



## A Preliminary Study on Anticancer and Antimicrobial Potential of Methanolic Extracts of *Verbascum napifolium*

Aylin TAŞKAYA<sup>1</sup>, Burcu ŞAHİN<sup>2</sup>, Nur CEYHAN GÜVENSEN<sup>3\*</sup>, Ramazan MAMMADOV<sup>4</sup>

<sup>1</sup>Muğla Sıtkı Koçman University, Faculty of Sciences, Department of Biology, Muğla

<sup>2</sup>Muğla Sıtkı Koçman University, Faculty of Sciences, Department of Molecular Biology and Genetics, Muğla

<sup>3</sup>Ege University, Tire Kutsan Vocational School, Food Technology Program, İzmir

<sup>4</sup>Muğla Sıtkı Koçman University, Faculty of Sciences, Department of Molecular Biology and Genetics, Muğla

\*Sorumlu Yazar (Corresponding author): nur.ceyhan.guvenesen@ege.edu.tr

### Abstract

Plants are used in the treatment of various diseases thanks to the active substances they contain. This study was conducted for the first time and evaluated the potential anticancer and antimicrobial activities of methanol extracts of the leaf and flower parts of *Verbascum napifolium*, an endemic species in the flora of Turkey. The antimicrobial activities were evaluated by the disc diffusion method and by determining the minimal inhibitory concentration (MIC). In all bacteria, flower methanol extract was found to be more sensitive. *Escherichia coli* was determined to be more sensitive to both extracts compared to all tested bacteria. These results suggest that *V. napifolium* possess potential important anticancer and antimicrobial activities. The anticancer activities the effect of *Verbascum napifolium* flower and leaf methanol extracts on cell viability in CaCo-2 and L929 cell lines were determined by MTT assay. Flower methanol extract of *Verbascum napifolium* showed a better anticancer potential than the leaf methanol extract.

### Research Article

### Article History

Received :11.11.2022  
Accepted :31.12.2022

### Keywords

*Verbascum napifolium*  
anticancer activity  
antimicrobial activity  
CaCo-2  
human cancer colon

## 1. Introduction

Plants have been used by people in all ages for health, beauty, youth and other purposes. With the knowledge accumulated over the years, traditional medicine has historically become widespread all over the world and still remains the main source of health for millions of people, despite the great advances in modern medicine (WHO, 2013). The interest in natural compounds obtained from plants with beneficial medicinal properties such as antitumor, antimicrobial, antioxidant and anti-inflammatory is increasing day by day (Bakkalı et al., 2008). Endemic medicinal plants have important and promising phytochemical compounds for new product development in the pharmaceutical industry; therefore research on them is very valuable. It has been reported that *Verbascum L.* species are used for different medical purposes such as diuretic, expectorant, laxative, mucolytic, sedative, sudorific and wound healing in Turkish folk medicine (Sen-Utsukarcı et al., 2018). *Verbascum* species have been reported as a source of abundant flavonoids and saponins. Biologically active molecules such as phenylethanoids, iridoid glycosides, neolignan glycosides and spermine alkaloids have also been found in several species of mullein (Scarpati and Delle, 1963; Yabalak et al., 2022). Plants of the genus *Verbascum*, known as mullein, are biennial or perennial herbaceous plants and sometimes shrubs. This genus is predominantly distributed in the temperate regions of the Northern Hemisphere, with approximately 360 species (248 species and 129 hybrids grow in Turkey). With 171 endemic species, the endemism rate in Turkey is 69% (Başer, 2008). In another study, this rate is given as 80% with 196 endemic species (Küçük et al., 2016).

Numerous biological properties have been proven in different *Verbascum* species: anti-inflammatory, antioxidant,

antimicrobial, antiviral, antinociceptive, antitussive, etc. All activities are related to plants' chemical constituents, so there are a lot of different compounds identified in *Verbascum* species, grouped into classes: saponins, iridoids, phenylethanoid glycosides, monoterpene glucosides, flavonoids, etc. (Angeloni et al., 2021; Grigorov et al., 2022). In many studies, the antimicrobial effects of methanol, ethanol and water extract obtained from *Verbascum* species were found to be quite good (Turker and Camper, 2002; Dulger et al., 2005; Khan et al., 2011; Ghasemi et al., 2015; Alahmer, 2017; Dulger and Dulger, 2018; Sen-Utsukarcı et al., 2018; Yabalak et al., 2022; Donn et al., 2023). This makes a potentially bioactive substance for many industrial products of different extracts of the plant. In this study, it aimed to determine the anticancer, antibacterial and antifungal activities of the methanol extract of the leaf and flower parts of *V. napifolium*. This is the first study of these extract, which is an endemic plant of the *V. napifolium* anticancer and antimicrobial potential of these extract, to the Muğla region and used by the public in traditional treatment.

## 2. Materials and Methods

### 2.1. Plant material and extraction

The flower and leaf parts of the boxed plant samples were separated into very small pieces with the help of a blender. Afterwards, 10 g of sample was weighed into erlenmeyer flasks and 100 mL of methanol was added. The mouth parts of the erlenmeyer flasks were first wrapped with aluminum foil and then with parafilm and the erlenmeyers were placed in a shaking water bath. It was kept in a shaking water bath at 48-50°C for 6 hours. Afterwards, it was filtered with the help of technical blotter. The filtered samples were placed in a lyophilizer operating at -54°C after the alcohol evaporated in the rotary evaporator. After 8-10 hours, the samples coming out of the lyophilizer were scraped with the help

of a spatula and transferred to capped glass bottles. The samples were stored at  $-20^{\circ}\text{C}$  until they were used during the experiment (Turan and Mammadov, 2018)

## 2.2. Antibacterial and antifungal activity test

Antibacterial and antifungal activity of the methanol extracts of *V. napifolium* was evaluated using the paper disc diffusion technique and by determining the minimal inhibitory concentration (MIC). Lyophilized bacteria and yeast were obtained from the culture collection of the Department of Basic and Industrial Microbiology, Faculty of Science, Ege University. *Staphylococcus aureus* ATCC 6538/P, *Bacillus cereus* CCM 99, *Escherichia coli* ATCC 35218, and *Pseudomonas aeruginosa* ATCC 27853 were used for antibacterial activity. *C. albicans* ATCC 10239 strains were used for antifungal activity.

### 2.2.1. Disc diffusion assay

The antibacterial activity of crude methanol extracts from *V. napifolium* was tested by the paper disc diffusion technique. (Collins and Lyne, 1987; Bradshaw, 1992; Karaalp et al., 2009; Kaya et al., 2010). The extracts were dissolved in DMSO, and then 20  $\mu\text{L}$  of each extract ( $0.8 \mu\text{g mL}^{-1}$  concentrations) of *V. napifolium* were absorbed onto sterile 6-mm diameter filter paper discs (Schleicher and Schüll, Nr 2668, Dassel, Germany). Bacterial strains were pre-cultured on Muller Hinton Broth medium (Merck) and incubated for 24 h at  $37 \pm 0.1^{\circ}\text{C}$ . *Candida* strains were pre-cultured on Sabouraud Dextrose Broth (Merck) and incubated for 48 h at  $25 \pm 0.1^{\circ}\text{C}$ . Autoclaved Mueller-Hinton Agar (Merck) was added to sterile plates under suitable conditions, and it was allowed to solidify under aseptic conditions. The turbidity of bacteria and fungi was prepared according to McFarland 0.5 scale to obtain a standard inoculum. Then 0.1 mL of the test

organisms were inoculated with a sterile drigalski spatula on the surface of the appropriate solid medium in the plates. The sterile disks impregnated with different extracts were then placed on the agar plates and incubated at  $37 \pm 0.1^{\circ}\text{C}$  for 24 h. The sterile disks impregnated with other extracts were then placed on the agar plates and incubated at  $25 \pm 0.1^{\circ}\text{C}$  for 48 h to measure antifungal activity. The inhibition zone (mm) of antibacterial and antifungal activity against test organisms was measured and evaluated. All experiments were done under sterile conditions in duplicated. Erythromycin and Ampicillin (Oxoid) ( $10 \text{ mg disc}^{-1}$ ) were used as positive controls. DMSO, methanol were used as negative control.

### 2.2.2 Determination of MIC values

Minimal inhibitory concentration (MIC) values of bacterial and fungal strains sensitive to *V. napifolium* were determined. In line with these objectives, a micro-dilution experiment was performed for *Verbascum* samples according to the procedures developed by the National Clinical Laboratory Standards Committee (Atlas, 1995; Karaalp et al., 2009; Kaya et al., 2010). Dilution series of the extracts were prepared by thawing from 5000 mL DMSO in test tubes and then transferred to the broth in 96-well microtiter plates. Plant extracts were tested at  $4/5000 \mu\text{g mL}^{-1}$  concentrations. Final concentrations in the medium were  $200 \mu\text{g mL}^{-1}$ . Before the inoculation of the test organisms, the bacteria strains and yeast strain were adjusted to 0.5 McFarland standards and diluted 1:100 ( $v v^{-1}$ ) in Mueller-Hinton broth and Sabouraud dextrose. The 96-well plates were prepared by dispensing extract into each well of broth and the inocula to obtain  $1 \times 10^8 \text{ CFU mL}^{-1}$ . Extract prepared at the concentration of  $128 \mu\text{g mL}^{-1}$  was added into the first wells. Then its serial dilutions (128, 64, 32, 16, 8, 4, 2, and  $1 \mu\text{g mL}^{-1}$ ) were transferred into the consecutive

wells. Plates were incubated at 37°C for 18–24 h and at 25°C for 48 h for the yeast. All the tests were performed in broth and repeated twice. The MIC was defined as the lowest concentration that showed clear against a black background (no visible growth). The MIC was defined as the lowest concentration of an extract or a substance to inhibit the growth of microorganisms after 18–24 h and 48 h for the yeast.

### 2.3. MTT assay

Caco-2 (colorectal cancer cell line, ATCC) and L929 (mouse fibroblast cell line, ATCC) cell lines were cultured in RPMI-1640 (Roswell Park Memorial Institute-1640) medium (Diagnovum, Germany) which was contained 10% heat-inactivated fetal bovine serum (Diagnovum, Germany) and penicillin-streptomycin (100 U mL<sup>-1</sup> - 100 µg mL<sup>-1</sup>) (Capricorn, Germany). The cells were maintained at 37°C in a humidified incubator with 5%

CO<sub>2</sub>. For the MTT test, the CaCo-2 and L929 cell lines were seeded at 2x10<sup>4</sup> cells/well with 200 µL of 96-well microplates. After the cells were incubated for 24 hours, 20 µL of the respective concentrations of each extract was added to the corresponding wells. After the 24-hour incubation period was completed, the medium in the wells was removed and 100 µL of the fresh medium was added to each well. Then, 10 µL of MTT (5 mg mL<sup>-1</sup>) was added to each well and the microplates were incubated in a CO<sub>2</sub> incubator for 4 hours. After the incubation period was completed, 100 µL of DMSO was added to each well. After shaking the microplates at 150 rpm for 6 minutes, absorbance (Abs) was measured with a spectrophotometer at a wavelength of 540 nm. The results were expressed as % cell viability according to the formula below (Uğur et al., 2017). IC<sub>50</sub> values were calculated using SPSS (version 22.0) using the obtained results.

$$\% \text{ Cell viability} = (\text{Average of Abs treated cells}) / (\text{Abs control group}) \times 100$$

### 2.4. Statistical analysis

All assays were performed in 2 replicates. The mean ± standard error and IC<sub>50</sub> values was analyzed using Microsoft Excel. IC<sub>50</sub> values in the HT-29 cell line for each test substance were calculated with SPSS (n.d.) statistical software (version 22.0).

## 3. Results and Discussion

### 3.1. Antibacterial and antifungal activity assays

The antibacterial and antifungal activities of *V. napifolium* methanolic extracts evaluated by disc diffusion and micro-well dilution techniques are reported in Tables 1 and 2, respectively. The disk diffusion test of both extracts of *V. napifolium* ranged from 0.00 ± 0.00 to 0.95 ± 0.00 mm across all bacterial strains. The

highest resistant to the leaf and flower extracts tested was demonstrated by the gram-negative bacteria *Pseudomonas aeruginosa* with an inhibition zone of 0.95 ± 0.00 mm and the gram-negative bacteria *E. coli* with an inhibition zone of, 0.70 ± 0.20 mm, respectively. The most sensitive bacteria to both extracts was gram (+) *Bacillus cereus*. When we look at the inhibition zones that we examined with different extracts for *Candida albicans*, we can say that the inhibition regions of flower extracts are more effective than leaf extracts. For both extracts, *C. albicans* disk diffusion experiment was found to be 0.80 ± 0.00 mm in leaf extracts and 0.95 ± 0.00 mm in flower extracts (Table 1). Turker and Camper (2002) evaluated the antibacterial activity of water, ethanol and methanol extract of *Verbascum thapsus* leaves and

their commercially available products against gram-negative (*Pseudomonas aeruginosa*, *Klebsiella pneumonia*, and *Escherichia coli*) and gram-positive bacteria (*Staphylococcus aureus*, *Staphylococcus epidermidis*, and *Streptococcus pyogenes*). They recommended that aqueous extract of decocted commercial leaf material exhibited inhibitory potential against all tested bacterial strains excluding *P. aeruginosa* and *S. pyogenes*. It maybe due to the difference in extraction methods or the difference in collecting time of plant samples. In previous studies, the methanol extracts of *Verbascum* species such as *V. gypsicola*, *V. chionophyllum*, *V. cilicium*, *V. trapifolium*, *V. meinckeanum* generally have been reported to be more active Gram-positive bacteria and the yeast than Gram-negative bacteria (Dulger et al., 2005).

Ghasemi et al. (2015) reported that among extracts their tested, *V. thapsus* methanolic extract of leaf (100–400 µg) had comparatively higher growth inhibitory potential against *E. coli* and *S. pyogenes* than other extracts (aqueous and ethanol). The ethanol leaf extract displayed growth inhibitory activity against *S. aureus*. No antibacterial or antifungal activities were observed by the essential oil (100–400 µg). Dulger and Dulger (2018) obtained methanol extracts of *Verbascum antinori* plant. The essences were effective against *Staphylococcus aureus* ATCC 6538P, *Bacillus cereus* ATCC 7064, *Listeria monocytogenes* ATCC 15313, *Micrococcus luteus* CCM169, but *Escherichia coli* ATCC 10538, found that the *Klebsiella pneumonia* UC57, *Pseudomonas aeruginosa* ATCC 27853, *Proteus vulgaris* ATCC 8427 bacteria.

**Table 1.** Antibacterial and antifungal activity of the methanolic extracts of *Verbascum napifolium* by the disc diffusion method

| Microorganism<br>(Gram reaction)                    | Inhibition zone (mm)        |                               |           |           |         |           |           |
|---|-----------------------------|-------------------------------|-----------|-----------|---------|-----------|-----------|
|   | Leaf<br>Methanol<br>Extract | Flower<br>Methanol<br>Extract | Standards |           |         |           |           |
|   |                             |                               | Amp.      | Erit.     | Nys.    | DMSO      | Meth.     |
| Antibacterial Activity                              |                             |                               |           |           |         |           |           |
| <i>Staphylococcus aureus</i><br>(ATCC 6538/P) G (+) | 0.90±0.00                   | 0.90±0.23                     | 2.10±0.06 | 2.85±0.00 | -       | 0.70±0.23 | 1.40±0.00 |
| <i>Bacillus cereus</i><br>(CCM 99) G (+)            | 0.80±0.20                   | 0.80±0.23                     | 2.20±0.00 | 3.20±0.00 | -       | 0±0.00    | 1.70±0.00 |
| <i>Escherichia coli</i><br>(ATCC 35218) G (-)       | 0.70±0.00                   | 0.80±0.20                     | 2.60±0.06 | 3.70±0.00 | -       | 0±0.00    | 1.70±0.00 |
| <i>Pseudomonas aeruginosa</i><br>(ATCC 27853) G (-) | 0.80±0.03                   | 0.95±0.00                     | 1.10±0.03 | 3.25±0.12 | -       | 0.7±0.20  | 1.20±0.00 |
| Antifungal Activity                                 |                             |                               |           |           |         |           |           |
| <i>C. albicans</i><br>ATCC 10239                    | 0.80±0.00                   | 0.95±0.00                     | -         | -         | 20±0.00 | 0.7±0.00  | 1.40±0.00 |

Amp.: Ampicillin (10 mg), Erit.: Erythromycin (10 mg), Nys: Nystatin (30 µg disc<sup>-1</sup>), DMSO: Dimethyl sulfoxide, Meth.: Methanol. Values (mean of two replicates) indicate zone of inhibition in mm and include filter paper disc diameter (6 mm); G: gram reaction; "0": no inhibition

In the micro-well dilution assay (Tables 2), the MICs of the extracts are between  $16 \pm 0.00$  and  $32 \pm 0.00 \mu\text{g mL}^{-1}$  and both extracts appear to show significant activity against the bacteria species tested and yeast. MIC value was determined as  $32 \pm 0.00 \mu\text{g mL}^{-1}$  in all bacteria tested with the leaf ethanol extract. Also a MIC value of  $32 \pm 0.00 \mu\text{g mL}^{-1}$  was detected in only *C. albicans* for both extracts. These results make us think that the reason why the extracts we obtained are more effective in fungi than all bacterial species is due to the different cell morphology of the fungi. In the literature, one of the first studies to evaluate this property analyzed the effects of methanolic extract of *V. sinuatum* inflorescences against four Gram (+) bacteria (*Staphylococcus epidermidis*, *S. aureus*, *Enterococcus faecalis*, and *Bacillus subtilis*) and eight Gram (-) bacteria (*Proteus vulgaris*, *Enterobacter aerogenes*, *Enterobacter cloacae*, *Klebsiella pneumoniae*, *Proteus mirabilis*, *Pseudomonas aeruginosa*, *Salmonella typhi*, and *Citrobacter diversus*). The results of this study showed that the methanolic extract is more effective against Gram (+) bacteria with minimum inhibitory concentrations (MICs) ranging from  $16 \mu\text{g mL}^{-1}$  for *S. epidermidis* to  $128 \mu\text{g mL}^{-1}$  for *Bacillus subtilis*. On the other hand, MIC values for Gram (-) bacteria varied between  $64$  to  $256 \mu\text{g mL}^{-1}$  (Senatore et al., 2007). Furthermore, Khan et al. (2011) studied the antimicrobial activity of *V. thapsus* aerial part methanolic extract ( $15 \text{ mg mL}^{-1}$ ) against selected Gram-positive and Gram-negative bacteria and reported the minimum inhibitory concentration (MIC) of *V. cholera* ( $10 \text{ mg mL}^{-1}$ ), *S. aureus* ( $1 \text{ mg mL}^{-1}$ ), *B. subtilis* ( $1 \text{ mg mL}^{-1}$ ), *P. aeruginosa* ( $1 \text{ mg mL}^{-1}$ ), *K. pneumoniae* ( $12.5 \text{ mg mL}^{-1}$ ) and *E.*

*coli* ( $15 \text{ mg mL}^{-1}$ ). Similarly, extract also exhibited growth inhibitory activity of tested fungal strains, such as *R. solani* (64%), *A. flavus* (58%), *A. niger* (25%) and *A. fumigatus* (23%) (Khan et al., 2011). Nofouzi et al. (2016), *Verbascum Speciosum* has obtained methanol extract by dusting the leaves of the plant. They observed that these extracts inhibited the development of *Staphylococcus Aureus* ATCC 6538, *Listeria Monocytogenes* ATCC 191118, *Bacillus Anthracis*, *Bacillus Cereus* and *Salmonella Typhimurium* ATCC 13311. In a previous study, represents a comparison of the activity profiles of 5 *Verbascum* species (*V. densiflorum Bertol.*, *V. gnaphalodes Bieb.*, *V. lagurus*, *V. phlomoides L.*, *V. xanthophoeniceum Griseb.*). All extracts were tested for their antimicrobial activity against Gram positive/Gram negative bacteria and yeast. The extracts of *V. phlomoides* and *V. densiflorum Bertol.* were active against all Gram positive bacteria (*S. aureus*, *S. epidermidis*, *E. faecalis*), generally. *V. phlomoides L.* methanol extracts and *V. densiflorum Bertol.* methanol extracts were found active against *S. aureus* and *S. epidermidis* as well as *V. densiflorum Bertol.* etroleum ether extracts against *S. epidermidis* and *E. faecalis*. The extracts of *V. phlomoides L.* were more active against Gram positive bacteria than the *V. densiflorum Bertol.* extracts (Sen-Utsukarcı et al., 2018). Yabalak et al. (2022) reported that among extracts their tested, a biological analysis was applied to evaluate the antimicrobial action of the extracts of *V. pseudoholotrichum* on several pathogens. The results showed that *V. pseudoholotrichum* is a good candidate for antimicrobial practices.

**Table 2.** Antibacterial and antifungal activity of the ethanolic extracts of *Verbascum napifolium* by micro- well dilution assay (MIC)

|  | Leaf Methanol Extract ( $\mu\text{g mL}^{-1}$ ) | Flower Methanol Extracts ( $\mu\text{g mL}^{-1}$ ) | Standards ( $\mu\text{g mL}^{-1}$ ) |              |              |
|--|---|--|-------------------------------------|--------------|--------------|
|  | Meth.   | Meth.  | DMSO                                | Control      | Meth.        |
| Antibacterial Activity                           |   |  |                                     |              |              |
| <i>Staphylococcus aureus</i> (ATCC 6538/P) G (+) | 32 $\pm$ 0.00                                   | 16 $\pm$ 0.00                                      | 0 $\pm$ 0.00                        | 0 $\pm$ 0.00 | 0 $\pm$ 0.00 |
| <i>Bacillus cereus</i> (CCM 99) G (+)            | 32 $\pm$ 0.00                                   | 16 $\pm$ 0.00                                      | 0 $\pm$ 0.00                        | 0 $\pm$ 0.00 | 0 $\pm$ 0.00 |
| <i>Escherichia coli</i> (ATCC 35218) G (-)       | 16 $\pm$ 0.00                                   | 16 $\pm$ 0.00                                      | 0 $\pm$ 0.00                        | 0 $\pm$ 0.00 | 0 $\pm$ 0.00 |
| <i>Pseudomonas aeruginosa</i> (ATCC 27853) G (-) | 32 $\pm$ 0.00                                   | 16 $\pm$ 0.00                                      | 0 $\pm$ 0.00                        | 0 $\pm$ 0.00 | 0 $\pm$ 0.00 |
| Antifungal Activity                              |   |  |                                     |              |              |
| <i>C. albicans</i> (ATCC 10239)                  | 32 $\pm$ 0.00                                   | 32 $\pm$ 0.00                                      | 0 $\pm$ 0.00                        | 0 $\pm$ 0.00 | 0 $\pm$ 0.00 |

G: gram reaction; DMSO: Dimethyl sulfoxide, Meth.: Methanol, Concentration range (128-1  $\mu\text{g mL}^{-1}$ ), '0': No inhibition

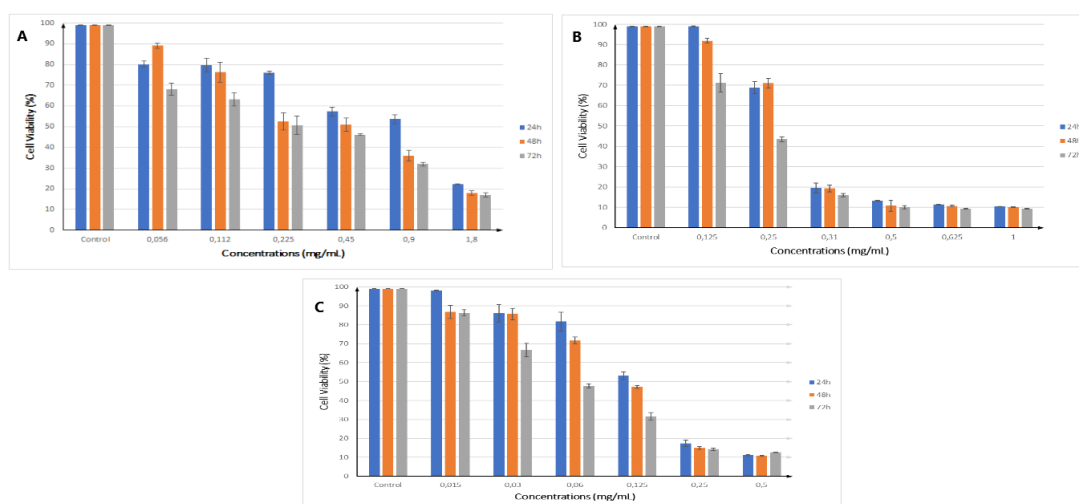
### 3.2 Effects of the extracts on cell viability of CaCo-2 and L929

It is known that plants have been used in the treatment of cancer since ancient times. Compounds found in extracts obtained from plants used for this purpose show significant anticancer activity (Iqbal et al., 2017). In previous studies, compounds found in extracts from plants have anticancer effects. (Cragg and Newman, 2005) Although there are studies on species belonging to different *Verbascum* genus in the literature, no anticancer studies have been found on the *V. napifolium* species used. In this study, the effects of methanol extracts of *Verbascum napifolium* leaf and flower part on cell viability in CaCo-2 and L929 cell lines were investigated by MTT assay for 24, 48 and 72 hours and the IC<sub>50</sub> values of the extracts in cells were calculated. Six different concentrations were tested for leaf and flower methanol extract in CaCo-2 and L929 cell lines. Leaf and flower methanol extracts of *V. napifolium* were found to decrease cell

viability depending on time and concentration in the CaCo-2 cell line, and the decreases in IC<sub>50</sub> value supported this. The IC<sub>50</sub> values of the leaf methanol extract at 24, 48 and 72 hours in this cell line were calculated as approximately 0.931, 0.664 and 0.250  $\text{mg mL}^{-1}$ , respectively. The IC<sub>50</sub> values of the flower methanol extract at 24, 48 and 72 hours in the CaCo-2 cell line were calculated as approximately 0.086, 0.075 and 0.049  $\text{mg/mL}$ , respectively. When the IC<sub>50</sub> values of the leaf and flower extracts are examined, it is seen that the flower methanol extract has a more cytotoxic effect than the leaf methanol extract against CaCo-2 cells. It was observed that the cell viability decreased depending on the concentration when treated with flower methanol extract at different concentrations and times in the L929 cell line. The IC<sub>50</sub> values of the flower methanol extract at 24, 48 and 72 hours in the L929 cell line were calculated as approximately 0.118, 0.170 and 0.062  $\text{mg/mL}$ , respectively. In addition, leaf methanol extract had a greater effect on the reduction of cell viability of CaCo-2 cell line

after 24 and 48 hours of incubation compared to the mouse fibroblast cell line L929. Cell viability did not fall below 50% in L929 cell line treated with leaf extract ( $0.9-0.028 \text{ mg mL}^{-1}$ ), and so  $IC_{50}$  value did not calculate. One study reported that although methanol extract obtained from *Verbascum* genus showed cytotoxic effect against SK-MEL cell line, it was ineffective against SK-OV-3 cell line (Tatlı and Akdemir, 2006). Iiescu et al. (2020) reported that methanol extract from aerial parts of *Verbascum nigrum* did not affect the

cytotoxicity of HaCaT cells, but showed a significant effect on A431 cells. In a different study, the cytotoxicity of *V. sinaiticum* flower extracts prepared with different solvents against MCF-7 was examined and it was seen that the ethanol extract showed the best cytotoxicity. It was also reported that the hydroethanolic leaf extract of the same plant showed the best cytotoxic effect against HepG2 and MRC-5 cell lines (Tauchen et al., 2015). Based on, the effects of extracts obtained from plants vary according to cell lines.



Effects of *Verbascum napifolium* leaf methanol extract on CaCo-2 cell viability depending on concentration and time (A) Effects of *Verbascum napifolium* flower methanol extract on CaCo-2 cell viability depending on concentration and time (B) Effects of *Verbascum napifolium* flower methanol extract on L929 cell viability depending on concentration and time (C)

**Figure 1.** Effect of *Verbascum napifolium* leaf and flower methanol extract on cell viability in CaCo-2 and L929 cell lines

#### 4. Conclusion

The anticancer and antimicrobial potential of methanol extracts of leaf and flower parts of *Verbascum napifolium* from Muğla has been demonstrated for the first time. Furthermore, a biological analysis was applied to evaluate the antimicrobial action of the extracts of *Verbascum napifolium* on several pathogens. The results showed that *Verbascum napifolium* is a good candidate for antimicrobial practices.

The highest resistant to the leaf and flower extracts tested was demonstrated by

the gram- negative bacteria *Pseudomonas aeruginosa* with an inhibition zone of  $0.95 \pm 0.00 \text{ mm}$  and the gram-negative bacteria *E. coli* with an inhibition zone of,  $0.70 \pm 0.20 \text{ mm}$ , respectively. The most sensitive bacteria to both extracts was gram (+) *Bacillus cereus*. The MICs of the extracts are between  $16 \pm 0.00$  and  $32 \pm 0.00 \mu\text{g mL}^{-1}$  and both extracts appear to show significant activity against the bacteria species tested and yeast. MIC value was determined as  $32 \pm 0.00 \mu\text{g mL}^{-1}$  in all bacteria tested with the leaf ethanol extract. Also a MIC value of  $32 \pm 0.00 \mu\text{g mL}^{-1}$  was detected in only *C. albicans* for both



extracts. In conclusion, when looking at its anticancer effects, flower methanol extract of *Verbascum napifolium* showed better anticancer potential than leaf methanol extract. As a result, it is predicted that future studies with *Verbascum napifolium* can be a research resource in the field of biotechnology. It is seen that this plant is a potential bioactive agent in many industrial application areas such as food, pharmacology, biotechnology and chemistry. It has been shown that it has suitable components in the treatment of virucidal diseases.

#### Declaration of Author Contributions

The authors declare that they have contributed equally to the article. All authors declare that they have seen/read and approved the final version of the article ready for publication.

#### Declaration of Conflicts of Interest

All authors declare that there is no conflict of interest related to this article.

#### Acknowledgements

We would like to thank Assoc. Prof. Dr. Mehlika Alper for their valuable comments.

#### Referanslar

Alahmer, N. 2017. *Verbascum* cinsine ait bazı türlerin antimikrobiyal aktivitesinin incelenmesi. Yüksek lisans tezi, Kastamonu Üniversitesi Fen Bilimleri Enstitüsü, Kastamonu.

Angeloni, S., Zengin, G., Sinan, K.I., Ak, G., Maggi, F., Caprioli, G., Kaplan, A., Bahs, M., Çakılcıoğlu, U., Bouyahya, A., Jugreet, S., Mahomoodally, M.F., 2021. An insight into *Verbascum bombyciferum* extracts: different extraction methodologies, biological abilities and chemical profiles. *Industrial Crops and Products*, 161: 113201.

Atlas R. M., Parks L. C., Brown A. E. 1995. Laboratory Manual of Experimental

Microbiology, Mosby-Year Book Inc., USA, 25.

Bakkalı, F., Averbek, S., Averbek, D., Idaomar, M., 2008. Biological effects of essential oils—a review. *Food And Chemical Toxicology*, 46(2): 446-475.

Başer, K.H.C., 2008. Sığırkuyruğu (*Verbascum sp.*). *Bağbahçe*. 61:22–23.

Bradshaw, L.J., 1992. Laboratory Microbiology, 4th ed. New York USA, Emeritus California State University, Saunders College Publishing, Fullerton.

Collins CM, Lyne PM. 1987. Microbiological Methods. Butterworths and Co. Ltd., London.

Cragg, G.M., Newman, D.J., 2005. Plants as a source of anti-cancer agents. *Journal of Ethnopharmacology*, 100(1-2): 72-79.

Donn, P., Barciela, P., Perez-Vazquez, A., Cassani, L., Simal-Gandara, J., Prieto, M.A., 2023. Bioactive Compounds of *Verbascum sinuatum* L.: Health Benefits and Potential as New Ingredients for Industrial Applications. *Biomolecules*, 13(3): 427.

Dulger, B., Dulger, G., 2018. Antibacterial Activity of *Verbascum antinori*. *Konuralp Tıp Dergisi*. 10(3): 395-398.

Dulger, B., Ugurlu, E., Aki, C., Suerdem, T. B., Camdeviren, A., Tazeler, G., 2005. Evaluation of antimicrobial activity of some endemic *Verbascum*, *Sideritis*, and *Stachys* species from Turkey. *Pharmaceutical biology*, 43(3): 270-274.

Ghasemi, F., Rezaei, F., Araghi, A., Abouhosseini, T.M., 2015. Antimicrobial activity of aqueous-alcoholic extracts and the essential oil of *Verbascum thapsus* L. *Jundishapur Journal of Natural Pharmaceutical Products*, 10(3): 3.

- Grigorov, M., Pavlović, D., Tadić, V., Nešić, I., Zlatković, B., 2022. Chemical composition of the ethanol extracts of *Verbascum niveum* flowers and leaves. *Macedonian Pharmaceutical Bulletin*, 68: 381-382.
- Iliescu, I.A., Peter, S., Albert, I., Skalicka-Woźniak, K., Miron, A., Luca, S.V., Wolfram, E., 2020. *Verbascum nigrum*: cytotoxicity evaluation in A431 epidermoid carcinoma cells and untargeted LC-HR-MS/MS metabolite profiling. *Chemistry & Biodiversity*, 17(12): e2000644.
- Iqbal, J., Abbasi, B.A., Mahmood, T., Kanwal, S., Ali, B., Shah, S.A., Khalil, A.T., 2017. Plant-derived anticancer agents: A green anticancer approach. *Asian Pacific Journal of Tropical Biomedicine*, 7(12): 1129-1150.
- Karaalp, C., Yurtman, A.N., Yavasoglu Karabay, N.Ü., 2009. Evaluation of antimicrobial properties of *Achillea L.* flower head extracts. *Pharmaceutical Biology*, 47(1): 86–91.
- Kaya, G.İ., Somer, Ü.N., Konyalıoğlu, S., Yalçın, T.H., Yavaşoğlu Karabay, N.Ü., Sarıkaya, B., Önür, M.A., 2010. Antioxidant and antibacterial activities of *Ranunculus marginatus var. trachycarpus* and *R. sprunerianus*. *Turkish Journal of Biology*, 34: 139-146.
- Khan, A.M., Qureshi, R.A., Gillani, S.A., Ullah, F., 2011. Antimicrobial activity of selected medicinal plants of Margalla Hills, Islamabad, Pakistan. *Journal of Medicinal Plants Research*, 5(18): 4665–4670.
- Küçük, S., Özdemir, F., İşcan, G., Incesu, Z., 2016. Determination of Cytotoxic and Anticandidal Activities of Three *Verbascum L.* Species from Turkey: *V. cheiranthifolium* Boiss. var. *asperulum* (Boiss.) Murb. Monorg., *V. pycnostachyum* Boiss. & Heldr and *V. orgyale* Boiss. & Heldr. *Turkish Journal of Pharmaceutical Sciences*, 13(3).
- Nofouzi, K., Mahmudi, R., Tahapour, K., Amini, E., Yousefi, K., 2016. *Verbascum speciosum* methanolic extract: Phytochemical components and antibacterial properties. *Journal of Essential Oil Bearing Plants*, 19(2): 499-505.
- Scarpati, M.L., Delle Monache, F., 1963. Isolation from *Verbascum sinuatum* of two new glucosides, verbascoside and isoverbascoside. *Annales de Chimie*, 53: 356–367.
- Senatore, F., Rigano, D., Formisano, C., Grassia, A., Basile, A., Sorbo, S., 2007. Phytogrowth-inhibitory and antibacterial activity of *Verbascum sinuatum*. *Fitoterapia*, 78(3): 244-247.
- Sen-Utsukarcı, B., Dosler, S., Taşkın, T., Abudayyak, M., Özhan, G., Mat, A., 2018. Türkiye'deki beş *Verbascum* türünün antioksidan, antimikrobiyal, antibiyofilm ve sitotoksik aktivitelerinin değerlendirilmesi. *Farmacia*, 66: 1014-1020.
- SPSS (n.d.) IBM SPSS Statistics for Windows, Version 22.0, Armonk, NY
- Tatlı, İ.İ., Akdemir, Z.S., 2006. Cytotoxic activity on some *Verbascum* species growing in Turkey. *Hacettepe University Journal of the Faculty of Pharmacy*, (2): 77-86.
- Tauchen, J., Doskocil, I., Caffi, C., Lulekal, E., Marsik, P., Havlik, J., ... & Kokoska, L., 2015. In vitro antioxidant and anti-proliferative activity of Ethiopian medicinal plant extracts. *Industrial Crops and Products*, 74: 671-679.
- Turan, M., Mammadov, R., 2018. Antioxidant, antimicrobial, cytotoxic, larvicidal and anthelmintic activities and phenolic contents of *Cyclamen alpinum*. *Pharmacology & Pharmacy*, 9(04): 100.

- Turker, A.U., Camper, N.D., 2002. Biological activity of common mullein, a medicinal plant. *Journal of Ethnopharmacology*, 82(2): 117–125.
- Uğur, D., Güneş, H., Güneş, F., Mammadov, R., 2017. Cytotoxic Activities of Certain Medicinal Plants on Different Cancer Cell Lines. *Turkish Journal of Pharmaceutical Sciences*, 14(3): 222–230.
- WHO 2013. WHO traditional medicine strategy: 2014–2023. Geneva: World Health Organization.
- Yabalak, E., Ibrahim, F., Eliuz, E.A.E., Everest, A., Gizir, A.M., 2022. Evaluation of chemical composition, trace element content, antioxidant and antimicrobial activities of *Verbascum pseudoholotrichum*. *Plant Biosystems-An International Journal Dealing with all Aspects of Plant Biology*, 156(2): 313-322.

---

**To Cite**

Taşkaya, A., Şahin, B., Ceyhan Güvensen, N., Mammadov, R., 2023. A Preliminary Study on Anticancer and Antimicrobial Potential of Methanolic Extracts of *Verbascum napifolium*. *ISPEC Journal of Agricultural Sciences*, 7(1): 135-145.  
DOI: <https://doi.org/10.5281/zenodo.7749361>.

---