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# Investigation of Anticancer and Anti-Migration Potentials of *Verbascum tenue* Murb. and Analysis of its Bioactive Compounds using LC–ESI–MS/MS

Israt JAHAN[1], Cumali KESKIN[2] ✉, Ayşe BARAN[3], Mehmet Fırat BARAN[4], Sevgi İRTEGÜN KANDEMİR[5, 6], Deniz Evrim KAVAK[7] Fatma MUNGAN KILIÇ[8], Murat KILIÇ[9]

[1] Department of Health Care Services, Vocational School of Health Services, Mardin Artuklu University, 47200 Mardin, Turkiye, [2] Department of Medical Services and Techniques, Vocational School of Health Services, Mardin Artuklu University, 47200 Mardin, Türkiye, [3] Department of Field Crops, Kızıltepe Faculty of Agricultural Sciences and Technologies, Mardin Artuklu University, Mardin, Türkiye, [4] Department of Food Processing, Vocational School of Technical Sciences, Batman University, Batman, Türkiye, [5] Department of Medical Biology, Faculty of Medicine, Dicle University, 21380, Diyarbakır, Türkiye, [6] Dicle University Science and Technology Application and Research Center, Dicle University, 21380, Diyarbakır, Türkiye, [7] Department of Molecular Biology and Genetics, Faculty of Science, Dicle University, 21380, Diyarbakır, Türkiye, [8, 9]Department of Plant Protection, Kızıltepe Faculty of Agricultural Sciences and Technologies, Mardin Artuklu University, 47060, Mardin, Türkiye

[1]https://orcid.org/0000-0003-4166-1617, [2]https://orcid.org/0000-0003-3758-0654, [3]https://orcid.org/0000-0002-2317-0489, [4]https://orcid.org/0000-0001-8133-6670 [5 6]https://orcid.org/0000-0001-6160-5626, [7]https://orcid.org/0000-0002-9681-4468, [8]https://orcid.org/0000-0001-6858-3458, [9]https://orcid.org/0000-0002-6408-9660

✉: cumalikeskin@artuklu.edu.tr

## ABSTRACT

*Verbascum* species (Scrophulariaceae) are recognized in ethnopharmacology for their diverse therapeutic properties. To investigate these claims scientifically, this study characterized a methanol extract of *Verbascum tenue* Murb., leaves (VTE) for its phytochemical composition, in vitro cytotoxicity, and anti-migratory activity. Phytochemical profiling via LC-ESI-MS/MS identified nine compounds, with vanillic acid, vanillin, and trans-ferulic acid as the major constituents. The cytotoxic effects of VTE were evaluated on a panel of cell lines—including SKOV-3 (ovarian adenocarcinoma), MCF-7 (breast carcinoma), U87 (glioblastoma), and HDF (human dermal fibroblast) cells—using the MTT assay across a concentration range of 15.625–500 µg/mL. VTE exhibited differential cytotoxicity, with $IC_{50}$ values of 236.74 µg/mL in SKOV-3 cells, 374.5 µg/mL in MCF-7 cells, and 386.68 µg/mL in HDF cells. The wound healing assay was conducted with 125 µg/mL VTE, a concentration selected for its high toxicity on SKOV-3 (viability 59.21%) and low toxicity on HDF (viability 91.06%). VTE significantly suppressed wound closure to 25.86% relative to the 40.36% observed in controls over 12 hours. This inhibition of cell migration, in conjunction with its cytotoxic profile, supports the potential of VTE as an agent with both anticancer and anti-metastatic properties.

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# *Verbascum tenue* Murb.'un Antikanser ve Anti-Göç Potansiyellerinin Araştırılması ve Biyoaktif Bileşiklerinin LC-ESI-MS/MS Kullanılarak Analizi

## ÖZET

*Verbascum* türleri (Scrophulariaceae), etnofarmakolojide çeşitli terapötik özellikleri ile bilinmektedir. Bu iddiaları bilimsel olarak araştırmak için, bu çalışma *Verbascum tenue* MURB., yapraklarının metanol özütünün (VTE) fitokimyasal bileşimini, in-vitro sitotoksisitesini ve anti-göç aktivitesini karakterize etmiştir. LC-ESI-MS/MS yoluyla yapılan fitokimyasal profilleme, vanilik asit, vanilin ve trans-ferulik asit ana bileşenleri olmak üzere dokuz bileşik tanımlamıştır. VTE'nin sitotoksik etkileri, SKOV-3 (yumurtalık adenokarsinomu), MCF-7 (meme karsinomu), U87 (glioblastoma) ve HDF (insan dermal fibroblastı) hücreleri de dahil olmak üzere bir dizi hücre hattı üzerinde, 15,625–500 µg/mL konsantrasyon aralığında MTT testi kullanılarak değerlendirilmiştir. VTE, SKOV-3 hücrelerinde 236,74 µg/mL, MCF-7 hücrelerinde 374,50 µg/mL ve HDF hücrelerinde 386,68 µg/mL $IC_{50}$ değerleriyle farklı sitotoksisite gösterdi. Yara iyileşme testi, SKOV-3 hücrelerinde yüksek toksisiteye (%59,21 canlılık) ve HDF

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hücrelerinde düşük toksisiteye (%91,06 canlılık) sahip olması nedeniyle seçilen 125 µg/mL VTE konsantrasyonu ile gerçekleştirildi. VTE, 12 saat boyunca kontrol grubunda gözlemlenen %40,36'ya kıyasla yara kapanmasını %25,86'ya kadar önemli ölçüde baskıladı. Hücre göçünün bu inhibisyonu, sitotoksik profiliyle birlikte, VTE'nin hem antikanser hem de anti-metastatik özelliklere sahip bir ajan olarak potansiyelini desteklemektedir.

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## INTRODUCTION

*Verbascum* L. is a genus within the Scrophulariaceae family that includes a substantial group of medicinal plants. Türkiye is recognized as the primary center of genetic diversity for this genus, featuring 257 species, 132 hybrids, and 13 partially artificial groups, along with six records that remain uncertain. Among these, 202 species are endemic to Türkiye, reflecting a high endemism rate of approximately 80% (Duman et al., 2021; Firat, 2022). The genus *Verbascum*, commonly referred to as mullein, has a long history of use in traditional medicine, where it has been employed for the management of gastrointestinal ailments, lowering cholesterol levels, alleviating flatulence, and treating inflammatory conditions and cough (Amin et al., 2020; Blanco-Salas et al., 2021; Yılmaz & Ecevit Genç, 2024). The therapeutic efficacy of various *Verbascum* species underpins their application in modern pharmacotherapy. Research has demonstrated that numerous species in this genus exhibit a broad spectrum of bioactivities, including antioxidant, anti-inflammatory, antimicrobial, antiviral, antinociceptive, antitussive, and immunomodulatory effects. These pharmacological properties are attributed to the diverse phytochemical constituents present in the plants, from which numerous specific bioactive compounds have been isolated and characterized (Angeloni et al., 2021; Grigorow et al., 2025). These plants, renowned for their medicinal properties, are considered promising for treating various human ailments. *Verbascum* is thought to possess a rich diversity of bioactive compounds, including proteins, phenolic acids, carbohydrates, sugars, tannins, carotenoids, flavonoids, terpenes, saponins, tetraglycosides, triterpenes, and minerals (Gupta et al., 2022). Owing to its potent phytoconstituents, mullein has been traditionally used by indigenous communities in various regions worldwide through folk medicine practices (Ercan et al., 2025). This species exhibits several pharmacological activities, including analgesic, nephroprotective, anthelmintic, antiviral, antibacterial, hepatoprotective, anti-inflammatory, anticancer, and cardiovascular properties (Jan et al., 2022; Lone et al., 2024). Research findings indicate that *Verbascum thapsus* L. is characterized by flavonoids such as verbascoside, hesperidin, and 3-methyl quercetin, along with volatile oils containing saponins. Additionally, the plant is rich in polysaccharides and iridoid glycosides, particularly aucubin, harpagide, and harpagoside, with higher levels in its leaves (Turker & Gurel, 2005). A distinct study revealed that *V. exuberans* possesses peripheral antinociceptive and anti-inflammatory activities (Eyiiş et al., 2020). Cancer currently poses a substantial public health burden, ranking as one of the leading causes of death worldwide (Wilkes, 2018). Chemotherapy is a prominent and well-recognized primary method for managing and preventing cancer among the various therapies available. However, contemporary chemotherapeutic agents encounter significant challenges due to pronounced side effects and the development of drug resistance (Alfarouk et al., 2015; Srivastava et al., 2016). Therefore, there is an urgent need to identify innovative, effective, and low-toxicity treatments for cancer. Plants hold considerable promise for providing chemoprotective benefits against cancer, as plant-derived compounds may modulate signaling pathways in cancer cells, including those that induce apoptosis and suppress cell growth (Srivastava et al., 2016). Over 60% of anti-cancer pharmaceuticals are derived from biological sources, including microorganisms, aquatic organisms, and plants (Sultana et al., 2014; Valiyari et al., 2020). A diverse range of plant-derived biocompounds has been extensively utilized as anticancer agents in both *in vitro* and *in vivo* studies, as well as in clinical trials. Notable examples include podophyllotoxin from *Podophyllum peltatum* L., vinca alkaloids from *Catharanthus roseus* (L.) G. Don, camptothecin from *Camptotheca acuminata* Decne., and taxol from *Taxus brevifolia* L. (Iqbal et al., 2017; Kuruppu et al., 2019). Furthermore, the concurrent use of naturally derived substances with conventional chemotherapeutic drugs shows significant promise in enhancing treatment efficacy through additive or synergistic effects (Fennell et al., 2004). This study introduces a novel investigation within the well-established field of *Verbascum* research. Prior work has primarily validated the genus's traditional uses, such as for antimicrobial or

anti-inflammatory purposes. In contrast, our research provides the first detailed analysis of the under-explored species *V. tenue* MURB., specifically for its anticancer potential. The originality of our approach is demonstrated by directly linking a defined chemical profile to two critical biological activities: cell death (cytotoxicity) and the inhibition of cancer spread (anti-migratory activity). By testing multiple cancer cell lines alongside healthy cells, we further identified a selective action against certain cancers. Consequently, this work uniquely positions *V. tenue* as a source of compounds that may not only inhibit cancer ce growthls but also impede metastasis.

## MATERIAL and METHOD

### Materials

In May 2023, the aerial parts of *V. tenue* MURB. were gathered from a calcareous slope along the Şanlıurfa-Adıyaman highway near the Mardin-Habur junction in Şanlıurfa, Turkiye, situated at an elevation of 717 meters. Identification of the plant species relied on authoritative botanical sources such as the Flora of Turkey by Davis et al. (1988) and the checklist of Flora of Turkey (Vascular Plants) by Güner et al. (2012). Botanical verification was conducted by Dr. Fatma Mungan Kılıç and Dr. Murat Kılıç from Mardin Artuklu University. A voucher specimen (Voucher No: M. Kılıç-MAU-2023-297) has been archived at the same institution for future reference.

### Preparation of Plant Extract

To initiate the extraction process, a precise amount of 20 grams of the dehydrated and pulverized *V. tenue* MURB. leaf was measured and carefully deposited into a Soxhlet cartridge. The solvent compartment was initially filled with 200 cc of methanol. The plant sample underwent extraction in a Soxhlet apparatus for 4 hours. The device was set to 65 °C to evaporate the methanol. A rotary evaporator at 40°C under reduced pressure was used to remove the solvent. The crude extract obtained was subjected to freeze-drying (lyophilization) using a Christ Alpha 1–2 LD freeze-dryer at the Sample Preparation Laboratory of Iğdır University. This process was carried out tcompletely o remove residual solvents, resulting in a dry, solvent-free extract suitable for subsequent analyses.

### Chemical Composition Analysis using LC-ESI-MS/MS

The phytochemical composition of the VTE was characterized using liquid chromatography-mass spectrometry (LC-ESI-MS/MS) to identify the compounds responsible for its biological activity. The data was also used to determine the chemical constituents of the extract. The phenolic standards, formic acid, ammonium formate, acetonitrile, and methanol were obtained from Merck (Germany). Upon measurement of the VTE solution in adequate amounts, it was dissolved in methanol, which was again serially diluted to a concentration of some volume through the dissolution of methanol and high-purity water. Subsequently, the solution was then filtered with the help of a microfilter and transferred to a vial for the LC-ESI/MS/MS process. An ideal chromatographic separation was achieved on a Poroshell 120 EC-C18 column (100 mm × 4.6 mm I.D., 2.7 µm particle size) using a flow rate of 0.4 mL/min and an injection volume of 4.0 µL. The mobile phase consisted of (A) 0.1% formic acid and 5 mM ammonium salt in water and (B) 0.1% formic acid and 5 mM ammonium salt in methanol. A linear gradient program was employed as follows: the composition of mobile phase B was increased from an initial 15% to 50%, then to 90%, and finally returned to 15% for column re-equilibration (Keskin et al., 2023).

### Cytotoxic Activities of VTE

Using the MTT assay, an experiment investigating the anticancer properties of VTE was conducted at the Cell Culture Laboratory of the Dicle University Scientific Research Center in Diyarbakir, Türkiye. The cell lines utilized in this study were sourced from the American Type Culture Collection (ATCC). The study aimed to assess the cytotoxic effects of the compound on human ovarian sarcoma (SKOV-3), human breast cancer (MCF-7), glioblastoma (U87), and cancer cell lines, as well as the healthy human dermal fibroblast (HDF) cell line. The U87, MCF-7, and HDF cell lines were cultured in 75 cm² flasks containing Dulbecco's Modified Eagle Medium (DMEM), enriched with 2 mM L-glutamine, 100 U/mL Penicillin-Streptomycin (Pen-strep), and 10% fetal bovine serum (FBS). Meanwhile, the SKOV-3 cell line was cultured in a similar configuration using Roswell Park Memorial Institute Medium (RPMI 1640) supplemented with 100 U/ml Pen-strep and 10% FBS in 75 cm² fl. The flasks of cells on the medium were positioned under optimal environmental conditions at 37 re, providing 95% humidity and 5% $CO_2$. The cells were counted through a hemocytometer at an 80% state of confluence. Subsequently, 90 µL of medium appropriate to the cell type containing $8 \times 10^3$ SKOV-3, $6 \times 10^3$ MCF-7, $1 \times 10^4$ U87, and $3 \times 10^3$ HDF cells was seeded into each well of 96-well plates and incubated overnight under optimal growth conditions (Kandemir & Ipek, 2023). Furthermore, VTE, dissolved in 5% dimethyl sulfoxide (DMSO) at concentrations ranging from 15.625 to 500 µg/mL, was added to wells containing cultured cells. The final DMSO concentration in both treatment and control groups was adjusted to 0.5% in the cell culture medium. The cells were then incubated

for 48 hours to allow interaction. Following the interaction period, 10 µL of MTT solution was added to each well in the microplate and incubated for an additional 3 hours. Afterwads, DMSO was added and left to incubate for 15 minutes. Following these procedures, the absorbance data of the cells were assessed at a wavelength of 540 nm using the MultiScan Go device by Thermo (Buranaamnuay, 2021). The percentage of cell viability was determined by utilizing the absorbance values and applying the following formula:

$$\% \text{ viability} = \frac{Absorbance\ of\ treated\ cells}{Absorbance\ of\ untreated\ (control)\ cells} \quad \%100 \qquad (1)$$

### Wound Healing Assay

The migratory potential of SKOV-3 cells exposed to a specific VTE concentration (125 µg/mL) was evaluated using a wound-healing assay. Cells were seeded in 6-well plates and incubated for approximately 24 hours to reach 90% confluence. A controlled scratch was delicately generated at the center of each well using a sterile 200 µL pipette tip, resulting in a single scratch on the confluent cell monolayer. Another scratch was made perpendicular to the initial one, forming a cross-shaped pattern at the centre of each well. Following the scratching procedure, the cells underwent two rounds of phosphate-buffered saline (PBS) washes after the cell culture medium was gently removed, and were subsequently incubated in DMEM supplemented with 10% FBS. Afterward, the VTE (125 µg/mL) was added to each well, excluding the control, and the wells were incubated for the subsequent experiment. Photographs were promptly captured immediately after the treatments were added (referred to as time point zero, or $t_0$), using a Zeiss inverted microscope (4X magnification). Subsequently, images were taken at 12-hour intervals over 36 hours to ensure consistent imaging of a predetermined region of interest. Each experimental condition was replicated three times to ensure robustness and bolster the reliability of the results. The migration rate was determined by measuring the wound closure area through image analysis using ImageJ software (NIH, USA), and subsequently calculated using the following formula:

$$\text{Wound closure } (\%) = \frac{measurment\ at\ 0\ h - measurment\ at\ specific\ time\ interval}{measurment\ at\ 0\ h} \times\ 100 \qquad (2)$$

### Statistical Analysis

Data obtained from replicated MTT and wound-healing assays were analyzed and graphically represented using GraphPad Prism 7. Statistical comparisons were performed using the t-test, and differences were considered statistically significant at $p < 0.05$.

## RESULTS and DISCUSSION

### Chemical composition of VTE

VTE was analyzed in terms of 23 standard phenolic compounds usine LC-MS/MS. A total of 9 components were detected. The main components of VTE were identified as vanillic acid (73.00 µg/L), vanillin (36.07 µg/L), and trans ferulic acid (26.70 µg/L). The % amounts of all components were given in Table 1. Vanillic acid, also known as 4-hydroxy-3-methoxybenzoic acid or VA is a phenolic compound that is synthesized through secondary metabolism in plants. Vanillic acid is widely used in the food industry as a flavoring agent, additive, and preservative, as well as in perfumery, and exhibits strong antioxidant, hypotensive, cardioprotective, hepatoprotective, and antiapoptotic properties, along with roles in gene regulation and snake venom inhibition (Almeida et al., 2016; Dhananjaya et al., 2006, 2009). Growing evidence indicates that vanillic acid and vanillin possess broad anticancer activity by inhibiting cell proliferation, inducing apoptosis, and suppressing migration, invasion, and metastatic signaling across diverse cancer types (Hao et al., 2025). Vanillic acid mediates these effects through oxidative stress–associated pathways and regulation of FN1A and COL1α1, whereas vanillin and its analogs inhibit migration, metastasis, and angiogenesis primarily via PI3K/Akt signaling (Chuang et al., 2020; Yeni, 2024). Similarly, ferulic acid and its derivatives exhibit significant preclinical anticancer efficacy by promoting apoptosis and cell cycle arrest, reducing proliferation and invasiveness, and modulating key oncogenic pathways, including PI3K/AKT, MAPK/ERK, AKT/mTOR, and NF-κB (Ahmed et al., 2025).

Vanillin ($C_8H_8O_3$) is further recognized for its potent antioxidant activity and its ability to protect against oxidative damage and promote wound healing, as demonstrated *in vitro* in epidermal keratinocyte and human dermal fibroblast models (Costantini et al., 2021; Melguizo-Rodríguez et al., 2021; Sinsuebpol et al., 2023; Zhao et al., 2017). Similarly, ferulic acid exhibits strong antioxidant and anti-inflammatory properties that support wound healing by scavenging reactive oxygen species and reducing oxidative stress–mediated inflammation (Ghaisas et al., 2014; Li et al., 2021).

Table 1. The LC-ESI-MS/MS quantification results of the VTE extract
*Çizelge 1. VTE ekstraktının LC-ESI-MS/MS kantifikasyon sonuçları*

| Detected Compounds | $R^2$ | RT | LOD (µg/L) | LOQ (µg/L) | Rec. (%) | Final Con. (µg/L) |
|---|---|---|---|---|---|---|
| Shikimic acid | 0.99 | 2,30 | 14.1 | 17.80 | 99.90 | N.D |
| Gallic acid | 0.99 | 2.90 | 15.00 | 18.60 | 99.90 | ND |
| Protocatechuic acid | 0.99 | 5.40 | 24.50 | 42.00 | 99.80 | 10.96±0.10 |
| Chlorogenic acid | 0.99 | 7.35 | 13.90 | 18.40 | 99.80 | ND |
| Hydroxybenzaldehyde | 0.99 | 7.60 | 24.30 | 40.30 | 99.50 | 6.66±0.05 |
| Vanillic acid | 0.99 | 7.74 | 22.90 | 38.90 | 99.50 | 73.00±0.05 |
| Caffeic acid | 0.99 | 7.79 | 10.10 | 11.90 | 100.10 | 12.20±0.08 |
| Vanillin | 0.99 | 8.62 | 10.20 | 12.00 | 99.90 | 36.07±0.12 |
| Coumaric acid | 0.99 | 9.43 | 35.10 | 43.10 | 99.70 | 7.87±0.04 |
| Polydatine | 0.99 | 9.63 | 12.3 | 19.5 | 99.80 | ND |
| Trans ferulic acid | 0.99 | 10.12 | 13.6 | 17.4 | 99.50 | 26.70±0.30 |
| Sinapic acid | 0.99 | 10.39 | 69.30 | 86.30 | 100.00 | ND |
| Scutellarin | 0.99 | 11.13 | 14.90 | 19.90 | 99.80 | ND |
| Hesperidin | 0.99 | 11.87 | 17.2 | 22.2 | 100.00 | ND |
| Isoquercetin | 0.99 | 11.89 | 16.10 | 23.10 | 100.00 | 2.93±0.10 |
| Kaempferol-3-glucoside | 0.99 | 13.25 | 10.0 | 12.6 | 90.90 | ND |
| Baicalin | 0.99 | 13.81 | 26.10 | 32.00 | 99.90 | ND |
| Chrysin | 0.99 | 14.11 | 00.020 | 00.30 | 99.90 | ND |
| Trans-cinnamic acid | 0.99 | 14.31 | 00.20 | 00.20 | 99.80 | 10.57±0.08 |
| Naringenin | 0.99 | 15.02 | 3.10 | 5.30 | 100.00 | ND |
| Kaempferol | 0.99 | 16.50 | 13.10 | 18.10 | 99.80 | ND |
| Biochanin A | 0.99 | 17.90 | 221.00 | 253.00 | 99.70 | ND |
| Dihydrocapcaicin | 0.98 | 18.70 | 58.10 | 85.20 | 99.90 | ND |
| Diosgenin | 0.99 | 23.32 | 12.40 | 17.40 | 99.80 | ND |

Note: $R^2$: Coefficient of determination; RT: Retention time (minutes); ND: Not determined; LOD: Limit of detection; LOQ: Limit of quantification; Rec: Recovery; LC-MS/MS; Liquid chromatography-tandem mass spectrometry

## Cytotoxic Activity Results

An *in vitro* MTT assay was conducted to evaluate the cytotoxicity of VTE (15.625, 31.25, 62.5, 125, 250, and 500 µg/mL) against three cancer cell lines (SKOV-3, MCF-7, and U87) and a healthy cell line (HDF). As depicted in Figures 1-3, the results indicate that VTE exhibited anticancer effects against various cancer cell lines in a concentration-dependent manner. Promisingly, VTE showed lower toxicity toward HDF cells than toward these cancer cells at the given concentrations (Figure 4). Moreover, significant cytotoxic effects were only observed ly inon HDF cells at VTE concentrations of 500 and 250 µg/mL (Figure 4), as cell viability percentages exceeding 80%, per ISO 10993-5 standardsindicateof non-cytotoxicity (Kandemir et al., 2024; López-García et al., 2014). The $IC_{50}$ values were determined to be 236.74 µg/mL for SKOV-3, 374.5 µg/mL for MCF-7, and 386.68 µg/mL for HDF cells. For U87 cells, viability was 52.37% at the highest VTE concentration (500 µg/mL), which is nearly equivalent to the $IC_{50}$ value (Figure 3). SKOV-3 showed the lowest cell viability among all cancer cell lines at VTE concentrations of 500, 250, and 125 µg/mL, with viability rates of 36.90%, 48.91%, and 59.21%, respectively (Figure 1).

The selectivity index (SI) is used to evaluate the selective action of anticancer compounds, which serves as a key metric in cytotoxicity and anticancer research, indicating the extent to which a compound preferentially targets cancer cells over normal, healthy cells. SI values were determined by dividing the $IC_{50}$ obtained in normal cells by the $IC_{50}$ measured in cancer cells (Nunes et al., 2016). Selectivity index values greater than 2 are generally interpreted as indicating strong preferential activity against cancer cells (Muñoz-Núñez et al., 2025). In SKOV-3 cells, the SI was calculated as the ratio of the $IC_{50}$ value in normal cells (386.68) to that in cancer cells (236.74), yielding an approximate value of 1.63. This finding suggests a moderate level of selectivity, demonstrating that the compound exerts greater inhibitory effects on cancer cells than on normal cells. However, the SI remaining below the optimal threshold implies that further refinement or optimization may be required to enhance therapeutic selectivity while reducing potential cytotoxic effects on healthy cells.

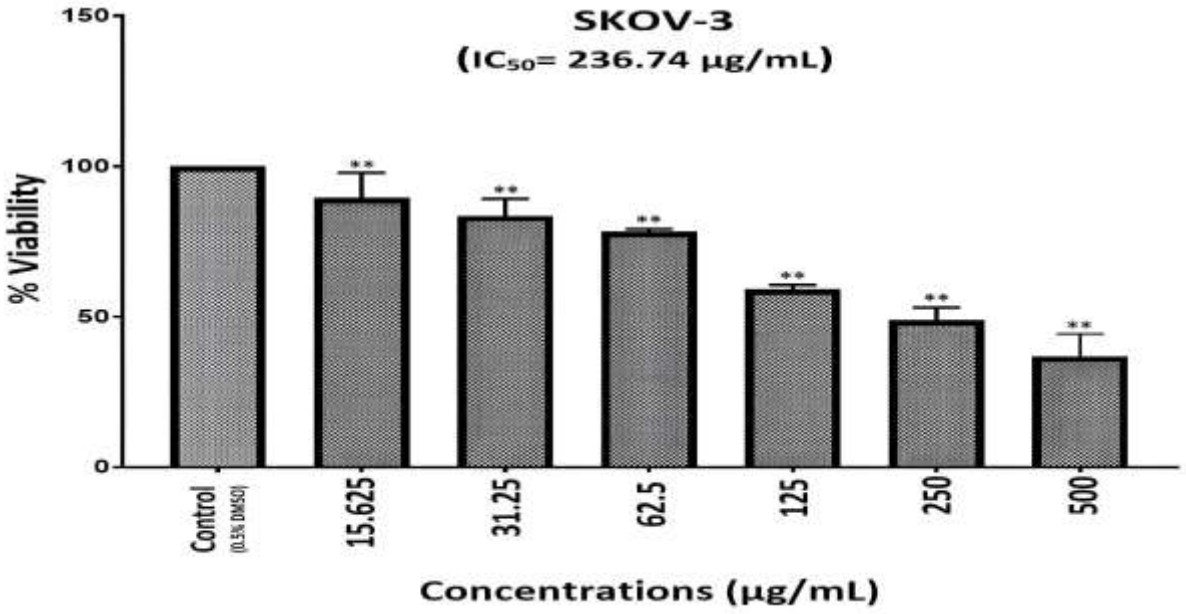


Figure 1. The cytotoxic impact of different concentrations of VTE on human ovarian sarcoma (SKOV-3) cells after a 48-hour incubation period. A notable reduction (** $P \leq 0.0015$) in cell viability was observed compared to the control group, and the data are shown as the average value along with ± standard deviation. The study was replicated at least four times (n=4).

*Şekil 1. 48 saatlik inkübasyon süresinden sonra farklı VTE konsantrasyonlarının insan yumurtalık sarkomu (SKOV-3) hücreleri üzerindeki sitotoksik etkisi. Kontrol grubuna kıyasla hücre canlılığında belirgin bir azalma (** $P \leq 0,0015$) gözlendi ve veriler ortalama değer ve ± standart sapma olarak gösterildi. Çalışma en az dört kez tekrarlandı (n=4).*

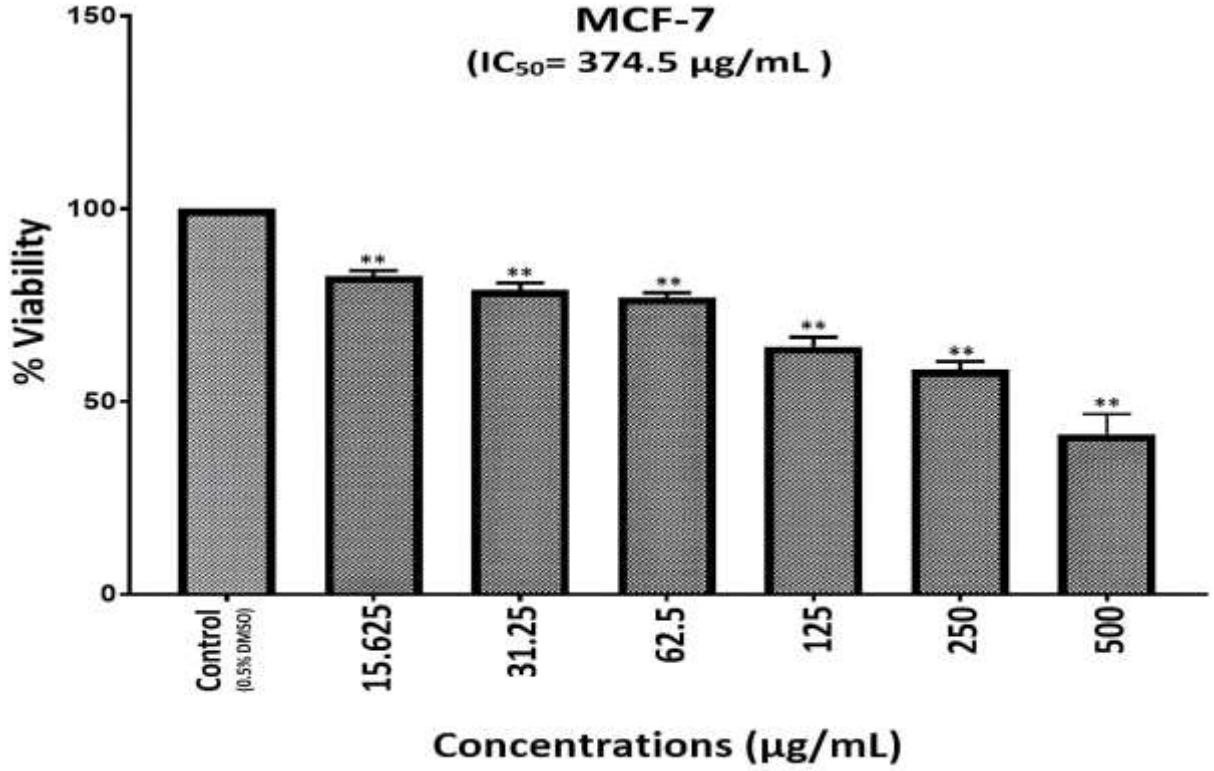


Figure 2. The cytotoxic impact of different concentrations of VTE on human breast cancer (MCF-7) cells after a 48-hour incubation period. A notable reduction (** $P \leq 0.0015$) in cell viability was observed compared to the control group, and the data are shown as the average value along with ± standard deviation. The study was replicated at least four times (n=4).

*Şekil 2. 48 saatlik inkübasyon süresinden sonra farklı VTE konsantrasyonlarının insan meme kanseri* (MCF-7) *hücreleri üzerindeki sitotoksik etkisi. Kontrol grubuna kıyasla hücre canlılığında belirgin bir azalma (** $P \leq 0,0015$) gözlendi ve veriler ortalama değer ve ± standart sapma olarak gösterildi. Çalışma en az dört kez tekrarlandı (n=4).*

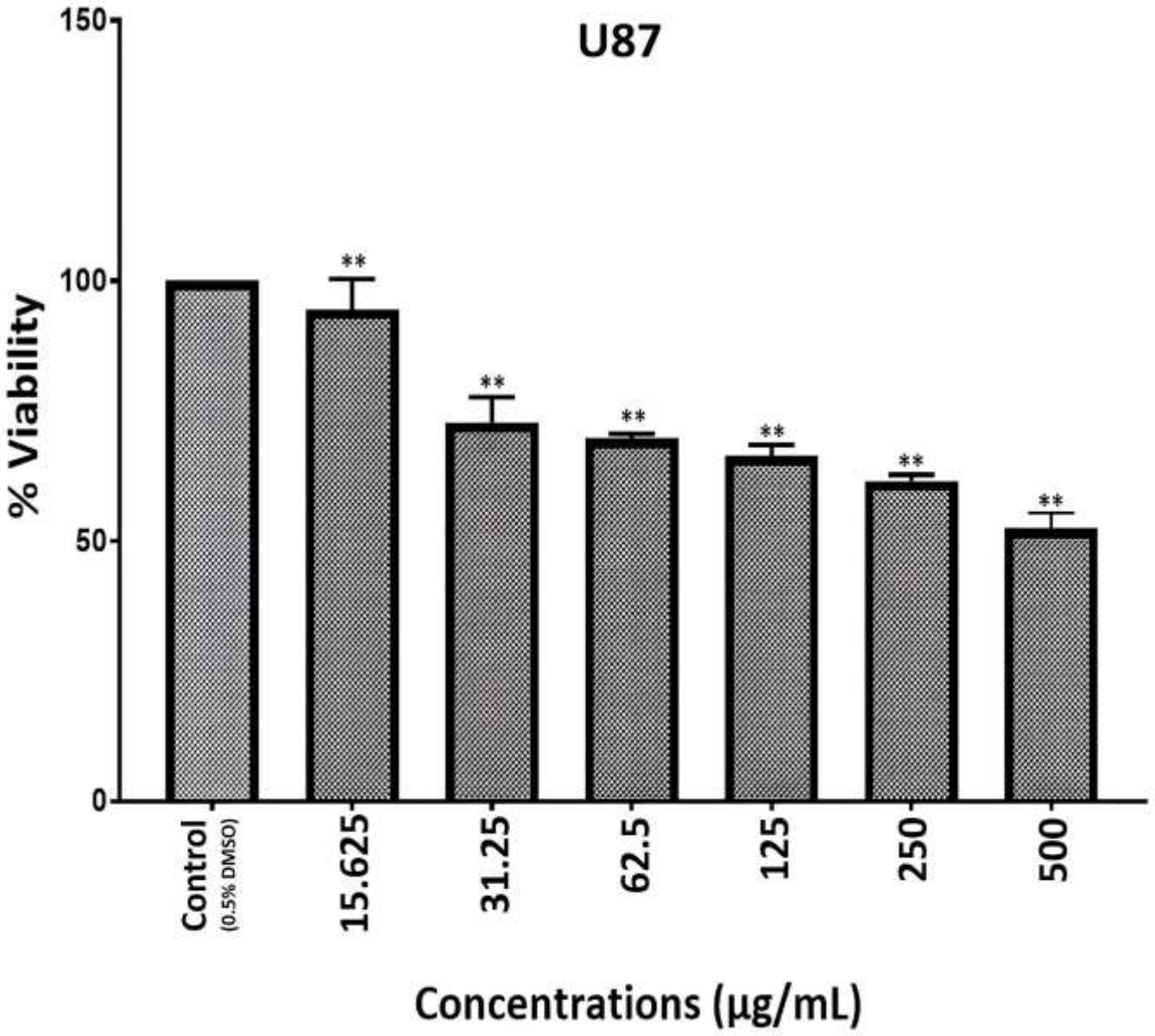


Figure 3. The cytotoxic impact of different concentrations of VTE on glioblastoma (U-87) cells after a 48-hour incubation period. A notable reduction (** $P \leq 0.0015$) in cell viability was observed compared to the control group and the data are shown as the average value along with ± standard deviation. The study was replicated at least four times (n=4).

*Şekil 3. 48 saatlik inkübasyon süresinden sonra farklı VTE konsantrasyonlarının* glioblastoma *(U-87) hücreleri üzerindeki sitotoksik etkisi. Kontrol grubuna kıyasla hücre canlılığında belirgin bir azalma (** $P \leq 0,0015$) gözlendi ve veriler ortalama değer ve ± standart sapma olarak gösterildi. Çalışma en az dört kez tekrarlandı (n=4).*

Dating back to prehistoric times, various species within the Scrophulariaceae family have long been utilized in traditional medicine for the treatment of various ailments, including fistula, constipation, goiter, erythema, fever, dry mouth, inflammatory diseases, psoriasis, eczema, wounds, and many infectious diseases (Stevenson et al., 2002; Orangi et al., 2016; Pasdaran & Hamedi, 2017). In addition to these extensive medicinal properties, numerous previous studies have well documented the anticancer potential of many plants in this family against various cancer cell lines via *in vitro* MTT assays, as detailed in Table 2.

### Wound Healing Assay Results

To assess the anti-migration potential of VTE, the human ovarian sarcoma (SKOV-3) cell line was selected due to its lowest $IC_{50}$ value of 236.74 µg/mL, which is siso significantly lower than the $IC_{50}$ value of 386.68 µg/mL observed in healthy HDF cells. Furthermore, a VTE concentration of 125 µg/mL was chosen for the wound healing assay because, at this concentration, HDF cells exhibited a high cell viability rate of 91.06%, indicating non-toxicity, whereas SKOV-3 cells showed a cell viability rate of 59.21%, indicating significant cytotoxicity (Kandemir et al.,

2024). The anti-migration activity was assessed by measuring the wound closure percentage, and the results showed a significant difference between the control and VTE-treated groups within the first 12 hours, with wound closure percentages of 40.36% and 25.86%, respectively (Figure 5A and 5B). However, this difference diminished ovef time, and after 36 hours of treatment, the control and VTE-treated groups exhibited nearly identical wound closure percentages of 77.94% and 77.91%, respectively (Figure 5A and 5B).

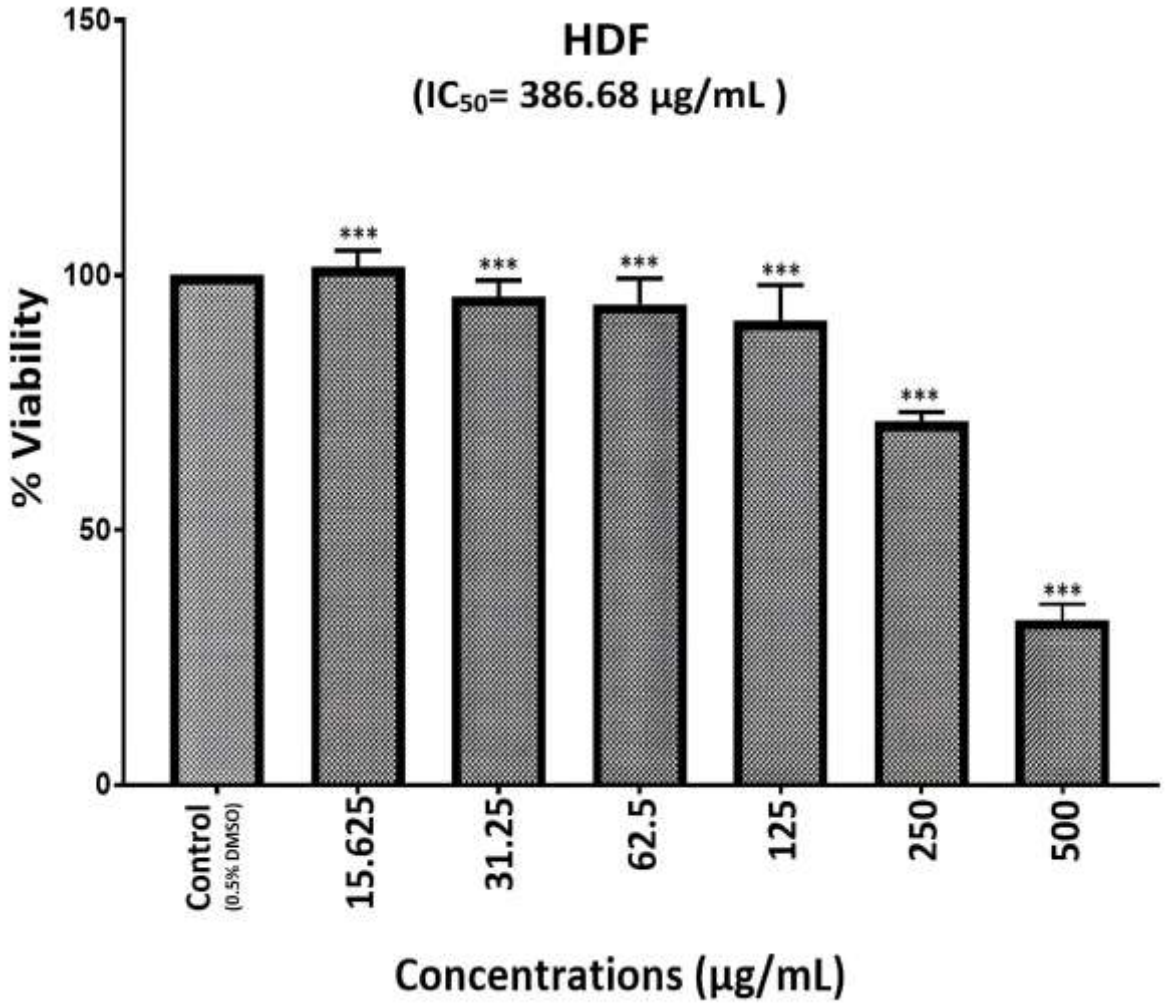


Figure 4. The cytotoxic impact of different concentrations of VTE on human dermal fibroblast (HDF) cells after a 48-hour incubation period. A notable reduction (** $P \leq 0.0015$) in cell viability was observed compared to the control group and the data are shown as the average value along with ± standard deviation. The study was replicated at least four times (n=4).

*Şekil 4. 48 saatlik inkübasyon süresinden sonra farklı VTE konsantrasyonlarının insan dermal fibroblast (HDF) hücreleri üzerindeki sitotoksik etkisi. Kontrol grubuna kıyasla hücre canlılığında belirgin bir azalma (** $P \leq 0,0015$) gözlendi ve veriler ortalama değer ve ± standart sapma olarak gösterildi. Çalışma en az dört kez tekrarlandı (n=4).*

Cancer metastasis is the process by which malignant cells migrate from their original site and subsequently invade and proliferate in distant organs (Mehlen & Puisieux, 2006). It poses significant challenges in the treatment of cancer patients and accounts for over 90% of all cancer-related deaths (Guan, 2015). Cancer metastasis encompasses a sequence of intricate and multifunctional biological events, including cell detachment, migration, invasion, and adhesion (Seyfried & Huysentruyt, 2013). Cell migration is a pivotal aspect of cancer progression, manifesting either as the movement of single cells or as coordinated groups of cells (Alizadeh et al., 2014). Consequently, targeting the disruption of this step could be a critical strategy for preventing cancer development and metastasis at any stage. Numerous prior studies have examined the anti-migratory properties of herbal medicine in the context of metastatic cancer. The delayed wound closure seen in the wound healing assay of this study reflects a suppression of cellular migratory capacity. This observation is consistent with the reported bioactivities of *Verbascum* species, which contain phenolic and flavonoid compounds associated with the regulation of cell movement and growth (Erdogan et al., 2024; Faris, 2024).

Table 2. Comparison of the anticancer potential of some plants belonging to the Scrophulariaceae family using the MTT method.

*Çizelge 2. Scrophulariaceae familyasına ait bazı bitkilerin antikanser potansiyelinin MTT yöntemi kullanılarak karşılaştırılması.*

| Plant species | Cancer cell lines | Cytotoxic effects | References |
|---|---|---|---|
| *Scrophularia oxysepala* | MCF-7; human breast carcinoma cells | subtractions Fa and Fb had $IC_{50}$ values of 52.9 µg/mL and 61.2 µg/mL, respectively, at 24 h. | Orangi et al., 2016 |
| *S. oxysepala* | Caco-2; human colon cancer cell line | after a 24 h incubation period, the $IC_{50}$ was 300 µg/mL. | Namvaran et al., 2017 |
| *S. floribunda* | HL-60 promyelocytic leukemia cells | at 72 h, the $IC_{50}$ value is 0.5 mg/mL. | Giessrigl et al., 2012 |
| *S. lucida* | HL-60 promyelocytic leukemia cells | at 72 h, the $IC_{50}$ value of 0.4 mg/mL | Giessrigl et al., 2012 |
| *S. pinardii*, and *S. libanotica* | HL-60 promyelocytic leukemia cells | the $IC_{50}$ value of 0.9 mg/mL at 72 h | Giessrigl et al., 2012 |
| *S. peregrine* | HL-60 promyelocytic leukemia cells | the $IC_{50}$ value of 3.7 mg/mL at 72 h | Giessrigl et al., 2012 |
| *S. amplexicaulis* | MCF-7 | the $IC_{50}$ values of dichloromethane (DCM) extract were observed as 117.5 ± 1.5, 71.8 ± 0.7, and 36.2 ± 0.6 µg/mL at 12 h, 24 h and 48 h, respectively. | Valiyari et al., 2020 |
| *S. atropatana* | MCF-7 | the $IC_{50}$ values of dichloromethane (DCM) extract were observed as 223.0 µg/mL at 24 h, 153.9 µg/mL at 36 h, and 114.7 µg/mL at 48 h. | Safarzadeh et al., 2017 |
| *S. striata* | astrocyte cancer cell (1321) | Lethal concentration 50 values ($LC_{50}$) of leaf extract were calculated as 6 and 9 µg/mL at 24 h and 48 h, respectively. | Ardeshiry Lajimi et al., 2010 |
| *S. deserti* | liver carcinoma (HepG2) | the alcoholic extract showed 15.43% cell viability | Musa et al., 2024 |
| *S. umbrosa* | MCF-7 | the $IC_{50}$ of methanolic extracts were calculated to be 530 µg/mL at 24 h | Mansouri et al., 2019 |
| *V. gypsicola* | human neuroblastoma cells (SH-SY5Y) | An $IC_{50}$ value of 50 µg/mL was observed after 48 hours of treatment. | Şirin, 2024 |
| *V. tenue* | human ovarian sarcoma (SKOV-3), human breast cancer (MCF-7) and glioblastoma (U87) | The $IC_{50}$ values were determined to be 236.74 µg/mL for SKOV-3, 374.5 µg/mL for MCF-7. effective cytotoxicity on U87 cells was determined by 52.37% viability at 500 µg/mL | This study |

Although specific evidence for Verbascum-mediated migration inhibition is scarce, the current results suggest that the extract may interfere with migration-related mechanisms, highlighting its potential relevance in limiting metastatic behavior. Supporting this, wound-healing assays with other plant extracts have demonstrated significant, dose-dependent inhibition of SKOV-3 cell migration. For instance, *Kaempferia parviflora* extract reduced wound closure to ~58–41% at 0.01–0.1 mg/mL after 24 h (Paramee et al., 2018), while *Emblica officinalis* extract markedly suppressed migration at ≥300 µg/mL within 24–48 h (De et al., 2016), compared with near-complete closure in controls. Similarly, in other cancer models, the methanolic leaf extract of *Clerodendrum inerme* significantly impaired the migratory capacity of A549 lung cancer cells, further supporting the anti-migration potential of plant-derived extracts (Tayeh et al., 2020). Likewise, ethanolic leaf extract of *Leucaena leucocephala* and the flower extract of *Dolichandrone serrulata* exhibited potential anti-migratory activities against human cervical cancer cells (HeLa) (Chatchanayuenyong & Sujayanont, 2020). On the other hand, in murine melanoma (B16F10 cells), the fruit extract of *Gardeniae fructus* has been shown to effectively suppress metastasis by impeding migration, invasion, and colonization (Im et al., 2016).

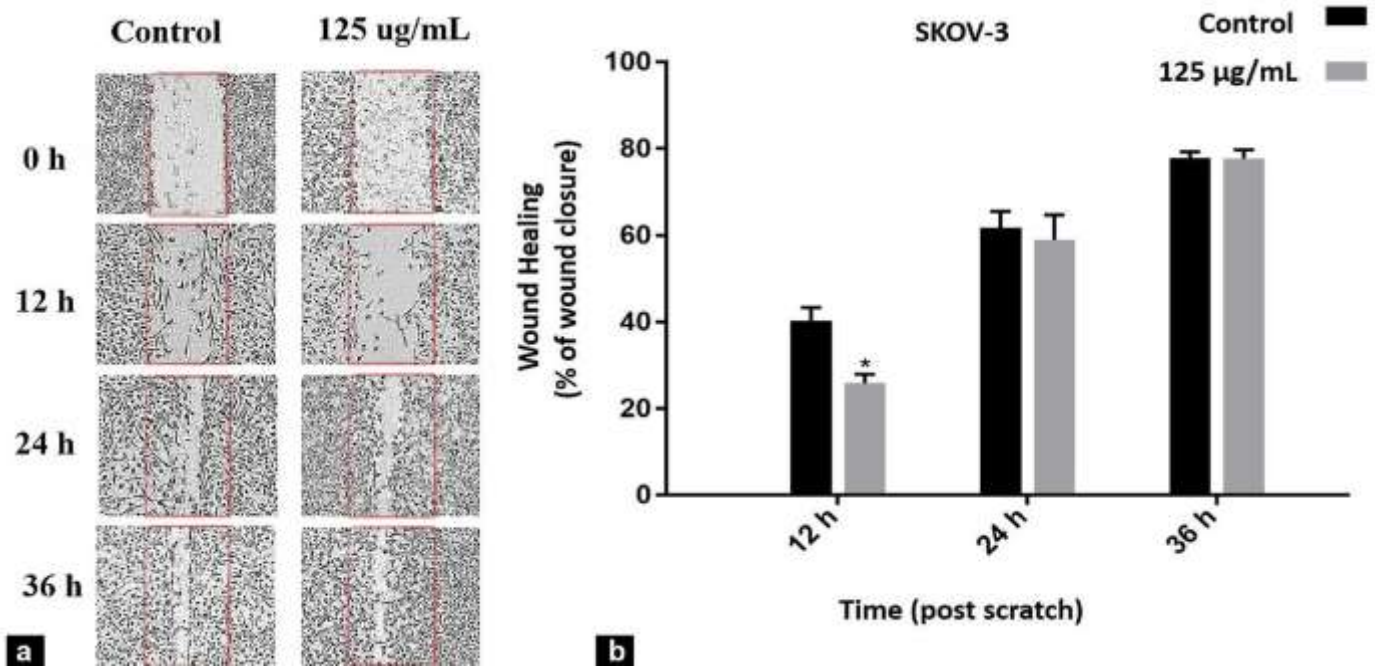


Figure 5. Wound healing assay for the assessment of the anti-migratory effects of VTE on the SKOV-3 cell line. (A) Illustrative images of wound closure at 0 h, 12 h, 24 h, and 36 h for both the control and the specified concentration of plant extract. (B) Quantification of wound closure at the designated time points, with *p < 0.05 compared to the control. The statistics are shown as the mean ± standard deviation (n = 3).

*Şekil 5. VTE'nin SKOV-3 hücre hattı üzerindeki anti-göçmen etkilerinin değerlendirilmesi için yara iyileştirme testi. (A) Hem kontrol hem de belirtilen bitki özütü konsantrasyonu için 0. saatte, 12. saatte, 24. saatte ve 36. Saatte yara kapanmasının açıklayıcı görüntüleri. (B) Kontrol ile karşılaştırıldığında *p < 0,05 ile belirlenen zaman noktalarında yara kapanmasının nicelleştirilmesi. İstatistikler, ortalama değer ve ± standart sapma (n = 3) olarak gösterildi.*

## CONCLUSIONS

Interest in bioactive compounds derived from medicinal plants has increased in recent years, particularly for their potential applications in cancer research due to their natural origin and relative accessibility. In this study, an initial *in vitro* investigation was conducted to examine the cytotoxic and anti-migratory effects of *Verbascum tenue* MURB at varying concentrations. leaf methanolic extract (VTE) on SKOV-3, MCF-7, and U87 cancer cell lines, alongside the normal HDF cell line. These findings suggest that VTE induces concentration- and time-dependent inhibitory effects on cell proliferation and migration in selected cancer cell lines, with a moderate level of selectivity toward human ovarian epithelial carcinoma (SKOV-3) cells, as indicated by the calculated Selectivity Index (SI). Although the observed selectivity does not meet the threshold commonly regarded as optimal, the findings indicate a preferential *in vitro* effect on cancer cells compared to normal cells. These results should be interpreted as preliminary and are not indicative of therapeutic efficacy. Accordingly, further optimization may be necessary to improve selectivity while minimizing potential cytotoxic effects on healthy cells. Further studies incorporating detailed mechanistic investigations, optimization of selectivity, and validation in appropriate *in vivo* models are necessary to elucidate the underlying biological mechanisms and to assess the potential relevance of VTE to future anticancer research.

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The authors have nothing to report.

### Contribution Rate Statement Summary of Researchers

The authors declare that they have contributed equally to the article.

### Conflict of Interest

The authors declare they have no conflict of interest.

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