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PHYTOCHEMICAL COMPOSITION, ANTIOXIDANT, ENZYME INHIBITORY AND CYTOTOXIC ACTIVITIES OF FLOWERS AND LEAVES OF *MALVA SYLVESTRIS* L.

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ABSTRACT. The objective of the study was to assess the biological functions and chemical composition of *Malva sylvestris* L. from Ida Mountain of Turkiye, a medicinal plant used for a variety of therapeutic applications. The antioxidant (DPPH, ABTS, Iron (II) chelate activity), enzyme inhibition (acetylcholinesterase), and cytotoxic properties of methanol extracts prepared from leaves and flowers were investigated. The chemical composition of the extracts was evaluated in terms of spectrophotometric (total phenol and total flavonoid) and chromatographic (HPLC) techniques. IC50 value of the DPPH radical scavenging effect of the flower extract, with the highest total phenol and flavonoid content, was found to be 0.5 mg/mL. The ABTS radical scavenging effect was 2.56 mmol/ L Trolox. While the extracts' chelating activity was not as great as that of EDTA, the enzyme inhibition of the flower extract was determined to be 37.67%. Flower extract was shown to have the most cytotoxic activity in both Hela and Hep G2 cell lines. In HPLC analysis; amounts of the detected phenolic compounds were determined, and method validation was performed. This research has given us a better understanding of the traditional use of the *M. sylvestris* plant from Turkiye, which stands out for its therapeutic properties.

Keywords: *M. sylvestris; Malvaceae; antioxidant; anticholinesterase activity; cytotoxic activity*

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INTRODUCTION

Humans have been using medicinal plants to heal ailments for centuries. Nowadays, there is a growth in the usage of medicinal plants across the world as a result of their demonstrated efficiency in treating specific ailments and assertions that their usage is safe. Medicinal plants, with the knowledge they have accumulated over time, also contribute to the production of medicines today [1]. Active compounds produced during secondary plant metabolism are generally responsible for the biological properties of medicinal plants. Studies on the chemical structures of plants and the mechanisms behind their biological processes are increasing day by day [2].

Malva sylvestris L. from the Malvaceae family is known as “ebegümeci” in Turkiye. The biennial-perennial herbaceous plant. *M. sylvestris* is often found in North Africa, Europe, and Southwest Asia [3,4]. The leaves are heart-shaped and have 5 to 7 lobes, and the flowers are a bright pink color with purple streaks [5]. This medicinal plant has historically been used to cure a variety of illnesses and disorders, including colds, burns, coughs, tonsillitis, bronchitis, digestive issues, dermatitis, and cut wounds [6]. The most often utilized components of *M. sylvestris*, the leaves, and flowers, contain a variety of bioactive substances, including, mucilages, phenol derivatives, flavonoids, tannins, coumarins, sterols, terpenoids, saponins, alkaloids. Many researches have demonstrated the antibacterial, anti-inflammatory, hepatoprotective, and antioxidant benefits of this plant given its rich composition [6-10].

Even though *M. sylvestris* is a plant with extensive traditional usage and has been the focus of several studies, there are still issues that require further investigation. Above all, it appears that this species’s effectiveness in several cancer cell lines has not been established. Determining the toxicity of both flower and leaf extracts to Hela and Hep G2 cell lines, as well as if there is a relationship with their antioxidant capabilities, is the primary objective of our investigation. It was also aimed to determine the chemical composition of the extracts with HPLC and to evaluate their activities on acetylcholinesterase enzyme. To the best of our knowledge, this study represents the first comprehensive investigation, encompassing not only the exploration of the anticancer effects of the plant on specified cells but also concurrent phytochemical analyses, alongside the examination of other biological activities. This research holds significance in the context of comparative analyses with previous studies conducted to date, as it pioneers the simultaneous examination of phytochemical profiles along with specific biological activities, particularly focusing on the anticancer potential of the plant in question.

RESULTS AND DISCUSSION

Chemical composition

The highest total phenol content was found in a flower extract with a value of 172.77±14.08 $mg_{GAE}/g_{extract.}$ In total flavonoid content, flower extract has the highest content with a value of 73.72±3.34 $mg_{CA}/g_{extract}$ also. The results are given in Table 1. The total phenol content of *M. sylvestris,* which was collected in Pakistan and made using methanol extract from the aerial parts, was 59.91±0.08 $mg_{GAE}/g_{extract}$. In contrast, the total flavonoid content was 61.12±0.117 $mg_{RE}/g_{extract}$ [11]. As compared to our findings, we may conclude that the species collected from the outskirts of Ida Mountain in Turkiye have higher phenol and flavonoid contents.

Table 1. Total phenol/flavonoid amount of extracts of *M. sylvestris* and contents of caffeic acid, chlorogenic acid, coumaric acid, ferulic acid, rutin, rosmarinic acid, and tannic acid in extracts (n=3)

	Flower extracts	**Leaves extracts**
Caffeic acid (%±SD*)	0.261±0.003	0.248±0.005
Chlorogenic acid (%±SD*)	0.164±0.008	0.199±0.003
Coumaric acid (%±SD*)	0.144±0.001	0.130±0.001
Ferulic acid (%±SD*)	0.061±0.004	0.056±0.008
Rutin (%±SD*)	0.304±0.003	ND*
Rosmarinic acid (%±SD*)	0.105±0.009	ND*
Tannic acid (%±SD*)	2.784±0.433	ND*
Total Phenol [mg_{GAE}/g_{extact}]	172.77±14.08	148.86±4.94
Total Flavonoid [$mg_{CA}/g_{extract}$]	73.72±3.34	40.19±1.37

ND*: Not determined

The levels of caffeic acid, chlorogenic acid, coumaric acid, ferulic acid, rutin, rosmarinic acid, and tannic acid in the plant extracts were calculated using HPLC analysis (Figure 1), and the results are shown in Table 1. In HPLC analysis performed; caffeic acid, chlorogenic acid, coumaric acid, ferulic acid, rutin, rosmarinic acid, and tannic acid were detected in the flower extract, while only caffeic acid, chlorogenic acid, coumaric acid, and ferulic acid were detected in the leaves extract. The amounts of the detected phenolic compounds were

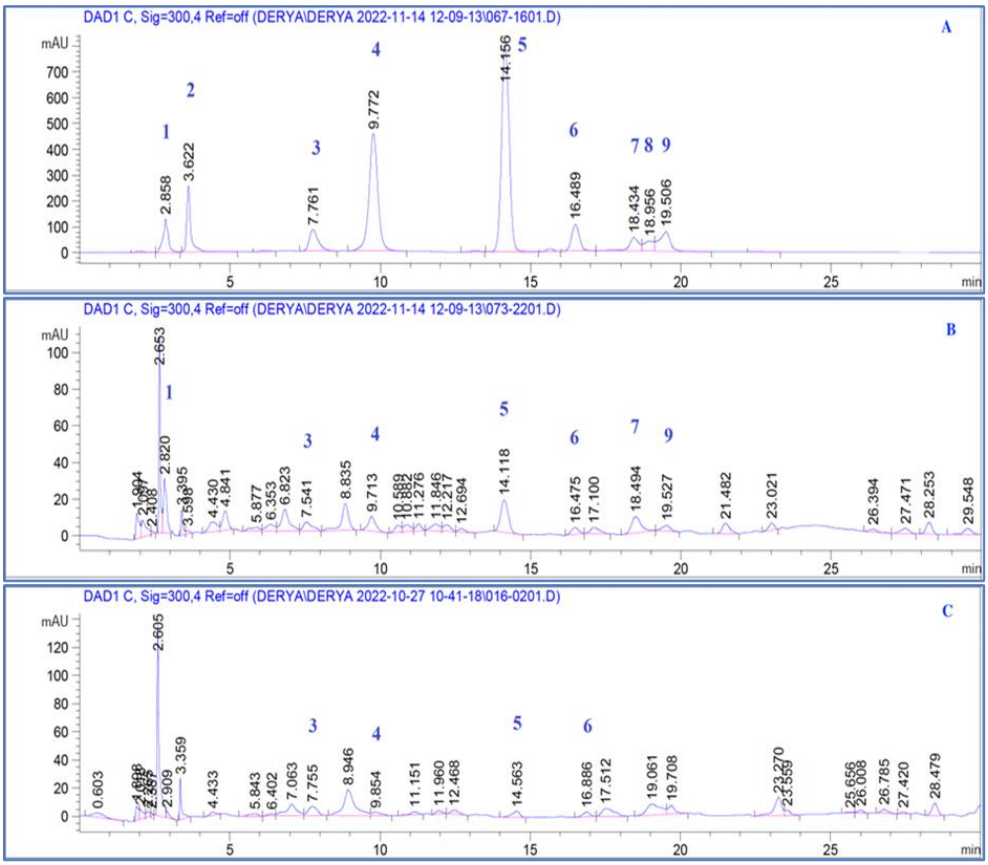

Figure 1. HPLC chromatograms. A. Standards: (1) Tannic acid, (2) Gallic acid, (3) Chlorogenic acid, (4) Caffeic acid, (5) Coumaric acid, (6) Ferulic acid, (7) Rutin, (8) Hyperoside, and (9) Rosmarinic acid; B. Flower extract; C. Leaf extract

determined, and method validation was performed. Calibration values, precision data, and statistical information from the recovery assays are included in Tables 2 and 3. The amounts of tannic acid (2.784±0.433 %) and rutin (0.304±0.003 %) in the flower extract were found to be higher than those of the other compounds. Likewise, caffeic acid content was found to be higher (0.248±0.005 %) in the leaf extract. Rutin, rosmarinic acid, and tannic acid were not detected in the leaf extract. In the study of Terninko et al. (2014), the presence of rutin and rosmarinic acid was also determined in flower extracts [12]. DellaGreca et al. (2009) isolated 4-hydroxybenzoic acid, 4-methoxybenzoic acid, 4-hydroxy-3-methoxybenzoic acid, 4-hydroxycinnamic acid, ferulic acid, methyl 2-hydroxydihydrocinnamate, scopoletin, N-trans-feruloyl

tyramine, and a sesquiterpene, (3R,7E)-3-hydroxy-5,7-megastigmadien-9-one from *M. sylvestris* leaf water extract [8]. In different studies, the presence of oxalic, malonic, fumaric, benzoic, malic, vanillic, ferulic, salicylic, and p-coumaric acids was detected in the leaves [13,14]. Luteolin, kaempferol, myricetin, apigenin, genistein, quercetin, kaempferol-3-*O*-rutinoside and quercetin-3-*O*-rutinoside and caffeoylquinic acid were detected in flowers [15]. In *M. neglecta* and *M. sherardiana* species, *p*-coumaric acid, caffeic acid, and rutin were found to be similar to our study [16].

Table 2. Calibration values for standards and precision data of the method

						Intra-day precision (RSD*%)			**Inter-day precision (RSD*%)**		
						Amount					
	Calibration range (µg/mL)	**Linear Equation**	**Correlation factor (r^2±SD*)**	**LOD µg/mL**	**LOQ µg/mL**	**50 µg/mL**	**100 µg/mL**	**200 µg/mL**	**50 µg/mL**	**100 µg/mL**	**200 µg/mL**
Caffeic acid	10-200	y=110424x-1591.6	0.9805±0.006	0.078	0.260	0.937	0.634	1.797	1.419	2.380	2.303
Chlorogenic acid	10-200	y=23710x-144.8	0.984±0.001	0.212	0.709	1.763	2.196	0.715	2.049	2.514	1.539
Coumaric acid	10-200	y=259913x-1950.2	0.997±0.008	0.014	0.047	1.445	0.395	1.244	3.835	3.423	0.948
Ferulic acid	10-200	y=24109x-27.692	0.999±0.003	0.067	0.225	0.583	0.869	0.558	1.118	3.253	1.127
Rutin	10-200	y=15370x-87.448	0.997±0.006	0.126	0.420	0.790	0.458	0.413	1.839	0.512	2.532
Rosmarinic acid	10-200	y=45771x-218.14	0.998±0.001	0.077	0.257	1.581	1.131	0.841	2.427	1.691	0.696
Tannic acid	10-200	y=1002.9x+43.687	0.990±0.002	0.418	1.394	2.625	2.993	1.243	3.846	1.544	0.741

Table 3. Recovery assay's statistical data of the method (n=3)

Standards	Concentration in sample (mg/mL)	Amount spiked (mg/mL)	Mean amount found in mixture (mg/mL)	Mean recovery (%±SD*)	RSD (%)
Caffeic acid	0.02	0.01	0.015	96.490±0.062	0.065
		0.02	0.02	96.259±0.282	0.293
		0.04	0.03	100.744±1.078	1.070
Chlorogenic acid	0.01	0.005	0.008	104.559±0.324	0.310
		0.01	0.01	103.645±0.233	0.224
		0.02	0.015	103.971±0.872	0.839
Coumaric acid	0.01	0.005	0.008	100.119±0.024	0.024
		0.01	0.01	95.332±0.205	0.215
		0.02	0.015	99.905±1.952	1.954
Ferulic acid	0.004	0.002	0.003	99.601±1.884	1.891
		0.004	0.004	103.39±0.285	0.276
		0.008	0.006	95.196±1.192	1.252
Rutin	0.02	0.01	0.015	100.539±0.591	0.587
		0.02	0.02	97.433±0.447	0.459
		0.04	0.03	101.028±1.072	1.061
Rosmarinic acid	0.006	0.003	0.005	106.398±0.004	0.004
		0.006	0.006	103.773±0.621	0.599
		0.012	0.009	98.784±0.699	0.708
Tannic acid	0.2	0.1	0.15	103.773±1.01	0.978
		0.2	0.2	103.587±0.430	0.415
		0.4	0.3	99.706±2.532	2.539

Antioxidant Activity

The scavenging effect of the extracts on both DPPH (1,1-Diphenyl-2-picrylhydrazyl radical) and ABTS (2,2′-Azino-bis (3-ethylbenzothiazoline-6-sulfonic acid) diammonium salt) radicals was evaluated. Flower extract with high total phenol and flavonoid content showed a stronger effect against both radicals. While none of the extracts could scavenge DPPH radicals as strongly as standard BHT (Butylated hydroxytoluene), the flower extract had the same significance as BHT against ABTS radical at 2 mg/mL concentration ($p>0.05$). The results are presented in Table 4. In the study by Irfan et al. (2021), the IC50 value of the dichloromethane fraction of the aerial part extract against the DPPH radical was found to be 22.11 µg/mL, and the IC 50 value of standard ascorbic acid was 7 µg/mL [11]. When the activity of the extract and the standard is proportioned, it corresponds to 3.13, while the ratio of extract/standard BHT corresponds to 5 in our study. Although the experimental procedures are different, it is seen that the activity rates are close to each other. In a different investigation, the butanol

fraction of the aerial part water extract was shown to have an IC50 value of 78.14 µg/mL against the DPPH radical and an IC50 value of 12.55 µg/mL for Trolox. In comparison, the IC 50 value against the ABTS radical was 166.79 µg/mL and that for Trolox was 188.16 µg/mL [17]. In this study, the extract and standard substance activity rates were found to be compatible with our study. Interestingly, in the study of Beghdad et al. (2014), leaf extract was emphasized as a stronger antioxidant than flower extract [18]. Similar to our results Petkova et al. (2019) discovered that flower extracts were more antioxidant than leaves in DPPH and FRAP assay [19].

The measurement of the chelating activity of the extracts for Fe^{2+} ions was studied at concentrations in the range of 250-8000 µg/mL, and Na_2EDTA at concentrations in the range of 100-350 µg/mL. It was observed that the Fe^{2+} chelating activity of the extracts increased depending on the concentration, but it was determined that no extract showed as much activity as EDTA used as a standard (Figure 2). Reducing their concentration in the medium by binding transition metal ions and thus delaying Fe^{2+} catalyzed lipid peroxidation is an important mechanism for measuring antioxidant capacity. Flavonols with 3-hydroxy-4-keto or 5-hydroxy-4-keto groups exhibit substantial metal chelation activities and form stable complexes with metals, according to the literature [20]. In this experiment, the stronger chelating of flower extracts was associated with the presence of anthocyanins present in the flowers [21]. Only the chelating activity of leaves has been previously assessed, even though there is no study in the literature that documents the metal chelating activity of flowers. The methanolic extract of *M. sylvestris* was shown to have remarkable activity in terms of chelating iron ions, with an IC50 value of 52.7±1.8 µg/mL [10].

Table 4. Radical scavenging effects of the *M. sylvestris* extracts

Extracts	DPPH IC50 (mg/mL)	TEAC mmol/ L Trolox
Flower extract	0.50±0.02*	2.56±0.08^{a} (2 mg/mL) 2.152±0.15^{b} (1 mg/mL)
Leaf extract	0.84±0.14**	2.48±0.16^{a,b}(2 mg/mL) 1.93±0.19^{c}(1 mg/mL)
BHT	0.1±0.05***	2.79±0.01^{a}(2 mg/mL) 2.63±0.03^{a}(1 mg/mL)

Values expressed as mean±standard error (n = 3), statistical analyses by Tukey comparison test. Bars with the same lower-case letters (a–b,) superscripts (*–***), are not significantly ($p>0.05$) different.

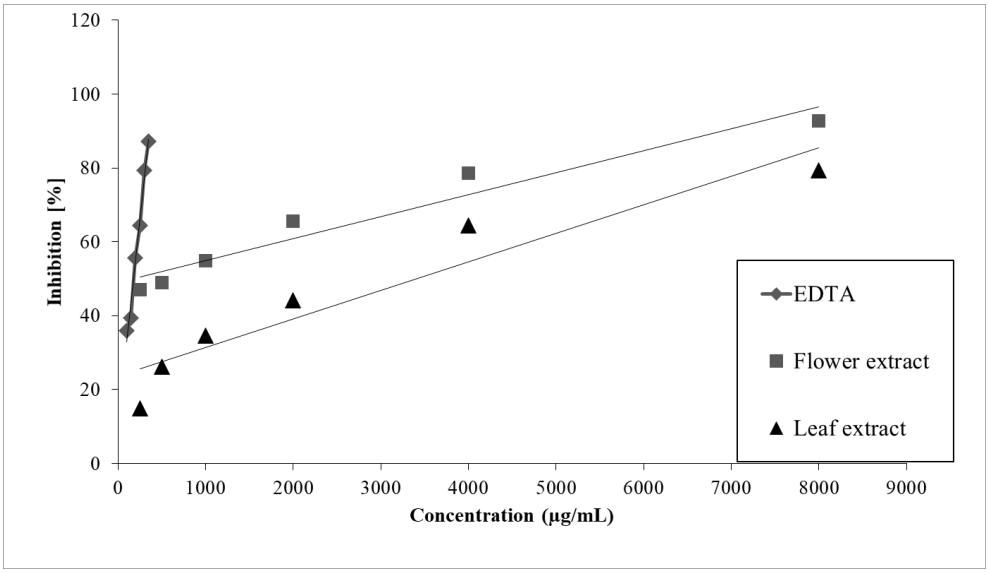

Figure 2. The chelating activity of extracts for Fe^{2+} ions

Acetylcholinesterase Enzyme Inhibition Activity

Acetylcholine is a neurotransmitter that is largely blocked by acetylcholinesterase (AChE) and is thought to have a role in the pathophysiology of Alzheimer's disease. Despite the fact that the cause of Alzheimer's disease is unknown, increasing acetylcholine levels through AChE enzyme inhibition is widely accepted as the most effective treatment strategy [22]. Table 5 provides a summary of the plant extracts' AChE inhibitory effects. Extracts were studied at a concentration of 8 mg/mL and standard galanthamine at a concentration of 1 mg/mL. Despite being at high concentrations, neither extract was statistically significant with galanthamine ($p>0.05$). While most acetylcholinesterase inhibitors contain nitrogen, the limited efficacy of these extracts might be attributed to a lack of alkaloid content [31]. Although there is little evidence of *M. sylvestris* AChE inhibition, the research found that a decoction of the aerial part decoction inhibited AChE by 25% at 5 mg/mL [7]. Results comparing enzyme inhibition of flower and leaf extracts are presented for the first time in this article.

Table 5. AChE inhibitory activities of the M. *sylvestris* extracts

	AChE Inhibition%
Flower extract (8 mg/mL)	37.67±2.15[a]
Leaf extract (8 mg/mL)	33.56±2.13[a]
Galanthamine(1 mg/mL)	63.43±1.64[b]

The values are exhibited as the mean ± standard error (SE, n = 3), and statistical comparisons were performed using the Tukey comparison test. Bars with the same lowercase letters (a–b) do not exhibit significant differences ($p > 0.05$).

Cytotoxic activity

Both flower and leaf extracts were more effective against the HeLa cell line. It was determined that flower extract inhibited viability significantly ($p<0.05$) even at 31.25 µg/mL concentration. The viability was found to be 25.57% at 1000 µg/mL in the group to which the flower extract was administered ($p<0.001$). Leaf extract showed a significant inhibition effect on viability in the concentration range of 62.5-1000 µg/mL. The viability was 38.47% in the leaf extract applied group at 1000 µg/mL ($p<0.001$). In the Hep G2 cell line, flower extract showed a significant inhibition effect on viability in the concentration range of 125-1000 µg/mL, while leaf extract showed significant inhibition only at 1000 µg/mL. Flower extract decreased the viability by 51.19% at 125 µg/mL ($p<0.01$). When all the results were examined, the flower extract was found to be more effective than the leaf extract in both cell lines. Results are given in Figure 3. Total phenol, flavonoid contents, as well as rutin, rosmarinic acid, and tannic acid, which are found in the flower extract different from the leaf extract, may be responsible for this effect. In a study with *M. sylvestris* leaf extract, it was reported that it showed a cytotoxic effect for B16 (murine melanoma) and A375 (human melanoma) cell lines. According to research, the extract had antiproliferative activity in B16 cells that was 61% and 97% higher than that of the control 1:200 and 1:40 dilutions, respectively. A 1:10 dilution resulted in a substantial 58% decrease in cell proliferation in A375 cells compared to the control [23]. To our knowledge, there is no study in the literature that determined the cytotoxic activity of flower and leaf extracts of *M. sylvestris* in HeLa and Hep G2 cell lines. *M. sylvestris* leaf hydro methanolic extract was studied in MCF-7 (human breast carcinoma), Hep G2 (human epiglottis cancer), and WEHI (mouse leukemia) cell lines in a study by Boutennoun et al. (2019), and its toxicity was found to be 45.20%, 62.62%, and 82.04%, respectively, at 125 µg/mL concentration [24]. More recently the cytotoxicity of Fe_3O_4 nanoparticles synthesized by *M. sylvestris* extract was analyzed by exposure to MCF-7 and Hep-G2 cancer cell lines, and the

IC50 value was reported as 100 µg/mL and 200 µg/mL, respectively [14]. Comparative toxicity of *M. sylvestris* leaf and flower extracts in HeLa and Hep G2 cell lines was elucidated for the first time in this study.

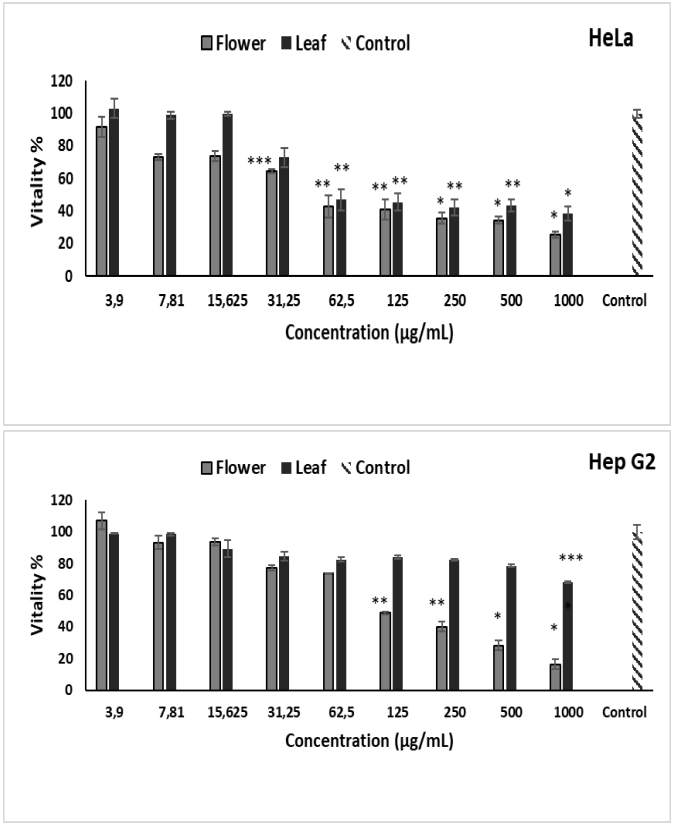

Figure 3. Cytotoxic activities of the extracts on HeLa and Hep G2 cell line. Values are given as mean ± sd (n = 3), statistical analyses by Dunnett's comparison test. *** p<0.05 ** p<0.01* p<0.001

CONCLUSION

We demonstrated, for the first time, the potential use of *M. sylvestris* flowers and leaves gathered from Ida Mountain of Turkiye as a functional food due to the presence of polyphenols and some biological activities exhibited *in vitro. M. sylvestris* flowers are a rich source of phenolic compounds. The extracts have demonstrated antioxidant and acetylcholinesterase-inhibiting

properties, as well as cytotoxic activity in cancer cell lines. This study supports the idea of using different parts (leaves and flowers) of traditionally used plants grown in different regions as edible, healthy ingredients with health-protective properties.

EXPERIMENTAL SECTION

Plant material and Extraction procedure

M. sylvestris were gathered in Altınoluk, Balıkesir. in May 2022. Dr. Derya Çiçek Polat authenticated the sample. *M. sylvestris* samples' flowers and leaves were separated and then dried individually. At room temperature, dry materials (each sample 100 g) were pulverized and extracted three times with methanol (24 h). An ultrasonic bath was used to complete the extraction (60 min.) After filtering, the extracts were concentrated to dryness in an evaporator. They were kept in the refrigerator throughout the study.

Total phenolic and flavonoid content

Total phenol and flavonoid levels were computed using gallic acid equivalents (GAE) and catechin equivalents (CA), respectively. The Folin-Ciocalteu method was employed to figure out the total quantity of phenolic substances in the extracts [25]. The total flavonoid level was measured using a colorimetric aluminum chloride assay [26].

High-performance liquid chromatography (HPLC) analysis

30 mg dry extracts were dissolved in 5 mL methanol (6 mg/mL) to make the sample solution. Vortex was used to homogenize the dissolution and ensure easy dissolution. Standards were prepared at a 500 µg/mL concentration for stock solution. A Waters Spherisorb C18 column (25 cm 4.6 mm, 5 m) was utilized for measurement. The gradient system delivered the mobile phase, which was 0.01% formic acid (A) and acetonitrile (B), at a 1 mL/min flow rate and maintained at 40 °C. The proportion of B was increased from 10% to 30% over 30 minutes, then returned to the initial conditions in 5 minutes. Measurements were carried out at a wavelength of 300 nm, because of obtained the best results in all samples.

Three injections of each of the five distinct standard concentrations (10, 25, 50, 100 and 200 mg/mL) were made. A calibration curve for quantification was created for each standard. Accuracy, precision, limit of detection (LOD), limit of quantitation (LOQ), and recovery values were computed for method validation [27,28]. It was determined using intra-day and inter-

day variance for precision assay. On the same day, triple injections of all standard solutions in three different concentrations were evaluated as precision of intra-day. The method used for intra-day precision was repeated on different days for inter-day precision, and differences were expressed by RSD. Ten injections of standards were done to analyze the LOD and LOQ values and the signal/noise ratio was determined. LOD, signal/noise value is 3:1, LOQ signal/ noise value is 10:1. Three different known concentrations of the standard were added to the sample for the recovery analysis, and the recovery percentage was computed.

For the robustness investigation, minor changes to the flow rate, column temperature, mobile phase, and wavelength were made and their effects were investigated.

Antioxidant Activity

DPPH• Radical Scavenging Activity

The method described by Hatano et al. (1989) was adapted and used to determine the extracts' DPPH radical scavenging properties [29]. In 96-well plates, 100 µL of extract solutions at various concentrations were dispersed, followed by 100 µL of DPPH (0.1 mM, in ethanol) solution. As a reference antioxidant, BHT (Butylated Hydroxytoluene) was utilized. The absorbance at 517 nm was recorded after 30 minutes in the dark at 37 °C to quantify the radical scavenging effect. The experiments were carried out three times. The percentage inhibition was calculated using Eq. 1. Non-linear regression curves were used to calculate IC50 values (Sigma Plot 2001 version 7.0, SPSS Inc., Chicago IL). (Sigma Plot 2001 version 7.0, SPSS Inc., Chicago IL).

$$\% \text{ Inhibition} = [(\text{Absorbance control} - \text{Absorbance sample}) / (\text{Absorbance control})] \times 100 \quad (1)$$

$ABTS^{\bullet+}$ Radical Scavenging Activity

By maintaining an aqueous solution of ABTS (7 mM) and potassium persulfate ($K_2S_2O_8$) (2.45 mM, final concentration) in the dark for 12-16 hours, an $ABTS^+$ radical was generated, and its absorbance at 734 nm was adjusted to be 0.700 (±0.020). Extracts were made in two concentrations (1 and 2 mg/mL). As a control antioxidant, BHT was utilized. The produced radical solution and extract were combined in an amount of 990 µL and 10 µL respectively. The reaction kinetics were measured at 734 nm once every minute for 30 minutes [30]. The percentages of inhibition examined versus concentration were found to be equal to Trolox (TEAC).

Iron (II) Chelate Activity

The extract solution was combined with 100 µL of 2.0 mM aqueous $FeCl_2$ and 900 µL of methanol in a volume of 200 µL. After five minutes, the reaction was accelerated by 400 µL of 5.0 mM ferrozine solution, and the absorbance at 562 nm was measured after ten minutes. Na_2EDTA was utilized as a control and the percentage inhibition of extracts was analyzed. The percentage inhibition was calculated using Eq. 1. Non-linear regression curves were used to calculate EC50 values (Sigma Plot 2001 version 7.0, SPSS Inc., Chicago IL). (Sigma Plot 2001 version 7.0, SPSS Inc., Chicago IL). The average of three parallel experiments was used to calculate the results [31].

Acetylcholinesterase Enzyme Inhibition Activity

With minor modifications, Ellman's method was employed to examine the extracts' ability to inhibit acetylcholinesterase (AChE) [32]. The 96-well plates were loaded with a sample (25µL), buffer (50 µL), and AChE solution (25 µL at 0.22 U/mL). The plates were then incubated for 15 minutes at 25 °C. Following that, 25 µL of ATCI substrate and 125 µL of DTNB (3.0 mM, 5,5-dithiol-bis-(2-nitrobenzoic acid)) were added. A microplate reader was used to read the mixture at 412 nm after it had been kept at 25 °C for 15 minutes. As a positive control, galantamine solution was prepared at a concentration of 1 mg/mL. A blank control was also created by blending the sample solution with all of the other solutions.

Cytotoxic Activity

The American Type Culture Collection provided the HeLa (CCL-2TM cervical cancer) and Hep G2 (HB-8065TM hepatocellular carcinoma) cell lines. The cells were cultured in RPMI and DMEM, respectively, with 1% combined antibiotics (penicillin and streptomycin) and 10% fetal bovine serum at 37 ºC and 5% CO_2.

HeLa and Hep G2 cells were sown at a density of $1x10^4$ cells/mL 100 (100 µL each well) in a 96-well plate and separated into three groups: blank, control, and extracts (3.9; 7.81; 15.6; 31.25; 62.5; 125; 250; 500, and 1000 µg/L). After 24 hours of incubation, cells were treated with 100 µL of vehicle or samples for 24 hours. The MTT 1-(4,5-Dimethylthiazol-2-yl)-3,5-diphenyl formazan) reagent (stock: 5 mg/mL in PBS) was then applied to each well and incubated for 4 hours at 37 °C. Each well received 100 µL of DMSO to dissolve the formazan crystals produced by MTT. After 10 minutes, each well was examined using a microplate reader with a 540 nm wavelength [33].

Statistical Analysis

For pairwise comparison tests, the Dunnett and Tukey tests were used at the p <0.05 level using the SPSS Version 11.0 statistic software package.

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