UDC 58.08; 58.04
DOI https://doi.org/10.32848/agrar.innov.2025.30.8

CHEMICAL CHARACTERIZATION, NEURODEGENERATIVE AND ANTIOXIDANT POTENTIALS OF TWO DIFFERENT HAWTHORN (*CRATAEGUS* SPECIES) FRUITS FROM AZERBAIJAN

GULIYEVA L. – PhD
orcid.org/0000-0002-3951-9083
Azerbaijan State Agricultural University
YILDIRIM Y. – PhD
orcid.org/0000-0002-3014-4510
Ege University, Turkiye
ABBASOVA T. – PhD
orcid.org/0000-0003-3455-0652
Azerbaijan State Agricultural University
İNCE I. – PhD
orcid.org/0000-0003-3045-6482
Ege University
GÜMÜŞTAŞ B. – Lecturer
orcid.org/0000-0002-3640-925X
Ege University
CUMAOĞLU A. – PhD
orcid.org/0000-0002-3997-7746
Erciyes University
YARARBAŞ G. – PhD
orcid.org/0000-0002-2993-5577
Ege University
BÖKE SARIKAHYA N. – PhD
orcid.org/0000-0002-8200-8561
Ege University

Introduction. Hawthorn is the common name of the *Crataegus* species in the *Rosaceae* family, and there are more than 1000 species in Asia, Europe, and North America, especially in the Northern hemisphere. The scientific name of hawthorn comes from the Greek word “kràtaigos” which means “strength and robustness” due to its hard and durable wood. The history of research and use of hawthorn goes back to ancient times. Even in the works of the ancient Greek philosopher Theophrastus (372-287 BC), hawthorns are described as medicinal and food plants. Today, hawthorn fruits, which are widely used as food and medicinal materials, are consumed fresh or processed in daily life in jams, jellies, soft drinks, confectionery and preserves [5, p. 5-26]. Many beneficial properties have also been attributed to hawthorn, including anticancer, antioxidant, anti-inflammatory [2, p. 315-324], atherosclerosis [11, p. 211-223], anti-diabetic, antihypertensive, effects and treatment of cardiovascular diseases. The pharmacological and therapeutic properties of hawthorn fruits are related to the quantity and quality of biologically active substances in their composition. The chemical composition of hawthorn fruits and flowers has been studied by many scientists. It has been reported that hawthorn contains many bioactive molecules, including phenolic acids, terpenoids, lignans, steroids, flavonoids, anthocyanins and sugars [4, p. 66-69; 5, p. 5-26].

Recent research shows that especially phenolic compounds are the main group of biologically active components in hawthorn [12, p. 67-71]. In the literature, there are many studies reporting that the hawthorn plant is rich in phenolics and that there is a linear relationship between the liberation of total phenols and total flavonoids and the inactivation of free radicals. They stated that the antioxidant capacity differs from the regions where the hawthorn plant is grown, from the parts of the fruit such as the peel, fruit or pulp from which it is extracted, and especially according to the different extraction methods and solvent systems used. Hakima et al. investigated the antioxidant effect of the hawthorn plant (*Crataegus monogyna*) collected from the Middle Atlas Mountains of Morocco as a potential source of new bioactive natural compounds. In this study, flowers, leaves, ripe and unripe fruits were analyzed by 2,2-diphenyl-1-picrylhydrazil (DPPH) free radical capture method. They suggested that the antioxidant activity showed a significant correlation with the total polyphenol content and that *C. monogyna* extracts exhibited significant antioxidant activity and thus it could offer great potential as a natural source of antioxidants [7, p. 30-35].

There are several of medicinally active phytochemicals that have been isolated from hawthorn with most of the data generated in studies of those species that are native to Europe and Asia. Comparatively little is known about the Azerbaijan *Crataegus* species. With this in mind, the aim of this paper is to provide a comprehensive review of the chemical content and biological potency of Azerbaijan hawthorns. In this study, the fruit samples from the branches of two different hawthorn tree (*C. pentagyna* and *C. caucasica*), from Azerbaijan were performed for their antioxidant activity by DPPH along with total flavonoid, sugar content

and fatty acid components were achieved by UV-Visible spectrofotometer, HPLC-RID and GC-FID, respectively. The capacity of hawthorn extracts to reduce H_2O_2 toxicity on SH-SY5Y cells at non-toxic concentrations was examined using the cell viability test. The determination of color index of the extracts was also examined by Lovibond Tintometer.

Material and methods. Quercetin, acetic acid and DPPH were obtained from the Sigma. Fructose, glucose, sucrose and aluminum chloride were purchased from Merck. Sorbitol used was of Phytoteclab. Methanol and ethanol were supplied from Supelco. Deioniozed water was generated using a Milli-Q Water Purification System. All other reagents used in this study were analytical grade.

Plant Materials

Two different fruit samples, hawthorns which exudate from the stem of the tree *Crataegus pentagyna* Waldst. do Kit. ex Willd. and *Crataegus caucasica* C. Koch. (Fig. 1) were collected from 1238 m altitude, northern part of the Lesser Caucasus, Goy-gol district (the borders of the Republic of Azerbaijan, N 40026'229'', E 046020'312'') in October 2022 between 11^{00}-12^{00} hours in the phase of biological maturity. The plant was identified by T.A. Kasumova and then was archived at Azerbaijan National Academy of Sciences Institute Botany Herbarium Foundation with the number *C. pentagyna* 91359, *C. caucasica* 91286. The fruits were harvested by hand and dried at room temperature, kept away sunlight.

Preparation of Extracts

After collecting all fruits from two different hawthorn trees (*Crataegus pentagyna* and *Crataegus caucasica*), they were grinded in laboratory grinder separately. The grinded fruits (100 g) were extracted using *n*-hexane, ethanol, acetone, and ethyl acetate by Soxhlet apparatus for 6 hours. The solvent was removed with a rotary vacuum evaporator at 40°C. All extracts were kept on dark at +4°C until LC-MS, GC-FID analysis, and biological activity studies [10, p. 116-123].

Determination of color index of the extracts

Color determination analysis was done by Lovibond Tintometer (PFX880). This instrument incorporates calibrated color standards for the particular scale of interest and is operated as a stand-alone instrument. The dried 50-100 mg of each extract was dissolved in their solvent in a 5 mL volumetric flask. The flask was kept in an ultrasonic bath mixed and warmly heated until a clear solution was obtained. Then, the solution was filtered through a 0.45 µm Millipore Millex-HV filter and was placed in the tube of the instrument. The samples were kept at +4°C until the analysis [13, p. 733-736].

GC-FID Analysis

Methyl esters of fatty acids were analyzed with an Agilent 6890 GC-FID combined system using Agilent HP-88 capillary column (100 m x 0.25 mm ID x 0.2 µm COI/T.20/Doc. No 33) for olive oils method was used for fatty acid methyl esters (FAME) determination. A thirty-seven (37) component mixture of FAME (Supelco) has been chosen as the standard for retention time to identify the fatty acids. 5 mg of each extract were mixed with 2ml 2M potassium hydroxide in methanol and vortexed. 2 ml of isooctane was added on the mixture and vortexed again. Then the samples were centrifuged at 3000 rpm for 4 min. Approximately 1 mL of the supernatant was vialed and injected 1 µL of sample into the GC-FID system. The temperature program was as follows; the oven temperature is held at 120°C for 1 min and then increased to 240°C at a rate of 4°C/min and held for 5 min. Injection and detector temperatures were 250°C and 260°C, respectively. Area ratio which is under the relevant peak was used for the quantitative analysis. Split ratio was 1:100. Helium was used as carrier gas at constant flow rate (1mL/min) [10, p. 116-123].

Total Flavonoid Content

The total flavonoid content method was created by modifying two different methods [6, p. 1-10]. 5 mL of 5% acetic acid and 0.25 mL of 2% aluminum chloride were added to 5 ml of diluted extract, respectively. After shaking for 30 sec, it was kept in the dark for 30 min. After this period, measurements were made at 425 nm against methanol blank in Shimadzu UV-2600 spectrophotometer. 5 mg of quercetin was weighed and dissolved in ethanol. Standard solutions (10, 25, 50, 100 and 200 µg/mL) were diluted from the stock solution. The sample preparation procedure was also applied to the standards.

Antioxidant Activity (DPPH• radical scavenging capacity)

The DPPH radical scavenging capacity was measured by using the method of Liu et al. [15, p. 76-85]. DPPH solution (0.1 mg/mL) dissolved in methanol was added to 0.5 mL of diluted extract sample. The solution was mixed by vortex. It was incubated at room temperature for 30 minutes. Absorbance of the solution was measured at 517 nm.

Fig. 1. Crataegus caucasica and Crataegus pentagyna, respectively

Determining of Carbohydrate Components

0.1 gram of the dried and grinded hawthorn fruit sample was weighed into the polypropylene tube for each species. 4 mL of water was added and mixed with vortex for 2 minutes, 1 ml of methanol was added and mixed again. It was centrifuged at 3000 g for 5 min. It was filtered into a vial and injected into the HPLC-RID. The HPLC system used was an Agilent 1100 series, consisting of a degasser (G1379A), isocratic pump (G1310A), autosampler (G1313A), column oven (G1316A) and refractive index detector (G1362A). Chromatographic separation was conducted using a Nova Gel Ca2+ 300 mm × 7.8, 9 µm column with a flow rate of 1 mL/min and injection volume of 20 µL. The column temperature was set at 30 °C. HPLC grade water was used as mobile phase.

Cell culture

In a 75 cm^2 culture flask at 37°C with a 5% CO_2 humidified environment, SH-SY5Y (Human Neuroblastama) cells were cultured using DMEM supplemented with 10% heat-inactivated FBS and 0.1% penicillin/streptomycin. The medium was replaced every 2-3 days, and cells were subcultured once they achieved 80-90% confluency. After the digestion process with 0.25% trypsin, cells were collected by centrifugation at 1000 rpm for five minutes before being resuspended in fresh medium. Cells were placed in adequate test plates, and they grew there overnight. Adherent cells were employed for additional investigation.

MTT assay

The MTT reduction test was used to analyze cytotoxicity. SH-SY5Y cells were seeded at a density of $1.5x10^3$ cells/well in 96-well plates with 2% FBS medium for the MTT test. H_2O_2 was incubated for 24 hours either by alone or in combination with the extract. At the end of incubation time the cells were treated with MTT (0.5 mg/mL) for an additional 2–3 hours. The medium in each well was removed out and DMSO was added to dissolve the purple formazan crystals, and then each well's solution's absorbance was assessed using a microplate reader at 570 nm.

Measurement of Intracellular ROS using H2DCF-DA assay

Intracellular ROS generation was measured using H_2DCF-DA reagent. To determine the concentration at which H_2O_2 increased intracellular ROS production, cells were first exposed to H_2O_2 at various concentrations (0-1000 µM) for 1 hour. To evaluate the ROS scavenging ability of the extract, 500 µm H_2O_2 was applied to the cells in the presence of various concentrations of the extract for 1 hour. Cells were then treated for 30 minutes in the dark with H_2DCF-DA reagent (25 M) in DMEM (without phenol red). Fluorescence spectroscopy, which uses 485 nm and 535 nm for excitation and emission, respectively is used to measure changes in fluorescence intensity.

Results and discussions. In this study, the fruit samples from the branches of two different hawthorn tree (*C. pentagyna* and *C. caucasica*) from Azerbaijan were performed for the toxicity of H_2O_2 on SH-SY5Y cells and antioxidant activities using a cell viability assay and DPPH along with total flavonoid content, carbohydrate components and fatty acid composition of *n*-hexane extract were achieved by UV-Visible spectrophotometer, HPLC-RID and GC-FID, respectively.

The fatty acid composition of the *n*-hexane extracts of fruits from *Crataegus pentagyna* and *Crataegus caucasica* species was investigated using GC-FID technique, for the first time. According to the results, the oil yields of the studied *C. pentagyna and C. caucasica* species were detected as 1.55% and 0.56% on the basis on dry weight of the plant materials, respectively. In total, 28 different fatty acid components were identified ranging their carbon numbers from C8 to C22. The total fatty acid percentages of the *n*-hexane extracts varied from 87.19% to 88.71% (Table 1).

The highest total fatty acid amount was detected in *C. caucasica* (88.71%, saturated and unsaturated). It can be seen clearly that a high amount of essential fatty acids (EFAs) was detected for two *Crataegus* species with a clear predominance of oleic and linoleic acids. Linoleic acid (LA) was the most abundant fatty acid as 47.00% and 49.95% for *C. pentagyna* and *C. caucasica* species, respectively. Another dominant fatty acid was oleic acid detected as 27.50% and 21.80% for *C. pentagyna* and *C. caucasica* species, respectively. Two hawthorn extracts contained also significant portions of *cis*-11,14-eicosadienoic acid and palmitic acid which varied between 10.13-14.20% and 4.71-6.76%, respectively. Linoleic acid (LA) and alpha linolenic acid (ALA) which are defined "essential" fatty acids since they are not synthesized in the human body and are mostly obtained from the diet, belong to the n-6 (omega-6) and n-3 (omega-3) series of polyunsaturated fatty acids (PUFA), respectively. The long chain omega-3 (n3) PUFAs are known for their beneficial health effects mainly with regard to cardiovascular and cognitive health.

Clinical studies have established that the n-6 fatty acid, linoleic acid (LA), and the n-3 fatty acid, linolenic acid (LNA) protect against coronary heart disease. When the fatty acid components of other fruits in the literature were examined, it was seen that the linoleic and oleic acid percentages in many of them are much lower than the hawthorn species. For example, oleic and linoleic acids amount in berries species were found in the range of 1.63-21.57%, 9.15-54.05%, respectively. These results show that hawthorn fruits harvested from Azerbaijan is higher than many fruit in terms of essential fatty acid content.

The ethanol, acetone, and ethyl acetate extracts of two different Azerbaijan hawthorns (*C. pentagyna* and *C. caucasica*) were investigated by means of total flavonoid, carbohydrate contents (dry fruit) and antioxidant activity by UV-Visible spectrophotometer, HPLC-RID and DPPH method, respectively. The results of the experiments are shown in Tables 2 and 3. The total flavonoid content in the two hawthorn fruit extracts was found as between 4.21-5.84 mg QUE/g. When the total flavonoid amounts of both species are compared, although there are approximately close results, it has been observed that the flavonoid content of the *C. pentagyna* species is slightly higher.

The carbohydrate contents of the dried Azerbaijan hawthorn fruit were measured in the range of 15.4 to 17.8 g/100 g fruit. The carbohydrate results in Table 3 show that sorbitol is present in high amounts in both species, while glucose and fructose are present in lower amounts. Filip et al. determined the amounts of glucose, fructose, sucrose and sorbitol in the leaves and fruits of apples in 2016. As

Table 1

Fatty acid composition (%) of two Crataegus species by GC-FID

R_T	Name (in symbols)	Fatty acids	*C. pentagyna*	*C. caucasica*
5.83	C8:0	Caprylic acid	0.04	0.14
7.05	C10:0	Capric acid	0.05	-
7.94	C12:0	Lauric acid	0.11	0.23
8.80	C13:0	Tridecanoic acid	0.03	-
9.74	C14:0	Myristic acid	0.10	0.17
11.15	C15:0	Pentadecanoic acid	0.04	0.12
12.18	C16:0	Palmitic acid	6.76	4.71
13.22	*cis* C16:1*w*	Palmitoleic acid	0.41	0.18
13.82	C17:0	Heptadecanoic A.	0.07	0.10
15.08	*cis* C17:1*w*	*cis*-10-heptadecanoic acid	0.07	0.06
15.90	C18:0	Stearic acid	1.65	1.52
16.90	*trans* C18:1*w*	Elaidic acid	0.03	0.04
17.25	*cis* C18:1 *w*	Oleic acid	27.50	21.80
18.77	all *trans* C18:2 *w*	Linolelaidic acid	0.02	0.03
19.17	all *cis* C18:2 *w*	Linoleic acid (LA)	47.00	49.95
20.35	C20:0	Arachidic acid	1.22	1.25
21.20	all *cis* C18:3 *w*	Alpha-linolenic acid (ALA)	1.01	0.92
21.32	*cis* C20:1 *w*	*cis*-11-eicosenoic acid	0.67	0.99
22.34	C21:0	Heneicosanoic acid	0.09	0.14
23.11	all *cis* C20:2 *w*	*cis*-11,14-eicosadienoic acid	10.13	14.20
23.95	C22:0	Behenic acid	0.75	1.03
24.67	all *cis* C20:3 *ω*6	*cis*-8,11,14-eicosatrienoic acid	0.11	0.17
25.71	all *cis* C20:3 *ω*3	*cis*-11,14,17-eicosatrienoic acid	0.14	0.30
26.59	C23:0	Tricosanoic acid	0.70	0.61
27.33	all *cis* C22:2 *ω*6	*cis*-13,16-docosadienoic acid	0.09	0.13
27.71	C24:0	Lignoceric acid	0.83	1.05
30.58	*cis* C24:1*w*	Nervonic acid	0.06	0.04
31.44	all *cis* C22:6 *ω*3	*cis*-4,7,10,13,16,19-docosahexaenoic acid	0.36	0.08
		Total saturated fatty acid %	12.85	11.25
		Total unsaturated fatty acid %	87.19	88.71

a result of their study with apple, which is one of the most consumed fruits in the world, glucose, fructose, and sorbitol amounts were compared with our results obtained from two different hawthorn species of Azerbaijan, the carbohydrate content of the hawthorn fruit of Azerbaijan was found to be higher. The results of the antioxidant content of hawthorn extracts were found in the range of 54-72% DPPH scavenging activities. When the results in Table 2 are examined, it is seen that the antioxidant capacity of the *C. pentagyna* species are higher than the *C. caucasica* species. While the % DPPH radical scavenging activity (0.5 mg /mL) values were maximum 72.40% ± 2.74 in *C. pentagyna* species, it reached a maximum value of 61.78% ± 0.50 in *C. caucasica* species.

According to our literature review, *in vitro* antioxidant properties of water and ethanol extracts of leaves, flowers and ripe hawthorn fruits based on DPPH, ABTS, superoxide radical capture, reducing power and metal chelating activity were determined by Keser et al. It was observed that aqueous and ethanol extracts of *Crataegus monogyna* subsp. *monogyna* fruits showed the highest activity in reducing power and metal chelating activity experiments. In addition, it was determined that the aqueous flower extract showed higher flavonoid content than the aqueous leaf extract. They attributed the antioxidant and pharmacological effects of the hawthorn plant to its polyphenolic content [8, p. 51-55]. Our study results also showed that antioxidant activity showed a significant correlation with the total flavonoid content in parallel with the studies in the literature, and the extracts of two hawthorn fruits exhibited significant antioxidant activity and therefore could offer great potential as a natural antioxidant source.

Table 2

Total flavonoid content and % DPPH radical scavenging activity of two Crataegus species

Plants	Extracts	mg QUE/g dry weight fruit	% DPPH radical scavenging activity (0.5 mg /mL)
C. pentagyna	Ethanol	5.54 ± 0.51	72.40 ± 2.74
	Acetone	5.23 ± 0.56	66.26 ± 2.28
	Ethyl acetate	5.84 ± 0.71	70.47 ± 3.14
C. caucasica	Ethanol	4.37 ± 0.38	54.54 ± 0.50
	Acetone	4.21 ± 0.43	58.37 ± 1.94
	Ethyl acetate	4.62 ± 0.29	61.78 ± 0.50

Table 3

Carbohydrate content of two Crataegus species (g/100 g fruit)

Plants	Glucose	Fructose	Sorbitol	Total
C. caucasica	3.32 ± 0.47	5.92 ± 1.01	6.12 ± 0.86	15.36 ±
C. pentagyna	4.34 ± 0.61	6.27 ± 1.07	7.19 ± 1.08	17.80 ±

Table 4

Color determination of two Crataegus species by Lovibond Tintometer

Plants	Extracts	Red	Yellow	Blue
C. pentagyna	ethanol	3.0	1.3	0.0
	acetone	1.3	2.6	2.4
	n-hexane	1.7	3.7	2.9
	ethyl acetate	2.0	14	0.0
C. caucasica	ethanol	2.0	2.3	0
	acetone	1.7	3.3	2.9
	n-hexane	1.9	4.1	2.9
	ethyl acetate	2.3	35.0	0

One of the important chemical characteristics used to describe commercial hawthorn fruit is its color. As we have seen in some studies in the literature, the color of dried and fresh hawthorn is an important factor for consumers in the food industry. It is known that the maturity of hawthorn fruit is classified according to the degree of color [3, p. 449-456]. The color index is also a significant indicator of biological activity and phenolic content. However, as far as we know, there is no method in the literature to define the color index of hawthorn fruit extracts. In addition, no study was found on the comparison of color indexes, chemical contents, and biological activities of hawthorn extracts. Thus, in this study the determination of color index of the extracts was investigated by Lovibond Tintometer (Table 4).

The Tintometer is a subtractive colorimeter, using red, blue and yellow color indexes. In our study red, blue and yellow glass standards in Lovibond Tintometer were used for color detection of 2 different *Crataegus* species. The units of red, blue and yellow varied from 1.3-3.0, 0-2.9 and 1.3-35 units indicating a color change, respectively. The highest color index for yellow color is seen as 35.0 in the ethyl acetate extract of *C. causica*. It is seen that antioxidant activity capacity and total flavonoid extract amount are in parallel with this result. This extract of hawthorn, which has the highest yellow index, also has the highest DPPH antioxidant capacity and total flavonoid content.

The effects of *Crataegus caucasia* and *Cretaegus pentagyna* extracts on the viability of SHSY5Y cells were examined. For this purpose, cells were treated with a series concentration of extract (0, 12.5, 25, 30, 100, 200 mg/mL) for 24 h. obtained results are summarized in Fig. 2.

The ability of the extracts of hawthorn fruits to reduce the toxicity of H_2O_2 on SH-SY5Y cells at non-toxic concentrations was examined using a cell viability assay. First, we investigated how varied H_2O_2 and extract concentrations affected the viability of cells (Fig. 3) shows the findings of the cell viability percentage following treatment with various concentration of H_2O_2 and extract.

At concentrations of 50 μM and higher, H_2O_2 dramatically decreased the viability of SH-SY5Y cells. While up to a concentration of 50 μg/ml, ethanol and acetone extracts of *Crataegus caucasia* showed no evidence of cytotoxicity, ethyl acetate extract of *Crataegus caucasia* didn't exhibit cytotoxicity up to a concentration 25 μg/ml. In the studies carried out on ethanol, acetone and ethyl acetate extracts of *C. pentagyna*, showed no evidence of cytotoxicity up to a concentration of 100, 100 and 50 μg/ml, respectively. As a result, non-toxic indicated concentrations were chosen to evaluate the protective effect of hawthorn extracts. The treatment with extract of hawthorn at the presence of 200 μM H_2O_2 significantly inhibited in a concentration-dependent manner the cytotoxicity induced by H_2O_2 in SH-SY5Y cells (Fig. 3) The next step was to test ROS (reactive oxygen species)

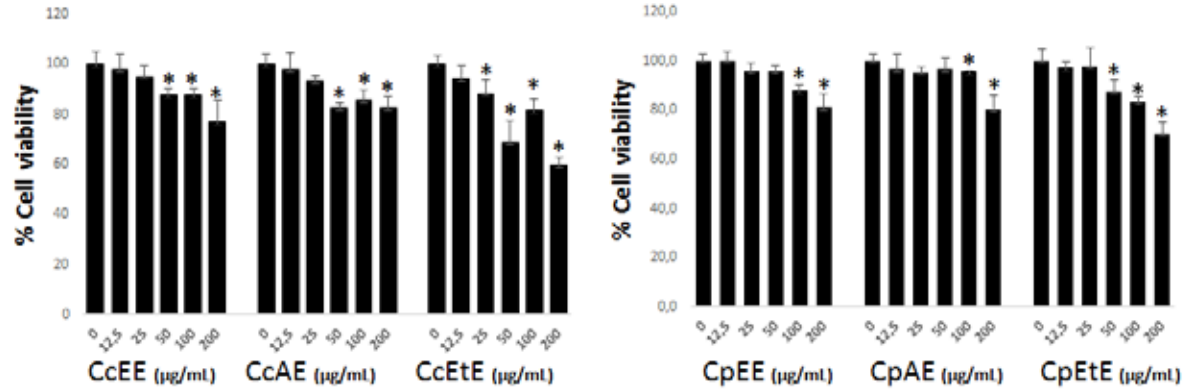

Fig. 2. The effects of Crataegus caucasia and Cretaegus pentagyna extracts on the viability of SHSY5Y cells. Cells were treated with a series concentration of extract for 24 h

Data are expressed as mean ± SD of five independent experiments (n = 5).

*CcEtE: C. caucasia ethyl acetate extract, CcEE: C.caucasia ethanol extract, CcAE: C. caucasia acetone extract, CpEtE: C. pentagyna ethyl acetate extract, CpEE: C. pentagyna ethanol extract, CpAE: C. pentagyna acetone extract. *p< 0.05 vs. Control cell.*

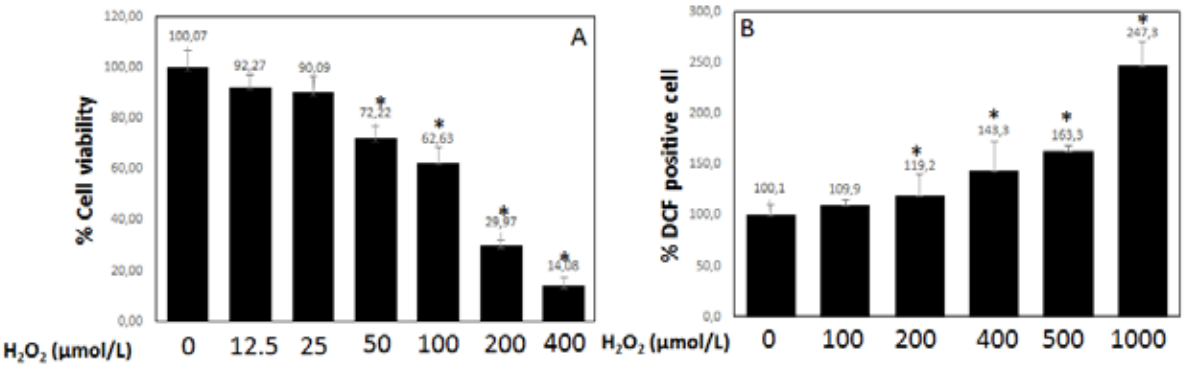

Fig. 3. (A) The effects of H2O2 on the viability of SH-SY5Y cells. Cells were treated with indicated concentration of H2O2 for 24h. (B) The effects of H2O2 on ROS production in SHSY5Y cells

*Cells were treated with indicated concentration of H_2O_2 for 1h. Data are expressed as mean ± SD of five independent experiments (n = 5). *p<0,05 vs. Control cell.*

production in cells using the H_2DCFDA (2',7'-dichlorodihydrofluorescein diacetate) reagent, a fluorescent dye that shows ROS, to see if hawthorn extracts could alleviate the oxidative stress caused by H_2O_2-induced ROS production. As seen in Fig. 4, cells treated with H_2O_2 (0-1000 µM) significantly increased the intensity of the DCF-released fluorescent signal in a dose-dependent manner, and the signal was markedly reduced in the presence of hawthorn extracts. In the presence of hawthorn extracts and H_2O_2 (500 µM) in the medium, DCF-liberated fluorescent signal decreased with varying extract concentration, suggesting the H_2O_2 scavenging effect of the extract (Fig. 5).

The hallmark of neurodegenerative disorders such as alzheimer's disease (AD), parkinson's disease (PD) and amyotrophic lateral sclerosis (ALS) is oxidative stress-induced progressive cell death in particular vulnerable neuronal cells, which is often linked to cytoplasmic protein aggregation in neurons. Natural products especially fruits have long been employed as traditional medicines for the treatment of neurodegenerative disorders due to their high antioxidant content. The protective properties of phenolic, flavonoid compounds obtained from natural products against neuronal cell damage caused by oxidative stress have been shown in several research over the past several decades [1, p. 81-90].

In another study; Culum et al. investigated the qualitative and quantitative determination of phenolic compounds in three *Crataegus* species growing in Bosnia region. They found that there are gallic acid 0.001-0.082 mg/g dry weight (DW), chlorogenic acid 0.19-8.70 mg/g DW and routine 0.03-13.49 mg/g DW in the extracts. Two flavonoids, vitexin and hyperoside, commonly found in chemotaxonomic studies of *Crataegus* species, were not detected in the studied extracts. As a result, they reported that the content of phenolic compounds in three different *Crataegus* extracts grown in Bosnia is quite high and can serve as a good source of bioactive compounds for medicinal purposes and food, and there are significant differences in the content of rutin, gallic acid and chlorogenic acid within and between species. Tohtahon et al. investigated the bioactivity of

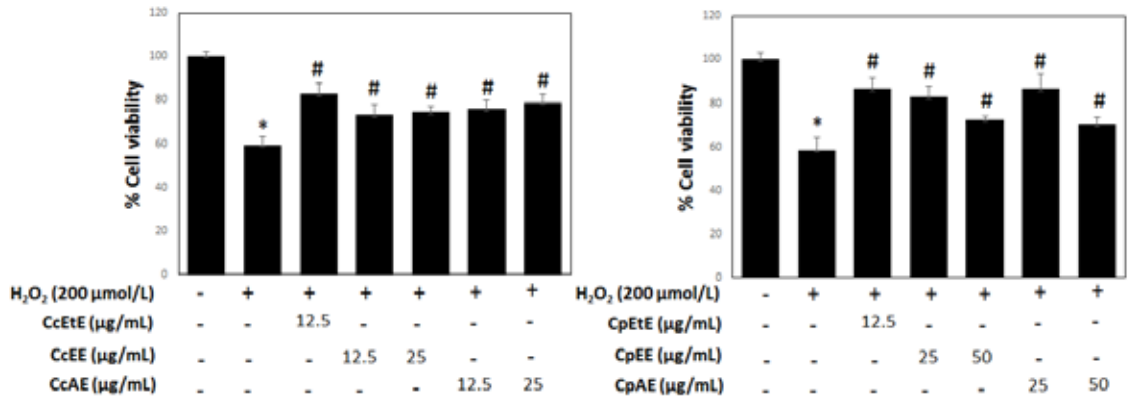

Fig. 4. Protective effects of Crataegus caucasia and Cretaegus pentagyna extracts on SHSY5Y cells against H2O2-induced cell death

*Cells were treated with extract (12.5, 25 and 50 µg/mL) in the presence of 200 µM H_2O_2 for 24 h. Data are expressed as mean ± SD of five independent experiments (n = 5). CcEtE: C. caucasia ethyl acetate extract, CcEE: C. caucasia ethanol extract, CcAE: C. caucasia acetone extract, CpEtE: C. pentagyna ethyl acetate extract, CpEE: C. pentagyna ethanol extract, CpAE: C. pentagyna acetone extract. *p< 0.05 vs. Control cell, #p< 0.05 vs. only H_2O_2 treated cell.*

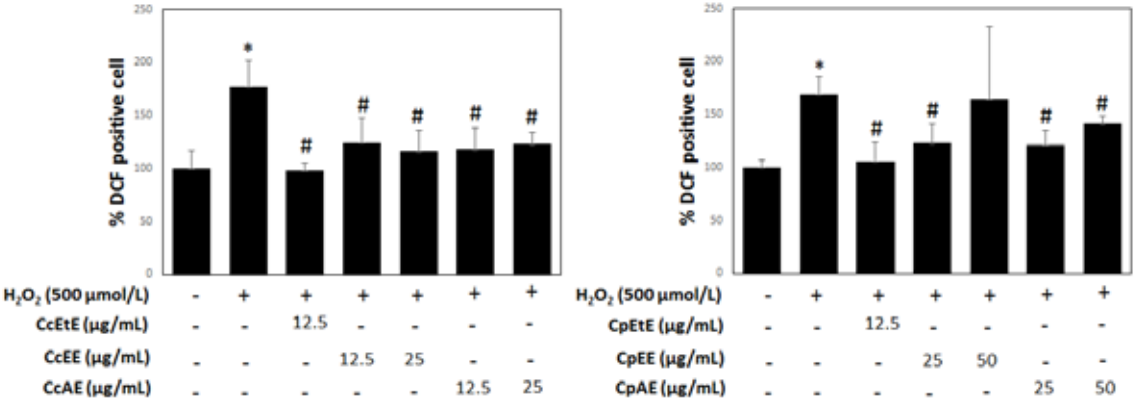

Fig. 5. Effects of C. caucasia and C. pentagyna extracts on intracellular ROS production

*Cells were treated with extract (12.5, 25 and 50 µg/mL) in the presence of 500 µM H_2O_2 for 1h. Data are expressed as mean ± SD of four independent experiments (n = 5). CcEtE: C. caucasia ethyl acetate extract, CcEE: C.caucasia ethanol extract, CcAE: C. caucasia acetone extract, CpEtE: C. pentagyna ethyl acetate extract, CpEE: C. pentagyna ethanol extract, CpAE: C. pentagyna acetone extract. *p< 0.05 vs. Control cell, #p< 0.05 vs. only H_2O_2 treated cell.*

triterpenoids from *Crataegus cuneata*. Triterpenoids are also a class of major compounds with broad biological activities in hawthorn species. The isolation and identification of six different triterpenoids (ursolic acid, maslic acid, corsolic acid, pomolic acid, eucaphic acid and 2a, 19a-dihydroxy-3-oxours-12-en-28 oic acid) were examined from *C. cuneata* fruit extracts enriched in triterpenoids. In addition, pomolic and eucaphic acids were found for the first time in the genus *Crataegus*. As a result of biological evaluation, they revealed that all isolated triterpenoids and triterpenoid-enriched *C. cuneata* fruits extracts showed significant xanthine oxidase and tyrosinase inhibitory activities, while some isolates showed significant acetylcholinesterase inhibitory activities [14, p. 1-9].

Hawthorn berries are a rich source of flavonoids compared to other fruits. Rosario et al. recorded in their study with 20 different genotypes of hawthorn fruit that hawthorn fruit contains higher phenolic compounds compared to other fruits such as lychee, peach, and strawberry [9, p. 1298-1304]. The results of this study clearly exhibited that the extract obtained from Azerbaijani *Crataegus* species utilized an antioxidant activity and neuroprotective effect against H_2O_2-induced cell death and ROS production in SH-SY5Y cells. Although, the cytotoxicity of *Crataegus caucasia* species was higher than that of *Crataegus pentagyna* species, their antioxidant properties were very close to each other. As a result, it is predicted that *C. pentagyna* species may be more suitable for human health due to its less cytotoxic and high antioxidant and carbohydrate contents.

Conclusion. The features such as chemical characterization, neurodegenerative and antioxidant potentials in different extracts of two different species of Azerbaijan hawthorn, *Crataegus – caucasia* and *Crataegus pentagyna*

were investigated, for the first time. It was concluded that these types hawthorns are good oleic and lineolic acids stores in terms of the essential fatty acid components. When the two species were evaluated in terms of cytotoxicity, it was found that they behaved differently from each other and the cytotoxicity of *C. caucasica* species is higher than that of *C. pentagyna* species. As a result of H_2O_2 experiments to examine the neurodegenerative effect, it has been proven that the extracts have a protective effect at different concentrations and the lethal effect of H_2O_2 is eliminated. It is seen that *C. pentagyna* suppresses the effect of H_2O_2 because it is not cytotoxic at high doses and its flavonoid content is richer when compared with *C. caucasica*. Consequently, it can be suggested that the presence of medicinal properties of two types of hawthorn fruits originating in Azerbaijan would be appropriate for use in the field of herbal supplements and food.

BIBLIOGRAPHY:

1. Ataie A, Shadifar M, Ataee R Polyphenolic antioxidants and neuronal regeneration. Basic Clinical Neuroscience, 2016, 7:81-90.
2. Bor Z, Arslan R, Bektas N, Pirildar S, Donmez AA (2012) Antinociceptive, antiinflammatory, and antioxidant activities of the ethanol extract of *Crataegus orientalis* leaves. Turkish Journal of Medical Sciences, 2012, 42: 315-324.
3. Coklar H, Akbulut M, Kılınç S, Yıldırım A, Alhassan I (2018) Effect of freeze, oven and microwave pretreated oven drying on color, browning index, phenolic compounds and antioxidant activity of hawthorn (*Crataegus orientalis*) fruit. Notulae Botanicae Horti Agrobotanici Cluj-Napoca, 2018, 46:449-456.
4. Čulum D, Čopra-Janićijević A, Vidic D, Klepo L, Tahirović A, Bašić N, Maksimović M (2018) HPLC-ED analysis of phenolic compounds in three Bosnian *Crataegus* species. Foods 2018, 7: 66-69.
5. Edwards JE, Brown PN, Talent N, Dickinson TA, Shipley PR A review of the chemistry of the genus Crataegus. Phytochemistry, 2012, 79:5-26. [in English]
6. Ferioli F, Giambanelli E, D'Antuono LF, Application of different analytical methods for the determination of phenolics and antioxidant activity in hawthorn (*Crataegus* spp.) bud and sprout herbal extracts. Journal of Applied Botany and Food Quality, 2020, 93:1-10.
7. Hakima B, Chaymae B, Bouchra T, Antioxidant activity of hawthorn (*Crataegus monogyna*) from Morocco. Academia Journal of Medicinal Plants, 2019, 7: 30-35.
8. Keser S, Celik S, Turkoglu S, Yilmaz Ö, Turkoglu İ, The investigation of some bioactive compounds and antioxidant properties of hawthorn (*Crataegus monogyna* subsp. *monogyna* Jacq). Journal of Intercultural Ethnopharmacology, 2014, 3:51-55.
9. Rosario GM, Emmanuel IE, Raúl NA (2013-12) Antioxidant compounds in hawthorn fruits (*Crataegus* spp.) of Mexico. Revista Mexicana Biodiversidad, 2013-12, 84: 1298-1304.
10. Sarikahya NB, Ucar EO, Kayce P, Gokturk RS, Sumbul H, Arda N, Kırmızıgül S Fatty acid composition and antioxidant potential of ten *Cephalaria* species. Records of Natural Products, 2015, 9:116-123.
11. Shanthi R, Parasakthy K, Deepalakshmi PD, Niranjali DS, Protective effect of tincture of *Crataegus* on oxidative stress in experimental atherosclerosis in rats. Journal of Clinical Biochemistry and Nutrition, 1996, 20:211-223.
12. Song SJ, Li LZ, Gao PY, Yuan YQ, Wang RP, Liu KC, Peng Y, Isolation of antithrombotic phenolic compounds from the leaves of *Crataegus pinnatifida*. Planta Med, 2012, 78: 67-71.
13. Tan YA, Kuntom A, Lee CK, Low KS, Comparative evaluation of palm oil color measurement using a prototype palm oil colorimeter. Journal of the American Oil Chemists' Society, 2004, 81:733-736.
14. Tohtahon Z, Zhang L, Han J, Xie X, Zongcai T, Yuan T, Extraction optimization, structural characterization and bioactivity evaluation of triterpenoids from hawthorn (*Crataegus cuneata*) fruits. Journal of Food Biochemistry, 2017, 41:1-9.
15. Zheng G, Deng J, Wen L, You L, Zhao Z, Zhou L, Release of phenolic compounds and antioxidant capacity of Chinese hawthorn "*Crataegus pinnatifida*" during in vitro digestion. Journal of Functional Foods, 2018, 40:76-85.

REFERENCES:

1. Ataie A, Shadifar M, Ataee R (2016) Polyphenolic antioxidants and neuronal regeneration. Basic Clinical Neuroscience 7:81-90.
2. Bor Z, Arslan R, Bektas N, Pirildar S, Donmez AA (2012) Antinociceptive, antiinflammatory, and antioxidant activities of the ethanol extract of *Crataegus orientalis* leaves. Turkish Journal of Medical Sciences 42: 315-324.
3. Coklar H, Akbulut M, Kılınç S, Yıldırım A, Alhassan I (2018) Effect of freeze, oven and microwave pretreated oven drying on color, browning index, phenolic compounds and antioxidant activity of hawthorn (*Crataegus orientalis*) fruit. Notulae Botanicae Horti Agrobotanici Cluj-Napoca 46:449-456.
4. Čulum D, Čopra-Janićijević A, Vidic D, Klepo L, Tahirović A, Bašić N, Maksimović M (2018) HPLC-ED analysis of phenolic compounds in three Bosnian *Crataegus* species. Foods 7: 66-69.
5. Edwards JE, Brown PN, Talent N, Dickinson TA, Shipley PR (2012) A review of the chemistry of the genus Crataegus. Phytochemistry 79:5-26.
6. Ferioli F, Giambanelli E, D'Antuono LF (2020) Application of different analytical methods for the determination of phenolics and antioxidant activity in hawthorn (*Crataegus* spp.) bud and sprout herbal extracts. Journal of Applied Botany and Food Quality 93:1-10.
7. Hakima B, Chaymae B, Bouchra T (2019) Antioxidant activity of hawthorn (*Crataegus monogyna*) from Morocco. Academia Journal of Medicinal Plants 7: 30-35.
8. Keser S, Celik S, Turkoglu S, Yilmaz Ö, Turkoglu İ (2014) The investigation of some bioactive compounds and antioxidant properties of hawthorn (*Crataegus monogyna* subsp. *monogyna* Jacq). Journal of Intercultural Ethnopharmacology 3:51-55.
9. Rosario GM, Emmanuel IE, Raúl NA (2013-12) Antioxidant compounds in hawthorn fruits (*Crataegus* spp.) of Mexico. Revista Mexicana Biodiversidad 84: 1298-1304.
10. Sarikahya NB, Ucar EO, Kayce P, Gokturk RS, Sumbul H, Arda N, Kırmızıgül S (2015) Fatty acid

composition and antioxidant potential of ten *Cephalaria* species. Records of Natural Products 9:116–123.
11. Shanthi R, Parasakthy K, Deepalakshmi PD, Niranjali DS (1996) Protective effect of tincture of *Crataegus* on oxidative stress in experimental atherosclerosis in rats. Journal of Clinical Biochemistry and Nutrition 20:211-223.
12. Song SJ, Li LZ, Gao PY, Yuan YQ, Wang RP, Liu KC, Peng Y (2012) Isolation of antithrombotic phenolic compounds from the leaves of *Crataegus pinnatifida*. Planta Med 78: 67-71.
13. Tan YA, Kuntom A, Lee CK, Low KS (2004) Comparative evaluation of palm oil color measurement using a prototype palm oil colorimeter. Journal of the American Oil Chemists' Society 81:733–736.
14. Tohtahon Z, Zhang L, Han J, Xie X, Zongcai T, Yuan T (2017) Extraction optimization, structural characterization and bioactivity evaluation of triterpenoids from hawthorn (*Crataegus cuneata*) fruits. Journal of Food Biochemistry 41:1-9.
15. Zheng G, Deng J, Wen L, You L, Zhao Z, Zhou L (2018) Release of phenolic compounds and antioxidant capacity of Chinese hawthorn "*Crataegus pinnatifida*" during in vitro digestion. Journal of Functional Foods 40:76-85.

Guliyeva L., Yildirim Y., Abbasova T., Ince I., Gümüştaş B., Cumaoğlu A., Yararbaş G., Böke Sarikahya N. Chemical characterization, neurodegenerative and antioxidant potentials of two different hawthorn (*Crataegus* species) fruits from Azerbaijan

Hawthorn (*Crataegus* spp.) is an edible wild fruit which is used in traditional medicine, food and beverage industries in many countries since ancient times. In this study, the fruits of two different *Crataegus* species (*C. pentagyna* and *C. caucasica*) from Azerbaijan were examined by means of chemical characterization, neurodegenerative and antioxidant potentials. The fruits were extracted using *n*-hexane, ethanol, acetone, and ethyl acetate by Soxhlet extractor. The fatty acid components (oleic and linoleic acids) of *n*-hexane extract were evaluated by GC-FID. Linoleic acid (LA) was the most abundant fatty acid as 47.00% and 49.95% for *C. pentagyna* and *C. caucasica* species, respectively. The ethanol, acetone, and ethyl acetate extracts of *C. pentagyna* and *C. caucasica* were investigated by means of total flavonoid, carbohydrate content and antioxidant activity by UV-Visible spectrophotometer, HPLC-RID and DPPH method, respectively. The total flavonoid and carbohydrate content in the two hawthorn fruit extracts were found as between 4.21-5.84 mg QUE/g and 15.4-17.8 g/100 g fruit, respectively. The results of the antioxidant content of hawthorn extracts were found in the range of 54-72% DPPH scavenging activities. It is seen that the antioxidant capacity of the *C. pentagyna* species are higher than the *C. caucasica* species. While the % DPPH radical scavenging activity (0.5 mg /mL) values were maximum 72.40% ± 2.74 in C. pentagyna species, it reached a maximum value of 61.78% ± 0.50 in C. caucasica species.

It was observed that aqueous and ethanol extracts of *Crataegus monogyna* subsp. *monogyna* fruits showed the highest activity in reducing power and metal chelating activity experiments. The hawthorn extracts exhibited 54-72% DPPH scavenging activities. The capacity of hawthorn extracts to reduce H_2O_2 toxicity on SH-SY5Y cells at non-toxic concentrations was examined using the cell viability test. It can be concluded that the cytotoxicity of *C. caucasica* species is higher than that of *C. pentagyna* species, but their antioxidant properties are approximately similar. According to these results, the extracts of two different *Crataegus* species (*C. pentagyna* and *C. caucasica*) from Azerbaijan can be used as easily available source of natural antioxidants and as a possible food supplement.

Key words: *Crataegus,* flavonoids, hawthorn fruit, hydrogen peroxide toxicity, antioxidant activity.

Гулієва Л., Йилдирим Є., Аббасова Т., Інце І., Гумусташ Б., Кумаоглу А., Ярарбаш Г., Боке Сарікахя Н. Хімічна характеристика, нейродегенеративний та антиоксидантний потенціал двох різних плодів глоду (Crataegus species) з Азербайджану

Глід (Crataegus spp.) – їстівний дикорослий фрукт, який з давніх часів використовується в традиційній медицині, харчовій промисловості та виробництві напоїв у багатьох країнах. У цьому дослідженні плоди двох різних видів Crataegus (C. pentagyna та C. caucasica) з Азербайджану були досліджені за допомогою хімічних характеристик, нейродегенеративного та антиоксидантного потенціалів. Плоди екстрагували н-гексаном, етанолом, ацетоном і етилацетатом за допомогою екстрактора Сокслета. Компоненти жирних кислот (олеїнова та лінолева кислоти) н-гексанового екстракту оцінювали методом ГХ-ФІД. Лінолева кислота (LA) була найпоширенішою жирною кислотою: 47,00% і 49,95% для видів C. pentagyna і C. caucasica відповідно. Етаноловий, ацетоновий та етилацетатний екстракти C. pentagyna та C. caucasica досліджували на загальний вміст флавоноїдів, вміст вуглеводів та антиоксидантну активність за допомогою УФ-видимого спектрофотометра, HPLC-RID та методу DPPH відповідно. Загальний вміст флавоноїдів і вуглеводів у двох екстрактах плодів глоду становив 4,21-5,84 мг QUE/г і 15,4-17,8 г/100 г плодів відповідно. Результати вмісту антиоксидантів в екстрактах глоду були виявлені в діапазоні 54-72% активності очищення DPPH. Видно, що антиоксидантна здатність видів C. pentagyna вища, ніж видів C. caucasica. У той час як значення % активності поглинання радикалів DPPH (0,5 мг/мл) були максимальними 72,40% ± 2,74 у видів C. pentagyna, воно досягало максимального значення 61,78% ± 0,50 у видів C. caucasica.

Було виявлено, що водний і етаноловий екстракти Crataegus monogyna subsp. Плоди monogyna показали найвищу активність у експериментах зі зниженням потужності та хелатною активністю металів. Екстракти глоду показали 54-72% поглинання DPPH. Здатність екстрактів глоду зменшувати токсичність H2O2 на клітини SH-SY5Y у нетоксичних концентраціях досліджували за допомогою тесту на життєздатність клітин. Можна зробити висновок, що цитотоксичність видів C. caucasica вища, ніж видів C. pentagyna, але їх антиоксидантні властивості приблизно однакові. Згідно з цими результатами, екстракти двох різних видів Crataegus (C. pentagyna та C. caucasica) з Азербайджану можуть бути використані як легкодоступне джерело природних антиоксидантів і як можлива харчова добавка.

Ключові слова: Crataegus, флавоноїди, плоди глоду, токсичність перекису водню, антиоксидантна активність.