

Evaluation of Antioxidant, Antimicrobial and Cytotoxic Activities of Endemic *Verbascum diversifolium*, which has Medical and Economic Value

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ABSTRACT

Background: *Verbascum diversifolium*, an endemic medicinal plant native to Türkiye, is recognized for its potential therapeutic and economic value. This study aimed to evaluate the antimicrobial, antioxidant, total phenolic, and cytotoxic activities of its leaf and flower extracts.

Materials and Methods: Antimicrobial activity was determined using the broth microdilution assay against three Gram-positive bacteria, three Gram-negative bacteria, and two fungal strains. Antioxidant activity was assessed by DPPH radical scavenging, ferric reducing power, and metal-chelating assays. Total phenolic content was measured using the Folin-Ciocalteu colorimetric method and expressed as Gallic Acid Equivalents (GAE). Cytotoxic activity was evaluated in MCF-7, A549, C6, and HUVEC cell lines using the MTT assay. **Results:** The leaf extract exhibited the strongest antimicrobial activity against *Escherichia coli* (MIC=0.079 mg/mL) and moderate activity against *Candida tropicalis* (MIC=0.207 mg/mL), whereas the flower extract showed moderate inhibition of *E. coli* (MIC=0.319 mg/mL). The flower extract demonstrated the highest DPPH radical scavenging activity (93.90%), while the leaf extract showed superior ferric reducing power and metal-chelating capacity. Total phenolic contents were 11.673 mg GAE/g and 10.782 mg GAE/g extract for the leaf and flower extracts, respectively. Both extracts exhibited limited cytotoxicity toward the tested cancer cell lines; however, low toxicity toward HUVEC cells and relatively greater responsiveness in MCF-7 cells indicated modest selectivity.

Conclusion: The findings suggest that *Verbascum diversifolium* is a promising natural source of antioxidant and antimicrobial compounds. Further phytochemical isolation, mechanistic studies, and *in vivo* investigations are required to identify the bioactive constituents responsible for its biological activities and to validate its therapeutic potential.

Keywords: Antimicrobial, Antioxidant, Total Phenolic Content, *Verbascum diversifolium*.

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INTRODUCTION

Due to the increasing global consumption of antibiotics, human pathogenic bacteria were detected to have developed resistance against drugs (Ruddaraju *et al.*, 2020). The emergence of drug-resistant bacterial and fungal pathogens has made the treatment of many infectious diseases increasingly challenging, particularly in immunocompromised patients, including those with AIDS and cancer. This circumstance has encouraged scientists to research for novel antimicrobial compounds from different sources (Newman and Cragg, 2016). The continuous emergence of antimicrobial-resistant bacteria and fungi has become a major concern for global healthcare, as it reduces the

therapeutic effectiveness of currently available antibacterial and antifungal agents. The rapid emergence of multidrug-resistant pathogens has intensified the search for alternative antimicrobial sources, particularly those derived from natural products and medicinal plants (Anand *et al.*, 2019). Because plants are rich sources from which new antimicrobial chemotherapeutic agents can be obtained (Brown and Wright, 2016). In this context, medicinal plants have regained scientific interest as sustainable and biologically safe alternatives for the development of new antimicrobial and antioxidant agents. Medicinal plants continue to constitute an essential component of primary healthcare systems and remain the principal source of traditional medicine for nearly 80% of the global population (Çakır and Akan, 2023). Among the plant families with recognized medicinal importance, Scrophulariaceae comprises approximately 200 genera and around 2,500 species of flowering plants. Members of this family are widely distributed across temperate and subtropical regions, where they naturally inhabit grasslands, roadsides, and other open environments, although several species are also cultivated for



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ornamental purposes. Notable genera include *Mimulus*, *Veronica*, *Verbascum*, *Penstemon*, and *Digitalis* (Ghahremani *et al.*, 2020). Among these, *Verbascum* L., commonly known as “mullein,” is one of the most prominent genera, comprising approximately 360 species worldwide. In Turkey, *Verbascum* locally called “sığır kuyruğu” is represented by 228 species, 185 of which are endemic, highlighting the country as an important center of diversity for the genus (Sengül *et al.*, 2005). Numerous *Verbascum* species have long been recognized in traditional medicine for the management of both internal and external ailments (Grigorov *et al.*, 2025). In Turkey, medicinal species including *Verbascum thapsus* L., *Verbascum phlomoides* L., and *Verbascum densiflorum* Bertol. are well known for their ethnopharmacological importance. Their floral parts have traditionally been used as expectorant, diuretic, and sedative remedies in folk medicine (Süntar *et al.*, 2010).

Verbascum diversifolium Boiss. is one of the endemic *Verbascum* species native to Turkey, yet information regarding its biological properties remains limited. While antimicrobial and antioxidant activities have been extensively investigated in several members of the genus, comparable data for *V. diversifolium* are still scarce. In particular, its antimicrobial efficacy, antioxidant potential, total phenolic content, and cytotoxic effects have not been systematically characterized. Although this species has been mentioned in ethnopharmacological records, experimental evidence supporting its medicinal value is still insufficient. Accordingly, this study aimed to evaluate the antimicrobial, antioxidant, and cytotoxic activities of *V. diversifolium* leaf and flower extracts and to examine their relationship with total phenolic content.

MATERIALS AND METHODS

Plant material collection

The *V. diversifolium* Boiss. were collected from the roadsides in Gözeli valley between June-July 2017 during the blooming period of the plants. The collected plant materials were identified for their species by Assoc. Prof. Dr. İsmail Türkoğlu, who is an academic member of the Science Teaching Department of Education Faculty in Firat University. Following collection, the Flowers (VDF) and Leaves (VDL) of *V. diversifolium* were separated and allowed to air dry under ambient laboratory conditions.

Plant material extraction

The dried plant samples' parts above the ground were extracted with the soxhlet device by using ethanol as the solvent (Redfern *et al.*, 2014). The extraction process was conducted until the last extract was colorless. The ethanol contents of the extracts obtained from the soxhlet device were removed in an evaporator, and the dry extracts obtained were placed in dark colored bottles with covers and stored at +4°C. When it was time to use the extracts, they were solved in the solvent/solvent/ in a way that the last concentration would be mg/mL.

Determining the antimicrobial effects

To evaluate their antimicrobial properties, extracts prepared from the Flowers (VDF) and Leaves (VDL) of *V. diversifolium* were subjected to a broth microdilution assay.

Broth microdilution method

The antimicrobial activity of *Verbascum diversifolium* Flower (VDF) and Leaf (VDL) extracts was evaluated using the broth microdilution assay to determine their Minimum Inhibitory Concentrations (MICs) against selected bacterial and fungal strains, following the protocol described by Wiegand *et al.* (2008). The antimicrobial panel included three Gram positive bacteria (*Staphylococcus aureus* ATCC 29213, *Enterococcus faecalis* ATCC 29212, and *Bacillus cereus* ATCC 11778), three Gram negative bacteria (*Escherichia coli* ATCC 25922, *Pseudomonas aeruginosa* ATCC 27853, and *Klebsiella pneumoniae* ATCC 13883), and two yeast strains (*Candida albicans* ATCC 10231 and *Candida tropicalis* DSM 11953).

Crude extracts were dissolved in 40% Dimethyl Sulfoxide (DMSO) to prepare stock solutions. Mueller-Hinton broth (Accumix®, AM1072) was used for antibacterial assays, whereas Sabouraud dextrose broth (HiMedia, ME033) was used for antifungal susceptibility testing. Serial twofold dilutions were prepared in sterile 96-well microplates to obtain final concentration ranges of 6.625-0.008 mg/mL for VDF and 5.115-0.009 mg/mL for VDL, corresponding to stock concentrations of 132.5 and 102.3 mg/mL, respectively. Fresh microbial inocula were adjusted to the 0.5 McFarland turbidity standard before use. The final inoculum densities were approximately 5×10^5 CFU/mL for bacterial strains and $0.5\text{--}2.5 \times 10^3$ CFU/mL for *Candida* spp. After inoculation, bacterial plates were incubated at 37°C for 16-24 hr, whereas fungal cultures were incubated at 35°C for the same period.

Following incubation, microbial growth was visualized by adding 50 µL of 2 mg/mL 2,3,5-triphenyltetrazolium chloride (TTC; Merck, Germany) to each well, followed by an additional 2 hr incubation. The MIC was defined as the lowest extract concentration that produced no visible color change, indicating complete inhibition of microbial growth. All experiments were performed independently in triplicate. For comparative evaluation and graphical presentation, MIC values were transformed to $\log_{10}$ values prior to statistical analysis.

Determining the antioxidant activities

Reducing power capacity

The ferric reducing antioxidant activity of the extracts was assessed following the procedure described by Oyaizu *et al.* (1986). Extract solutions prepared at different concentrations (1.0 mL) were mixed with 2.5 mL of 0.2 M phosphate buffer (pH 6.6) and an equal volume of 1% potassium ferricyanide. After incubation at 50°C for 20 min, the reaction was terminated by adding 2.5 mL of 10% Trichloroacetic Acid (TCA). The mixtures

were then centrifuged at 5000 rpm for 15 min, and 2.5 mL of the resulting supernatant was combined with 2.5 mL of distilled water and 0.5 mL of 0.1% ferric chloride solution. The absorbance of the final reaction mixture was measured at 700 nm. A higher absorbance value indicated a greater ferric reducing capacity of the extract, reflecting the reduction of Fe^{3+} to Fe^{2+} in the reaction system, with FeCl_3 serving as the ferric ion source.

DPPH radical scavenging activity

The free radical scavenging activity of the extracts was determined using the DPPH assay described by Blois (1958), with slight modifications. A freshly prepared 1 mM DPPH solution in ethanol was used throughout the experiment. Aliquots of the aqueous and ethanolic plant extracts (50, 100, and 250 $\mu\text{L mg}^{-1} \text{mL}^{-1}$) were mixed with 3 mL of ethanol, followed by the addition of 1 mL of the DPPH solution. The reaction mixtures were incubated in the dark at room temperature for 30 min before measuring the absorbance at 517 nm. A mixture containing 3 mL of ethanol and 1 mL of DPPH solution without extract served as the control.

The antioxidant activity of the extracts was assessed by monitoring the decrease in absorbance, which reflects the reduction of the stable purple DPPH radical to its yellow-colored non-radical form. Radical Scavenging Activity (RSA) was calculated according to the following equation:

$$\text{RSA (\%)} = [(\text{A}_{\text{control}} - \text{A}_{\text{sample}}) / \text{A}_{\text{control}}] \times 100$$

Metal chelating activity

The ferrous ion-chelating capacity of the extracts was evaluated according to the procedure described by Dinis *et al.* (1994). Briefly, 0.2 mL of extract solution at different concentrations (50-250 μL) was mixed with 0.05 mL of 2 mM $\text{FeCl}_2 \cdot 4\text{H}_2\text{O}$ and 0.35 mL of distilled water. Ethanol was then added to adjust the total reaction volume to 4.0 mL. Chelation was initiated by adding 0.2 mL of 5 mM ferrozine solution, followed by thorough vortex mixing. The reaction mixtures were allowed to stand at room temperature for 10 min before the absorbance was measured at 562 nm. A reaction mixture lacking ferrozine served as the blank, whereas the control contained all reagents except the plant extract.

Ferrozine readily forms a stable magenta-colored complex with Fe^{2+} ions. In the presence of compounds possessing metal chelating activity, formation of the ferrozine Fe^{2+} complex is inhibited, resulting in a reduction in absorbance at 562 nm. The percentage of metal chelation was calculated using the following equation:

$$\text{Metal chelating activity (\%)} = [(A_0 - A_1) / A_0] \times 100$$

where A_0 represents the absorbance of the control and A_1 represents the absorbance of the reaction mixture containing the extract.

Total phenolic content

The Total Phenolic Content (TPC) of the extracts was quantified using the Folin-Ciocalteu colorimetric assay according to the method described by Singleton *et al.* (1965). Briefly, 1 mL of the extract solution was mixed with 1 mL of Folin Ciocalteu reagent. Following a 5 min incubation, 3 mL of 2% sodium carbonate (Na_2CO_3) solution was added, and the mixture was incubated at room temperature for 90 min to allow color development. The absorbance was then measured at 760 nm using a UV-vis spectrophotometer. Gallic acid was employed as the reference standard to construct the calibration curve, and the results were expressed as milligrams of gallic acid equivalents per gram of extract (mg GAE/g extract).

Cell culture and cell viability assay

The cytotoxic potential of the *V. diversifolium* extracts was evaluated using the MTT colorimetric assay. Human breast adenocarcinoma (MCF-7), human lung carcinoma (A549), rat glioma (C6), and Human Umbilical Vein Endothelial (HUVEC) cell lines were selected for the study. Cells were cultured in Dulbecco's Modified Eagle Medium (DMEM; Invitrogen) supplemented with 10% Fetal Bovine Serum (FBS), 2 mM L-glutamine, 100 U/mL penicillin, and 100 $\mu\text{g/mL}$ streptomycin under humidified conditions at 37°C in an atmosphere containing 5% CO_2 . For the cytotoxicity assay, cells were seeded into 96-well plates at a density of 1×10^4 cells per well and allowed to attach before treatment. Plant extracts were administered in culture medium containing 1% DMSO at final concentrations of 1, 10, 100, and 1000 $\mu\text{g/mL}$. Following a 24 hr incubation period, cell viability was assessed by adding 10 μL of 12 mM MTT solution (Vybrant®, Invitrogen) to each well and allowing the reaction to proceed for an additional 4 hr incubation under the same experimental conditions.

The resulting purple formazan formation was quantified by measuring the absorbance at 570 nm using a microplate reader (Epoch, USA). Cell viability was determined from the absorbance values, and half-maximal inhibitory concentration (IC_{50}) values were calculated using GraphPad software. All treatments were performed in triplicate.

Statistical analyses

All experimental procedures were carried out independently in triplicate, and the data are expressed as mean $\pm$ Standard Deviation (SD). Half-maximal Inhibitory Concentration (IC_{50}) values were calculated using GraphPad Prism version 8 (GraphPad Software, San Diego, CA, USA), which was also employed for graphical representation of the data. Statistical analyses were performed with IBM SPSS Statistics version 22.0 (IBM Corp., Armonk, NY, USA). Statistical significance was established at $p < 0.05$.

Ethical Statement

The present study exclusively involved established commercial cell lines (MCF-7, A549, C6, and HUVEC) together with standard reference microbial strains. Since no human participants, patient-derived specimens, experimental animals, or animal-derived tissues were included, approval from an institutional ethics committee was not required.

RESULTS

Antimicrobial activity

The antimicrobial activities of *V. diversifolium* flower (VDF) and leaf (VDL) extracts against the tested microorganisms are summarized in Table 1. Based on the classification proposed by Kuete *et al.* (2010) and Awouafack *et al.* (2013), plant extracts with MIC values ≤ 0.1 mg/mL are considered to exhibit strong antimicrobial activity, those with MIC values between 0.1 and 0.625 mg/mL are regarded as moderately active, whereas MIC values exceeding 0.625 mg/mL indicate weak antimicrobial activity.

Among the tested microorganisms, the leaf extract demonstrated the strongest inhibitory effect against *E. coli*, with an MIC value of 0.079 mg/mL, indicating potent antimicrobial activity. In contrast, a moderate inhibitory effect was observed against *C. tropicalis* (MIC = 0.319 mg/mL). The flower extract also exhibited moderate activity against *E. coli*, with an MIC value of 0.207 mg/mL.

Log-transformed ($\log_{10}$) Minimum Inhibitory Concentration (MIC, mg/mL) values of *V. diversifolium* extracts against tested microorganisms. Lower $\log_{10}$ (MIC) values indicate stronger antimicrobial activity. $\log_{10}$ transformed MIC values were used for heatmap visualization (Figure 1).

Antioxidant activity

The DPPH assay demonstrated the radical scavenging potential of the extracts by reflecting their ability to donate electrons or hydrogen atoms. The observed decrease in absorbance indicated the reduction of the stable DPPH radical to diphenylpicrylhydrazine, confirming the antioxidant capacity of the tested extracts. Low absorbance indicates a reducing in the

amount of DPPH radical (Bensmira *et al.*, 2015). According to the % DPPH free radical scavenging capacity results in Table 2, plant extracts demonstrated sound radical scavenging activities, while VD flowers were determined to be higher compared to the other extract (93.90%).

The reducing power assay evaluates the electron-donating ability of antioxidant compounds by measuring their capacity to reduce ferric ions (Fe^{3+}) to ferrous ions (Fe^{2+}). The resulting Fe^{2+} complex produces a blue colored reaction product, the intensity of which is determined spectrophotometrically at 700 nm. Higher absorbance values indicate greater reducing power and, consequently, stronger antioxidant potential (Ebrahimzadeh *et al.*, 2009).

The reducing capacities of *V. diversifolium* extracts at concentrations of 50, 100, and 250 $\mu\text{g/mL}$ are presented in Table 2. The reducing activity of both extracts increased in a concentration dependent manner. Among the tested samples, the leaf extract (VDL) exhibited the highest reducing power at all tested concentrations. A similar trend was observed in the metal chelating assay, where VDL also demonstrated the greatest ferrous ion-chelating activity among the plant extracts. However, the chelating capacity of the extracts remained lower than that of the positive control, EDTA, which showed approximately 95% chelating activity.

Total phenolic content

The total phenolic contents of the *V. diversifolium* Leaf (VDL) and Flower (VDF) extracts were quantified using the Folin Ciocalteu colorimetric assay and expressed as milligrams of gallic acid equivalents per gram of extract (mg GAE/g). The calibration curve constructed with gallic acid showed excellent linearity (Figure 2). Based on this calibration, the total phenolic contents were calculated as 11.673 mg GAE/g for the leaf extract and 10.782 mg GAE/g for the flower extract.

Cytotoxic Activity

The cytotoxic activity of *V. diversifolium* extracts was evaluated against MCF-7 (human breast cancer), A549 (human lung carcinoma), C6 (rat glioma) and HUVEC (human umbilical cord vascular endothelial cells) cell lines using the MTT assay after 24

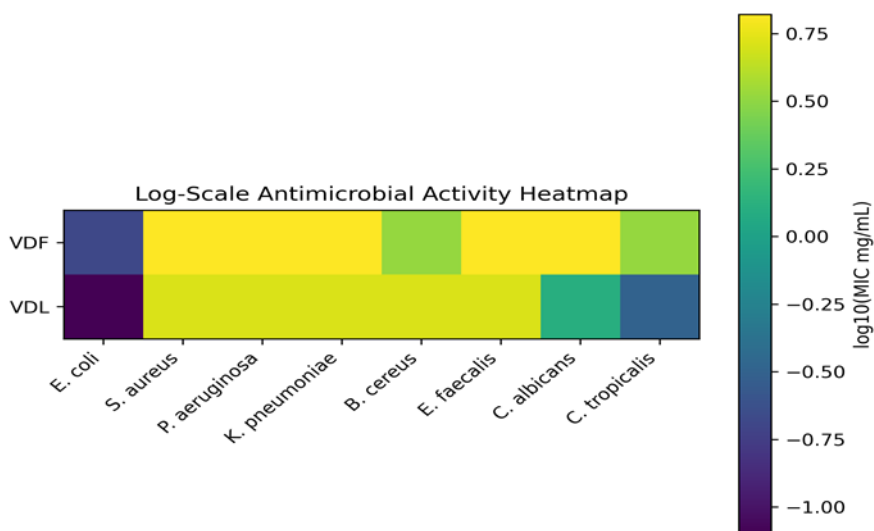
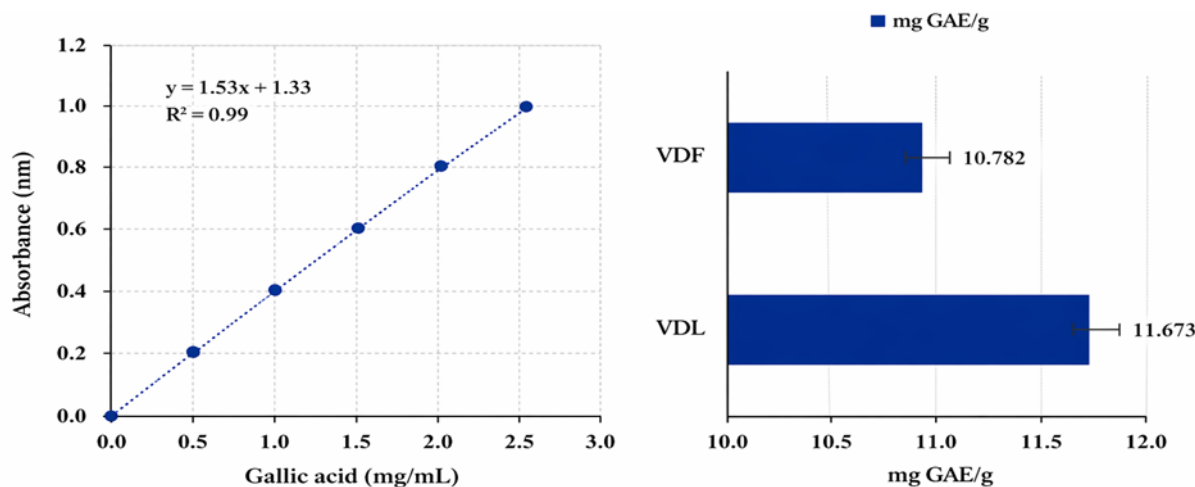
Table 1: Antimicrobial Activities of *V. diversifolium* Extracts.

Extract	<i>Escherichia coli</i>	<i>Staphylococcus aureus</i>	<i>Pseudomonas aeruginosa</i>	<i>Klebsiella pneumonia</i>	<i>Bacillus cereus</i>	<i>Enterococcus faecalis</i>	<i>Candida albicans</i>	<i>Candida tropicalis</i>
VDF	0.207*	>6.625	>6.625	>6.625	3.310	>6.625	6.625	3.310
VDL	0.079**	>5.115	>5.115	>5.115	>5.115	>5.115	1.278	0.319*

* moderate effect, ** strong effect.

Table 2: Antioxidant Activity Results of *V. diversifolium*.

Concentration (µg/mL)	DPPH Scavenging Activity (%)		Reducing Power (nm)		Metal Chelating Activity (%)	
	VDF	VDL	VDF	VDL	VDF	VDL
50	67.97 ± 1.03	80.77 ± 4.07	0.195 ± 0.027	0.264 ± 0.04	45.68 ± 0.99	60.86 ± 2.7
100	82.83 ± 0.94	81.78 ± 1.89	0.353 ± 0.051	0.435 ± 0.06	58.87 ± 1.63	71.2 ± 3.58
250	93.90 ± 3.21	92.97 ± 2.41	0.679 ± 0.085	0.927 ± 0.072	66.21 ± 2.47	76.4 ± 3.97

**Figure 1:** Log10 transformed MIC values were used for heatmap visualization.**Figure 2:** Gallic acid standard calibration curve and total phenolic contents of *V. diversifolium* extracts determined using the Folin Ciocalteu assay.

h of treatment. Both extracts exhibited generally high IC_{50} values (>100 µg/mL), indicating low cytotoxic activity. The leaf extract showed no detectable cytotoxicity against HUVEC, MCF-7, and A549 cells, while weak activity was observed only in C6 cells. According to the National Cancer Institute (NCI) criteria, compounds with IC_{50} values above 500 µg/mL are considered inactive (Mehdinezhad *et al.*, 2016). Based on these thresholds, the extracts demonstrated weak cytotoxicity overall, with MCF-7 cells showing relatively limited selectivity, suggesting a weak to moderate cytotoxic potential in this line.

Selectivity index

The Selectivity Index (SI) was determined by dividing the IC_{50} value obtained for HUVEC cells by the corresponding IC_{50} value of each cancer cell line. As shown in Figure 3, SI values ranged from 0.93 to 1.36 for flower extracts and from 1.05 to 1.77 for leaf extracts. The highest selectivity was observed in MCF-7 cells for the leaf extract.

The observed cytotoxic responses suggest that the biological activity of *V. diversifolium* may be associated with specific bioactive

constituents rather than the crude extracts as a whole. Therefore, further phytochemical fractionation and characterization studies are warranted to identify the compounds responsible for the observed selective cytotoxic effects. As illustrated in Figure 3, both VDF and VDL extracts exhibited distinct cytotoxic profiles across the tested cell lines. The calculated IC_{50} values revealed differences in cellular sensitivity, with higher IC_{50} values indicating lower cytotoxic activity. In addition, the Selectivity Index (SI) analysis demonstrated that the extracts displayed varying degrees of selectivity toward cancer cells relative to HUVEC cells.

DISCUSSION

An integrated assessment of the antimicrobial, antioxidant, and cytotoxic activities of *V. diversifolium*, an endemic medicinal plant of Türkiye, was carried out in the present study. Given the limited data available for this species, the findings contribute meaningful evidence to its pharmacological potential. Overall, the results indicate that *V. diversifolium* extracts exhibit selective antimicrobial activity, considerable antioxidant capacity, and dependent cytotoxic effects against selected cancer cell lines.

The antimicrobial results indicated a selective rather than broad spectrum activity profile. Among the tested microorganisms, *E. coli* exhibited the highest susceptibility, while *C. tropicalis* was more sensitive than *C. albicans* among yeast strains. Both flower and leaf extracts were active against *E. coli*, with the leaf

extract showing comparatively stronger inhibition. Comparable observations have been reported for other *Verbascum* genus. For instance, Boğa *et al.* (2016) reported weak to moderate antimicrobial activity in *V. flavidum*, particularly against *E. coli*, with methanolic extracts showing the lowest MIC values. Similarly, Saltan *et al.* (2011) attributed the antimicrobial effects of *Verbascum*-derived phenolics to mechanisms such as membrane disruption and oxidative stress induction. Additional studies (Şengül *et al.*, 2005) further support the antimicrobial relevance of this genus. Moreover, a recent study on *V. diversifolium* demonstrated measurable antioxidant, cytotoxic, and genotoxic activities in different solvent extracts, highlighting the influence of extraction conditions and phytochemical composition on biological activity (Keser *et al.*, 2025). Collectively, these findings indicate that *Verbascum* species represent a potential source of plant derived antimicrobial agents, although their efficacy appears to be species and compound dependent.

The antioxidant activity of *V. diversifolium* was evaluated using DPPH radical scavenging, iron reducing power, and Fe^{2+} chelation assays, providing complementary insights into different antioxidant mechanisms. The flower extract exhibited the strongest DPPH radical scavenging activity, suggesting a high capacity for electron or hydrogen donation. Similar antioxidant profiles have been reported for other *Verbascum* species. For example, Boğa *et al.* (2016) observed strong DPPH inhibition

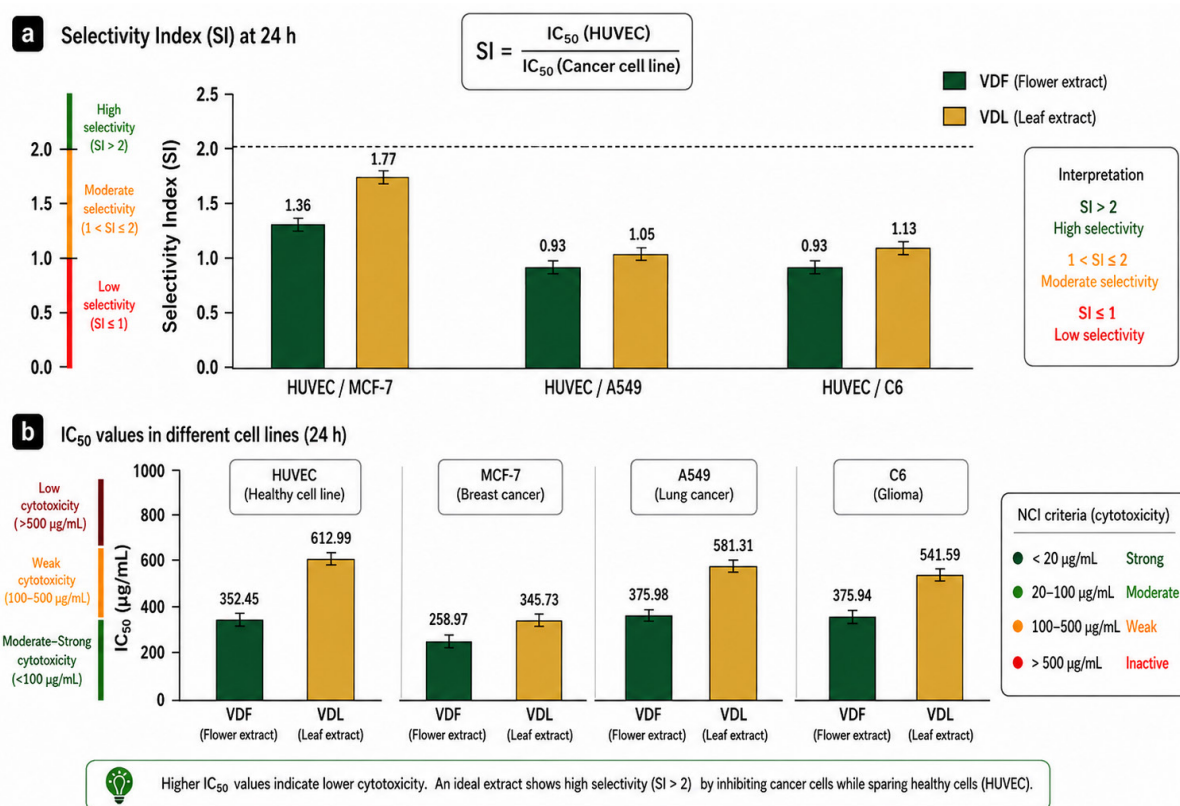


Figure 3: Cytotoxic activity and Selectivity Index (SI) of *V. diversifolium* extracts after 24 hr. (a) SI values. (b) IC_{50} values ($\mu\text{g/mL}$) of VDF and VDL extracts (MTT assay). Data are mean $\pm$ SD ($n=3$).

in *V. flavidum* methanolic extracts, while Shakeri and Farokh (2015) reported enhanced activity in ethyl acetate fractions of *V. sublobatum*. Consistent with these findings, Akşit and Şimşek (2025) demonstrated high antioxidant potential in *V. sinuatum* using multiple assays (DPPH, ABTS, FRAP). The Fe^{2+} chelation results demonstrated a concentration-dependent increase in metal chelating activity; however, none of the extracts reached the activity level of EDTA. One possible mechanism underlying the antioxidant activity of the extracts is their capacity to sequester transition metals, particularly iron, thereby limiting Fenton-type reactions responsible for reactive oxygen species formation. Therefore, the ability of the extracts to bind ferrous ions may represent an important mechanism underlying their antioxidant potential. The antioxidant properties observed for *V. diversifolium* are likely attributable, at least in part, to its phenolic constituents. The total phenolic contents of the leaf and flower extracts were determined as 11.673 and 10.782 mg GAE/g extract, respectively, demonstrating that both plant parts contain appreciable amounts of phenolic compounds. Reports published on different *Verbascum* species have consistently shown that increased phenolic content is accompanied by enhanced antioxidant capacity, a trend also observed in the present study (Paun *et al.*, 2016; Alali *et al.*, 2017; Alkowni *et al.*, 2023). The antioxidant effectiveness of phenolic compounds is largely influenced by their molecular structure, particularly the number and position of hydroxyl substituents as well as the extent of conjugation within the aromatic ring system (Cai *et al.*, 2006).

Cytotoxicity assays demonstrated that both the Flower (VDF) and Leaf (VDL) extracts exerted generally weak cytotoxic effects against MCF-7, A549, and C6 cancer cell lines while exhibiting minimal toxicity toward the non cancerous HUVEC cell line. The present results further reinforce the notion that extracts from *Verbascum* species are associated with relatively weak cytotoxic effects, as previously reported (Boontha *et al.*, 2024). Among the tested samples, the flower extract showed the greatest inhibitory activity against MCF-7 cells, with an IC_{50} value of approximately 259 $\mu\text{g/mL}$, indicating a greater sensitivity of this cell line to the extract. Nevertheless, according to the NCI classification, crude extracts with IC_{50} values above 500 $\mu\text{g/mL}$ are generally regarded as weakly cytotoxic, suggesting that the overall cytotoxic potential of *V. diversifolium* extracts is limited. The Selectivity Index (SI) analysis further indicated a modest preference for cancer cells over normal HUVEC cells, with the highest SI value observed for the leaf extract against MCF-7 cells ($\text{SI}=1.77$). Collectively, these findings suggest that the cytotoxic properties of *V. diversifolium* are relatively mild and are likely influenced by the phytochemical composition of the extracts as well as the extraction procedure. For example, Keser *et al.* (2025) reported significant antioxidant and cytotoxic effects of *V. diversifolium* extracts in MCF-7 cells, while Erdoğan *et al.* (2024) demonstrated inhibitory effects of *V. gimgimense* on A549 cell

proliferation. Similarly, Faris (2024) showed that *V. ponticum* extracts induce apoptosis via mitochondrial pathways, suggesting a mechanism-based antiproliferative effect. Comparable results have been reported for other *Verbascum* species. In addition, Ercan and Çalışkan (2026) reported that *V. andrusii* displays high antioxidant activity (DPPH, ABTS, CUPRAC, DMPD) along with significant cytotoxic effects against A549 cells. Likewise, Akşit and Şimşek (2025) documented strong antioxidant capacity in hydromethanolic flower extracts of *V. sinuatum*.

The differences observed between flower and leaf extracts in the present study suggest that the distribution of bioactive compounds is not uniform across plant tissues. This observation is supported by recent comparative studies showing that antioxidant and cytotoxic properties vary significantly among *Verbascum* species due to differences in metabolite profiles (Grigorov *et al.*, 2025). Overall, *V. diversifolium* exhibits biologically relevant antioxidant activity, likely driven by its phenolic composition. In contrast, its cytotoxic activity remains limited, indicating that crude extracts may not be sufficiently potent for direct anticancer applications. Taken together, these findings highlight that the biological activity of *Verbascum* species is strongly dependent on phytochemical composition, plant part, and extraction conditions.

CONCLUSION

The findings of the present study demonstrate that *V. diversifolium* possesses considerable antioxidant capacity together with selective antimicrobial activity, whereas its cytotoxic effects remain limited when evaluated as crude extracts. These results suggest that the observed biological activities are likely associated with specific phytochemical constituents that may become more effective following purification and fractionation. Accordingly, future investigations should emphasize the isolation and structural characterization of the active compounds, clarification of their molecular mechanisms, and confirmation of their biological activities using appropriate *in vivo* models.

The increasing demand for novel plant-derived bioactive compounds, particularly in the context of antimicrobial resistance, highlights the pharmaceutical and nutraceutical potential of *V. diversifolium*. Collectively, the present findings expand current knowledge of the biological and ethnopharmacological value of this endemic species and provide a scientific basis for future studies aimed at developing natural antioxidant and antimicrobial products.

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ABBREVIATIONS

DMSO: Dimethyl sulfoxide; **DPPH:** 2,2-diphenyl-1-picrylhydrazyl; **GAE:** Gallic Acid Equivalents; **MIC:** Minimum Inhibitory Concentration; **MTT:** 3-(4,5-dimethylthiazolyl-2)-2,5-diphenyltetrazolium bromide; **SI:** Selectivity Index; **VDF:** *Verbascum diversifolium* flowers; **VDL:** *Verbascum diversifolium* leaves; **CFU:** Colony Forming Units; **EDTA:** Ethylenediaminetetraacetic Acid; **GAE:** Gallic Acid Equivalents; **MCF-7:** Human Breast Adenocarcinoma Cell; **A549:** Human Lung Carcinoma; **C6:** Glioma cell Line from Rats; **HUVEC:** Human Umbilical Vein Endothelial Cell; **NCI:** National Cancer Institute.

CONFLICT OF INTEREST

The authors declare that there is no conflict of interest.

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