MARMARA
PHARMACEUTICAL JOURNAL

Research Article

www.marmarapharmj.com

In vitro antioxidant and cytotoxic activity of *Muscari neglectum* growing in Turkey

Esra EROĞLU ÖZKAN[1*], Serpil DEMİRCİ KAYIRAN[2], Turgut TAŞKIN[3], Mahmoud ABUDAYYAK[4]

[1] Istanbul University, Faculty of Pharmacy, Department of Pharmacognosy, 34116, Beyazit, Istanbul, Turkey
[2] Cukurova University, Faculty of Pharmacy, Department of Pharmaceutical Botany, Balcali, 01330, Adana, Turkey
[3] Marmara University, Faculty of Pharmacy, Department of Pharmacognosy, 34668, Haydarpasa, Istanbul, Turkey
[4] Karadeniz Technical University, Faculty of Pharmacy, Department of Pharmaceutical Toxicology, Trabzon, Turkey

ABSTRACT: *Muscari* species have been used as antirheumatic, stomachic, diuretic, expectorant and anti verruca in folk medicine. The species have also been used as food for humans and animals in Turkey. In this study, the antioxidant and cytotoxic activities of different extracts obtained from *Muscari neglectum* were studied. The antioxidant capacities of the extracts were evaluated with DPPH free radical scavenging activity, metal chelating capacity and ABTS cation radical scavenging capacity assays. The cytotoxic potentials of the extracts were determined by MTT and LDH assays on HeLa-cells (human cancer cell line) and NRK-52E-cells (rat kidney cell line). The aqueous extracts from *M. neglectum* bulbs (IC_{50}: 2.83± 0.54 mg/mL) and herba (IC_{50}: 8.52± 1.3 mg/mL) exhibited stronger DPPH free radical scavenging activity than other extracts, respectively. The ethanol extract from *M. neglectum* bulbs showed the highest ABTS (27.88 %) cation radical scavenging activity. According to the metal chelating activity test results, the aqueous extracts from *M. neglectum* herba and bulbs showed the strongest metal chelating activity (28.99% and 28.07%, respectively).

KEYWORDS: *Muscari neglectum*, antioxidant activity, cytotoxic activity, Turkey.

1. INTRODUCTION

Muscari Mill. (Asparagaceae) is a genus of bulbous plants and has a wide distribution in Mediterranean region, Europe, Iran, Iraq and Afghanistan [1, 2]. In Turkey, it is represented by 37 species, 25 of which are endemic [2, 3]. Among them, *Muscari neglectum* Guss. ex Ten. have been used in traditional medicine as antirheumatic, stomachic, diuretic, expectorant and anti verruca. The fruit of *M. neglectum* have been used for curing of rheumatism disease [4, 5]. The leaves, flowers and flower buds are edible as raw, boiled, grilled or pickled [6, 7]. It has also been used as food for humans and animals, dye, toys, ornamental plants in parks and gardens. The bulbs of *M. neglectum* have pectoral stimulatory effects, anti-inflammatory, anti-allergic and aphrodisiac effects [8].

The chemical composition of the *M. neglectum* is composed of anthocyanins, flavonoids, homoisoflavanones, spirocyclic nortriterpenoid glycosides, polyhydroxylated pyrrolizidine alkaloids and essential oil [9, 10]. Homoisoflavonoid compounds had been reported to exhibit antiinflammatory, estrogenic, antiestrogenic, anticancer and angioprotective bioactivities [6].

The aim of this study was to investigate the antioxidant and cytotoxic activities of the several extracts obtained from herba and bulbs of *M. neglectum* collected from Kahramanmaraş, Turkey. The antioxidant capacities of the extracts were evaluated with DPPH free radical scavenging activity, metal chelating capacity and ABTS cation radical scavenging capacity assays. The cytotoxic potentials of the extracts were determined by MTT and LDH assays on HeLa-cells (human cancer cell line) and NRK-52E-cells (rat kidney cell line).

2. RESULTS AND DISCUSSION

2.1. Antioxidant activity of *M. neglectum* extracts

The aqueous extracts from *M. neglectum* bulbs (IC_{50}: 2.83 ± 0.54 mg/mL) and herba (IC_{50}: 8.52± 1.3 mg/mL) exhibited stronger DPPH free radical scavenging activity than other extracts. BHT and ascorbic acid were used as positive controls. As shown in Table 1, the DPPH free radical scavenging activities of the all extracts were lower than those of BHA and ascorbic acid.

The effects on ABTS cation radical scavenging of *M. neglectum* extracts were shown in Table 1. The ethanol extract from *M. neglectum* bulbs showed the highest ABTS (27.88 %) cation radical scavenging activity.

Received: 19.09.2017 / **Accepted:** 29.11.2017
Corresponding Author: Esra Eroğlu Özkan
E-mail: esraeroglu@gmail.com; eseroglu@istanbul.edu.tr
Phone: +90-212-4400000 / 13594
ORCID No: https://orcid.org/0000-0002-1569-2535

How to cite this article: Eroğlu Özkan E, Demirci Kayıran S, Taşkın T, Abudayyak M. *In vitro* antioxidant and cytotoxic activity of Muscari neglectum growing in Turkey. Marmara Pharm J. 2018; 22 (1): 74-79.

http://doi.org/10.12991/mpj.2018.43
Marmara Pharm J 2018;22(1): 74-79

Table 1. DPPH, ABTS radical scavenging and metal chelating activity of different extracts from *M. neglectum* bulbs and herba

Extracts/ Standards	DPPH (IC_{50}: mg/mL)	ABTS (%) (10 µg/mL)	Metal chelating activity (%) (50 µg/mL)
BH	9.6± 1.1[a]	15.52[a]	0.47[a]
BDC	4.09± 0.46[b]	24.50[b]	5.99[b]
BE	4.53± 0.49[c]	27.88[c]	9.50[c]
BM	4.09± 0.73[d,c]	18.08[d]	9.36[d,c]
BA	2.83± 0.54[e]	10.51[e]	28.07[e]
HH	13.02± 0.1[f]	14.05[f]	13.05[f]
HDC	23.48± 1.4[g]	11.98[g]	2.08[g]
HE	9.60± 1.2[h]	10.45[h,e]	9.31[h]
HM	22.49± 0.71[i]	21.73[i]	18.40[i]
HA	8.52± 1.3[i]	20.58[i]	28.99[j]
Ascorbic acid	0.09± 0.006[j]	-	-
BHT	0.32± 0.03[k]	54.38[j]	-
EDTA	-	-	96.51[j]

BH/HH: Bulb/Herba Hexane; BDC/HDC: Bulb/Herba Dichloromethane; BE/HE: Bulb/Herba Ethanol; BM/HM: Bulb/Herba Methanol; BA/HA: Bulb/Herba Aqueous extract. BHT: Butylhydroxytoluene. EDTA: Ethylenediaminetetraacetic acid.
Values are mean of triplicate determination (n = 3) ± standard deviation
Different superscript letters in each column exhibit significant differences in mean values at P< 0.05 according to Tukey's Multiple Comparison test.

According to the results obtained from the metal chelating activity test, the aqueous extract from *M. neglectum* herba showed the strongest metal chelating (28.99%) activity. Also, this extract exhibited a very close result to aqueous extracts from *M. neglectum* bulbs (28.07%).

In the present study, we used three different antioxidant assays and the results of all three assays showed that the aqueous and ethanol extracts from *M. neglectum* bulbs had the highest DPPH and ABTS radical scavenging activity, respectively. Also, the aqueous extracts of *M. neglectum* herba and bulbs had the strongest metal chelating activity.

2.2. Cytotoxic Activity of *M. neglectum* extracts

The cytotoxic activity results of *M. neglectum* extracts were shown in Table 2 and Table 3. Some of the extracts induced cell cytotoxicity in a concentration-dependent manner in HeLa cell line (Figure 1-8). The result showed that the BE, BM, HDC and HM extracts of the *M. neglectum* exhibited high cytotoxic activity against HeLa cell line. These extracts should not be used as cytotoxic agent because they are more toxic to NRK-52 cell lines even at low concentrations. According to results, aqueous and hexane extracts obtained from bulbs and herba didn't show

Table 2. Cytotoxic activities of *M. neglectum* bulb extracts

Extracts	MTT				LDH			
	HeLa Cells		NRK-52 Cells		HeLa Cells		NRK-52 Cells	
	Conc (mg/mL)	% Death	Conc (mg/mL)	% Death	Conc (mg/mL)	% Death	Conc (mg/mL)	% Death
BH	nd	nd	nd	nd	nd	nd	nd	nd
BDC	0.10	50	0.04	50	0.18	50	0.03	60
BE	0.04	50	0.06	50	0.20	32	0.03	90
BM	0.05	50	0.03	67	0.07	50	0.03	72
BA	nd	nd	nd	nd	nd	nd	nd	nd

BH: Bulb Hexane; BDC: Bulb Dichloromethane; BE: Bulb Ethanol; BM: Bulb Methanol; BA: Bulb Aqueous extract. (nd: non determined).

Table 3. Cytotoxic activities of *M. neglectum* herba extracts

Extracts	MTT				LDH			
	HeLa Cells		NRK-52 Cells		HeLa Cells		NRK-52 Cells	
	Conc (mg/mL)	% Death	Conc (mg/mL)	% Death	Conc (mg/mL)	% Death	Conc (mg/mL)	% Death
HH	nd	nd	nd	nd	nd	nd	nd	nd
HDC	0.03	50	0.03	50	0.20	30	0.03	67
HE	nd	nd	nd	nd	nd	nd	nd	nd
HM	0.05	50	0.03	64	0.07	50	0.03	74
HA	nd	nd	nd	nd	nd	nd	nd	nd

HH: Herba Hexane; HDC: Herba Dichloromethane; HE: Herba Ethanol; HM: Herba Methanol; HA: Herba Aqueous extract. (nd: non determined).

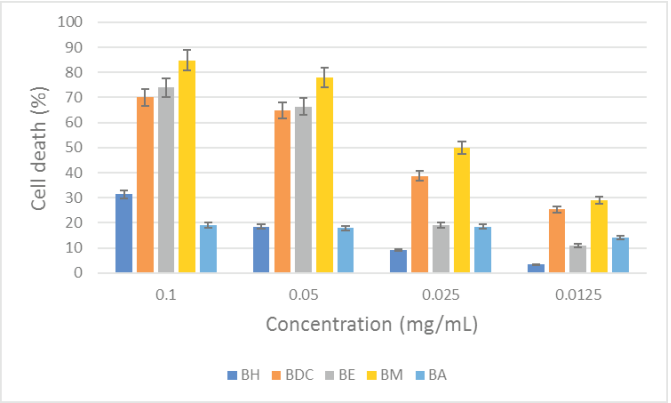

Figure 1. Cytotoxic activities of *M. neglectum* bulb extracts by MTT Assay in Hela cells.

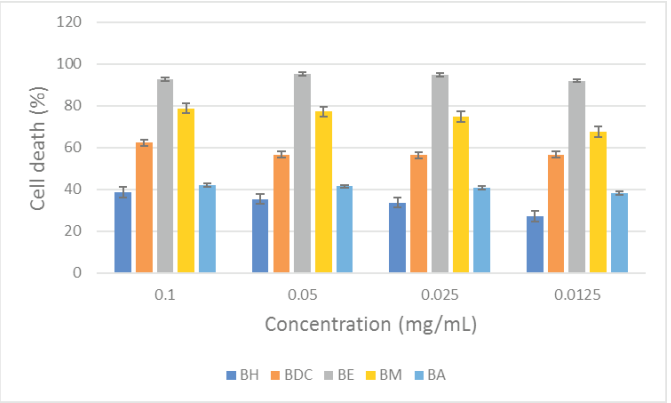

Figure 4. Cytotoxic activities of *M. neglectum* bulb extracts by LDH Assay in NRK-52E cells.

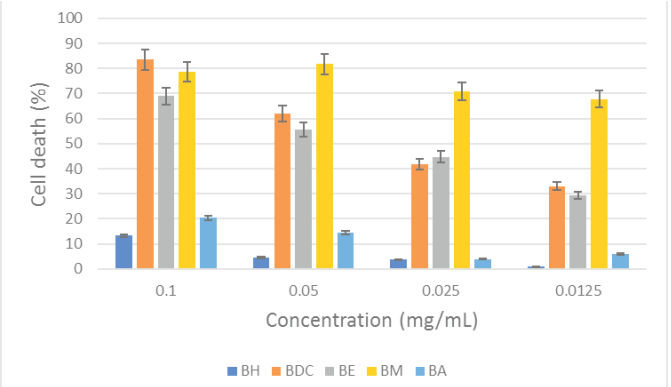

Figure 2. Cytotoxic activities of *M. neglectum* bulb extracts by MTT Assay in NRK-52E cells.

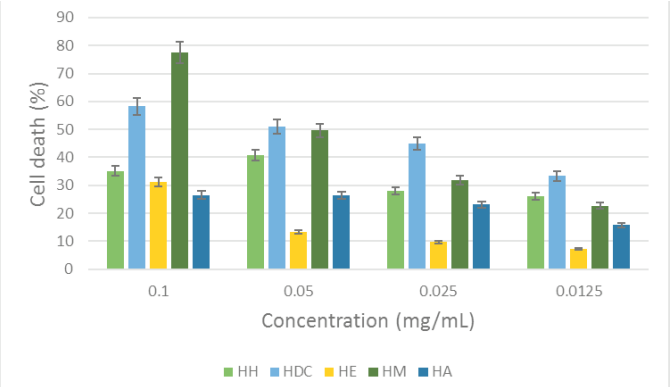

Figure 5. Cytotoxic activities of *M. neglectum* herba extracts MTT Assay in Hela cells.

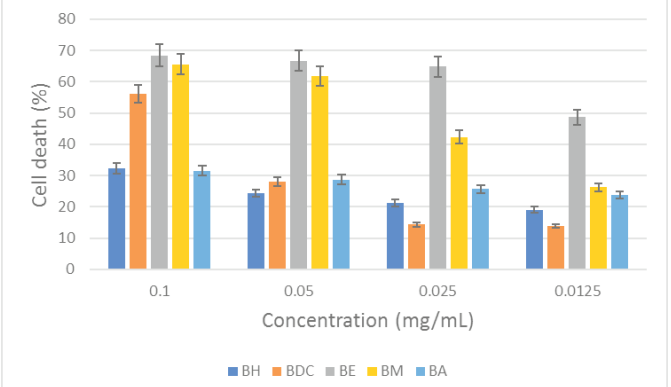

Figure 3. Cytotoxic activities of *M. neglectum* bulb extracts by LDH Assay in Hela cells.

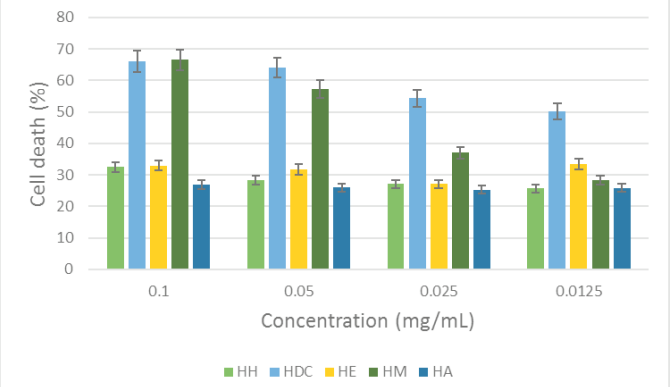

Figure 6. Cytotoxic activities of *M. neglectum* herba extracts LDH Assay in Hela cells.

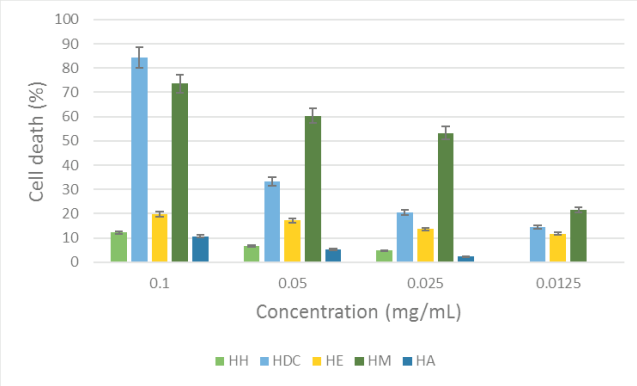

Figure 7. Cytotoxic activities of *M. neglectum* herba extracts MTT Assay in NRK-52E cells.

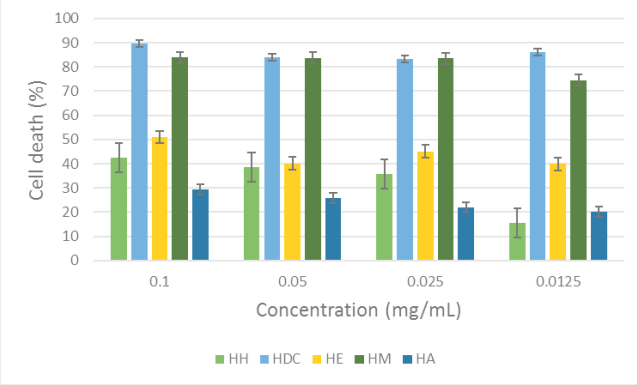

Figure 8. Cytotoxic activities of *M. neglectum* herba extracts LDH Assay in NRK-52E cells.

any cytotoxicity against both cell lines. There was no correlation between antioxidant and cytotoxic activity.

3. CONCLUSION

It is concluded that the report represents the first study on biological activities of *M. neglectum* growing in Turkey. Further phytochemical and biological studies are needed to determine the active constituents of *M. neglectum*.

4. MATERIAL AND METHODS

4.1. Plant Material

The aerial parts and bulbs of *Muscari neglectum* were collected from Kahramanmaraş in April, 2015 and dried at room temperature. Specimens were identified and vouchers were deposited in the Herbarium of Istanbul University, Faculty of Pharmacy (ISTE) under code number of ISTE 100115.

4.2. Preparation of Extracts

Dried and powdered aerial parts and bulbs (5g) of *M. neglectum* were macerated with ethanol (100 mL) and water (100 mL) at room temperature, separately. The extracts were filtered and dried under reduced pressure at a temperature below 30 °C. Dried and powdered bulbs and aerial parts of *M. neglectum* were exhausted with hexane, dichloromethane and methanol by using Soxhlet, respectively. The extracts were dried under reduced pressure.

4.3. Antioxidant Activity

4.3.1. DPPH. Free radical scavenging assay

The stable radical DPPH was used to measure the free radical scavenging activity according to literature [11]. The DPPH solution (3.9 mL) was added to the different extracts of plant and standards (BHT and ascorbic acid) (0.1 mL). After incubating at 25 °C for 30 min, the absorbance of the samples was read at 517 nm against the blank using a spectrophotometer.

4.3.2. ABTS•+cation scavenging assay

The $ABTS^{\bullet+}$ cation scavenging activity of the extracts and synthetic antioxidant substances were determined in accordance with the literature [12]. $ABTS^{\bullet+}$ (3.96 mL) solution was added plant extracts (0.4 mL). After incubating at 25 °C for 6 min, absorbance of the mixture was recorded at 734 nm against a blank.

4.3.3. Metal chelating power assay

The activity of the extracts to chelate iron (II) was evaluated according to the literature [13]. The extracts and standard (EDTA) (200µL) were added to 50 µL of $FeCl_2$ (2 mM) solution. The reaction was initiated by the addition 200 µL of ferrozine (5 mM), left standing at ambient temperature for five minutes. Then, ethanol was added to this mixture until a final volume of 4 mL was achieved. After incubating at 25 °C for 10 min, absorbance of the mixture was recorded at 562 nm against a blank.

4.4. Cytotoxic Activity

4.4.1. Cell culture and Exposure

Rat kidney epithelial cell line (NRK-52E) (CRL-1571™) and Human cervix adenocarcinoma cell line (Hela) (CCL-2™) were obtained from the American Type Culture Collection (ATCC, Manassas, VA). NRK-52E cells were cultured in Dulbecco's modified Eagles medium-F12 containing 5.5 mmol/L D-glucose supplemented with 10% FBS and 100 U/mL penicillin–streptomycin. Hela cells were cultured in low glucose Dulbecco's modified Eagles medium (1 mmol/L D-glucose) supplemented with 10% FBS and 100 U/mL penicillin–streptomycin. The cells were incubated at 5% CO2, 90% humidity and 37 ˚C.

Cells were seeded in 96-well plates at 10^3 – 10^4 cells /well, after 24 h incubation the culture medium was removed, and new cell culture medium with various concentrations of the extracts were added and incubated for 24 h. The exposure concentrations were determined as 0.0125 – 0.2 mg/ml for each extracts. The cytotoxic potential of extracts was investigated using MTT [14] and LDH [15] assays.

In every test, negative (untreated, culture medium), solvent (1% DMSO) controls and positive (1% triton X-100) were used. For each extract, four concentrations were tested in triplicates and each test was repeated three times.

4.4.2. MTT Assay

MTT, 3-[4,5-dimethylthiazol-2-yl]-2,5-diphenyl-tetrazolium bromide, is a water soluble yellow pigment reduced by the mitochondrial succinate dehydrogenase enzyme to insoluble purple formazan product. Mitochondrial succinate dehydrogenase is only active in viable cells, thus, colour changes by the activity of the enzyme can be used as a cytotoxicity endpoint. Following the exposure for 24h, MTT solution (20 µL) (5 mg/mL in PBS) was added to each well and the plates were incubated for farther 2 h. After that, the supernatant containing MTT was removed, and 100 µL DMSO was added to each well, the resulting formazan crystals were solubilized in by shaking for 5 min at 150 rpm and the optical density (OD) of every well was read at 590 nm (against the reference wavelength of 670 nm) using a microplate spectrophotometer system (Epoch, Germany). The inhibition of enzyme activity in cells was calculated as compared to the solvent control. The results were expressed as the maximal cell death (%) – related concentration [14].

4.4.3. LDH assay

Lactate dehdyrogenase (LDH) is a stable cytoplasmic enzyme present in all cells, and rapidly released into the cell culture supernatant upon membrane damage or cell lysis. In this study the LDH activity in culture supernatants was measured spectrophotometricaly at 490 nm according to the kit's instructions (Roche Cytotoxicity Detection Kit – Mannheim, Germany). Briefly, the exposed cells were centrifuged for 3 minutes at 1000 rpm. 100 µL supernatant was mixed with freshly prepared reaction mixture (prepared by mixing two separate solutions diaphorase/NAD+ mixture and iodotetrazolium chloride/sodium lactate mixture) in new plates and incubated at room temperature away from light for 30 min. After that, a stop solution was added to the mixtures and the absorbance was measured at 490 nm. The results were compared with the absorbance values of the triton X-100 %1 (positive control) which give the maximum cell death. The results were expressed as the maximal cell death (%) – related concentration [15].

4.5. Statistical analysis

The significance of differences between the control and experimental groups was calculated by one-way ANOVA Dunnett t-test using SPSS version 17.0 for Windows (SPSS Inc., Chicago, IL) and expressed as mean±standard deviation (SD) and standard error (SE). p values of less than 0.05 were selected as the levels of significance.

Authorship statement

Author contributions: Concept – E.E.O., S.D.K.; Design – E.E.O., S.D.K.; Supervision – E.E.O.; Resource – E.E.O., S.D.K., T.T., M.A.; Materials – E.E.O., S.D.K.; Data Collection and/or Processing – E.E.O., S.D.K., T.T., M.A.; Analysis and/or Interpretation – E.E.O., S.D.K., T.T., M.A.; Literature Search – E.E.O., S.D.K., T.T., M.A.; Writing – E.E.O., S.D.K., T.T., M.A.; Critical Reviews – E.E.O., S.D.K., T.T., M.A.

Conflict of interest statement

The authors declared no conflict of interest.

REFERENCES

[1] Speta F. Hyacinthaceae. In: Kubitzki K, editor. Flowering Plants · Monocotyledons. The Families and Genera of Vascular Plants. 3. Berlin, Heidelberg: Springer; 1998.

[2] Demirci S, Özhatay N, Koçyiğit M. *Muscari erdalii* (Asparagaceae, Scilloideae), a new species from Southern Turkey. Phytotaxa. 2013;154(1):38-46.

[3] Yildirim H. Muscari elmasii sp. nova (Asparagaceae): a new species from western Anatolia, Turkey. Turk J Bot. 2016;40(4):380-387.

[4] Erol M, Tuzlacı E. Eğirdir (Isparta) yöresinin geleneksel halk ilacı olarak kullanılan bitkileri. XI BİHAT. 1995:22-24.

[5] Ugurlu E, Secmen O. Medicinal plants popularly used in the villages of Yunt Mountain (Manisa-Turkey). Fitoterapia. 2008;79(2):126-131.

[6] Lim T. *Muscari neglectum*. Edible medicinal and non-medicinal plants: Springer; 2014. p. 122-5.

[7] Mahboubi M, Taghizadeh M. The antimicrobial and antioxidant activity of M*uscari neglectum* flower ethanol extract. Herba Pol. 2016;62(4):39-48.

[8] Usher G. A dictionary of plants used by man: Constable and Company Ltd.; 1974.

[9] Mulholland DA, Schwikkard SL, Crouch NR. The chemistry and biological activity of the Hyacinthaceae. Nat Prod Rep. 2013;30(9):1165-1210.

[10] Kayıran SD, Özkan EE. The ethnobotanical uses of Hyacinthaceae species growing in Turkey and a review of pharmacological activities. IJTK. 2017; 16(2): 243-250.

[11] Taskin T, Bitis L. *In vitro* antioxidant activity of eight wild edible plants in Bursa province of Turkey. Farmacia 2016;64(5): 706-711.

[12] Re R, Pellegrini N, Proteggente A, Pannala A, Yang M, Rice-Evans C. Antioxidant activity applying an improved ABTS radical cation decolorization assay. Free Radic Biol Med. 1999;26(9):1231-1237.

[13] Dinis TC, Madeira VM, Almeida LM. Action of phenolic derivatives (acetaminophen, salicylate, and 5-aminosalicylate) as inhibitors of membrane lipid peroxidation and as peroxyl radical scavengers. Arch Biochem Biophys. 1994;315(1):161-169.

[14] Alley MC, Scudiero DA, Monks A, Hursey ML, Czerwinski MJ, Fine DL, Abbott BJ, Mayo JG, Shoemaker RH, Boyd MR. Feasibility of drug screening with panels of human tumor cell lines using a microculture tetrazolium assay. Cancer Res. 1988;48(3):589-601.

[15] Mayo JG, Shoemaker RH, Boyd MR. Feasibility of drug screening with panels of human tumor cell lines using a microculture tetrazolium assay. Cancer Res. 1988;48(3):589-601.

[16] Legrand C, Bour J, Jacob C, Capiaumont J, Martial A, Marc A, Wudtke M, Kretzmer G, Demangel C, Duval D. Lactate dehydrogenase (LDH) activity of the number of dead cells in the medium of cultured eukaryotic cells as marker. J Biotechnol. 1992;25(3):231-243.