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Original Research

Anticancer and Immunomodulatory Effects of Colchicum Speciosum Bulb Extract on MCF-7 Breast Cancer Cell

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ABSTRACT

Objective: Cancer is a pathophysiological condition that develops as a result of DNA damage caused by environmental mutagens and is becoming increasingly prevalent. Genetic factors, the immune system, and cytokines are reported important task the pathophysiological mechanisms of breast cancer, mostly cancer among women today. Natural plants such as *Colchicum speciosum (C. speciosum)* are gaining prominence in alternative therapy research due to their colchicine content and anticancer properties. Our study was conducted to evaluate the immunomodulatory and anticancer effects of *C. speciosum* bulb extract on MCF-7 cell.

Methods: *C. speciosum* was harvested and extracted; then the dose ratio was determined using the MTT method, and cytotoxicity, apoptotic level, and antibacterial activity were evaluated in MCF-7 cell. Cytokine levels were measured using commercially available kits via the ELISA method, and apoptotic assessment was examined using a commercial kit containing Annexin V-FITC/PI via fluorescence microscopy. The data was analysed statistically using GraphPad Prism (v10.2.0; GraphPad Software, ABD).

Results: In the MTT evaluation, a significant decrease in cell proliferation was observed in the 240 and 500 µg/mL applications compared to the control group, with increasing dose rate and incubation time. The cytokine profile of *C. speciosum* bulb was characterized by decrease of pro-inflammatory cytokines (TNF-α, IL-1β) and increase in anti-inflammatory cytokines (IL-10), while a decrease in TGF-β1 and BDNF levels was detected depending on the applied dose. Annexin V-FITC/PI analyses demonstrated that *C. speciosum* bulb extract elicited dose- and time-dependent apoptotic responses in MCF-7 cell. In addition, antibacterial effects of *C. speciosum* bulb extract were observed.

Conclusions The *C. speciosum* bulb was observed to have regulatory effects on cell proliferation via cytokines and apoptotic mechanisms in a dose and time-dependent manner. Additionally, its antibacterial effect was observed independently of our findings.

Keywords: antimicrobial activity, antiproliferative effect, apoptosis, *Colchicum speciosum*

INTRODUCTION

It has been reported that in developing societies, approximately one in three people may develop cancer and approximately one in five may die from this disease [1]. Approximately 10–15% of cancers can be passed on genetically, while 85–90% result from errors caused by exposure of DNA in living cells to mutagens and structural changes in cellular DNA [2,3]. Cancer cells are structures that can multiply indefinitely without capsules and generally consist of continuously and rapidly multiplying tumor structures that metastasizen [4]. The World Health Organization reports that mostly cancers in women are breast, uterine, and colon cancer, while in men they are lung, prostate, stomach, and colon cancer [5]. Breast cancer is shown to be more likely to occur among women than other types of cancer [6]. It is stated that patients have a high chance of survival if breast cancer is detected early [7]. Breast cancer develops based on a number of risk factors, including genetic predisposition, family history, diet and lifestyle, hormones, age and reproductive factors [8,9]. It is presented as a pathological condition with different prognoses and heterogeneous molecular subtypes of breast cancer [10]. Inherited mutations in tumor suppressor and DNA repair genes such as BRCA1, BRCA2, and TP53 contribute to the development of breast cancer [11]. The assessment of breast cancer is based on different pathological entities, histological grade showing genomic alterations, lymph node metastasis, and clinical pathological evaluations [12]. Recent studies on cytokines and breast cancer have attracted attention. Cytokines, which constitute the fundamental components of the immune system, play a role in the regulation of cellular biological processes. They also exert both tumor-suppressive and tumor-promoting effects in the tumor microtissue environment [13]. The role of cytokines in breast cancer progression and their relationship is gaining prominence. Interleukin-10 (IL-10) has been suggested to enhance the immune response in the tumor microenvironment and inhibit tumor formation by suppressing chronic inflammation through immune modulation, cellular proliferation, and anti-apoptotic processes via the JAK-STAT3 pathway [14,15]. In breast cancer biology, IL-1β (Interleukin-1 beta) and TNF-α (tumor necrosis factor-alpha) have been reported to potentially affect metastasis and proliferation by altering the tumor microenvironment as inflammatory cytokines [16]. TNF-α and IL-1β affect the biological processes of breast carcinoma by activating NF-κB and MAPK signaling cascades. Activation of NF-κB promotes cell proliferation and migration via the MAPK pathway by transporting cytokines to the nucleus and increasing the expression of proinflammatory genes [17]. Brain-derived neurotrophic factor (BDNF), a member of the neurotrophin superfamily, exerts its biological effects through the tropomyosin receptor kinase B (TrkB) receptor. Overexpression of TrkB in breast cancer cells has been associated with several biological processes, including tumor proliferation, metastasis, and resistance to chemotherapy [18]. However, the development of resistance to cancer drugs, the side effects that arise, and the high costs are increasing the need for new cancer drugs. Natural products are making significant contributions to the development of therapeutic agents. *Colchicum speciosum (C. speciosum)* are cultivated specifically for medicinal purposes and are currently used in the treatment of certain diseases [19]. *C. speciosum* is commonly known as the vargit flowers. It is a perennial plant belonging to the Colchicaceae family that produces purple-violet or purple-pink flowers. With approximately 160 species, this plant grows in the northern parts of Turkey and in the mountainous regions of the Caucasus, North Africa, West Asia, South Africa, and Northern Iran [20,21]. Some *Colchicum* species are preferred in the treatment of diseases such as thalassemia, Hodgkin's lymphoma, cirrhosis, psoriasis, amyloidosis, Behçet's disease, gout, and myeloid leukemia due to their tropoloid alkaloid content [22]. *Colchicum* species native to Turkey are divided into two groups: spring-flowering and fall-flowering. The bulb and seed parts of the plant contain high levels of colchicine and its derivatives. [23]. Since no economical and effective synthesis method has been found for colchicine alkaloids, colchicine and other alkaloids are obtained through extraction from plant sources [24]. Colchicine has been shown to be used conservatively in the treatment of certain disorders, and this plant has been demonstrated to possess anticancer, antimicrobial, anti-tumor, antioxidant and antidiabetic activities, and to be effective in numerous diseases, including those with

Main Points

- Colchicum speciosum onion exhibited anticancer and antimicrobial activity.
- Colchicum speciosum onion was observed to increase anti-inflammatory mediators while suppressing proinflammatory responses.
- Colchicum speciosum onion exhibited antiproliferative effects on cancer cells.
- Colchicum speciosum onion increased apoptotic findings in a time- and dose-dependent manner.

a genetic basis. [21,22,25]. The main objective of our research is to investigate the molecular effects of the extract obtained from the bulb of *C. speciosum* on the MCF-7 cell line in vitro. Additionally, to enhance the integrity of the study and the scope of the findings, the antibacterial activity of the extract against various bacterial species was evaluated.

MATERIALS AND METHODS

Our experimental research was carried out at the physiology and multidisciplinary scientific laboratories of Haliç University and Istanbul University-Cerrahpaşa. The cell line used was provided that American Type Culture Collection (ATCC-CRL-2088, USA) as MCF-7 (ATCC catalog no: HTB-22). The cells used were cultured in the 9th passage. DMEM (Dulbecco's Modified Eagle Medium, Gibco) was used to support the culture environment. DMEM was supplemented 10% FBS (Fetal Bovine Serum, Gibco) and 1% antibiotics (Penicillin/Streptomycin, Gibco). Cells retrieved from a -196 °C liquid nitrogen tank were thawed in a sterile laminar flow cabinet. After thawing, they were seeded into 25 cm² flasks. After seeding, they were incubated in an incubator (CO_2 Incubator, MCO-170AC-PE, PHC Corporation, Japan) set at 5% CO_2 and 37 °C. When the cells reached 85–90% confluence after incubation, this was verified using a PrimoVert-Inverted microscope (Carl Zeiss Microscopy). For the other stages of the study, the cells were removed from the flask using Trypsin-EDTA (Gibco) and passaged. By passage, the cells were multiplied and prepared for subsequent experimental stages.

Preparation of Plant Material

In this study, *C. speciosum* was used as the plant material. The bulbs of the plant were collected from İkizdere Village in Rize, Türkiye and were not obtained through commercial purchase. The collected plant parts were placed on blotting paper and left to dry in a dark, well-ventilated environment, away from direct sunlight. Throughout the three-week drying period, the blotting paper was replaced at regular intervals to ensure uniform and effective drying.

Plant Material and Extraction of *C. speciosum*

For extraction, the bulbs of *C. speciosum* were first cut into small pieces and accurately weighed. A total of 20 g of plant material was transferred into a glass beaker, and 200 mL of 70% ethanol (1:10 w/v) was added. To protect the solution from light exposure, the beaker was wrapped with aluminum foil, and its mouth was sealed with parafilm to prevent solvent evaporation. The mixture was then placed on a magnetic stirrer and stirred continuously at room temperature for 24 hours. Following the incubation period, the mixture was filtered to separate the liquid extract from the plant residue. The residue was then re-extracted under identical conditions two more times (three extractions in total). All filtrates were pooled, and subsequently, the combined ethanolic extracts were concentrated using a rotary evaporator at 40–45 °C under reduced pressure to remove the solvent and obtain the crude extract of *C. speciosum*. The crude extract was stored at −20 °C until use in further experiments.

Antibacterial Assay

The antibacterial evaluation of CS extracts was performed using the microdilution method against gram-positive and gram-negative standard pathogenic bacteria (*Staphylococcus aureus* ATCC 29213, *Enterococcus faecalis* ATCC 29212, *Escherichia coli* ATCC 25922, *Pseudomonas aeruginosa* ATCC 27853). A sterile microplate with 96 wells was used to locate Muller Hinton suspensions, different doses of extracts, and suspensions of bacteria. 0.5 McFarland turbidity standard solutions (1 x 10^8 CFU/mL) were prepared in tubes containing sterile saline using bacterial subcultures on 5% sheep blood agar. These bacterial suspensions were diluted at a 1/10 rate with sterile saline. 100 µl of Mueller Hinton agar suspension, used to feed the bacteria, was added to the wells using a micropipette. Liquid extracts were sterilized with filters (0.22 µm) and then diluted with sterile distilled water at different percentages.100 µl of each dose were spread into the line for each other bacteria. 100 µl of each bacteria's diluted suspension was spread into their wells. Finally, the first lines were used as negative control containing only Muller Hinton broth suspension. The second line was used as positive control to see bacterial growth, containing Muller Hinton broth and diluted bacterial suspension. The plate was incubated at 37°C for 20 hours. Figure 1 shows before the incubation of the microplate. The optical densitometry (OD) values of the wells were measured at 450 nm in a microplate reader (SPECTRO star Nano, BMG Labtech, Ortenberg, Germany) after the end of the incubation. The mean values of negative control and positive control were calculated, and values of wells smaller than positive control were accepted as negative and aggregations at the bottom of the wells were observed. Wells with aggregation were accepted as positive. Observation and OD values were coherent.

Cell Viability Test: MTT Assay (3–[4,5-dimethylthiazol-2-yl]-2,5-diphenyl-tetrazolium bromide)

Cell viability was determined using the MTT test. As a first step,

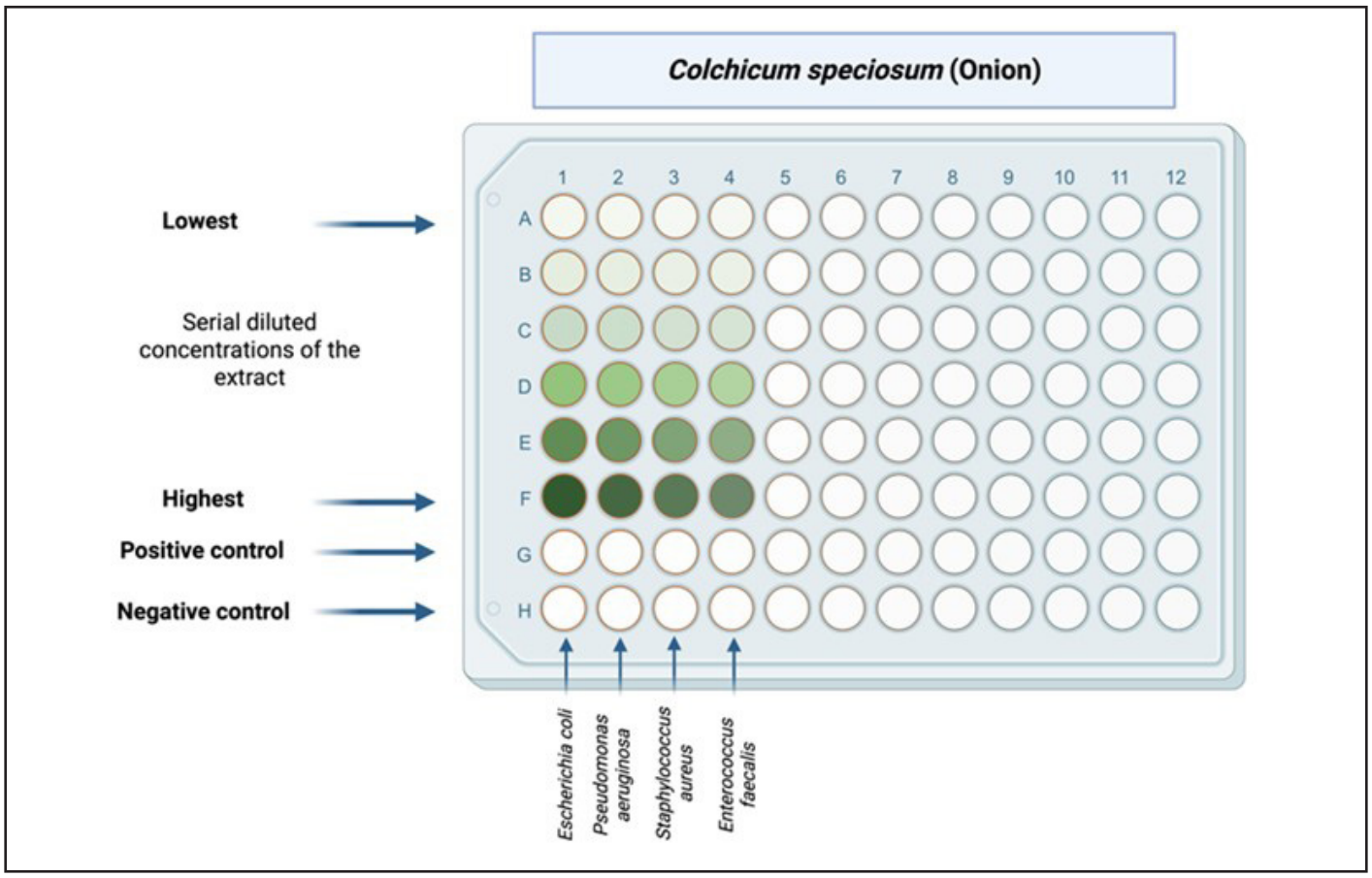


Figure 1. Microplate before the incubation. (Visual assessment of the antibacterial activity of serially diluted *Colchicum speciosum* bulb extract against *Escherichia coli (E. coli), Pseudomonas aeruginosa* (*P. aeruginosa*), *Staphylococcus aureus (S. aureus),* and *Enterococcus faecalis (E. faecalis)* using a 96-well microtiter plate assay. Rows A–F represent increasing concentrations of the extract from lowest to highest prepared by serial dilution, while row G corresponds to the positive control (standard antibacterial agent) and row H represents the negative control (bacterial culture without extract). Each column corresponds to a specific bacterial strain. Extract concentrations were prepared in mg/mL and applied using a serial dilution method).

96-well plates were used for cell plating, with 5 × 10^4 cells per well. One day after cell plating, cells were exposed to different doses (15, 30, 60, 120, 240, 500 µg/mL) of C. speciosum onion extract for 24, 48, and 72 hours. After the incubation periods of the added C. speciosum onion extract, 0.1 mg/ml of MTT reagent was added to each well. After the MTT reagent, the cells were incubated for three hours at 37°C and 5% CO2 humidification. The formation of formazan crystals in the cells was ensured. After incubation, all liquid medium in the wells was removed. Formazan crystals were separated using dimethyl sulfoxide (300:1 µl). After the plates were left in a dark environment for 15 minutes, cell viability was measured spectrophotometrically (Thermo Multiskan GO, Fisher Scientific) at different wavelengths of 540, 570 and 590 nm.

Apoptosis

The Annexin V-FITC/PI Apoptosis Kit was provided from a commercial supplier (Elabscience Bionovation Inc., Cat. No. E-CK A211, Texas, USA). Annexin V is a protein that exhibits high affinity for Ca^+2-dependent phosphatidylserine. Propidium iodide (PI) binds to nucleic acids and is used in apoptotic staining [26]. Cells were first seeded into a 6-well plate at a ratio of 1x10^5 cells/well. Each seeded well was set up with three replicates, and five randomly scanned wells were selected from each well for quantitative evaluation in Annexin V-PI fluorescence staining and visualized at 20–40x magnification using an inverted fluorescence microscope (Carl Zeiss TM Axio Vert.A1 FL-LED Inverted Microscope, Germany) and software (Carl Zeiss Axiocam 506 ZEN 2 Lite Software, Germany). Automated cell counts were evaluated using ImageJ (ImageJ, NIH, USA) and

verified with manual counting. Test conditions were performed according to commercial manufacturer instructions [27].

Enzyme-Linked Immunosorbent Assay (ELISA) Tests

Supernatants obtained from MCF-7 cell underwent differents treatments were analyzed for BDNF (Brain-Derived Neurotrophic Factor), IL-10(Interleukin-10), IL-1β(Interleukin-1β), TNF-α (Tumor Necrosis Factor-alpha), and TGF-β1 (Transforming Growth Factor-beta1) levels were measured using the relevant ELISA kits. The kits used were, respectively: BDNF (Cat.No. E1302Hu), IL-10 (Cat.No. E0102Hu), IL-1β (Cat. No. E0143Hu), TNF-α (Cat.No. E0082Hu), and TGF-β1(Cat.No. E0134Hu) (BT LAB, China). Samples were collected at 24, 48, and 72 hours and centrifuged at 3000 rpm (≈11,000 × g) for 20 minutes. The cell solution was adjusted to pH 7.2–7.4 and diluted with PBS. Forty microliters were added to ELISA plates coated with the relevant antibodies in 96-well plates, followed by the addition of biotinylated antibody for attachment. It was incubated by adding Streptavidin-HRP solution. The finally, unconnetted streptavidin-HRP was washed away. Subsequently, A and B substrate solutions were applied. Colorimetric changes were detected. Finally, the reaction was terminated by adding stop solution and measurement was made at 450 nm via spectrophotometer.

Statistical Analyses

Normal distribution and homogeneity of the data were evaluated via Shapiro-Wilk and Levene's tests. ANOVA and post-hoc Bonferroni test were used for intergroup comparisons. Statistical evaluations were performed using GraphPad Prism Version 10.2.0 (GraphPad Software, USA). Values of $p < 0.05$ were considered statistically significant.

RESULTS

Antibacterial Assay

All positive and negative controls were properly included and functioned as expected throughout the antibacterial assays. No antibacterial activity was observed for the extract against the two tested Gram-negative bacterial strains: *Escherichia coli* ATCC 25922 and Pseudomonas aeruginosa ATCC 27853. In contrast, antibacterial activity was detected against the Gram-positive bacterium *Enterococcus faecalis* ATCC 29212 at a concentration of 0.625% (w/v) of the bulb extract. Additionally, inhibitory activity was observed against *Staphylococcus aureus* ATCC 29213 at a higher concentration of 5% (w/v). Figure 2 presents the visual appearance and aggregation patterns of the wells from the microdilution assay. Table 1 optical density (OD) values of the wells, yellow coloured wells display antibacterial activities. Table 2 reports the minimum inhibitory concentrations (MICs) determined in this study.

Table 1. OD values of the wells, yellow coloured wells displays antibacterial activities

Onion	Onion	Onion	Onion
2,279	3,166	2,547	1,135
2,288	3,132	2,923	0,598
2,263	3,121	2,375	0,52
2,16	3,166	2,35	0,46
2,317	2,917	1,437	0,646
2,298	3,079	0,709	0,846
(PC) 1,553	3,122	2,258	1,867
(NC) 0,113	0,092	0,157	0,11
E. coli	***P. aureginosa***	***S. aureus***	***E. faecalis***

Table 2. MIC values as concentrations detected in the study

Bacteria	Onion of CS
E. coli	>10%
P. aureginosa	>10%
S. aureus	5%
E. faecalis	0.625%

MTT Assay

MTT test results obtained at different concentrations (15, 30, 60, 120, 240, 500 µg/mL) and different time points (24 hours, 48 hours, and 72 hours) for *C. speciosum* bulb were evaluated. MTT test data obtained after 24, 48, and 72 hours of incubation showed a significant reduction in cell proliferation, especially at concentrations of 240 and 500 µg/mL, compared to control groups at the respective time intervals ($p < 0.001$). Inhibition of cell proliferation was more pronounced at concentrations of 240 and 500 µg/mL, and due to the most significant biological effect, application doses of 240 and 500 µg/mL were selected for use in subsequent analyses (Figure 3). *C. speciosum* bulb extract was evaluated at a wavelength of 570 nm for statistical analysis.

Time-Dependent Cell Concentration

The antiproliferative effects of *C. speciosum* bulb at concentrations of 240 and 500 µg/mL were evaluated microscopically at 24, 48,

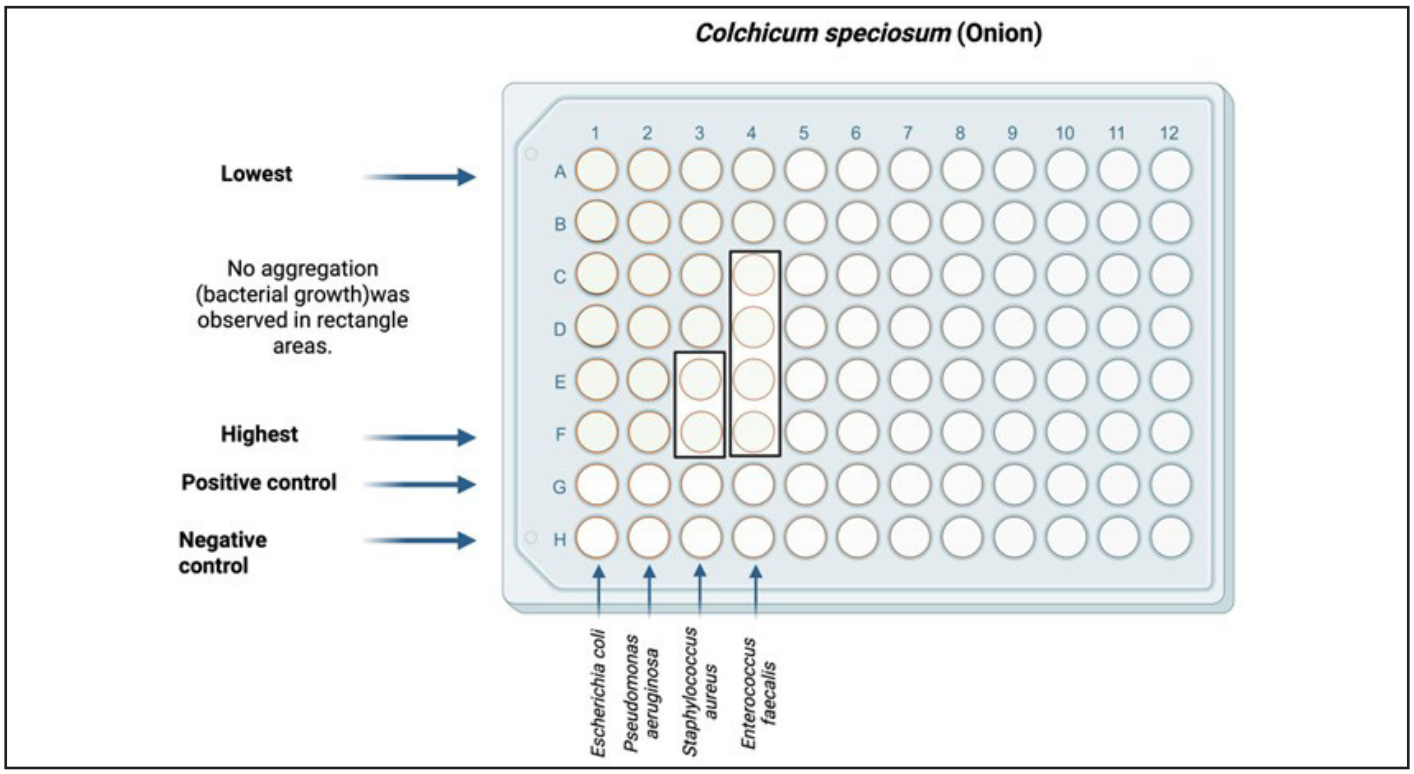


Figure 2. Microplate after the incubation. (Evaluation of the antibacterial activity of *Colchicum speciosum* bulb extract against *Escherichia coli (E. coli)*, *Pseudomonas aeruginosa (P. aeruginosa)*, *Staphylococcus aureus (S. aureus),* and *Enterococcus faecalis (E. faecalis)* using a 96-well microtiter plate assay. Rows A–F represent serially increasing concentrations of the extract from lowest to highest (expressed in mg/mL), while row G indicates the positive control (standard antibacterial agent) and row H represents the negative control (bacterial culture without extract). Columns correspond to the tested bacterial strains. Wells highlighted by rectangles indicate the absence of bacterial aggregation or visible growth

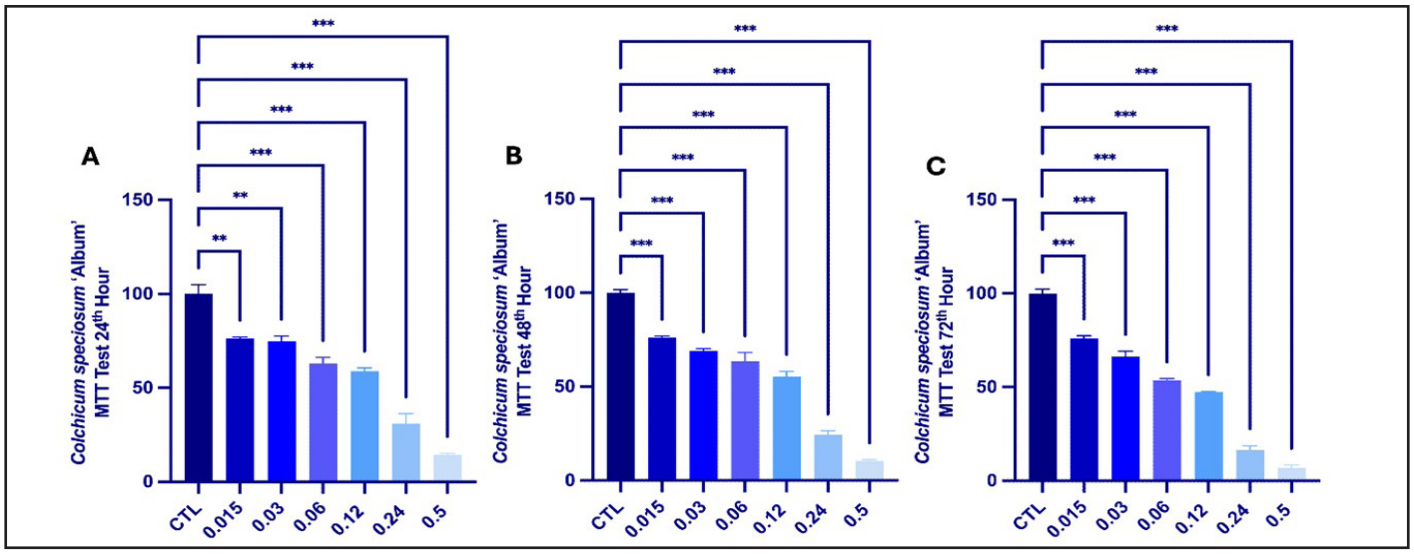


Figure 3. MTT analysis. (Effects of 15, 30, 60, 120, 240, 500 µg/mL concentrations of *C. speciosum* extract on cell viability at 24, 48, and 72 hours. Cell viability in the MCF-7 cell line was assessed using the MTT assay. (A) graphic shows 24-hour; (B) graphic shows 48-hour; (C) graphic shows 72-hour treatment results. CTL: Control group. Statistical analysis was performed by one-way ANOVA and the Bonferroni post-hoc test. Values are given as the mean ± standard deviation (SD) obtained from three independent experiments (n = 3 technical replicates). $p<0.050$ was considered significant. $p<0.050$*, $p<0.010$** , $p<0.001$***

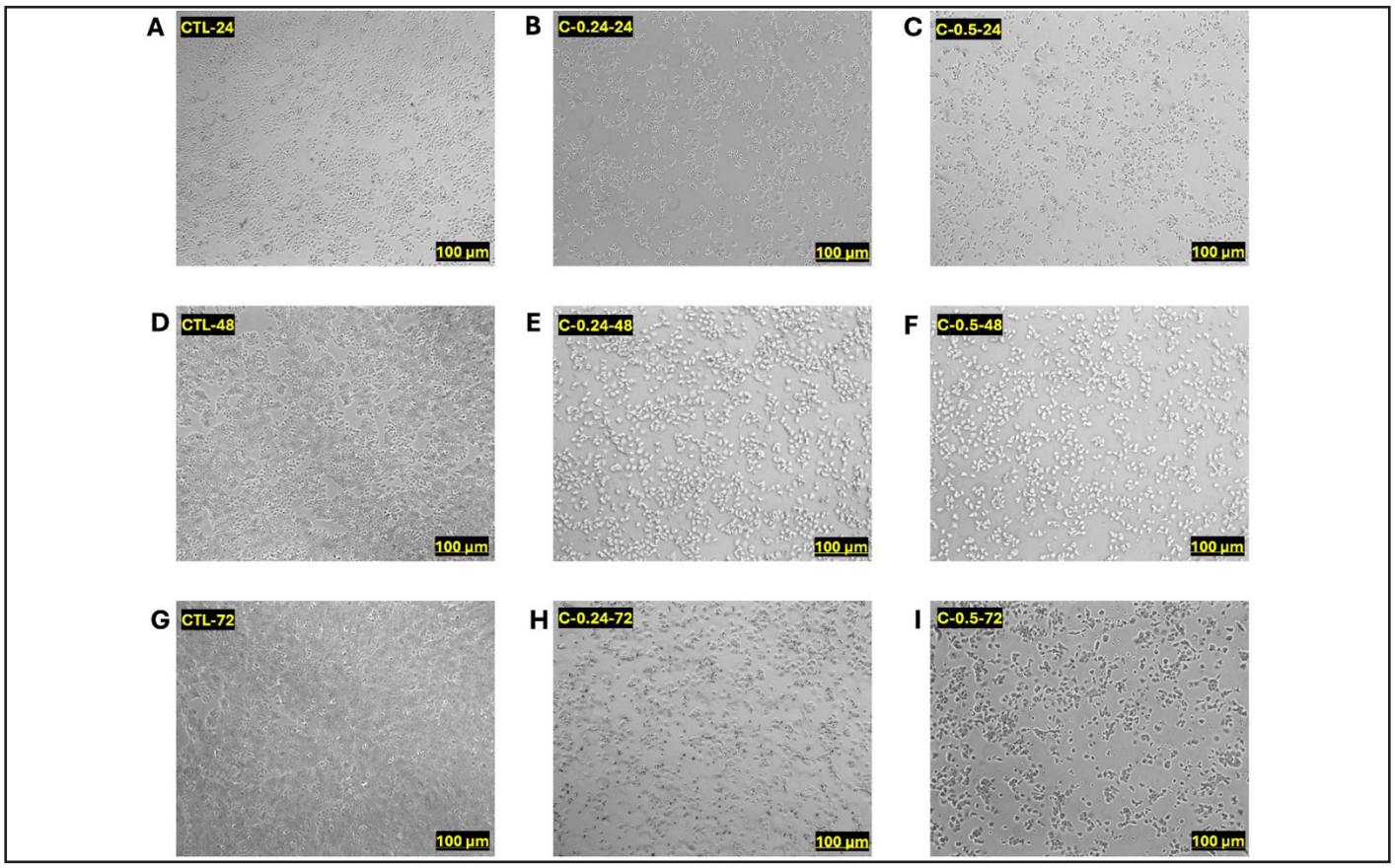


Figure 4. *C. speciosum* bulb 24-48-72 microscopic image. (Cell morphology in the control (CTL) group. The MCF-7 cell line were observed at 24 (A), 48 (B), and 72 (C) hours. n = 3, technical replicates. Scale bar: 100 µm.

and 72 time intervals. The obtained microscopic images support the findings of the MTT assay. In conclusion, the antiproliferative effect of *C. speciosum* bulb was clearly observed microscopically as time and dose increased according to the doses we applied (Figure 4).

Apoptosis (%) Cell Concentration

The cell concentration in the apoptosis mechanism of *C. speciosum* bulb was evaluated based on the application dose ratio (240, 500 µg/mL, CTL) and incubation period (24, 48, 72 hours). C-240 µg/mL and C-500 µg/mL incubation groups were compared with CTL groups. A statistically notable increase was ıdentified as incubation time increased between the groups ($p < 0.001$). However, depending on the dose ratio, a statistically significant decrease was detected in comparison of C-500 µg/mL application with C-240 µg/mL application within each incubation period ($p < 0.001$) (Figure 5).

Apoptotic Evaluation

In the CTL-24 group, apoptotic and necrotic rates in cells remained at physiological basal levels. In the C-240 µg/mL–24 group, Annexin V-positive (+) cells indicating the early apoptosis stage were observed, while a smaller number of PI-positive (+) cells indicating late apoptosis signals were also present. In the C-500 µg/mL -24 group, the apoptotic response was detected at lower levels. In the CTL-48 group, apoptotic and necrotic rates remained at physiological basal levels. In the C-240 µg/mL-48 group, a significant decrease in the number of Annexin V positive (+) cells was observed. In the C-500 µg/mL-48 group, an increase in Annexin V positive (+) signal and late apoptosis/necrosis in PI positive (+) cells were detected. In the CTL-72 group, apoptotic and necrotic rates remained at physiological basal levels. In the C-240 µg/mL-72 and C-500 µg/mL-72 groups, a decrease in the number of Annexin V-positive (+) cells was observed, and cellular integrity was preserved. However, in the C-500 µg/mL-72 group, intense PI-positive (+) cells were observed, indicating that the cells were in the late apoptosis or necrosis stage (Figure 6).

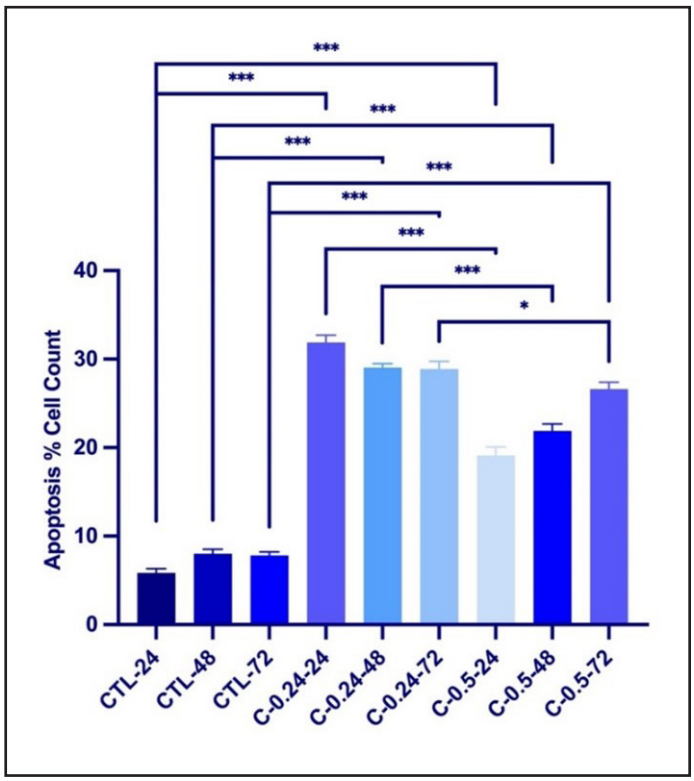


Figure 5. Apoptotic cell concentration. (At the 24, 48 and 72 hour, 240 µg/mL and 500 µg/mL of *C. spesiosum* bulb extract on the apoptotic % cell count on MCF-7 cell line. Apoptosis rate quantified as the % Annexin V (+) cells among the total cell number. Annexin V-FITC/PI–stained fluorescence images were analyzed using ImageJ (n = 3 technical replicates per group). CTL: control group. Data were analyzed by one-way ANOVA with Bonferroni post hoc test and are expressed as mean ± SD (three independent experiments; n = 3 technical replicates). $p < 0.050$ was considered statistically significant. $p < 0.050$*, $p < 0.010$** , $p < 0.001$***

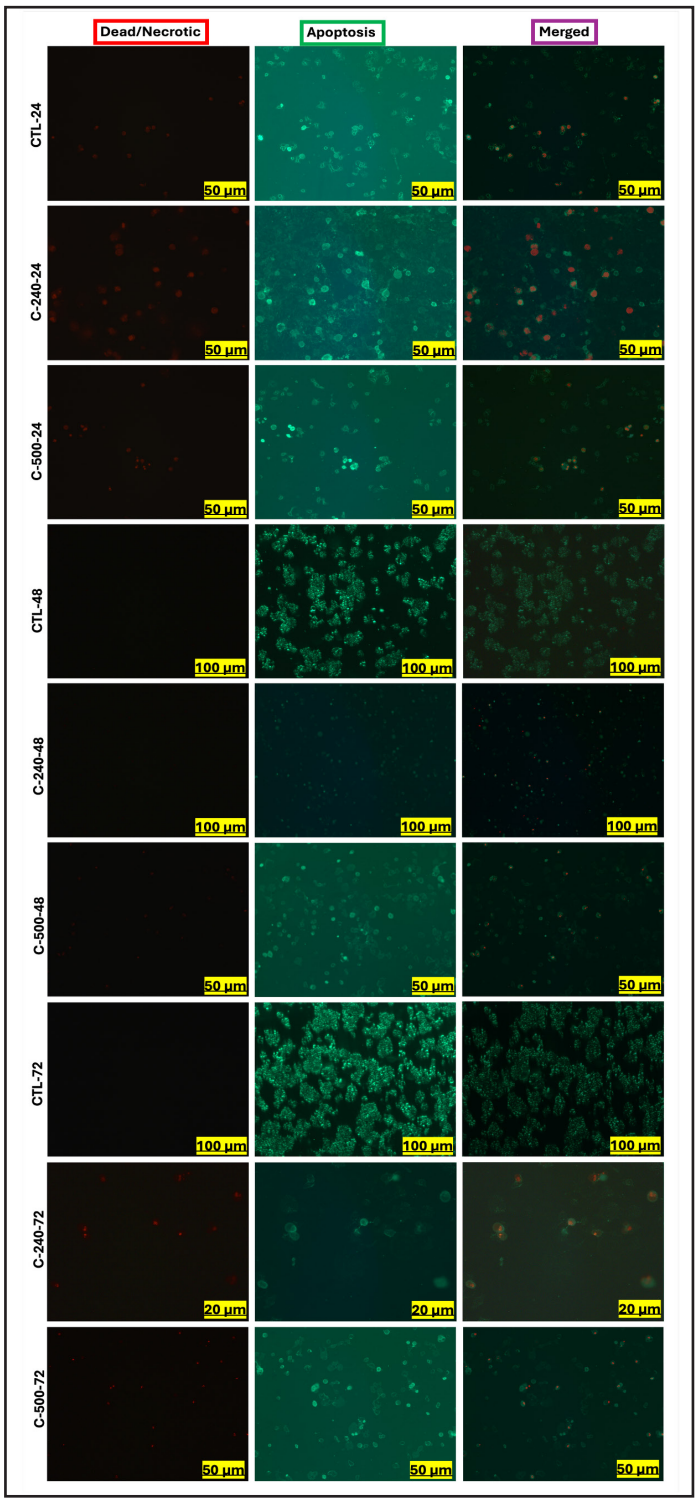


Figure 6. Fluorescent microscopic images. (Annexin V-FITC/PI double staining was performed to evaluate apoptosis and cell death in MCF-7 cells treated with 240 µg/mL and 500 µg/mL *C. speciosum* bulb extract. Cells were examined at 24, 48, and 72 h using a fluorescence microscope. Annexin V-positive cells (green) indicate apoptotic cells, PI-positive cells (red) indicate dead/necrotic cells, and merged images show overlapping signals representing late apoptotic (Annexin V/PI double-

ELISA Evaluation

In intergroup comparisons of TNF-α, only numerical changes were observed between different time periods in the CTL group, and no statistically significant difference was found. Compared to the CTL group, TNF-α levels in the C-240 and C-500 µg/mL groups at all time periods (24, 48, 72) showed a statistically significant decrease with increasing time period ($p < 0.010$ $p < 0.001$). Intergroup evaluation of the C-240 µg/mL and C-500 µg/mL doses at different time points revealed that the C-500 µg/mL group was more effective in MCF-7 cell than the C-240 µg/mL group with increasing time (Figure 7). IL-1β levels showed an increase over time in the CTL group's internal evaluation during intergroup comparisons, but no statistically significant

positive) cells (n = 3 technical replicates). Images are raw and were only assembled as panels without digital processing affecting cell morphology. All images were acquired at 20×-100x magnification; scale bar = 20, 50, 100 µm)

difference was found. Compared to the CTL group, both the C-240 and C-500 µg/mL groups showed a statistically significant decrease over time ($p < 0.010$ and $p < 0.001$, respectively). In the evaluation between the C-240 and C-500 µg/mL groups, it was determined that the IL-1β level decreased over time in the C-500 µg/mL group and that this decrease was statistically significant compared to the C-240 µg/mLgroup ($p < 0.010$). We believe that the high dose of C. speciosum bulb plant extract provides an anti-cancer effect by suppressing inflammation (Figure 7). TGF-β1 levels were analyzed temporally between groups, and in the CTL group-internal evaluation, only numerical changes were observed between different time intervals, with numerical fluctuations detected over time. An increase in time was associated with a numerical decrease in the C-240 µg/mL and C-500 µg/mL group-internal evaluation. In the C-240 and C-500 µg/mL group-interval evaluation, the C-500 µg/mL group was found to have lower TGF-β1 levels in all time intervals. C. speciosum bulb treatment led to a decrease in TGF-β1 levels, which may indicate potential modulation of pathways associated with cancer progression. Additionally, the extract may exert modulatory effects on inflammatory responses in cancer treatment (Figure 7). IL-10 levels were evaluated in terms of groups and time intervals. In the internal evaluations performed in the CTL group, only numerical changes were observed between different time intervals, and numerical fluctuations were detected between times. Compared to the C-240, C-500 µg/mL, and CTL groups, an increase in IL-10 levels was observed as time progressed. ($p < 0.050$, $p < 0.001$). In the evaluation between the C-240 and C-500 µg/mL groups, an increase in IL-10 levels was observed in the C-500 µg/mL group with increasing time ($p < 0.050$). (Figure 7). When comparing BDNF levels between groups, numerical changes were observed in the CTL group's internal assessment at increasing time intervals, but no statistically significant difference was found. In the within-group assessment, a decrease over time was detected in the C-240 and C-500 µg/mL groups ($p < 0.050$, $p < 0.010$). In the comparison between the C-240 and C-500 µg/mL groups, the BDNF level was lower in the C-500 µg/mL group (Figure 7).

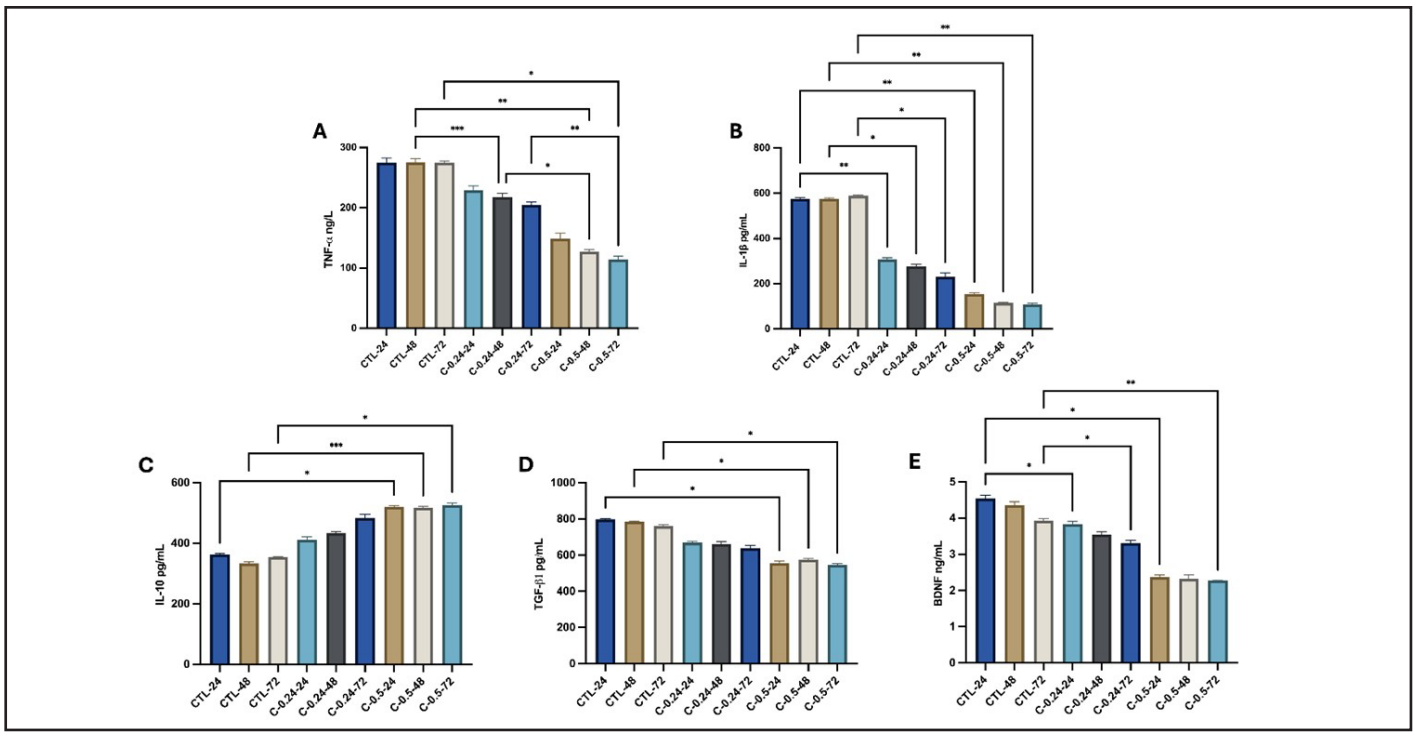


Figure 7. *C. speciosum bulb ELISA assay.* (ELISA analyses of samples collected at 24, 48, and 72 h following treatment with *C. speciosum* bulb extract (240 µg/mL and 500 µg/mL). Pro-inflammatory cytokines IL-1β (A, pg/mL) and TNF-α (B, ng/L), and anti-inflammatory cytokines BDNF (C, ng/mL), IL-10 (D, pg/mL), and TGF-β1 (E, pg/mL) were evaluated. CTL: control. Data are mean ± SD (n = 3). One-way ANOVA with Bonferroni post-hoc test; $p < 0.050$*, $p < 0.010$** , $p < 0.001$***)

DISCUSSION

Cancer is among the most common causes of death globally, and more effective treatment methods are being developed using biological and molecular approaches. In this context, natural products are at the forefront of research. In our study, we evaluated the antitumor, antimicrobial, and anticancer effects of *C. speciosum* bulb extract on the MCF-7 breast cancer cell line. The antibacterial activity of *C. speciosum* bulb plant extract was evaluated against gram-positive and gram-negative bacteria. According to the results, no antibacterial activity was observed against the gram-negative bacteria *Escherichia coli* and *Pseudomonas aeruginosa*. However, a significant antibacterial activity was detected on the gram-positive bacterium *Enterococcus faecalis*. Furthermore, an inhibitory effect was detected at high doses of the plant extract on *Staphylococcus aureus*. These findings indicate that *C. speciosum* bulb possesses potential antibacterial properties against certain Gram-positive bacteria and exhibits an effective inhibitory effect on these bacteria. Baltacı et al. [28] demonstrated in their study that the plant extract obtained from *C. speciosum* flowers possesses antimicrobial activity against *Bacillus cereus*. A separate study has also demonstrated the antibacterial activity of *Colchicum luteum* against gram-positive bacteria [29]. In our study, the antimicrobial activity of *C. speciosum* bulb was observed. *C. speciosum* bulb showed a significant decrease in MCF-7 cell proliferation by inhibiting cell proliferation at doses of 240 and 500 µg/mL. Consequently, *C. speciosum* bulb demonstrates potential anticancer effects and the capacity to inhibit the growth of cancer cells. In a study, the alkaloid-rich extract of the *Colchicum autumnale* plant was shown to inhibit breast cancer cell growth (MCF-7) [30]. In another study, methanolic extracts obtained from *C. speciosum* seeds were found to contain tropolone alkaloids such as colchicine and colchicoside. These compounds were reported to exhibit cytotoxic effects on cancer cells, with colchicine inhibiting microtubule polymerization and thereby preventing cell division [31]. Artun et al. [32] demonstrated in their study that the *Colchicum sanguicolle* plant has anticancer potential and could be an effective compound against cancer cells. In our study, we observed the antiproliferative activity of *C. speciosum* bulb in cancer cells following the MTT. The effects of *C. speciosum* bulb on ig fin the MCF-7 cell were examined in detail at 24, 48, and 72-hour time intervals using the Annexin V-FITC/PI dual staining technique. We observed that *C. speciosum* bulb induced early apoptosis (Annexin V^+ cells) at low doses, while at high doses, late apoptosis and necrosis (PI^+ cells) progressed. Foumani and colleagues (2022) evaluated the apoptotic effects of colchicine isolated from the *Colchicum autumnale* plant on MCF-7 and 4T1 breast cancer cell lines. MCF-7 cell were incubated with colchicine at doses of 0.5 and 1.0 µg/mL for 24 hours; apoptosis analysis using the Annexin V-FITC/PI staining method revealed a dose-dependent, statistically significant increase in both early and late apoptosis rates at both doses [33]. Another study suggested that the antiproliferative effect of *Colchicum autumnale* extract may trigger apoptosis through microtubule destabilization [30]. Our findings that *C. speciosum* bulb induces a dose-dependent increase in both early and late apoptosis in MCF-7 cell support studies conducted with isolated colchicine and similar compounds. Annexin V/PI analyses indicate that *C. speciosum* bulb may possess anticancer potential by inducing apoptosis. In addition to findings related to apoptosis, time- and dose-dependent changes in TNF-α, IL-1β, TGF-β1, IL-10, and BDNF levels were analyzed to evaluate the regulatory potential of *C. speciosum* bulb on the immune response. The results obtained demonstrate that *C. speciosum* bulb exhibits a multifaceted biological effect that affects not only cell death but also the immune system. In our findings, TNF-α and IL-1β levels were observed at lower levels over time compared to CTL at C-240 and C-500 µg/mL application doses. However, the C-500 µg/mL dose had a higher effect ratio compared to C-240 µg/mL. A study also showed that TNF-α initiates apoptotic signaling in MCF-7 cell via the p53 pathway through HDAC3/ERα regulation. High TNF-α levels promote apoptosis via caspase-7, while low levels tend to suppress it [34]. Picarda et al. [35] reported that TNF-α plays multiple roles in luminal (ER^+) breast cancer in addition to apoptosis, and that high TNF-α levels in MCF-7 promote cell death. Preclinical molecular studies have demonstrated that colchicine strongly inhibits NLRP3 inflammasome assembly via microtubule disruption, thereby inhibiting IL-1β production [36]. TNF-α suppression is thought to enhance anti-inflammatory effects and activate apoptotic signaling pathways; IL-1β suppression is thought to have both anti-inflammatory and tumor-suppressive effects. The relationship between TGF-β1 expression and cancer development and progression is complex. In a different study, colchicine use was shown to reduce TGF-β1 levels after heart damage [37]. We believe that the decrease in TGF-β1 levels may be related to the modulatory effects of colchicine-like compounds contained in *C. speciosum* bulb on the immune system by triggering the cellular stress response and modulating cell signaling pathways. In our findings, BDNF levels decreased with increasing dose and time compared to the CTL group. In the study, the presence of BDNF was analyzed in MDA-MB-231,

MCF-7 and ZR-751 cells by RT-PCR. The presence of BDNF was shown in all three cell lines. It was shown that inhibition of BDNF in MDA-MB-231 cells caused a decrease in cell growth [38]. In addition, it was reported that BDNF had a pro-apoptotic effect in MDA-MB-231 cells and that the apoptosis rates increased with BDNF deletion [39]. Our study suggests that similar mechanisms to those involved in BDNF proliferation in breast cancer may also apply to the MCF-7 cell line.

Limitations

The limitations of our study are confined to the evaluation of only two doses. The effects of *C. speciosum* bulb should be investigated in detail on different breast cancer and other cancer cell lines.

CONCLUSION

Within the scope of this study, the antiproliferative, apoptotic, antimicrobial, and immunomodulatory effects of *C. speciosum* bulb on MCF-7 cell were comprehensively evaluated. MTT assay results revealed that the extract statistically significantly suppressed metabolic activity at both 240 and 500 µg/mL doses, with a marked decrease in cell viability observed particularly at the high dose. *C. speciosum* bulb was found to exhibit antimicrobial effects on gram-positive bacteria. Apoptosis analyses revealed that early apoptosis rates increased at a dose of 240 µg/mL, while late apoptosis became predominant at a dose of 500 µg/mL. *C. speciosum* bulb has been shown to induce dose-dependent programmed cellular death. Additionally, when changes in cytokine levels were examined, decreases in TNF-α, IL-1β, TGF-β1, and BDNF levels were identify in the treatment groups in comparison with the CTL groups. When comparing the treatment groups, increasing time and increasing dose ratio were found to be more effective. IL-10 levels remained high over time and with increasing application doses. When all these results were evaluated together, it was observed that *C. speciosum* bulb produced antiproliferative and apoptotic effects in MCF-7 cell, while also causing significant dose and time-dependent changes in immune response regulatory cytokines. In this context, the plant extract in question has the potential to be evaluated as a natural agent with anticancer potential. The evidence of our research shows that the biological effects of *C. speciosum* bulb are not limited to the current data, and that advanced in vitro and in vivo research are required to investigate the biomolecular mechanisms of action and to more definitively confirm therapeutic potential.

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