Journal of Physical Chemistry and Functional Materials

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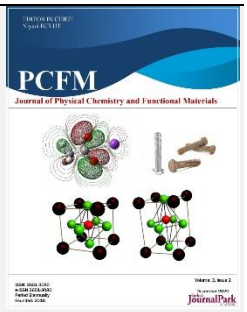

Investigation of *in vitro* genotoxic, cytotoxic, antiradical and phytochemical properties of endemic *Verbascum diversifolium* Hochst.

Serhat Keser[1,*], Ebru Bahar Ciftci[2], Samet Öz[3], Suat Tekin[4], Omer Kaygili[5]

[1]*Department of Chemical Technology, EOSB Vocational School, Firat University, 23119 Elazig, Türkiye serhatkeser@gmail.com Orcid ID: 0000-0002-9678-1053*

[2]*Department of Chemistry, Faculty of Science, Firat University, 23119 Elazig, Türkiye ebrubaharciftci@gmail.com Orcid ID: 0009-0001-9594-7285*

[3]*Department of Veterinary, Health Services Vocational School, Osmaniye Korkut Ata University, 80000 Osmaniye, Türkiye sametoz@osmaniye.edu.tr Orcid ID: 0000-0002-4549-4388*

[4]*Department of Physiology, Faculty of Medicine, Inonu University, 44280 Malatya, Türkiye tekinsuat@gmail.com Orcid ID: 0000-0002-2757-1802*

[5]*Department of Physics, Faculty of Science, Firat University, 23119 Elazig, Türkiye okaygili@firat.edu.tr Orcid ID: 0000-0002-2321-1455*

** Corresponding author: E-mail: serhatkeser@gmail.com Fax: +90-424-2555367, Phone: +90-424-2370000 / 8234*

ABSTRACT

Verbascum species are consumed as tea among the public, and these species used as a breast softener, expectorant, in the treatment of hemorrhoids and rheumatism, and as a wound healer. To investigate the antiradical, cytotoxic, genotoxic and phytochemical compounds of ethanol, methanol and water extracts of endemic *Verbascum diversifolium* Hochst. (Nizip mullein) plant grown in Elazig is the main aim of this study. Antiradical activity of plant was determined against ABTS, DPPH and OH radicals, and cytotoxic and genotoxic activities were determined against human cancer cell lines of A2780 (ovarian) and MCF-7 (breast) with MTT and Comet assays. All extracts of *V. diversifolium* were in the ABTS radical scavenging test; *V. diversifolium* ethanol and methanol extracts in the DPPH radical scavenging test; *V. diversifolium* methanol extract in the OH radical scavenging test showed higher antiradical activity than the standard antioxidant BHT. This plant was determined to be rich in total phenolic, total flavonoid, total proanthocyanidin, α-tocopherol, phytosterols and unsaturated fatty acids. DNA damage studies of *V. diversifolium* extracts were carried out by the Comet assay method using the parameters of the olive tail moment, tail density, tail length, head length and head density. The cell death occurred through the DNA damage mechanism, and it can be said that this plant shows effective cytotoxic activities

ARTICLE INFO

Keywords:
Verbascum diversifolium Hochst.; genotoxicity; cytotoxicity; antiradical

Received: January 3, 2025
Accepted: February 2, 2025
ISSN: 2651-3080
DOI: 10.54565/jphcfum.1612825

1. INTRODUCTION

It is known that many compounds and/or compound groups that have natural antioxidant properties are found in abundance in the fruit, leaf, flower, stem, bark, shoot, seed, etc. parts of plants and that these have very important potential bioactive properties [1-4]. Studies have shown that people who consume plenty of vegetables and fruits are less likely to develop cancer and cardiovascular diseases that the mortality rate is lower in these people due to the aforementioned diseases, and that there is an inverse proportion between the risk of developing these diseases and fruit and vegetable consumption. It is suggested that this situation is due to the bioactive compounds that plants contain in abundance (e.g. phenolic acids, phenolic compounds, flavonoids, tocopherols, carotenes,

phytosterols). In the last 20 years, studies on determining the anticancer, antitumor, antimicrobial, antibacterial, antioxidant and antiradical properties of plants and products obtained from these plants, as well as the isolation and characterization of compounds and/or compound groups that provide these properties have gained great interest and the same situation continues today. At the center of such studies are plants consumed by the public for treatment purposes and/or as food [1, 5-8].

It is known that approximately 650 medicinal plants are grown in Turkey, and all of them can grow naturally. The number of these plants cultivated is very small, and they are produced in very limited areas [9]. Since medicinal and aromatic plants are used in food and beverage production, pharmaceutical industry, perfumery and cosmetic production, their consumption has also increased rapidly. When looked at from the perspective of the pharmaceutical industry, the tendency towards plant-based drugs has increased significantly due to their lower cost and fewer side effects compared to synthetic drugs. It is stated that the active ingredient of at least 1/4 of the drugs produced and developed by today's pharmaceutical industry is of plant origin [10].

The genus *Verbascum* L. is a perennial herbaceous plant in the family Scrophulariaceae. Species belonging to this genus grow in temperate regions of the Northern Hemisphere and are represented by 360 species. The genus *Verbascum* L., represented by approximately 250 species, 185 of which are endemic in Turkey, is also commonly called mullein [11]. Preparations prepared from the leaves and flowers of *Verbascum* species are consumed as tea among the public. In addition, it is known to be used as a breast softener, expectorant, in the treatment of hemorrhoids and rheumatism, and as a wound healer. Studies have shown that many *Verbascum* species have antimalarial, antiviral, antitumor, antioxidant, anti-inflammatory, antibacterial, antimicrobial, etc. properties [12,13].

The goal of this study is to investigate the in vitro antiradical, cytotoxic, genotoxic and phytochemical contents of ethanol, methanol and water extracts of *Verbascum diversifolium* Hochst. (Nizip mullein) flowers.

2. MATERIAL AND METHOD

2.1. Collection Details of Plants Used in the Study

Verbascum diversifolium (Nizip mullein): **10.05.2020** Between Elazig and Keban, around Pul Village, Roadside. Altitude: 1.5 km. The voucher specimen number is Keser 001. It was stored in the herbarium of Firat University, Faculty of Science, Department of Biology, Elazig/Türkiye.

In the identification of plant samples, the works titled "Flora of Turkey and the East Aegean Islands" [14,15] and "List of Plants of Turkey (Vascular Plants)" [16] were used. The collected plant materials were dried in a laboratory environment, out of sunlight and at ambient temperature, and extraction processes were started without wasting time.

2.2. Extraction Process

The flowers of the *Verbascum diversifolium* (Nizip mullein) plant were first crushed in a blender and turned into powder for the extraction process. 20 grams of each plant sample was weighed, homogenized with 200 mL of solvent (ethanol, methanol and water) and centrifuged at 5000 rpm. At the end of the process, supernatants were taken and solvents were removed with appropriate procedures. The obtained extracts were stored in the deep freezer at -18 °C and dissolved at µg/mL concentration for analysis [17].

2.3. Determination of Antiradical Activity

Antiradical activity tests were performed according to procedures detailed in previous studies [18-20]:

$$RSA(\%) = \left(\frac{A_0 - A_1}{A_0} \right) \times 100 \quad (1)$$

where A_0 and A_1 are the absorbance of the control and extract.

2.4. Determination of Phytochemical Contents

Phytochemical contents analyses were performed according to procedures detailed in previous studies [18-20].

2.5. Determination of Anticancer Properties

Anticancer properties analyses were performed according to procedures detailed in previous studies [18-20].

2.6. Determination of the Genotoxicity with Comet Assay

Determination of the genotoxicity with Comet assay tests were performed according to procedures detailed in previous study [21].

2.7. Statistical Analyses

Statistical evaluations of the results obtained from the analyses were made with the help of the SPSS Statistics 22.0 program. The evaluation of antiradical tests was carried out with ANOVA and DMRT tests (for $p<0.05$). The results were presented as mean ± standard deviation. Anticancer analyses were done using the KOLMOGOROV SMIRNOV test. While group comparisons were made with a one-way analysis of variance and variance homogeneities were made with the LEVENE test, it was observed that the variances were not homogeneous. Therefore, while multiple comparisons were made with the TAMHANE T2 test (for $p<0.05$).

3. RESULTS AND DISCUSSIONS

3.1. Antiradical Activity Results

The antiradical activity results of water, methanol and ethanol extracts of *Verbascum diversifolium* flowers are presented in Table 1. According to these test results, all *V. diversifolium* extracts showed higher antiradical activity than the standard antioxidant BHT in ABTS radical scavenging test; *V. diversifolium* methanol extract in OH

radical scavenging test, *V. diversifolium* ethanol and *V. diversifolium* methanol extracts in DPPH radical scavenging test. Radical scavenging percentages for ABTS radical scavenging test are listed from highest to lowest as *V. diversifolium* Methanol (VDM) (%98.55) > *V. diversifolium* Ethanol (VDE) (%98.42) > *V. diversifolium* Water (VDW) (%90.98) > BHT (%90.33). For the OH radical scavenging test, radical scavenging percentages are listed from largest to smallest as VDM (%90.42) > BHT (%89.28) > VDE (%87.97) > VDW (%84.89). For the DPPH radical scavenging test, radical scavenging percentages are listed from largest to smallest as VDM (%91.45) > VDE (%90.88) > BHT (%89.98) > VDW (%82.11).

Table 1 Antiradical activities of *Verbascum diversifolium* extracts (500 µg/mL)

Samples	$ABTS^{+\bullet}$ (%)	$OH^{\bullet}$ (%)	$DPPH^{\bullet}$ (%)
***V. diversifolium* Water**	90.98±0.23[b]	84.89±0.42[b]	82.11±0.15[b]
***V. diversifolium* Ethanol**	98.42±0.44[a]	87.97±0.35[a]	90.88±0.73[a]
***V. diversifolium* Methanol**	98.55±0.27[a]	90.42±0.11[a]	91.45±0.24[a]
BHT	90.33±0.32[b]	89.28±0.19[a]	89.98±0.67[a]

Groups with the same letter are statistically similar; $p<0.05$

No antiradical studies were found related to the taxon *Verbascum diversifolium*. However, when studies on the genus *Verbascum* were examined, Yilmaz [12] claimed that *V. antiochium* methanol extracts scavenged DPPH radicals by 100%. In another study, it was determined that *V. caesareum*, *V. galilaeum*, *V. gaillardotii*, *V. pinetorum*, *V. tripolitanum*, and *V. sinuatum* extracts had DPPH radical scavenging activity [22]. Marian et al. [23] found that *V. phlomoides* extract 86.36%, Amin et al. [24] found that *V. calvum* flowers 60-70%, and Gupta et al. [25] found that *V. thapsus* extract scavenged DPPH radicals at a high rate. The results of the above-mentioned studies obtained from our study prove that plants belonging to the *Verbascum* species have antiradical activity.

3.2. Phytochemical Analysis Results

The amounts of phenolic compounds, flavonoids and proanthocyanidins in water, methanol and ethanol extracts of *Verbascum diversifolium* flowers are presented in Table 2. According to these results, total phenolic compound contents of the extracts were ranked as VDW (69.03 mg GAE/g extract) > VDM (67.53 mg GAE/g extract) > VDE (67.40 mg GAE/g extract); while total flavonoid contents were ranked as VDM (6897.43 µg CE/g extract) > VDE (6593.50 µg CE/g extract) > VDW (6405.41 µg CE/g extract); Total proanthocyanidin contents are listed as VDM (1719.67 µg CE/g extract) > VDE (1349.67 µg CE/g extract) > VDW (1208.56 µg CE/g extract).

Table 2 Amounts of total proanthocyanidins, total flavonoids and total phenolic compounds of *Verbascum diversifolium*

Samples	Total Flavonoid	Total Proanthocyanidin	Total Phenolic
***V. diversifolium* Water**	6405.41±4.27	1208.56±1.19	69.03±0.82
***V. diversifolium* Ethanol**	6593.50±3.88	1349.67±2.10	67.40±0.36
***V. diversifolium* Methanol**	6897.43±5.09	1719.67±1.99	67.53±0.55

The amounts of flavonoids and proanthocyanidins are presented as µg catechin equivalent/g extract, and the amount of phenolic compounds are presented as mg gallic acid equivalent/g extract. Fat-soluble vitamin, plant sterols and free fatty acid contents of *Verbascum diversifolium* flowers are presented in Table 3. According to these results, *V. diversifolium* extracts contained 0.033 µg/g retinol, 1.067 µg/g δ-tocopherol, 126.133 µg/g α-tocopherol, 5.133 µg/g vitamin K, 0.333 µg/g vitamin D, 39.533 µg/g ergosterol, 25.233 µg/g stigmasterol, 243.967 µg/g β-sitosterol, 1.58%

caprylic acid (8:0), 0.71% myristic acid (14:0), 0.91% pentadecanoic acid (15:0), 30.00% palmitic acid (16:0), 7.09% palmitoleic acid (16:1), 8.95% It was found that it contains stearic acid (18:0), 12.88% oleic acid (18:1), 11.59% linoleic acid (18:2), 22.93% linolenic acid (18:3), 2.66% arachidonic acid (20:4), 0.15% eicosapentaenoic acid (20:5), 0.55% lignoceric acid (24:0), 42.70% saturated, 57.30% unsaturated fatty acids.

Table 3 Vitamin, phytosterol and free fatty acid contents *Verbascum diversifolium*

Phytochemical Contents (µg/g)	***Verbascum diversifolium***
Retinol	0.033±0.02
δ-tocopherol	1.067±0.13
α-tocopherol	126.133±1.35
Vitamin K	5.033±0.29
Vitamin D	0.333±0.15
Ergosterol	39.533±1.29
Stigmasterol	25.233±1.54
β-Sitosterol	243.967±2.03
Fatty Acids (%)	
8:0	1.58±0.03
14:0	0.71±0.02
15:0	0.91±0.01
16:0	30.00±0.72
16:1	7.09±0.24
18:0	8.95±0.71
18:1	12.88±0.31
18:2	11.59±0.85
18:3	22.93±0.63
20:4	2.66±0.26
20:5	0.15±0.02
24:0	0.55±0.08
Saturated FA	42.70
Unsaturated FA	57.30

When phytochemical studies related to *V. diversifolium* taxon were examined, it was understood that this species was not the subject of any study examining total phenolic, flavonoid, proanthocyanidin, vitamin, sterol and free fatty acid contents; however, the amounts of phenolic compounds and flavonoids of many other species of the *Verbascum* genus were tried to be determined. Danahaliloğlu [22] determined that the extracts of *V. sinuatum*, *V. gaillardotii*, *V. caesareum*, *V. pinetorum*, V. *galilaeum*, and *V. tripolitanum* extracts contained total phenolic compounds in the range of 120.5 to 316.3 mg GAE/g extract. Amin et al. [24] determined that *V. calvum* flowers contained 74.91 to 79.56 mg GAE/g total phenolic, Marian et al. [23] determined that *V. phlomoides* contained 471.33 mg GAE total phenolics and 5.36 mg QE total flavonoids. The results obtained from this thesis and the results of the studies mentioned above show that species belonging to the *Verbascum* genus are rich in phytochemical content.

3.3. Anticancer Analysis Results

The IC_{50} values of anticancer activity results of *V. diversifolium* ethanol, water and methanol extracts on the A2780 and MCF-7 cancer cell lines are given in Table 4 and Figure 1. *V. diversifolium* ethanol extract (2.28 µg/mL) has better anticancer activity for the A2780 cell lines than all the other extracts; *V. diversifolium* methanol extract (28.75 µg/mL) has a better anticancer activity for the MCF-7 cell lines than all the other extracts. To our best knowledge, there is no report about anticancer properties in *V. diversifolium*.

Table 4 The IC_{50} values of endemic *V. diversifolium* flowers extracts against A2780 and MCF-7 cancer cell lines for the anticancer activity assay at 24 hours

Samples (µg/mL)	A2780	MCF-7
***V. diversifolium* Water**	18.98	284.70
***V. diversifolium* Ethanol**	2.28	138.70
***V. diversifolium* Methanol**	4.54	28.75

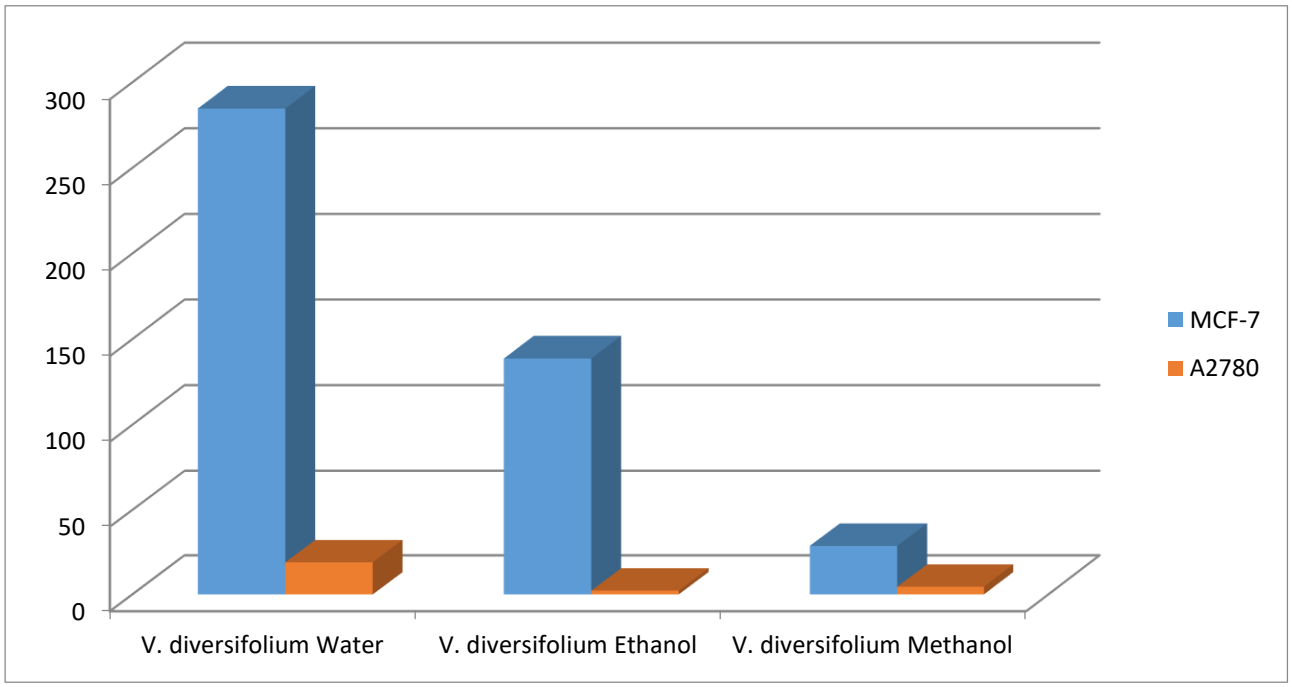

Figure 1 The IC_{50} values of *V. diversifolium* extracts against MCF-7 and A2780 cancer cell lines

Although no anticancer studies have been found on *V. diversifolium* species, some species belonging to the *Verbascum* genus have been determined to be the subject of anticancer research. For example, Marian et al. [23] found that *V. phlomoides* had high anticancer activity against B16-F10 melanoma cell lines, Amin et al. [24] found that *V. calvum* flowers had high anticancer activity against human cancers of MCF-7 (breast) and A549 (lung). Gupta et al. [25] found that *V. thapsus* plant had high anticancer activity against liver and lung cancer cells. In another study, Dinani et al. [26] investigated the cytotoxic activities of *V. alceoides* extracts against HeLa (human cervical carcinoma) and HUVEC (human umbilical vein endothelial cell) lines, and suggested that this plant had high cytotoxicity against HeLa cells and low cytotoxicity against HUVEC cells. The results we obtained and the anticancer studies related to the above-mentioned *Verbascum* have shown that species belonging to this plant genus have anticancer activity.

3.4. Genotoxic Analysis Results

The results of DNA damage analysis of ethanol, methanol and water extracts of *V. diversifolium* flowers in effective doses according to IC50 values in MCF-7 and A2780 cell lines are given in Table 5, Table 6 and Figure 2. As a result of Comet experiments, it was determined that the plant extracts added to the culture medium were effective on the parameter values of the cells in the mentioned tables and that this situation was statistically significant ($p<0.05$). It was determined by Comet Assay that the decrease in cell viability after 24 hours of incubation was mainly due to the death of the cells due to DNA damage.

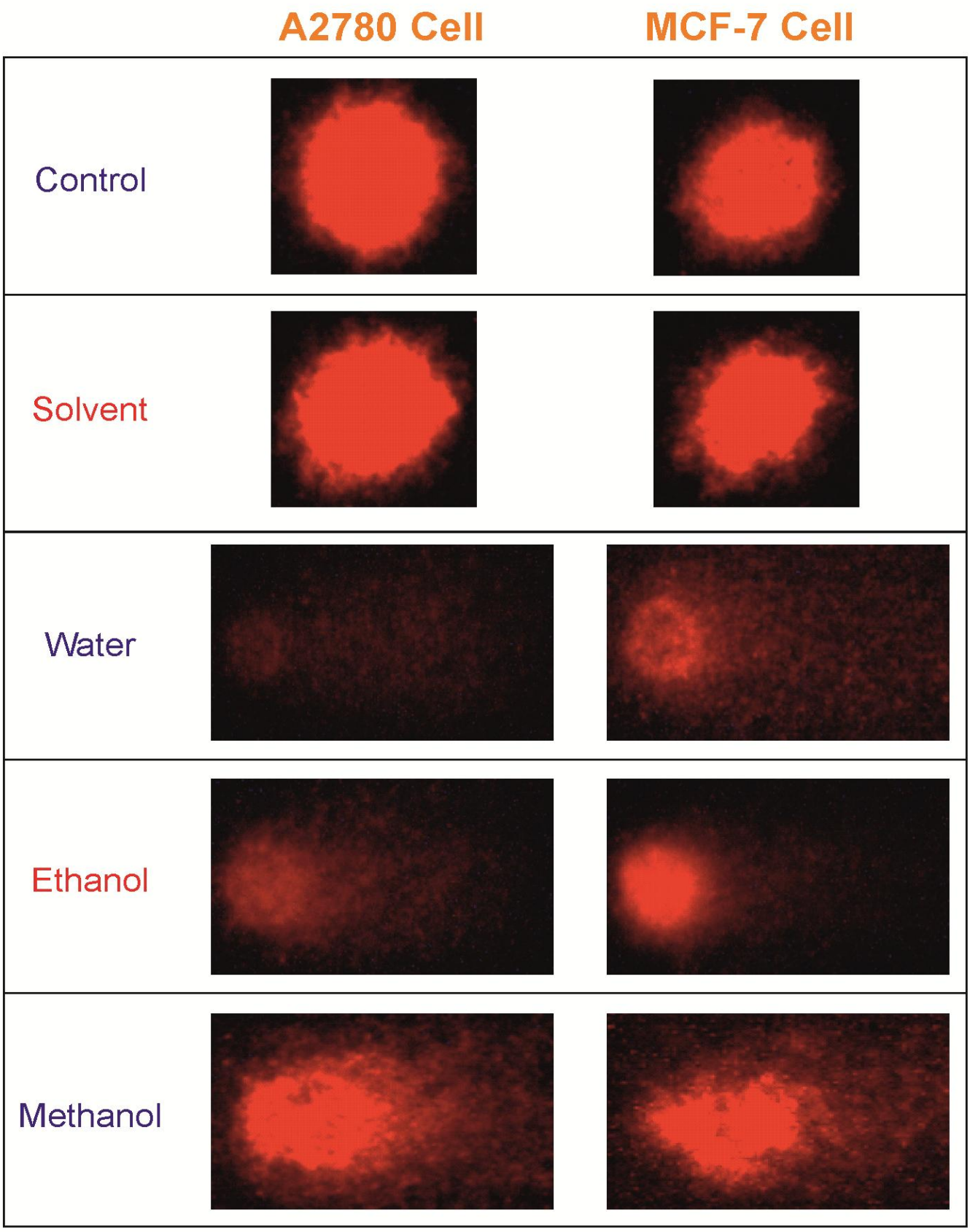

Figure 2 Images obtained from Comet Assay trials for *V. diversifolium* extracts against MCF-7 and A2780 cancer cell lines

Table 5 DNA damage of *V. diversifolium* extracts on A2780 cell line at 24 hours

	Control	Solvent	VDW	VDE	VDM
Tail Length (TL)	4007.40±15 52.64	3696.29±84 5.56	11238.46±1 3643.57*	5466.66±18 32.71*	7813.79±95 5.65
Tail Intensity (TI)	104.25±63. 22	80.39±31.3 8	313.81±586 .83*	158.38±70. 04*	257.37±16. 86
Olive Tail Moment (OTM)	970.77±613 .81	647.17±250 .93	4948.59±74 49.1*	1673.27±13 04.89*	3170.47±27 4.9*
Head Length (HL)	18992.59±4 025.1	19659.25±3 180.13	10400±358 1.28*	17800±521 0.02	11158.62±2 107.64*
Head Intensity (HI)	1298.67±69 9.1	1451.53±49 4.27	423.94±281 .44*	1290.65±73 1.36	507.19±228 .71*

(Results are presented as Mean ± Standard Deviation. *p<0.05)

Table 6 DNA damage of *V. diversifolium* extracts on MCF-7 cell line at 24 hours

	Control	Solvent	VDW	VDE	VDM
Tail Length (TL)	3560±1284 .81	3928.57±1 553.21	17051.85± 13128.78*	9820.51±5 693.72*	9878.26±7 005.38*
Tail Intensity (TI)	406881.63 ±264873.38	534828.21 ±258781.27	261045.62 ±277646.97*	235369.92 ±170904.99*	329247.69 ±253746.81
Olive Tail Moment (OTM)	391257.26 ±271381.57	434532.92 ±299851.59	281611.74 ±219823.58	413668.07 ±249119.23	424428.39 ±274980.73
Head Length (HL)	15080±199 6.27	15242.85± 1533.19	16444.44± 4305.75	13446.15± 4052.92	16886.95± 4860.16
Head Intensity (HI)	431583.56 ±205834.31	614303.85 ±335831.93	329983.55 ±281673.23	390399.46 ±303734.56	322369.91 ±285595.04

(Results are presented as Mean ± Standard Deviation. *p<0.05)

4. CONCLUSION

The presented study is the first study to determine the antiradical, anticancer and phytochemical properties of *V. diversifolium*. According to the antiradical analysis results, all extracts of *V. diversifolium* showed higher antiradical activity than the standard antioxidant BHT in the ABTS radical scavenging test; *V. diversifolium* ethanol and methanol extracts in the DPPH radical scavenging test; *V. diversifolium* methanol extract in OH radical scavenging test. According to phytochemical content analyses, it was observed that *V. diversifolium* methanol extract contained higher amounts of total flavonoids, total phenolics and total proanthocyanidins compared to other extracts. It was determined that the plant is rich in α-tocopherol, phytosterols and unsaturated fatty acids. According to the anticancer analysis results, it was understood that *V. diversifolium* plant has anticancer activity against human breast cancer (MCF-7) and human ovarian cancer (A2780) cell lines depending on the increasing dose. In addition, as a result of the genotoxic analysis performed, it was understood with the Comet test that the plant performs its anticancer activities through DNA damage.

Acknowledgement: This work was supported by Firat University (FÜBAP), Project Number: FF.19.34. This study was derived from the master thesis of Ebru Bahar Ciftci from Firat University, Institute of Sciences, Department of Chemistry. The authors thank Dr. O. Yilmaz and Dr. A. Kocak for their contributions.

Competing interests

The authors declare that they have no conflict of interest.

REFERENCES

[1] Keser F. 2018. Bazı Yenilebilir Bitkilerin Antiradikal, Antibakteriyel ve Antikanser Özellikleri ile İçerdikleri Fitokimyasal Bileşiklerin Saptanması, (In Turkish). Master's Thesis, Fırat University, Institute of Science.

[2] Larson RA. 1988. The antioxidants of higher plants. Pytochemistry. 27: 969–978.

[3] Ramarathnam N, Osawa T, Namiki M, Kawakishi S. 1988. Chemical studies on novel rice hull antioxidants. 1. Isolation, fractionation, and partial characterization. Journal of Agricultural and Food Chemistry. 36: 732–737.

[4] Pratt DE. Hudson BJF. 1990. Natural antioxidants not exploited commercially in food antioxidants. Hudson B.J.F. Ed., Elsevier, Amsterdam. pp. 171–192.

[5] Ames BM, Shigena MK, Hagen TM. 1993. Oxidants, antioxidants and the degenerative diseases of ageing. Proceedings of the National Academy of Sciences of the United States of America. 90: 7915–7922.

[6] Dragsted LO, Strube M, Larsen JC. 1993. Cancer-protective factors in fruits and vegetables: Biochemical and biological background. Pharmacology & Toxicology. 72: 116–135.

[7] Gülçin İ. 2002. Isırgan Otunun (*Urtica dioica*) Antioksidan Aktivitesinin Belirlenmesi, Oksidatif Enzimlerin Karakterizasyonu ve Bazı *İn Vivo* Etkilerinin İncelenmesi, (In Turkish). PhD Thesis, Ataturk University, Institute of Science.

[8] Çiçek EB. 2022. *Astragalus compactus* subsp. *compactus* (Guni) ve Endemik *Verbascum diversifolium* (Nizip Sığırkuyruğu) Taksonlarinin *in vitro* Biyoaktif ve Fitokimyasal Özelliklerinin Belirlenmesi, (In Turkish). Master's Thesis, Firat University, Institute of Science.

[9] Baytop T. 1999. Türkiye'de Bitkilerle Tedavi (In Turkish). 2. Baskı. Nobel Tıp Yayınevi. İstanbul, Türkiye.

[10] Çöllü Z. 2007. *Urtica pilulifera* L. Bitkisinin Antioksidan Aktivitesinin Araştırılması, (In Turkish). Master's Thesis, Ondokuz Mayis University, Institute of Science.

[11] Davis PH. 1988. Flora of Turkey and the East Aegean Islands. Vol. 10, Edinburgh Univ. Press, Edinburgh**.**

[12] Yilmaz M, 2009. *Verbascum antiochium* Boiss. (Scrophulariaceae) Bitki Ekstraktının Antimikrobiyal ve Antioksidan Aktivitesinin Belirlenmesi, (In Turkish). Master's Thesis, Mustafa Kemal University, Institute of Science.

[13] Civelek ED. 2018. *Verbascum pyramidatum* Bieb. Üzerinde Farmakognozik Araştırmalar, (In Turkish). Master Thesis, Hacettepe University, Institute of Health Sciences.

[14] Davis PH. 1969. Flora of Turkey and the East Aegean Islands. Vol. 3, Edinburgh Univ. Press, Edinburgh.

[15] Davis PH. 1978. Flora of Turkey and the East Aegean Islands. Vol. 6, Edinburgh Univ. Press, Edinburgh.

[16] Güner A, Aslan S, Ekim T. Vural M. Babaç MT. 2012. Türkiye Bitkileri Listesi (Damarlı Bitkiler), (In Turkish). Nezahat Gökyiğit Botanik Bahçesi ve Flora Araştırmaları Derneği Yayını. İstanbul.

[17] Keser S. 2014. Antiradical activities and phytochemical compounds of firethorn (*Pyracantha coccinea*) fruit extracts. Natural Product Research. 28: 1789–1794.

[18] Keser S, Keser F, Kaygili O, Tekin S, Demir E, Turkoglu I, Turkoglu S, Parlak AE, Yilmaz O, Karatepe M, Sandal S, Kirbag S. 2020. Phytochemical compounds and antiradical, antimicrobial, and cytotoxic activities of the extracts from *Hypericum scabrum* L. flowers. Natural Product Research. 34: 714–719.

[19] Keser S, Keser F, Karatepe M, Kaygili O, Tekin S, Turkoglu I, Demir E, Yilmaz O, Kirbag S, Sandal S. 2020. Bioactive contents, *in vitro* antiradical, antimicrobial and cytotoxic properties of rhubarb (*Rheum ribes* L.) extracts. Natural Product Research. 34: 3353–3357.

[20] Keser S, Kak O. 2021. In vitro antimicrobial, antiradical, anticancer evaluation, and phytochemical contents of endemic *Scorzonera semicana* DC. Journal of Food Processing and Preservation. 45: e15971.

[21] Koran K, Çalıskan E, Öztürk DA, Çapan I, Tekin S, Sandal S, Görgülü AO. 2023. The first peptide derivatives of dioxybiphenyl-bridged spiro cyclotriphosphazenes: *In vitro* cytotoxicity activities and DNA damage studies. Bioorganic Chemistry. 132: 106338.

[22] Danahaliloğlu H. 2014. Hatay Bölgesinde Yetişen Çeşitli *Verbascum* Türlerinin Bazı Kimyasal ve Biyolojik Özelliklerinin Belirlenmesi, (In Turkish). PhD Thesis, Mustafa Kemal University, Institute of Science.

[23] Marian E, Vicas LG, Jurca T, Muresan M, Pallag A, Stan RL, Sevastre B, Diaconeasa Z, Ionescu CML, Hangan AC. 2018. *Salvia officinalis* L. and *Verbascum phlomoides* L. chemical, antimicrobial, antioxidant and antitumor investigations. Revista De Chimie. 69: 365–370.

[24] Amin HIM, Hussain FHS, Gilardoni G, Thu ZM, Clericuzio M, Vidari G. 2020. Phytochemistry of *Verbascum* species growing in Iraqi Kurdistan and bioactive iridoids from the flowers of *Verbascum calvum*. Plants. 9: 1066.

[25] Gupta A, Atkinson AN, Pandey AK, Bishayee A. 2022. Health-promoting and diseasae-mitigating potential of *Verbascum thapsus* L. (common mullein): A review. Phytotherapy Research. 36: 1507–1522.

[26] Dinani MS, Malakooti S, Akbari V. 2020. *In vitro* cytotoxic activity of *Verbascum alceoides* against cervix carcinoma cells. Journal of Reports in Pharmaceutical Sciences. 9: 19–24.