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Araştırma Makalesi

Muscari armeniacum Bitkisinin Farklı Ekstraktlarının Antioksidan, Antimikrobiyal ve Sitotoksik Aktivitelerinin İncelenmesi

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ÖZ

Bu çalışmanın amacı *Muscari armeniacum* bitkisinin toprak üstü ve soğanlarının maserasyon yöntemiyle elde edilen farklı ekstraktların antioksidan ve antimikrobiyal aktivitelerini karşılaştırmalı olarak değerlendirmektir. Ayrıca çalışma, etanol ekstraktlarının insan fibroblastlarının ve MCF-7 meme kanseri hücre hatlarının çoğalması üzerindeki etkisini belirlemeyi amaçlamaktadır. *Muscari armeniacum* bitkisinin toprak üstü kısımlarından ve soğanlarından elde edilen petrol eteri, kloroform ve etanol ekstraktlarının antioksidan aktiviteleri DPPH, CUPRAC ve FRAP yöntemleriyle incelenmiştir. Ekstraktların içerdiği toplam fenolik bileşik miktarı FCR yöntemi kullanılarak belirlendi. Antimikrobiyal aktivite agar kuyusu difüzyon yöntemiyle belirlendi. Bitkinin etanol ekstraktlarının sitotoksik aktiviteye yeteneği MTT tahlili kullanılarak belirlendi. Soğanların kloroform ekstraktının diğer ekstraktlara göre daha yüksek DPPH (IC_{50}:0,056 mg/mL), FRAP (2,738 mM FeSO4/mg ekstrakt) ve CUPRAC (3,640 mM trolox eşdeğeri/mg ekstrakt) aktivite potansiyeline sahip olduğu belirlendi. Ayrıca toprak üstü kısım kloroform ekstraktının en yüksek DPPH (IC_{50}: 0,889 mg/mL), CUPRAC (1,166 mM trolox eşdeğeri/mg ekstrakt) ve FRAP (0,823 mM $FeSO_4$/mg ekstrakt aktivitesi) antioksidan aktivitesini gösterdiği belirlendi. Soğanlardan elde edilen kloroform ekstraktının toprak üstü kısımlara göre daha yüksek miktarda fenolik içerik içerdiği ve buna bağlı olarak en yüksek antioksidan aktiviteyi gösterdiği belirlendi. Toprak üstü petrol eteri ekstraktının *S. aureus* ATCC 25923 ve *S. epidermidis* ATCC 11228'e karşı antimikrobiyal aktiviteye sahip olduğu, soğanların etanol ekstraktının ise yalnızca *S. epidermidis* ATCC 11228'e karşı antimikrobiyal aktiviteye sahip olduğu bulundu. Çalışma, 500 µg/mL dozundaki toprak üstü ve soğan etanol ekstraktlarının insan fibroblastları ve MCF-7 meme kanseri hücre dizileri üzerinde sitotoksik potansiyeli olmadığını gösterdi. Bitkinin farklı kısımlarının insan normal hücre hatları üzerinde sitotoksik aktivite göstermemesi, bu türün antioksidan ve antimikrobiyal ajan olarak kullanılabileceğini düşündürmektedir.

Anahtar kelimeler: *Muscari armeniacum*, antimikrobiyal, antioksidan, sitotoksik

Investigation of Antioxidant, Antimicrobial and Cytotoxic Activities of Different Extracts of *Muscari armeniacum*

ABSTRACT

The aim of this study is to comparatively evaluate the antioxidant and antimicrobial activities of different extracts obtained by the maceration method of the aerial and bulbs of the *Muscari armeniacum*. Additionally,

the study aims to determine the effect of ethanol extracts on the proliferation of human fibroblasts and MCF-7 breast cancer cell lines. The antioxidant activities of petroleum ether, chloroform and ethanol extracts from the aerial parts and bulbs of the *Muscari armeniacum* were examined by DPPH, CUPRAC and FRAP methods. The amount of total phenolic compounds contained in the extracts was determined using the FCR method. Antimicrobial activity was determined by the agar well diffusion method. The ability ethanol extracts of plant to cytotoxic activity was determined using the MTT assay. It was determined that the chloroform extract of bulbs had higher DPPH (IC_{50}:0.056 mg/mL), FRAP (2.738 mM FeSO4/mg extract) and CUPRAC (3.640 mM trolox equivalent/mg extract) activity potential than other extracts. In addition, it was determined that the aerial parts chloroform extract showed the highest DPPH (IC_{50}: 0.889 mg/mL), CUPRAC (1.166 mM trolox equivalent/mg extract) and FRAP (0.823 mM FeSO4/mg extract activities) antioxidant activity. It was determined that the chloroform extract obtained from bulbs contained higher amounts of phenolic contents than the aerial parts and accordingly showed the highest antioxidant activity. Aerial parts petroleum ether extract was found to have antimicrobial activity against S. aureus ATCC 25923 and S. epidermidis ATCC 11228, and the ethanol extract of bulbs was found to have antimicrobial activity only against S. epidermidis ATCC 11228.

The study showed no cytotoxic potential of aerial parts and bulbs ethanol extracts at a dose of 500 µg/mL on human fibroblasts and MCF-7 breast cancer cell lines. The fact that different parts of the plant do not show cytotoxic activity on human normal cell lines suggests that this species can be used as an antioxidant and antimicrobial agent.

Key words: *Muscari armeniacum, antimicrobial, antioxidant, cytotoxic*

INTRODUCTION

The use of plants for medicinal purposes dates back to ancient times. In our country, as well as all over the world, medicinally important plants have been used by people for centuries. In aerobic organisms, free radicals, that is, reactive oxygen species, are formed during the body's normal use of oxygen or under the influence of various external factors such as environmental agents (pesticides, aromatic hydrocarbons, toxins, solvents, etc.), stress and radiation. The resulting free radicals can damage basic cellular components such as lipids, proteins and nucleic acids in the organism. Our body uses the antioxidant system that prevents the formation of free radicals and prevents damage. Therefore, our body's natural defense system should be supported with antioxidant molecules. Some medicinal plants have antimicrobial properties and are widely used in the treatment of some diseases. With the development of technology, the production of synthetic antimicrobial agents has increased. However, due to misuse and overuse of these agents, microorganisms may gain resistance (Vanderbank, 1949; Shinde et al., 2012). The origin of the Asparagaceae family is thought to be Eurasian or African. The family is widely distributed throughout the world (Gürsoy, 2016). The *Muscari* genus, located in the Asparagaceae family, was introduced to the scientific community in 1754 by Botanist Philip Miller (1691- 1771) in his work titled "The Gardeners Dictionary" (Eroğlu, 2020). The origin of the name *Muscari* is based on the fact that some members of the *Muscari* genus are fragrant. It is derived from the Latin word “muscus” (musk). The word musk came to our language from the Arabic language (Eroğlu, 2020). According to the Turkish Language Association, the word musk means "a fragrant substance and mist extracted from a gland under the abdominal skin of a type of male gazelle that lives in the high mountains of Asia." It is recorded in Turkey that the name *Muscari*, meaning "musk scent, musk hyacinth", was used in ancient Istanbul because the flowers of the *Muscari macrocarpum* species were fragrant (Gürsoy, 2009). *Muscari* genus is distributed in a wide region around the world. It extends throughout the Mediterranean basin to the Caucasus, as well as in the temperate regions of Europe, North Africa and the southwestern parts of Asia. Although there is variation according to species, plants generally grow in meadows, steppes, snow-melt areas, stony-rocky areas, slopes, watersides, saline areas and various forest undergrowth (Eroğlu, 2020).The *Muscari armeniacum* H.J.Veitch plant is generally known as grape hyacinth (Suzuki and Nakano, 2000). At the same time, its other common names are Arabian Hyacinth, Catnip, Müşkürüm, Gavurbaşı, Turkish Hyacinth, Viper Onion, Garden Grape Hyacinth and Armenian Grape Hyacinth (Kahraman, 2019; Bokov, 2019; Arslan, 2021). The plant is called Grape Hyacinth and Turkish Hyacinth in English (Kahraman, 2019). *Muscari armeniacum* is a herbaceous, bulbous, perennial plant with blue-purple flowers that resemble showy bunches of grapes (Kahraman, 2020). M. armeniacum is widely used as an ornamental plant in parks, gardens and pots due to its eye-catching flowers and striking appearance (Kahraman, 2022; Bokov, 2019). Grape hyacinth is a honey plant and attracts bees, butterflies and bumblebees to the garden with its pleasant scent (Bokov, 2019; Azad and Amin, 2012). It is also used in the perfumery and cosmetics industry with its pleasant smell (Bokov, 2019; Azad and Amin, 2012). In Turkey, the bulbs of the plant are traditionally consumed as food (Kıyıcı, 2021; Tuzlacı, 2011). It is known among the public that the oil obtained after soaking the above-ground

parts of the plant in olive oil for 1 week is used externally in the treatment of eczema and as a skin moisturizer (Arslan, 2021; Tuzlacı, 2016). In addition, it is known that the external use of the plant supports wound healing, has antiseptic properties, and provides an anesthetic effect on dermatological inflammatory processes, wounds and burns (Bokov, 2019). M. armeniacum contains various groups of biologically active substances. These groups include flavonoids, mucilages, saponins and alkaloids. Flavonoids include anthocyanins (definidin and cyanidin derivatives) and homoisoflavonoids (Bokov, 2019). Anthocyanins are the floral pigments that give the plant's flowers their sky blue color (Lou et al., 2014; Bokov, 2019). It is known that anthocyanins, which have antioxidant properties, also reduce the risk of cancer, diabetes, cardiovascular and neurological disorders (Serrano et al., 2020). In addition, it is thought that homoisoflavonoids found in the plant can be used to prevent cancer cells thanks to their strong antioxidant and potential antimutagenic effects (Bokov, 2019). The flowers of the M. armeniacum plant have a floral, fruity and slightly peach-like scent. The main components of this fragrance have been identified as 2-phenylethanol and 2-(4-methoxyphenyl)-ethanol. At the same time, grape hyacinth contains muscarosides (A12, B13, G3, J6, K7, L8 and M9), which are in the group of oligoglycosides, and musarmins, which are proteins that inactivate the ribosome (Bokov, 2019).

The objective of this study is to compare the antioxidant and antimicrobial properties of various extracts derived from the bulbs and aerial parts of M. armeniacum. Additionally, the study aims to determine the effect of ethanol extracts on the proliferation of human fibroblasts and MCF-7 breast cancer cell lines.

MATERIAL AND METHODS

Plants material

Muscari armeniacum was identified by Dr. Gizem Emre. The identified sample was kept at the Marmara University Faculty of Pharmacy Herbarium with the code MARE: 22684 until the day the study started.

Preparation of Extracts

The aerial parts and bulbs of the *Muscari armeniacum* were dried under room conditions. The dried parts were turned into powder with the help of a plant grinder. Crude extracts were obtained from the powdered aerial parts and tubers (18 g) by sequential maceration method using petroleum ether, chloroform and ethanol solvents. The organic solvents obtained were evaporated in a rotary evaporator (Heidolph, 45 °C and low pressure) to obtain crude extracts. The obtained dry extracts were stored in the refrigerator at +4 °C until the day the study started.

Antioxidant activity assays

DPPH assays: 240 µL of DPPH solution (0.1 mM) were added to the 10 µL of extracts that had been obtained at various doses (0.5-3 mg/mL). Before being incubated for 30 minutes at 25 °C, the produced mixtures were stirred for 1 minute. Every day at 517 nm, the mixes' absorbance values were measured. Under identical circumstances, the absorbance of the control sample was measured using 10 µL of methanol rather than the extract. The information gathered throughout the investigation is provided as IC_{50} = mg/mL (Wei et al., 2010).

CUPRAC assays: The extracts' ability to reduce copper (II) ions while still having antioxidant activity was assessed using a method created by Apak et al. in 2004. In a nutshell, 60 µL of Cu(II)2H2O, 60 µL of neocuproine, and 60 µL of 1 M NH4Ac were mixed, then 60 µL of the extracts were added, and finally 10 µL of ethanol was added to the mixture. The mixes' absorbance was spectrophotometrically evaluated at 450 nm after 60 min against a reference solution that was made by substituting ethanol for the plant extracts. The extracts' CUPRAC values were provided as mg Trolox equivalent/mg extract (Apak et al., 2004).

FRAP assays: The FRAP reagent was stored at 37 oC for 30 min. It consisted of 25 mL of 300 mM acetate buffer (pH 3.6), 2.5 mL of TPTZ solution, and 2.5 mL of 20 mM FeCl3.6H2O. 10 µL of extract were combined with 190 µL of FRAP reagent, and after 4 minutes, the mixture's absorbance at 593 nm was measured. The extracts' FRAP values were presented as mM Fe2+/mg extract (Taskin et al., 2021).

Assay for total phenolic content: Briefly, 0.1 mL of the diluted plant extracts, 4.5 mL of water, and 0.1 mL of the Folin-Ciocalteu reagent were combined, and then 0.3 mL of sodium carbonate solution (2%) was added. One minute of medium-continuous shaking was then performed. After two hours at room temperature, the absorbance at 765 nm was measured using a UV/Vis Spectrophotometer. Total phenolic content was calculated as mg gallic acid equivalents (GAE) per milligram of plant extract (Sembiring et al., 2018).

Antimicrobial effectiveness test

Antimicrobial activity potential of plant extracts was determined primarily by the agar well diffusion method. Minimal inhibitory concentration (MIC) was determined for extracts showing antimicrobial activity in agar well diffusion method.

Agar well diffusion test: As bacteria; Staphylococcus aureus ATCC 25923, Staphylococcus epidermidis ATCC 11228, Escherichia coli ATCC 25922, Pseudomonas aeruginosa ATCC 27853, Proteus mirabilis ATCC 14153,

Klebsiella pneumoniae ATCC 4352 and as yeast; Candida albicans ATCC 10231 strains were used. Bacteria were inoculated on tryptic soy agar and C. albicans on sabouraud dextrose agar, incubated at 37 °C for 24 hours. Microorganism suspensions were prepared from colonies in 0.85% NaCl physiological saline solution (PSS). Bacterial suspensions were adjusted to 108 cfu/ml and C. albicans suspensions to a concentration of 106 cfu/mL according to Mc Farland 0.5 standard turbidity. The microorganism suspensions were spread over the surface of the Mueller hinton agar for bacteria and sabouraud dextrose agar (SDA) for C. albicans by sterile swabs under aseptic conditions and then 5 mm diameter wells were made surface of the medium with a sterile punch. The wells were filled with 50 µL (50 mg/mL) of the extracts dissolved in appropriate solvents. In addition, meropenem (10 µg/well) for bacteria, amphotericin B (100 µg/well) as a positive control for the yeast, solvent (DMSO) and PSS will be used as negative control. Inoculated petri dishes will be incubated at 37°C for 18-24 hours for bacteria, for 24-48 hours at 35 °C for yeast, and at the end of incubation time, inhibition zones will be measured in mm. The trials will be performed with three replicates and will be averaged.

Detection of minimal inhibitor concentration for bacteria: Detection of MIC for bacteria were performed in accordance with the standards of the Clinical and Laboratory Standards Institute (CLSI). Cation Adjusted Mueller Hinton Broth (CAMHB) was used as medium. Bacteria suspension was prepared from the colonies in the 24-hour bacterial culture according to Mc Farland 0.5 turbidity and the final inoculum concentration shall be diluted to 5x105 cfu/ml. The sterile U-based microdilution plates were placed 100 µL of the CAMHB. Soluble extracts were placed 100 µl in the first wells and serial dilutions were made respectively. Then 5µL of bacterial suspension was added to the wells containing the extract and the plates incubated at 37 °C for 24 hours. At the end of the incubation, the lowest extracting concentrations with no growth was determined as minimal inhibitory concentration (MIC). Escherichia coli ATCC 25922 was used as a quality control microorganism. CAMHB, DMSO and PSS were used as negative control. Meropenem was used as positive control (Perez et al., 1990; NCCLS, 2003; CLSI 2005).

Cell Culture Studies

Human fibroblasts and MCF-7 breast cancer cells were grown in DMEM medium, containing L-glutamine, 10% fetal bovine serum (FBS), and 1% penicillin-streptomycin (P/S). The cells were incubated at 37°C in a 5% CO_2 humidified atmosphere. When the cells reached 70-80% confluence, they were washed with phosphate-buffered saline (PBS) and treated with trypsin-EDTA to detach them from the culture flask. After trypsinization, the cells were centrifuged at 1200 rpm for 5 minutes to pellet them. The cell pellet was resuspended in fresh medium by gentle pipetting. Following cell counting, the cells were subcultured into new flasks at an appropriate dilution, and the process was repeated every 3 days.

MTT Assay

The study assessed the viability of ethanol extracts from the aerial parts and bulbs of *Muscari armeniacum* using the 3-(4,5-Dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) assay after 24 hours. Cells were seeded in 96-well plates at a density of 1×10^4 cells per well (n=4) and were incubated for 24 hours. After that, extracts were applied to the cells and left for another 24 hours. Next, 10 µL MTT was added and incubated for 3 hours. The plates were then incubated on a shaker for 10 minutes, and the percentage of living cells was determined at 570 nm using a multi-plate reader (EnSpire Manager, Perkin Elmer). For the fibroblast cell line, four different concentrations (62.5, 125, 250, and 500 µg/mL) were used, and the IC_{50} value was determined. To calculate the fractional effect (Fa) (1-cell viability) values, absorbance values were obtained via the MTT experiment. The doses corresponding to these Fa values were entered into the CompuSyn software (Paramus, NJ). In the MCF-7 cell line, the % viability values were compared at a dose of 500 µg/mL. (Goler et al., 2023).

Statistical analysis: The results were computed using the mean standard deviations (SD) of three parallel, independent measurements. One-way analysis of variance was conducted using ANOVA test, and significant differences between means were identified using the Tukey Multiple Comparison test, with a p-value of 0.05 being regarded as statistically significant.

RESULTS and DISCUSSION

Determination of DPPH radical scavenging activity

The free radical scavenging activities of different extracts from the plant were determined using the DPPH method. The antioxidant activities of the extracts and the compound used as standard (ascorbic acid) were evaluated by comparing their IC_{50} values. The results obtained were shown in Table 1. According to the results, it was determined that the chloroform extracts from the bulbs (IC_{50}: 0.056 mg/ml) and aerial parts (IC_{50}: 0.889 mg/ml) of the plant showed higher DPPH radical scavenging activity compared to other extracts. When the activities of bulbs and aerial parts extracts were compared, it was determined that the chloroform extract of bulbs showed the highest DPPH radical scavenging activity. In the study, when the activities of extracts and

standard were compared, it was determined that all extracts showed lower activity than the ascorbic acid compound (IC_{50}: 0.004 mg/ml) used as standard.

Table 1. DPPH radical scavenging activities of different extracts from the plant

	DPPH (IC_{50}: mg/ml)	
Extracts	Bulbs	Aerial parts
Petroleum ether	0.229 ± 0.029*	1.085 ± 0.198*
Chloroform	0.056 ± 0.005*	0.889 ± 0.453*
Ethanol	0.110 ± 0.004*	1.056 ± 0.257*
Ascorbic acid	0.004 ± 0.007	

**p <0.05* compared to ascorbic acid.

Determination of iron (III) ion reduction antioxidant power (FRAP)

The Iron (III) ion reducing power of plants is very important in evaluating their antioxidant potential. The iron reducing power is based on the reduction of Fe^{+3} of the herbal extract to Fe^{+2} and examining this situation spectrophotometrically at 593 nm. In this method, high absorbance indicates high iron reduction potential. For this purpose, in this study, the Iron (III) ion reducing antioxidant power of different extracts from the plant was examined comparatively. According to the obtained FRAP values it was determined that the chloroform extracts from the bulbs (2.738 mM $FeSO_4$/mg extract) and aerial parts (0.823 mM $FeSO_4$/mg extract) of the plant showed higher FRAP values compared to other extracts. When the activities of bulbs and aerial part extracts were compared, it was determined that bulbs chloroform extract had a stronger iron (III) ion reducing power than all other extracts. In addition, in this study, it was determined that all extracts showed lower FRAP values than the standard BHA compound (5.8 mM $FeSO_4$).

Table 2. FRAP values of different extracts obtained from the plant

	FRAP (mM $FeSO_4$/mg extract)	
Extracts	Bulbs	Aerial parts
Petroleum ether	2.224 ± 0.159*	0.709 ± 0.055*
Chloroform	2.738 ± 0.261*	0.823 ± 0.025*
Ethanol	1.121 ± 0.045*	0.683 ± 0.041*
BHA	5.8± 0.104	

**p <0.05* compared to BHA.

Copper (II) ion reducing antioxidant capacity method (CUPRAC)

The copper(II) ion reducing antioxidant capacity of different extracts from the plant was evaluated by the CUPRAC method. In this study, it was determined that the bulbs (3.640 mM TE/ mg extract) and aerial parts (1.166 mM TE/ mg extract) of the plant have a stronger copper(II) ion reducing antioxidant capacity potential than other extracts. When all extracts obtained from different parts of the plant were compared among themselves, it was determined that bulbs chloroform extract had stronger CUPRAC values than all other extracts. Findings showed that all extracts prepared from the plant exhibited lower copper(II) ion-reducing antioxidant capacity than the BHT compound (5.683 mM TE).

Table 3. CUPRAC values of different extracts from the plant

CUPRAC (mM trolox equivalent/mg extract)		
Extracts	Bulbs	Aerial parts
Petroleum ether	2.675 ± 0.808*	0.581 ± 1.375*
Chloroform	3.640 ± 1.070*	1.166 ± 0.130*
Ethanol	1.407 ± 0.423*	0.421 ± 0.017*
BHT	5.683 ± 0.337	

**p <0.05* compared to BHT

Determination of total phenolic content in extracts

Total phenolic compounds contained in the different extracts obtained by using the maceration method of the bulb and aerial parts of the plant were determined using the FCR method. The results obtained are given in Table 4 as mg gallic acid equivalent /mg extract. This study determined that chloroform extracts from the bulb and aerial parts of the plant contained higher amounts of phenolic compounds than other extracts. When the results of the plant different parts were evaluated, it was determined that bulb chloroform extracts (0.099 mg GAE/ mg extracts) contained the highest amount of phenolic compounds.

Tablo 4. The amount of phenolic compounds contained in different extracts from the plant

Total phenolic contents (mg GAE/ mg extract)		
Extracts	Bulbs	Aerial parts
Petroleum ether	0.032 ± 0.004	0.020 ± 0.003
Chloroform	0.099 ± 0.008	0.024 ± 0.003
Ethanol	0.012 ± 0.001	0.010± 0.004

GAE: Gallic acid equivalent

Antimicrobial Activity

The antimicrobial activities of different extracts obtained from different parts of the plant were determined according to the agar well diffusion method and the findings are shown in Table 5.

The petroleum ether extract of aerial part was found to have antimicrobial activity against S. aureus ATCC 25923 and S. epidermidis ATCC 11228 and ethanol extract of bulbs was found to have antimicrobial activity only against S. epidermidis ATCC 11228. Other extracts do not have antimicrobial activity (Table 5). The MIC of meropenem against E. coli ATCC 25922 used as a quality control microorganism was within the CLSI quality control limits.

Table 5. Antimicrobial activity of different extracts from the plant

	S. aureus ATCC 25923		*S. epidermidis* ATCC 11228	
Extracts	Zone Diameter (mm)	MIC (mg/ml)	ZoneDiameter (mm)	MIC (mg/ml)
Bulbs-EtOH	0	ND	3.01 ± 0.14	> 3.25
Aerial parts-PE	12.57 ± 0.23	> 3.25	5.21 ± 0.18	> 3.25
Merpopenem	27.75±0.32	2	38.32±0,28	0.25

ND: Not determined, EtOH: Ethanol, PE: Petroleum ether, MIC: Minimal inhibitory concentration

Cytotoxic Activity

Human fibroblasts and MCF-7 breast cancer cells were subjected to indicated concentrations of *M. armeniacum* extracts for 24 hours. Assessment of cell viability was conducted using the MTT assay.

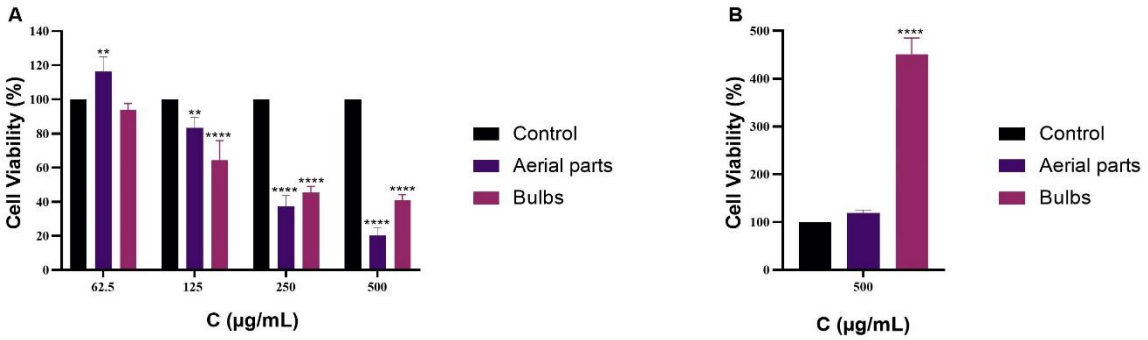

Figure 1. Effects of aerial parts and bulbs of the *M. armeniacum* on the viability of human fibroblasts and MCF-7 breast cancer cells. Error bars represent the mean ±S.E.M. **$p < 0.01$ and ****$p < 0.0001$ compared to control group.

To determine the IC_{50} value, four different concentrations (62.5, 125, 250, and 500 µg/mL) of aerial parts and bulbs of *M. armeniacum* were applied to human fibroblast cells. The viability of aerial parts was found to be 116.43 ± 7.55%, 83.38 ± 6.17%, 37.29 ± 6.44%, and 20.20 ± 4.66%, respectively. For bulbs, the viability was 93.87 ± 3.69%, 64.49 ± 11.45%, 45.47 ± 3.60%, and 41.06 ± 3.15%, respectively. As stated in the method, the IC_{50} for the aerial parts was 239.444 µg/mL, whereas that for the bulbs was 285.903 µg/mL.

After that, 500 µg/mL aerial parts and bulbs of *M. armeniacum* were applied to human MCF-7 breast cancer cells, and their viability was evaluated and compared to the control group. The viability of aerial parts was found to be 118.80 ± 6.45%, and for bulbs, it was 451.28 ± 33.92%. The study showed that ethanol extracts from aerial parts and bulbs at a dose of 500 µg/mL do not have any anticancer properties. Additionally, it was determined that both plant parts had no cytotoxic effect on the normal cell line at the same concentration.

No detailed biological activity study was found in the literature review regarding the *M. armeniacum*. However, various biological activity studies have been carried out on other *Muscari* species within the same genus. According to the LC-MS/MS results of the *Muscari neglectum* Guss. ex Ten plant, the aerial parts ethanol extracts of the plant contain quinic acid (1.726 µg/g), fumaric acid (5.771 µg/g), gentisic acid (0.138 µg/g), caffeic acid (0.052 µg/g), kemferol (0.035 µg/g) and apigenin (0.092 µg/g). It was observed that bulbs ethanol extract contains quinic acid (0.411 µg/g), fumaric acid (1.567 µg/g), caffeic acid (0.619 µg/g) and kemferol (0.300 µg/g) (Eroğlu 2021). In another study, *Muscari parviflorum* Desf. It has been observed that the plant has antioxidant activity (Mammadov et al., 2012l), and the *Muscari muscarimi* plant has potential antiproliferative and apoptotic activity on cancer cells (Özay et al., 2021).
In the study conducted by Loizzo et al. (2010) with the *Muscari comosum* (L.) Mill., it was determined that the ethanol extract showed the highest DPPH radical scavenging activity. It was also observed that this extract showed good hypoglycemic activity for α-amylase and α-glucosidase (Casacchia et al., 2017). In the study conducted by Onaran and Bayram (2018), on the *Muscari aucheri* (Boiss.) Baker, which grows endemic in Turkey, the methanol extract from the plant has been shown to have antifungal activity against Fusarium oxysporum f. sp. cucumerinum, Alternaria solani, Verticillium dahliae, Rhizoctonia solani ve Botrytis cinerea.
In this study, unlike the above studies, petroleum ether, chloroform and ethanol extracts were prepared from the aerial parts and bulbs of M. armeniacum, using the maceration method. For the first time, our team examined in detail the antioxidant and antimicrobial activities of different extracts obtained from this plant. Additionally, no studies have been found in the literature regarding the effects of aerial parts and bulbs on the viability of human fibroblast cells and the MCF-7 breast cancer cell line. Therefore, in this study, the cytotoxic activities of different parts were comparatively examined.

CONCLUSION

This study showed that bulbs and aerial parts chloroform extracts had higher antioxidant activity than other extracts. It was also determined that the bulbs parts of the plant exhibited higher antioxidants activity and total phenolic compounds than the aerial parts. Aerial parts petroleum ether and bulbs ethanol extracts was found to have stronger antimicrobial activity than other extracts. The study showed no cytotoxic potential of aerial parts and bulbs ethanol extracts at a dose of 500 µg/mL on human fibroblasts and MCF-7 breast cancer cell lines. These findings suggested that the bulbs and aerial parts of the plant could be used as a natural agents of antioxidants and antimicrobials.

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Contribution Rate Statement Summary of Researchers: TT was responsible for antioxidant activity and prepared the manuscript. EA and BNY examined the antioxidant activity. Plant identified by GE. ER examined the antimicrobial activity. SA and AMYG examined the cytotoxic activity All authors contributed to writing and editing the manuscript. All authors read and approved the final manuscript.

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