Cumhuriyet Sci. J., 45(3) (2024) 537-542
DOI: https://doi.org/10.17776/csj.1497258

Cumhuriyet Science Journal

| csj.cumhuriyet.edu.tr | Founded: 2002 ISSN: 2587-2680 e-ISSN: 2587-246X Publisher: Sivas Cumhuriyet University

Determination of Cytotoxic Activity of *Aronia melanocarpa* (Michx.) Elliot Fruit Extracts on Breast Cancer (MCF-7) and Cervical Cancer (HeLa) Cell Lines

Eda Sönmez Gürer[1,a,*], Ayhan Altıntaş[2,b]

[1] *Department of Pharmacognosy, Faculty of Pharmacy, Sivas Cumhuriyet University, 58140, Sivas, Türkiye*
[2] *Department of Pharmacognosy, Faculty of Pharmacy, Anadolu University, 26470, Eskişehir, Türkiye*
**Corresponding author*

Research Article

History
Received: 07/06/2024
Accepted: 23/09/2024

ABSTRACT

Aronia (chokeberry) fruits are consumed as fresh fruit due to their high antioxidant activity, and are also preferred among the public in the production of natural medicines. *Aronia melanocarpa* (Michx.) Elliot, a species of the Rosaceae family, contains many phytochemical compounds such as flavonoids, phenolic compounds, lignans, terpenes, tocopherols, phospholipids, organic acids and high amounts of anthocyanins. In this study, it was aimed to determine the *in vitro* anticarcinogenic activities of *A. melanocarpa* (Michx.) Elliot fruit extracts prepared with 6 different solvents. In the study, the cytotoxic effects of the fruits were investigated using breast cancer cell line (MCF-7) and cervical cancer cell line (HeLa), and their effects on healthy cells were investigated using human endothelial cells (HUVEC) and mouse fibroblast cells (L929) by the MTT method. As a result of the study; It was determined that the highest cytotoxicity on the breast cancer (MCF-7) cell line was observed in the ethanol extract (IC_{50}=111.44 µg/mL) and the lowest cytotoxicity was observed in the hexane extract (IC_{50}=661.80 µg/mL). It was determined that the highest cytotoxicity on the cervical cancer (HeLa) cell line was observed in the ethanol extract (IC_{50}=95.14 µg/mL) and the lowest cytotoxicity was observed in the ethyl acetate extract (IC_{50}=319.51 µg/mL). According to these values; It was determined that all extracts of Aronia fruit had cytotoxic effects on MCF-7 and HeLa cell lines, selectivity index values were higher in HeLa cells, and they did not have cytotoxic effects on HUVEC and L929 healthy cell lines (IC_{50}=411.25-663.27 µg/mL). Thus, it has been determined that the fruits of the *Aronia melanocarpa* species are promising in the development of new natural resources, new drugs and therapeutic agents in cancer treatment, thanks to their anticancer activities and low cytotoxicity. It is recommended that further research be conducted on the mechanisms of anticancer activity in the future.

Keywords: *Aronia melanocarpa,* Antioxidant, Anticancer, Cytotoxicity.

[a] *edagurer@cumhuriyet.edu.tr* *https://orcid.org/0000-0003-0319-6312* [b] *aaltinta@anadolu.edu.tr* *https://orcid.org/0000-0002-9413-0636*

Introduction

Of late years, the preference for native produces in the protection and therapy of illnesses has increased. *Aronia melanocarpa* grows in the form of a bush, its fruits are small, dark purple or almost black in color, and like other berry-derived fruits, it attracts the attention of researchers because it has high antioxidant potential. It is known that the most important structure that makes aronia attractive is its high phenolic compounds, most importantly anthocyanins in the form of cyanidin derivatives. Fruits contain high amounts of polyphenolic compounds, as well as many biologically active vitamins, especially vitamins C and E, various minerals, carotenoids, pectins and organic acids [1]. Aronia; Chokeberry, commonly known as Chokeberry, is a fruit belonging to the Aronia genus of the Maloideae subfamily of the Rosaceae family. There are two species of this genus consumed as fruit: *Aronia melanocarpa* (Michx.) Ell. (black currant) and *Aronia arbutifolia* (L.) Pers. (red chokeberry) [1].

Phenolic compounds, especially those commonly found in fruits, have a significant impact on human health as they prevent diabetes, allergies, vascular diseases, hypertension, thrombosis, have cardioprotective, neurological damage, and anticancer properties [2]. These secondary metabolite groups, which are important antioxidant compounds of plant origin, are the main functional compound groups that enable plants to be used for medicinal purposes and contribute to people's healthy lives and the continuity of their current state of health [3].

Berries such as blueberries, blackberries, blueberries, aronia and raspberries are food sources with high antioxidant activity [4,5]. The fact that consumption rates in the world have been increasing in recent years has significantly increased the value of these fruits in exports and imports [6]. Medicinal and aromatic plants serve humanity because they are edible and drinkable resources. Plants contain natural antioxidants, antimicrobial, antiviral and antineoplastic, etc. There are various active ingredients that act as such, and therefore they are at the center of many pharmacological activity studies. It has been shown in various studies that various functional compounds such as phenolic compounds, flavonoids and anthocyanins naturally found in plant contents provide great benefits in maintaining health in people with their antioxidant, antimicrobial, preventing Alzheimer's disease, preventing pulmonary diseases, preventing diabetes and anticarcinogenic potentials [7–9]. It has been determined that aronia, which is in the

berry class, is more valuable than other berry fruits (such as blueberries, blackberries, blueberries, raspberries) due to its high content of antioxidant phytochemical compounds [10]. A study conducted with chokeberry juice, green tea and apple juice shows that; with the addition of chokeberry juice to apple juice, the content becomes richer in terms of vitamin C, phenolic acids and anthocyanins. The addition of green tea extract resulted in a much more significant enrichment of apple juice in phenolic acids and flavonoids than chokeberry juice [11].

If we look at the sources of the World Health Organization (WHO); cancer ranks second among the causes of death in the world. Mostly lung, breast, colorectal, prostate, skin and stomach cancers are seen. Lifestyles known to be related to cancer development include excessive weight, Inadequacy of consumption of fruits and vegetables, inactivity and consumption of tobacco products and alcohol [12].

The reason why Aronia species are more popular among other fruits for scientists is; It can be listed as having antiatherosclerotic, hypotensive and antiplatelet activities, primarily heart and cardiovascular diseases, protective effect on stomach ulcers, preventing liver damage, cancer diseases prevention and antiproliferative effects [1]. In this study, it was aimed to determine the *in vitro* anticarcinogenic activities of *A. melanocarpa* (Michx.) Elliot fruit extracts prepared with 6 different solvents. In the study, the cytotoxic effects of the fruits were investigated using breast cancer cell line (MCF-7) and cervical cancer cell line (HeLa), and their effects on healthy cells were investigated using human endothelial cells (HUVEC) and mouse fibroblast cells (L929) by the MTT method.

Materials and Methods

Materials

DMEM (Sigma–Aldrich), L-Glutamine Streptomycin (Sigma–Aldrich), MTT Solution (Applichem A-1080), Absolute Ethyl alcohol (Sigma), Tris-Acetic Acid-EDTA (TAE, 5x) to be used in experiments.), Dimethyl sulfoxide (DMSO), Trypsin (Applichem), Potassium chloride (KCl), phosphoric acid (H_3PO_4), sodium chloride (NaCl), sodium phosphate (NaH_2PO_4), ethyl alcohol (C_2H_5OH), FBS (Sigma–Aldrich), RPMI 1640 (Sigma–Aldrich), acetonitrile (Me-CN), Tris-Boric Acid-EDTA (TBE, 10x), Trypsin (Applichem), Sodium hydroxide (NaOH), sodium chloride (NaCl), sodium phosphate (NaH_2PO_4), thiobarbituric acid (TBA), 2,3,5-Triphenyltetrazolium chloride (TTC) (Merck, Germany) were provided.

In this study, as plant material, fruits obtained from *Aronia melanocarpa* (Michx.) Elliot type plant was purchased from the producer company named Dr. Aronia and dried in the shade.

Plant Extraction

It was added from 100 ml solvents on a 20 g of ground dry plant and kept at room temperature 24 hours a day, then drained and the same process was repeated for 3 days. The collected extracts were blown in the evaporator (40 ° C) using vacuum, the extract was combined with dark glass bottles and % yield calculation was performed for use in bioactivity studies and kept at -20 ° C.

For water extract; 100 ml of hot distilled water was added on the 20 g of ground dry plant and kept at for 10-15 minutes, then drained and the same process was repeated 3 times. Lyophilization process was applied to the collected extract, combined in dust dust dark glass bottles and % yield calculation was made and kept at -20 ° C for use in bioactivity studies.

Stock solutions were prepared from each extract at a concentration of 1 mg/mL to be used in biological activity studies. Stock solution concentrations to be used in the studies were prepared by dissolving the water extract in distilled water and the other extracts in dimethylsulfoxide (DMSO).

Determination of Antitumor Activity In vitro

Within the scope of this study, to define the *in vitro* anticancer potential of aronia plant fruit extracts; breast cancer (MCF-7) and human cervical adenocarcinoma (HeLa) cell lines were used. Cancer cell lines were incubated in DMEM in 75 flasks by placing them in a CO_2 incubator to proliferate sufficiently [13,14].

Cell lines were cultured in media containing DMEM and 10% FBS in 25 cm^2 flasks in an oven with 5% CO_2 at 37 °C. The flasks that reached the passaging state were selected and the sowing process was started in a sterile laminar Flow-cabinet. 5 mL Trypsin-EDTA solution (0.25%) and phosphate buffer (PBS) were added to the medium to remove cells adhering to the flask. 15 mL of DMEM was added onto the trypsin and the inside of the flasks were washed thoroughly by pipetting. The flasks were then transferred to the falcons, one flask to the other. In order to determine whether the cells proliferated appropriately for transplanting into 96-well cell culture plates and to count them, they were stained with Trypan blue and counted using a Thoma slide and an inverted microscope [15,16]. The falcons were centrifuged at 2000 rpm for 8 minutes, and after the centrifugation, the cells were collected at the bottom of the falcons. The upper phase was poured, the cells were removed by tapping the falcons slowly, and 20 mL DMEM and 5 mL FBS were added to the cells. 200 µL of the mixture was placed in each of 96 wells ($5x10^3$ cells in 100 µl/plate space). At the end of the process, the 96-well plates were removed to the incubator (Autoflow IR) [13,14].

Extracts at different concentrations were added to the cells planted in 96-well plates, in triplicate, and at the end of the process, the 96-well plates were placed in the incubator and the cell cultures were incubated for 24 hours. At the end of the period, 10 microliters of 12 mM MTT (Vybrant, Invitrogen) solution was added to the wells and incubated for 4 hours at 37°C in an oven with 5% CO^2 capacity. At the end of the period, the absorbance was measured in 570 nm in the microbial reader (Thermo, USA). As a result of the MTT determination, IC_{50} values were determined using the Graphpad program and

graphics were drawn. components (SI) has been calculated by obtaining the IC_{50} ratio on the IC_{50}/cancer line on the healthy cell line [15].

Determination of Cytotoxic Activity In Vitro

In this study, MTT test was used on human endothelial cell (HUVEC) line and mouse fibroblast cell (L929) line for *in vitro* cytotoxicity research. In the first stage of viability tests performed with tetrazolium compounds, cells were incubated with plant extracts for 24 hours. MTT solution was prepared (5mg/ml), for which MTT chemical was dissolved in dH_2O and filtered before use. In the second stage, the media incubated in 96-well plates were emptied and 50 mL of MTT tetrazolium compound and 50 microliters of new medium were added to the cell cultures. It was incubated at 37°C for an average of 1-4 hours. For the spectrophotometer, 200 microliters of DMSO solvent was added to dissolve the formazan crystals and homogenized by gentle mixing. Finally, the amount of living and non-living cells was detected by color differences at a wavelength of 570 nm using the spectrophotometric method [16–18]. The obtained spectrophotometric data were converted to IC_{50} values in the Graphpad program and it was evaluated whether 6 different extracts made from aronia plant fruits had any lethal toxic effects on HUVEC and L929 cells.

Statistical Analysis

SPSS 23.0 (IBM Corporation, Armonk, New York, United States) program was used to analyze the data. While Independent-Samples T test is used to compare two groups, One-Way Anova test is used to compare more than two groups; LSD, Dunnett and Games Howell tests were used for post hoc analyses. The Kruskal-Wallis Test technique, one of the non-parametric conditions, was used. IC_{50} values were calculated using the Graphpad program. Quantitative data are mean ± S.D. (standard deviation) values are expressed in tables. Categorical data were expressed as n (number) and percentage (%). The results were evaluated at the 95% confidence level and considered important if the p value was less than 0.05.

Results and Discussion

In our study, firstly extractions of Aronia fruits with different solvents were prepared and biological activity analyzes were carried out according to the aim of the study. The yield calculations of the extracts prepared with different solvents were calculated as a percentage based on the dry plant. Extraction yields of the fruit vary between 68.6% g and 1.25% g. It was observed that the highest yield was in the methanol solvent and the lowest in the hexane solvent (Table 1).

Table 1. Extract yields of *Aronia melanocarpa* fruits prepared with different solvents

Extract	% Extraction efficiency
Methanol	%68,6
70% Ethanol	%36,84
Ethanol	%5,72
Ethyl acetate	%2,15
Hexane	%1,25
Water	%30,85

The extracts obtained from the fruit with 6 different solvents were applied on two separate cancer cell lines (MCF-7 and HeLa) at different concentrations between 0.1 µg/mL and 1000 µg/mL, and their cytotoxic activities were examined using the MTT test after 24 hours of incubation, all ext. It was observed that all extracts had moderate cytotoxic activities on MCF-7 and HeLa cell lines, and the highest cytotoxic activity was in the ethanol extract (Table 2). It was determined that aronia fruit extracts were more effective on the HeLa cell line than on the MCF-7 cell line, and ethanol extracts and methanol extracts had a more cell viability-reducing effect on these cells than others (Figure 1 and Figure 2).

Table 2. IC_{50} levels of *Aronia melanocarpa* fruit extracts in MCF-7 and HeLa cell lines

Extract	MCF-7 IC_{50} Values µg/mL	HeLa IC_{50} Values µg/mL
Methanol	268,42 ± 54,63	101,55 ± 24,27
70% Ethanol	237,18 ± 42,19	98,98 ± 28,31
Ethanol	111,44 ± 39,67	95,14 ± 22,87
Ethyl acetate	259,50 ± 54,43	319,51 ± 88,25
Hexane	661,80 ± 78,70	163,98 ± 73,48
Water	346,12 ± 84,56	116,12 ± 58,63

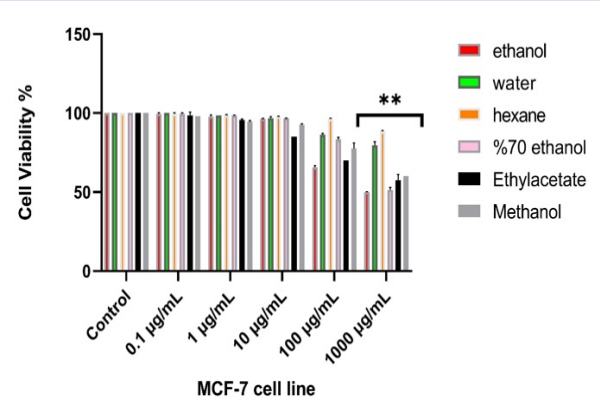

Figure 1. % Cell viability results of *Aronia melanocarpa* fruit extracts in MCF-7 cell line. Activities were evaluated after 24 hours of incubation. (**p<0.01)

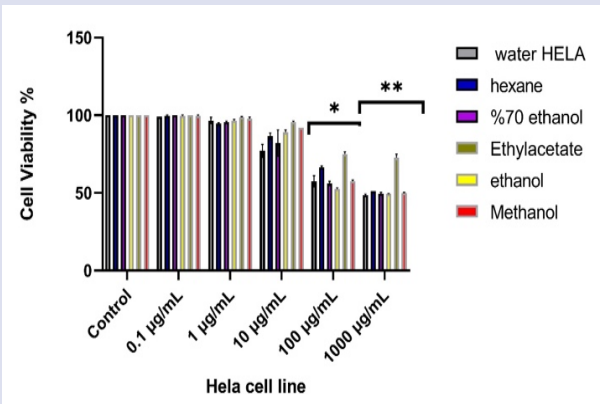

Figure 2. % Cell viability results of *Aronia melanocarpa* fruit extracts in HeLa cell line. Activities were evaluated after 24 hours of incubation. (**p<0.01)

The extracts obtained from aronia fruit with 6 different solvents were applied on two separate healthy cell lines (HUVEC and L929) at concentrations between 0.1 µg/mL and 1000 µg/mL, and after 24 hours of incubation, their cytotoxic activities were examined using the MTT test. As a result of the MTT reaction, readings were made on the Elisa device (Thermo). IC_{50} values showing the effects of extracts on different cell lines are presented in Table 2. There was no statistically important difference between the control group and the dose groups in both cell lines ($p>0.05$; Table 3.). In the light of these data, it was concluded that aronia fruit extracts did not have a cytotoxic effect on healthy cell lines HUVEC and L929.

Table 3. IC_{50} values of *Aronia melanocarpa* fruit extracts in HUVEC and L929 cell lines ($p>0,05$)

Extract	HUVEC IC_{50} Values µg/mL	L929 IC_{50} Values µg/mL
Methanol	468,72± 79,11	501,22± 54,45
70% Ethanol	437,59± 51,49	497,18± 77,28
Ethanol	411,25± 49,13	459,27± 85,46
Ethyl acetate	569,47± 74,16	521,55± 96,08
Hexane	458,85± 78,27	663,27± 103,12
Water	463.27± 84,73	506,42± 114,13

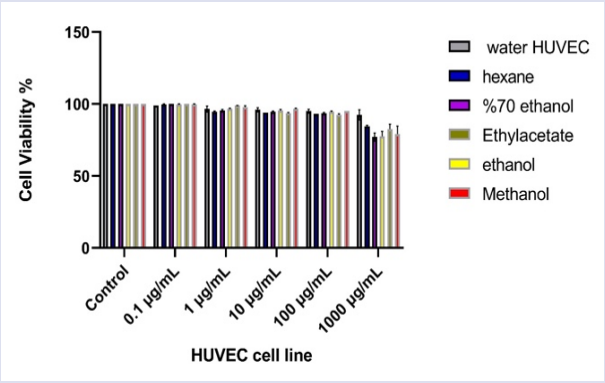

Figure 3. % cell viability results of *Aronia melanocarpa* fruit extracts in HUVEC cell line

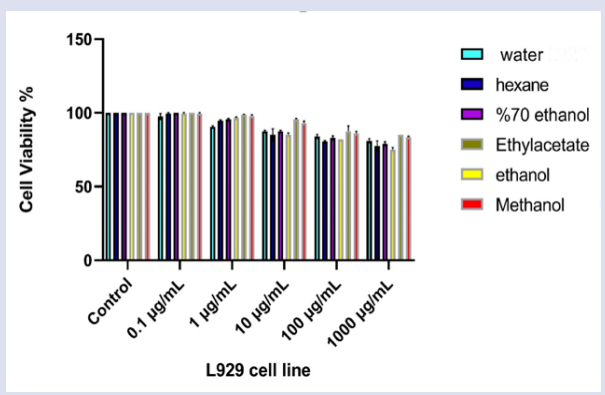

Figure 4. % cell viability results of *Aronia melanocarpa* fruit extracts in L929 cell line

By comparing the IC_{50} values of the MCF-7 and HeLa cell lines, which are cancer cell lines to which aronia fruit extracts were applied, and the healthy HUVEC cell line, comparison results were obtained regarding which cells the plant extracts are most effective on (Table 4). In this comparison, defined as the selectivity index, aronia plant ethanol extract showed the highest anticarcinogenic potential in the MCF-7 cell line with a selectivity index value of 3.69, and aronia plant methanol extract showed the highest anticarcinogenic activity in the HeLa cell line with a selectivity index value of 4.62 can be seen. In Table 4, the selectivity index values of plant extracts in the hala cells are higher.

Table 4. Selectivity Index obtained from the comparison of IC_{50} values of *Aronia melanocarpa* fruit extracts in HUVEC, MCF-7 and HeLa cell lines

Selectivity Index		
Extract	IC_{50} (HUVEC/MCF-7)	IC_{50} (HUVEC/HeLa)
Methanol	1,75	4,62
70% Ethanol	1,84	4,42
Ethanol	3,69	4,32
Ethyl acetate	2,19	1,78
Hexane	0,69	2,80
Water	1,34	3,99

For many years; The fruits were first consumed fresh, then made into rusks, and brewed as tea for influenza infections. As fresh fruit or dried, freshly squeezed juice, jam, sherbet, sauce, cake. Conditions where it is most frequently used in treatment. *Aronia melanocarpa* is a high antioxidant capacity and rich in anthocyanins. In both *in vitro* and *in vivo* studies; Stomach ulcers have been found to have healing potentials on diseases such as many cancer and diabetes.

Sharif et al.; The effects of *Aronia melanocarpa* fruit juice on the stem cell line P19 in mouse embryonal carcinoma cells were investigated; As a result of the experiment, it was concluded that aronia fruit juice inhibited cell proliferation and caused the induction of apoptosis [19]. In a study investigating the biological effects of *Aronia melanocarpa* extracts on the human colon adenocarcinoma cell line; The anticancer activity of extracts was detected by MTT test. In conclusion; It was thought that *A. melanocarpa* leaf extract may show anticancer activity [20]. In the study conducted by Mcdougall et al.; Berry fruit extracts, rich in polyphenols, were screened for their antiproliferative activity using human cervical cancer (HeLa) cells grown in microtiter plates. Extracts of mountain ash, raspberry, blueberry, cloudberry, arctic thornbush and strawberry have shown anticancer activity[21]. In the study conducted on 5 different berry juices, the antiproliferative activity of Konic'-Ristic et al. fruit juices on HeLa cells, Fem X cells and MCF-7 cells was examined. In the results of working, it was determined that all fruit juices showed antiproliferative activity in a dose-dependent manner, with IC_{50} ranging from 10.2 to 70.5 l/ml [22].

Cyanidin glycosides, one of the major components of *Aronia melanocarpa*, have been found to inhibit HeLa human cervical tumor cell proliferation, thereby causing antiproliferative activity [23]. There are studies showing

the anti-proliferative or protective effects of aronia fruit extracts on many types of cancer, especially colon cancer [10], pancreatic cancer [24], breast cancer [25], colon cancer [26]. In the study investigating the biological effects of *A. melanocarpa* leaf extracts on the human colon adenocarcinoma cell line Caco-2; extracts have been found to show anticancer activity [20].

Gao et al [27], reported that *A. melanocarpa* fruit extract showed antiproliferative activity (IC_{50} = 338.36 µg/mL) by inhibiting the growth of HepG2 human liver cancer cells.

A recent study investigated the potential effect of *Aronia melanocarpa* extract on cell viability in human colon cancer cell line (HT-29) and healthy human umbilical cord endothelial cell line (HUVEC). MTT assay showed that Aronia extract induced 50% cell death (IC_{50}) at a concentration of 186 µg/mL in HT-29 cell line 48 h after treatment. Cytotoxicity results showed a dose-dependent decrease in cell viability in HT-29 cell line. However, increasing concentrations of Aronia extract did not show a similar effect in HUVEC cell line [28]. The results obtained support our study.

Cvetanovic et al. [29], the cytotoxic activity of *A. melanocarpa* extracts on malignant cell lines (A-549, LS-174T and HeLa) and normal lung fibroblasts (MRC5) was examined. The results obtained showed that the growth of the malignant cells used was inhibited by the effect of the extracts. In addition, HeLa cells were shown to be much more sensitive to extracts than the other three cell lines. In our study, the results were found to be compatible with this study, as the extracts showed the highest anticarcinogenic activity in the HeLa cell line. Šavikinetal et al., who conducted a study similar to ours [30], reported that aronia fruit water extracts prepared in the form of infusion and decoction showed cytotoxic activity against HeLa cells and the IC_{50} values obtained were 86.99 and 11.16µg/mL, which was consistent with the IC_{50} values found in our study (116.12 ± 58.63 µg/mL) has been observed.

Conclusion

For *in vitro* anticancer activity determination, it was applied to breast cancer (MCF-7) and cervical cancer (HeLa) cell lines, and cytotoxic activities were examined using the MTT test. In conclusion; It has been determined that all extracts of aronia fruits have moderate cytotoxic activities on MCF-7 and HeLa cell lines, all extracts are more effective on the HeLa cell line, and ethanol and methanol extracts have a more cell viability-reducing effect on these cells than others.

For *in vitro* cytotoxic activity determination, application was performed on human endothelial (HUVEC) and mouse fibroblast (L929) cell lines, cytotoxic activities were examined using the MTT test and the extracts were compared in terms of effectiveness. In conclusion; It has been determined that all extracts of aronia fruits have no cytotoxic effect on healthy cell lines HUVEC and L929. The fact that any natural substance or extract obtained from plants or a single active substance does not harm healthy cells, but has a cytotoxic effect on cancerous cells, suggests that it may have potential in the development of drug substances in the future. In this context, it is promising that aronia plant fruit extracts do not have cytotoxic effects on the tested HUVEC and L929 healthy cell lines.

If we make a comparison in terms of selectivity index; It is seen that aronia plant ethanol extract shows the best anticarcinogenic activity in the MCF-7 cell line with a selectivity index value of 3.69, and aronia plant methanol extract shows the highest anticarcinogenic activity in the HeLa cell line with a selectivity index value of 4.62. It is also seen that the selectivity index values of plant extracts are higher in HeLa cells.

As a result of the data we obtained from our study; It has been shown that fruits belonging to the *Aronia melanocarpa* species can contribute to the literature in the development of new natural resources, new drugs and treatment agents in cancer treatment, thanks to their anticancer activities and low cytotoxicity. However, further research on the anticancer activity mechanisms of these extracts is recommended by the application of more advanced and comprehensive techniques, including *in vivo* experiments.

Conflicts of Interest

There are no conflicts of interest in this work.

Acknowledgment

This article is derived from Eda Sönmez Gürer's 2022 pharmacognosy doctoral thesis. The study was funded by the Anadolu University Scientific Research Project Fund (ANABAP) (Project number: 2105S218).

References

[1] Jurikova T., Mlcek J., Skrovankova S., Sumczynski D., Sochor J., Hlavacova I., Fruits of Black Chokeberry *Aronia melanocarpa* in the Prevention of Chronic Diseases, *Molecules*, 22 (2017) 944.

[2] Gorzynik-Debicka M., Przychodzen P., Cappello F., Kuban-Jankowska A., Marino Gammazza A., Knap N., Potential Health Benefits of Olive Oil and Plant Polyphenols, *Int J Mol Sci.*, 19 (2018) 686.

[3] Şekeroğlu Z.A., Şekeroğlu V., Oksidatif Mitokondrial Hasar ve Yaşlanmadaki Önemi, *Türk Bilimsel Derleme Dergisi,* 2 (2009) 69–74.

[4] Benvenuti S., Pelleti F., Melegari M., Bertelli D., Polyphenols, Anthocyanins, Ascorbic Acid and Radical Scavenging Activity of Rubus, Ribes, and Aronia, *J Food Sci.,* 69(3) (2004) 164-169.

[5] Çağlar M.Y., Demirci M., Üzümsü Meyvelerde Bulunan Fenolik Bileşikler ve Beslenmedeki Önemi, *Avrupa Bilim ve Teknoloji Dergisi*, 7 (2017) 18–26.

[6] Ağaoğlu Y.S., Türkiye'de Üzümsü Meyvelerin Dünü Bugünü ve Yarını, Ulusal Kivi ve Üzümsü Meyveler Sempozyumu Ordu*,* (2003) 1–14.

[7] Akalın G., Selamoglu Z., Nutrition and Foods for Skin Health, *Journal of Pharmaceutical Care*, 7(1-2) (2019) 31-33

[8] Deng H., Zhu J., Tong Y., Kong Y., Tan C., Wang M., Antibacterial Characteristics and Mechanisms of Action of *Aronia melanocarpa* Anthocyanins Against *Escherichia coli*, *LWT*, 150 (2021) 112018.

[9] Kaloudi T., Tsimogiannis D., Oreopoulou V., *Aronia melanocarpa*: Identification and Exploitation of its Phenolic Components, *Molecules,* 27 (2022) 4375.

[10] Kulling S., Rawel H., Chokeberry *(Aronia melanocarpa)* – A Review on the Characteristic Components and Potential Health Effects, *Planta Med.,* 74 (2008) 1625–1634.

[11] Średnicka-Tober D., Kazimierczak R., Rembiałkowska E., Strok T., Świąder K., Hallmann E., Bioactive Compounds in Organic Apple Juices Enriched with Chokeberry and Green Tea Extract, *Journal of Research and Applications in Agricultural Engineering,* 62 (2017) 173–177.

[12] Cancer in World Health Organization Available at: http://www.who.int/news-room/fact-sheets/detail , Retrieved January, 2024.

[13] Saraç H., Daştan T., Demirbaş A., Durna Daştan S., Karaköy T., Durukan H., Madımak (*Polygonum cognatum* Meissn.) Bitki Özütlerinin Besin Elementleri ve İn Vitro Antikanserojen Aktiviteleri Yönünden Değerlendirilmesi, *Süleyman Demirel Üniversitesi Ziraat Fakültesi Dergisi*, 1. Uluslararası Tarımsal Yapılar ve Sulama Kongresi, (2018) 340–347.

[14] Saraç H., Daştan T., Durukan H., Durna Daştan S., Demirbaş A., Karaköy T., Kırmızı Gelincik (Fam: Papaveraceae, *Glaucium grandiflorum* Boiss.&Huet *var.* grandiflorum) Bitkisinin Farklı Özütlerinin Besin Elementi İçeriğinin ve *In Vitro* Antiproliferatif Etkilerinin Değerlendirilmesi, *Ziraat Fakültesi Dergisi*, (2018) 417–428.

[15] Al-Qubaisi M., Rozita R., Yeap S.K., Omar A.R., Ali A.M., Alitheen N.B., Selective Cytotoxicity of Goniothalamin Against Hepatoblastoma HepG2 Cells, *Molecules*, 16 (2011) 2944–2959.

[16] Tokur O., Aksoy A., *In Vitro* Sitotoksisite Testleri, Harran Üniversitesi Veteriner Fakültesi Dergisi, 6 (2017) 112–118.

[17] Mosmann T., Rapid Colorimetric Assay for Cellular Growth and Survival: Application to Proliferation and Cytotoxicity Assays, *J Immunol Methods*, 65 (1983) 55–63.

[18] Riss T.L., Moravec R.A., Use of Multiple Assay Endpoints to Investigate the Effects of Incubation Time, Dose of Toxin, and Plating Density in Cell-Based Cytotoxicity Assays, *Assay Drug Dev Technol,* 2 (2004) 51–62.

[19] Sharif T., Stambouli M., Burrus B., Emhemmed F., Dandache I., Auger C., The Polyphenolic-Rich *Aronia melanocarpa* Juice Kills Teratocarcinomal Cancer Stem-Like Cells, but Not Their Differentiated Counterparts, *J Funct Foods,* 5 (2013) 1244–1252.

[20] Efenberger-Szmechtyk M., Nowak A., Nowak A., Cytotoxic and DNA-Damaging Effects of *Aronia melanocarpa*, *Cornus mas*, and *Chaenomeles superba* Leaf Extracts on the Human Colon Adenocarcinoma Cell Line Caco-2, *Antioxidants*, 9 (2020) 1030.

[21] McDougall G.J., Ross H.A., Ikeji M., Stewart D., Berry Extracts Exert Different Antiproliferative Effects against Cervical and Colon Cancer Cells Grown *In Vitro*, *J Agric Food Chem.,* 56 (2008) 3016–3023.

[22] Konić-Ristić A., Šavikin K., Zdunić G., Janković T., Juranic Z., Menković N., Biological Activity and Chemical Composition of Different Berry Juices, *Food Chem*., 125 (2011) 1412–1417.

[23] Ruginǎ D., Sconţa Z., Leopold L., Pintea A., Bunea A., Socaciu C., Antioxidant Activities of Chokeberry Extracts and the Cytotoxic Action of Their Anthocyanin Fraction on HeLa Human Cervical Tumor Cells, *J. Med. Food*, 15 (2012) 700–706.

[24] Thani N.A.A., Keshavarz S., Lwaleed B.A., Cooper A.J., Rooprai H.K., Cytotoxicity of Gemcitabine Enhanced by Polyphenolics from *Aronia melanocarpa* in Pancreatic Cancer Cell Line AsPC-1, *J. Clin. Pathol*., 67 (2014) 949–954.

[25] Kedzierska M., Olas B., Wachowicz B., Stochmal A., Oleszek W., Jeziorski A., The Nitrative and Oxidative Stress in Blood Platelets İsolated from Breast Cancer Patients: The Protectory Action of *Aronia melanocarpa* Extract, *Platelets*, 21 (2010) 541–548.

[26] Bermudez-Soto M., Tomasbarberan F., Garciaconesa M., Stability of Polyphenols in Chokeberry (*Aronia melanocarpa*) Subjected to *In Vitro* Gastric and Pancreatic Digestion, *Food Chem*., 102 (2007) 865–874.

[27] Gao N., Wang Y., Jiao X., Chou S., Li E., Li B., Preparative Purification of Polyphenols from *Aronia melanocarpa* (Chokeberry) with Cellular Antioxidant and Antiproliferative Activity, *Molecules*, 23 (2018) 139.

[28] Caliskan Z., Yucel M.F., Celikok Y., Guler V., Duranay S. A., Preliminary Study of the Anti-Proliferative Effect of *Aronia melanocarpa* Extract on Human Colon Cancer Cells and its Relation with Human TERT Protein, *Experimental Biomedical Research*, 6(2) (2023) 88-98.

[29] Cvetanović A., Zengin G., Zeković Z., Švarc-Gajić J., Ražić S., Damjanović A., Comparative *In Vitro* Studies of the Biological Potential and Chemical Composition of Stems, Leaves and Berries *Aronia melanocarpa*'s Extracts Obtained by Subcritical Water Extraction, *Food and Chemical Toxicology*, 121 (2018) 458–466.

[30] Šavikin K., Zdunić G., Janković T., Gođevac D., Stanojković T., Pljevljakušić D., Berry Fruit Teas: Phenolic Composition and Cytotoxic Activity, *Food Research International*, 62 (2014) 677–683.