HPLC) chromatogram of the hydroalcoholic extract of *Nigella sativa*; the identified peaks are: (1) Hydroxybenzoic acid; (2) Syringic acid; (3) Ferulic acid

The results of the statistical analysis indicated a significant and concentration-dependent decrease in the viability of HDF cells following exposure to aqueous extract concentrations of 100 µg/mL and higher, compared to the control group ($P < 0.05$). Accordingly, at concentrations of 100, 200, and 400 µg/mL, HDF cell viability decreased significantly, reaching 83.3 ± 3.3%,

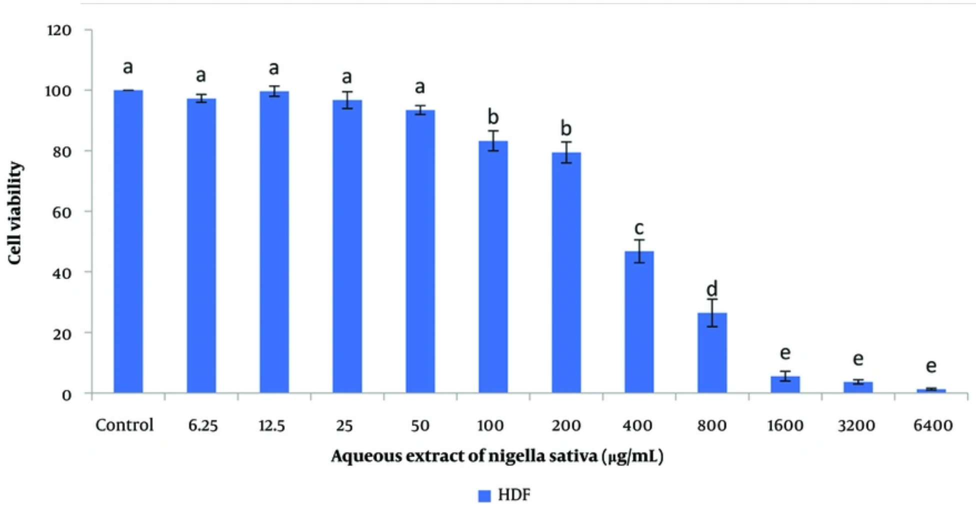

Figure 3. The effect of different concentrations of the aqueous extract of *Nigella sativa* on the viability of human dermal fibroblasts (HDF) cells (P < 0.05) (different small letters above each bar indicate statistically significant differences between groups at the level of P < 0.05. Groups sharing common letters do not differ significantly).

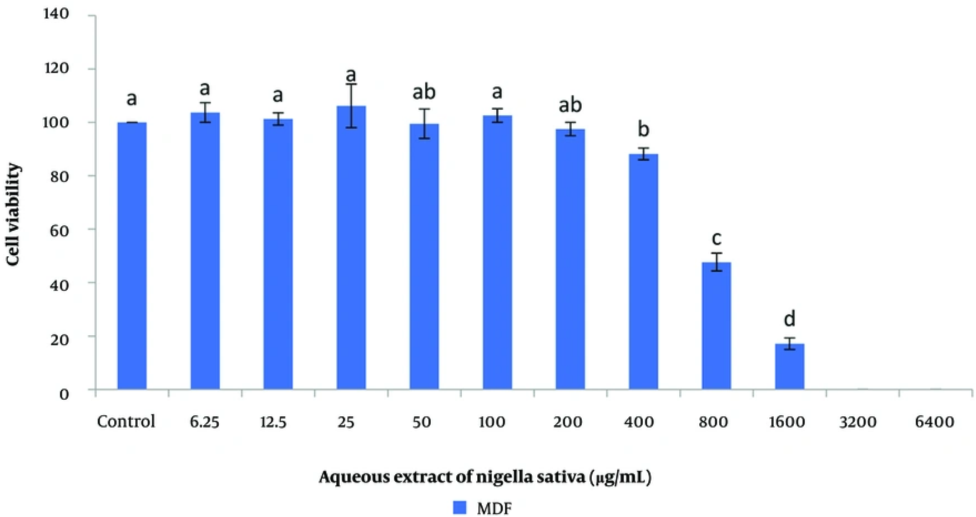

Figure 4. Effect of different concentrations of aqueous *Nigella sativa* extract on the viability of MCF-7 cells (P < 0.05) (different small letters above each bar indicate statistically significant differences between groups at the level of P < 0.05. Groups sharing common letters do not differ significantly).

79.4 ± 4.3%, and 46.7 ± 8.3% respectively, while the viability in the control group was 100% (Figure 3).

4.3. Evaluation of the Cytotoxic Effect of the Aqueous Extract of Nigella sativa on the Viability of MCF-7 Cells

In an investigation of the effect of different concentrations of aqueous *N. sativa* extract on MCF-7, the results showed that at concentrations of 100 and 200 µg/mL, no statistically significant difference in cell survival was observed compared to the control group.

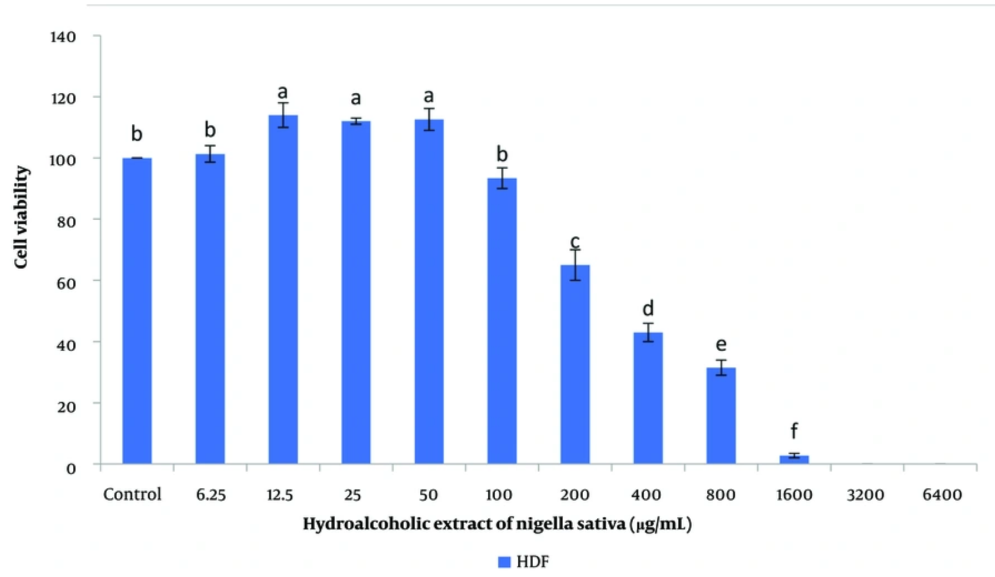

Figure 5. Effect of different concentrations of hydroalcoholic *Nigella sativa* extract on the viability of human dermal fibroblasts (HDF) cells ($P < 0.05$) (different small letters above each bar indicate statistically significant differences between groups at the level of $P < 0.05$. Groups sharing common letters do not differ significantly).

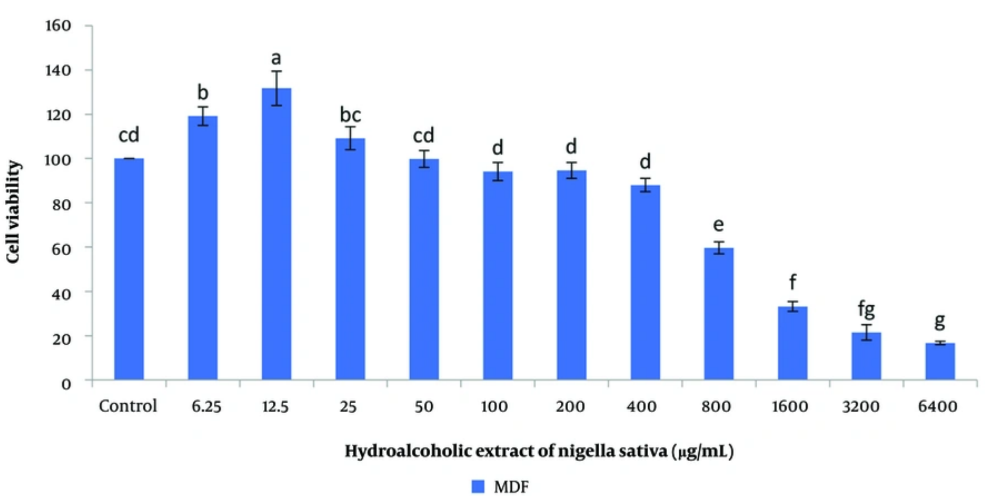

Figure 6. Effect of different concentrations of hydroalcoholic *Nigella sativa* extract on the viability of MCF-7 cells ($P < 0.05$) (different small letters above each bar indicate statistically significant differences between groups at the level of $P < 0.05$. Groups sharing common letters do not differ significantly).

However, at concentrations of 400 µg/mL and higher, the aqueous *N. sativa* extract induced a significant, concentration-dependent reduction in the viability of MCF-7 cells compared to the control group ($P < 0.05$).

Accordingly, the percentage survival of MCF-7 cells at concentrations of 100, 200, and 400 μg/mL was measured as 102.5 ± 2.5%, 97.5 ± 2.5%, and 88.2 ± 2.2%, respectively, whereas this value was considered 100% in the control group (Figure 4).

4.4. Evaluation of the Cytotoxic Effect of the Hydroalcoholic Extract of Nigella sativa on the Viability of Human Dermal Fibroblasts Cells

Data regarding the effect of different concentrations of hydroalcoholic *N. sativa* extract on normal (HDF) and cancerous (MCF-7) cells are presented in Figures 5. and 6. The results indicated that this extract, in the concentration range of 12.5 to 50 μg/mL, caused a significant increase in the viability of normal HDF cells. In contrast, at concentrations of 200 μg/mL and higher, a significant decrease in the survival of these cells compared to the control group was observed ($P < 0.05$).

Based on the findings, the percentage viability of HDF cells at concentrations of 12.5, 25, 50, 100, 200, 400, and 800 μg/mL was measured as 114.1 ± 4.0%, 112.0 ± 1.0%, 112.6 ± 3.6%, 93.4 ± 3.4%, 64.9 ± 5.0%, 42.9 ± 3.0%, and 31.5 ± 2.5%, respectively, while this value was considered 100% in the control group (Figure 5).

4.5. Evaluation of the Cytotoxic Effect of the Hydroalcoholic Extract of Nigella sativa on the Viability of MCF-7 Cells

The results showed that only at concentrations of 800 μg/mL and higher (toxic but non-selective concentrations), the hydroalcoholic extract of *N. sativa* caused a significant reduction in the viability of MCF-7 cancer cells compared to the control group ($P < 0.05$). In contrast, at lower concentrations (6.25 and 12.5 μg/mL), this extract led to a significant increase in the survival of MCF-7 cells ($P < 0.05$). Based on this, the percentage viability of MCF-7 cells at concentrations of 6.25, 12.5, and 800 μg/mL was measured as 119.1 ± 4.1%, 131.7 ± 7.7%, and 59.7 ± 2.6%, respectively, while the corresponding value for the control group was considered 100% (Figure 6).

The present study evaluated the efficacy, which is dependent on dose and type, of aqueous and hydroalcoholic extracts of *N. sativa* on MCF-7 and HDF cell lines. The findings indicate that although both extracts show concentration-dependent inhibitory effects on the growth of MCF-7 cells, they lack the necessary selective property for targeted anticancer application. This is because, within the concentration range effective against cancer cells, they also exert significant toxicity on normal HDF cells, and thus no significant difference is observed in the selective death of cancer cells compared to the preservation of healthy cell viability. These results emphasize the complexity of the cellular response and its dependence on factors such as the type of extract, concentration, and cell nature, and reveal the need for more in-depth pharmacological studies to determine an optimal dose that can realize the anticancer potential of *N. sativa* with minimal side effects on healthy cells.

5. Discussion

This study reveals complex and concentration-dependent outcomes of *N. sativa* aqueous and hydroalcoholic extracts on normal and cancerous cells. Although both extracts exhibited anti-proliferative effects on MCF-7 cells at high concentrations, they lacked the requisite therapeutic selectivity, as they simultaneously induced significant toxicity in healthy HDF cells. Furthermore, dual responses (growth stimulation or inhibition) were observed depending on the extract type and concentration used, underscoring the pharmacological complexity of these extracts. These findings highlight the need for a deeper understanding of the structure-activity relationship and the determination of an optimal dose.

A growing body of scientific evidence supports the potent anticancer potential of *N. sativa* and its bioactive compounds, particularly TQ. Studies indicate that these agents exhibit multifaceted and promising anticancer effects against a broad spectrum of cancers, including breast cancer. Numerous in vitro studies have investigated the molecular mechanisms of this activity on various breast cancer cell lines, such as MDA-MB-231 and MCF-7 (2, 14, 15).

For instance, studies on MDA-MB-231 breast cancer cells have shown that a methanolic extract of *N. sativa* at low concentrations (2.5 and 5 μg/mL) induces dose-dependent apoptosis by increasing pro-apoptotic gene expression and decreasing anti-apoptotic gene expression (14). This finding is consistent with a study on SiHa cervical cancer cells, where a methanolic extract of *N. sativa* (125 μL/mL) led to apoptosis through the activation of caspase- and p53-dependent pathways (16). Furthermore, methanolic, hexane, and chloroform extracts of *N. sativa* demonstrated strong antiproliferative effects on HeLa (cervical) cells with very low IC50 values (ranging from ng/mL to μg/mL) (17).

This apoptotic mechanism has also been confirmed for the pure compound TQ (13, 15). Specifically, TQ exerts its effects by inhibiting cell proliferation, inducing oxidative stress (increasing ROS), and inhibiting inflammatory pathways (9, 13). Additionally, thymol, another derivative of *N. sativa*, disrupts the growth of MCF-7 cells at an IC50 of 200 μM by inhibiting the expression of key cell cycle genes (Cyclin D1 and PCNA) (18).

This study clearly demonstrates that both aqueous and hydroalcoholic extracts of *N. sativa* exert distinct and significant effects on HDF and MCF-7 cell lines. These effects are highly dependent on the extract type, concentration, and specific cell line. *N. sativa* and its compounds also influence tumor progression processes. Studies indicate that these compounds can inhibit cancer cell invasion and metastasis by suppressing the expression of matrix metalloproteinase enzymes (MMP-2 and MMP-9) and the angiogenic factor (VEGF), while simultaneously increasing the expression of the adhesion molecule E-cadherin (19, 20). For example, *N. sativa* oil at a concentration of 200 μg/mL significantly reduced MMP2-9 gene expression in MCF-7 and AGS cell lines. Furthermore, these compounds can modulate the cellular stress response by regulating the expression of heat shock proteins (HSPs). The same study showed that *N. sativa* oil decreased HSP60-70 expression (9).

One of the challenges in using natural compounds like *N. sativa* is their limited therapeutic selectivity and concurrent toxicity to healthy cells at effective concentrations, as also observed in the present study. To overcome this limitation, novel formulations such as nano emulsions and nanoparticles have been developed. These formulations significantly enhance anticancer efficacy by improving the bioavailability and penetration of active compounds (such as TQ) (21). For example, a nanoemulsion formulation of *N. sativa* oil extract demonstrated potent cytotoxicity against MCF-7 cells (22). Additionally, silver nanoparticles synthesized with *N. sativa* extract (N-AgNPs) at concentrations of 10 - 100 μg/mL affected MCF-7 cells by inducing apoptosis and regulating inflammatory markers (such as COX-2) (4).

Another promising approach is combination therapy with common chemotherapeutic drugs. Evidence suggests that the concurrent use of *N. sativa* extracts or its nano formulations with drugs such as doxorubicin can have a synergistic (enhancing) effect, increasing treatment efficacy (22). This highlights the importance of developing combined formulations (e.g., nano emulsions containing doxorubicin and *N. sativa* extract) as a potential therapeutic strategy.

In summary, the findings of this study indicate that the concentrations required to inhibit MCF-7 cancer cells simultaneously cause significant cytotoxic effects on normal HDF cells. These observations underscore the necessity for future research to focus on identifying and optimizing specific active compounds, precisely determining the therapeutic dose, and developing novel formulations to achieve selective efficacy. Considering the statistical power limitation of the present study, confirmation of these findings in future studies with a more robust statistical design is essential.

5.1. Conclusions

In summary, this study demonstrates that *N. sativa* extracts, particularly the aqueous form, exhibit anticancer potential against MCF-7 cells at intermediate concentrations. A key finding is the strongly concentration-dependent response pattern: Very low concentrations are ineffective or even stimulate cell growth, intermediate concentrations suggest potential selective activity, while high concentrations are generally cytotoxic. Furthermore, the extraction solvent (aqueous versus hydroalcoholic) significantly alters the biological activity profile of the extract. Given these results, precise therapeutic dose determination and formulation optimization are essential prerequisites for developing any therapeutic application of *N. sativa* to achieve maximum anticancer efficacy with minimal toxicity to healthy cells. As this research aimed at preliminary screening and delineating the effective concentration range, future studies should focus on lower, physiologically more relevant concentrations, likely achievable through advanced drug delivery technologies.

Acknowledgements

The authors acknowledge the Agricultural Institute of the Iranian Research Organization for Science and Technology (IROST) for providing laboratory facilities and technical support.

Footnotes

AI Use Disclosure: The authors declare that no generative AI tools were used in the creation of this article.

Authors' Contribution: Roghayeh Daneshyar: Investigation, writing-original draft; Mohammad Zandi: Supervisor, conceptualization, methodology, resources, formal analysis, visualization; Fariba Sharifnia: Advisor, conceptualization, visualization; Mohammad Reza Sanjabi: Advisor, visualization; Annahita Ghaedrahmati: Writing-review and editing.

Conflict of Interests Statement: The authors declare no conflict of interest.

Data Availability: The dataset presented in the study is available on request from the corresponding author during submission or after publication.

Funding/Support: The present study received no funding/support.

References

1. Baig WA, Alwosaibai K, Al-Jubran KM, Chaudhry TM, Al-Dowish N, Alsaffar F, et al. Synergistic anti-cancer effects of Nigella sativa seed oil and conventional cytotoxic agent against human breast cancer. *Drug Metab Pers Ther*. 2022;**37**(3):315-21. [PubMed ID: 35405048]. https://doi.org/10.1515/dmpt-2021-0229.
2. Friyoga S, Wirdah A, Nur Q, Irsan S, Yenny Dian A; Irfanuddin, et al. Effectiveness of Nigella sativa Addition against TNF-Alpha in Stage III and IV Breast Cancer Undergoing Doxorubicin and Cyclophosphamide Chemotherapy at Dr. Mohammad Hoesin General Hospital, Palembang, Indonesia. *Biosci Med J Biomed Transl Res*. 2023;**8**(2):4009-14. https://doi.org/10.37275/bsm.v8i2.924.
3. Alshaibi HF, Aldarmahi NA, Alkhattabi NA, Alsufiani HM, Tarbiah NI. Studying the Anticancer Effects of Thymoquinone on Breast Cancer Cells through Natural Killer Cell Activity. *Biomed Res Int*. 2022;**2022**:9218640. [PubMed ID: 36199754]. [PubMed Central ID: PMC9527111]. https://doi.org/10.1155/2022/9218640.
4. Rohini B, Akther T, Waseem M, Khan J, Kashif M, Hemalatha S. AgNPs from Nigella sativa Control Breast Cancer: An In Vitro Study. *J Environ Pathol Toxicol Oncol*. 2019;**38**(2):185-94. [PubMed ID: 31679281]. https://doi.org/10.1615/JEnvironPatholToxicolOncol.2019027318.
5. Ardeshir RA, Moarefvand K. Selective apoptosis induction by antimicrobial peptide TP4 in MCF-7 breast cancer cells. *Sci Rep*. 2025;**15**(1):15061.
6. Saini D, Chaudhary PK, Chaudhary JK, Kaur H, Verma GK, Pramanik SD, et al. Molecular mechanisms of antiproliferative and pro-apoptotic effects of essential oil active constituents in MCF7 and T24 cancer cell lines: in vitro insights and in silico modelling of proapoptotic gene product-compound interactions. *Apoptosis*. 2025;**30**(3-4):805-25. [PubMed ID: 39738801]. https://doi.org/10.1007/s10495-024-02065-x.
7. Khurshid Y, Syed B, Simjee SU, Beg O, Ahmed A. Antiproliferative and apoptotic effects of proteins from black seeds (Nigella sativa) on human breast MCF-7 cancer cell line. *BMC Complement Med Ther*. 2020;**20**(1):5. [PubMed ID: 32020890]. [PubMed Central ID: PMC7076859]. https://doi.org/10.1186/s12906-019-2804-1.
8. Sharma B, Shekhar H, Sahu A, Haque S, Kaur D, Tuli HS, et al. Deciphering the anticancer potential of thymoquinone: in-depth exploration of the potent flavonoid from Nigella sativa. *Mol Biol Rep*. 2025;**52**(1):268. [PubMed ID: 40016603]. https://doi.org/10.1007/s11033-025-10375-9.
9. Cinar I, Gidik B, Dirican E. Determination of anti-cancer effects of Nigella sativa seed oil on MCF7 breast and AGS gastric cancer cells. *Mol Biol Rep*. 2024;**51**(1):491. [PubMed ID: 38578469]. https://doi.org/10.1007/s11033-024-09453-1.
10. Sharfaraz A, Ferdoushi A, Hossain MA, Nupur AS, Salma U, Karim MF. Computer-based discovery of SIRT7 inhibitors from Nigella sativa for cancer treatment. *J Mol Graph Model*. 2026;**142**. https://doi.org/10.2139/ssrn.5293319.
11. Sourav C, Chong KT, Tayara H. Exploring Nigella sativa anticancerous properties using network pharmacology, molecular docking and molecular dynamics simulation approach for non-small cell lung cancer. *Food Biosci*. 2025;**63**. https://doi.org/10.1016/j.fbio.2024.105525.
12. Korak T, Ergul E, Sazci A. Nigella sativa and Cancer: A Review Focusing on Breast Cancer, Inhibition of Metastasis and Enhancement of Natural Killer Cell Cytotoxicity. *Curr Pharm Biotechnol*. 2020;**21**(12):1176-85. [PubMed ID: 32351178]. https://doi.org/10.2174/1389201021666200430120453.
13. Kurnia D, Dharsono HDA, Takaya A, Padilah R, Apriyanti E. Potential of Secondary Metabolite Compounds of Black Cumin Seeds (Nigella sativa L.) as Therapeutic Agents for Chronic Diseases. *Drug Des Devel Ther*. 2025;**19**:9603-26. [PubMed ID: 41169705]. [PubMed Central ID: PMC12571005]. https://doi.org/10.2147/DDDT.S554704.
14. Dilshad A, Abulkhair O, Nemenqani D, Tamimi W. Antiproliferative properties of methanolic extract of Nigella sativa against the MDA-MB-231 cancer cell line. *Asian Pac J Cancer Prev*. 2012;**13**(11):5839-42. [PubMed ID: 23317266]. https://doi.org/10.7314/apjcp.2012.13.11.5839.
15. Esmaeli M, Ghanbari A, Barazesh M, Dehabadi MD. Nigella sativa (Black Seed) and Breast Cancer: A Systematic Review. *Indian J Gynecol Oncol*. 2024;**22**(4). https://doi.org/10.1007/s40944-024-00920-y.
16. Hasan TN, Shafi G, Syed NA, Alfawaz MA, Alsaif MA, Munshi A, et al. Methanolic Extract of Nigella sativa Seed Inhibits SiHa Human Cervical Cancer Cell Proliferation Through Apoptosis. *Nat Prod Commun*. 2013;**8**(2). https://doi.org/10.1177/1934578x1300800221.
17. Shafi G, Munshi A, Hasan TN, Alshatwi AA, Jyothy A, Lei DK. Induction of apoptosis in HeLa cells by chloroform fraction of seed extracts of Nigella sativa. *Cancer Cell Int*. 2009;**9**:29. [PubMed ID: 19943925]. [PubMed Central ID: PMC2794855]. https://doi.org/10.1186/1475-2867-9-29.
18. Vahitha V, Lali G, Prasad S, Karuppiah P, Karunakaran G, AlSalhi MS. Unveiling the therapeutic potential of thymol from Nigella sativa L. seed: selective anticancer action against human breast cancer cells (MCF-7) through down-regulation of Cyclin D1 and proliferative cell nuclear antigen (PCNA) expressions. *Mol Biol Rep*. 2024;**51**(1):61. [PubMed ID: 38170326]. https://doi.org/10.1007/s11033-023-09032-w.
19. Sujuti H, Wulandari LR, Ulhaq ZS. The Effect of Nigella sativa Linn. Extract on E2F1 Expression and Apoptosis in Retinoblastoma Y79 Cells. *J Herb Med*. 2025;**49**:100974.
20. Arif R, Bukhari SA, Mustafa G, Ahmed S, Albeshr MF. Network Pharmacology and Experimental Validation to Explore the Potential Mechanism of Nigella sativa for the Treatment of Breast Cancer.

Pharmaceuticals. 2024;**17**(5). [PubMed ID: 38794187]. [PubMed Central ID: PMC11124279]. https://doi.org/10.3390/ph17050617.

21. Periasamy VS, Athinarayanan J, Alshatwi AA. Anticancer activity of an ultrasonic nanoemulsion formulation of Nigella sativa L. essential oil on human breast cancer cells. *Ultrason Sonochem*. 2016;**31**:449-55. [PubMed ID: 26964971]. https://doi.org/10.1016/j.ultsonch.2016.01.035.

22. Mahmoud SS, Torchilin VP. Hormetic/cytotoxic effects of Nigella sativa seed alcoholic and aqueous extracts on MCF-7 breast cancer cells alone or in combination with doxorubicin. *Cell Biochem Biophys*. 2013;**66**(3):451-60. [PubMed ID: 23242945]. https://doi.org/10.1007/s12013-012-9493-4.