ANTIMICROBIAL, ANTIOXIDANT, ANTIPROLIFERATIVE AND CYTOTOXIC ACTIVITIES OF ARONIA FRUIT EXTRACT

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Dilaranur TANAĞARDI

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We approve the thesis of **Dilaranur TANAĞARDI**

Examining Committee Members:

Assoc. Prof. Dr. Ayşe Handan Baysal

Department of Food Engineering, İzmir Institute of Technology

Assoc. Prof. Dr. Ahsen Rayman

Department of Food Engineering, Ege University

Assoc. Prof. Dr. Çağatay Ceylan

Department of Food Engineering, İzmir Institute of Technology

06 July 2023

Assoc. Prof. Dr. Ayse Handan Baysal

Supervisor, Department of
Food Engineering
Izmir Institute of Technology

Assoc. Prof. Dr. Efe Sezgin

Co-advisor, Department of
Food Engineering
Izmir Institute of Technology

Assoc. Prof. Dr. Oğuz Büyükkileci

Head of the Department of Biotechnology

Prof. Dr. Mehtap EANES

Head of the Graduate School of
Engineering and Sciences

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ABSTRACT

ANTIMICROBIAL, ANTIOXIDANT, ANTIPROLIFERATIVE AND CYTOTOXIC ACTIVITIES OF ARONIA FRUIT EXTRACT

Aronia melanocarpa contains high amounts of phenolic compounds, especially anthocyanins. Because of its high chemical content and significant antioxidant action, this food is known as a functional food, and its use is spreading worldwide. Within the scope of this thesis research, studies were carried out on *Aronia melanocarpa* dry extract and liquid extract.

For these purposes, chromatographic and chemical profile were determined in detail by HPLC (High-Pressure Liquid Chromatography), Fourier-Transform Infrared Spectroscopy (FTIR) and Q-TOFF- MS (Quadrupole Time of Flight Mass Spectrometry), and significant bioactive were determined.

Spectroscopic methods were used to characterize phenolic, anthocyanin, and flavonoid components. *Aronia melanocarpa* dry and liquid extracts were tested for antioxidant activity using DPPH and ABTS methods. The antioxidant potential of the *Aronia melanocarpa* dry and liquid extracts studied is high.

To test the antiproliferative and cytotoxic effects, cytotoxic studies were performed on the CaCo2 cell line. Cell migration was also studied in HUVEC and HaCat cell lines.

The antimicrobial activity of *Aronia melanocarpa* dry extract and liquid extract was tested against *Saccharomyces cerevisiae*, a yeast species, and bacterial strains of *Staphylococcus aureus*, *Escherichia coli* O157:H7, *Salmonella Typhimurium*. The extracts analyzed showed an antimicrobial effect on the tested bacteria at different concentrations. The results obtained in this study emphasize that the tested *Aronia melanocarpa* dry extract and liquid extract have antimicrobial properties.

Chromatographic, chemical, and cytological data reveal that *Aronia melanocarpa* liquid and dry extracts can be used as antioxidative and antiproliferative products as food supplements in the health field.

ÖZET

ARONYA MEYVE ESKTRAKTININ ANTİBAKTERİYEL, ANTİOKSİDAN, ANTİPROLİFERATİF VE SİTOTOKSİK AKTİVİTELERİ

Aronia melanocarpa yüksek miktarda fenolik bileşikler, özellikle de antosiyaninler içerir. Zengin kimyasal içeriği ve güçlü antioksidan aktivitesi nedeniyle fonksiyonel gıda olarak adlandırılmakta, ve kullanımı dünya çapında yaygınlaşmaktadır. Bu tez araştırması kapsamında *Aronia melanocarpa* kuru ekstraktı ve sıvı ekstraktı üzerinde çalışmalar yapılmıştır.

Bu amaçlar doğrultusunda kromatografik ve kimyasal profili HPLC (Yüksek Basınçlı Sıvı Kromatografisi), Fourier Dönüşümlü Kızılötesi Spektroskopisi (FTIR) ve LC-Q-TOFF- MS (Sıvı Kromatografisi-Uçuş Zamanlı Kütle Spektrometresi) ile detaylı olarak belirlenmiş ve önemli biyoaktifler tespit edilmiştir.

Fenolik, antosiyanin ve flavonoid bileşenlerin karakterizasyonu için spektroskopik yöntemler kullanıldı. *Aronia melanocarpa* kuru ve sıvı ekstraktları DPPH ve ABTS yöntemleri kullanılarak antioksidan aktivite açısından test edildi. Araştırılan *Aronia melanocarpa* kuru ve sıvı ekstraktlarının antioksidan potansiyeli yüksektir.

Antiproliferatif ve sitotoksik etkiler için CaCo2 hücre hattı üzerinde sitotoksik analizler yapıldı, aynı zamanda HUVEC ve HaCat hücre hatlarında hücre migrasyonu incelendi.

Aronia melanocarpa kuru ekstraktının ve sıvı ekstraktının antimikrobiyal aktivitesi, bir maya türü olan *Saccharomyces cerevisiae* ve *Staphylococcus aureus*, *Escherichia coli* O157:H7, *Salmonella* Typhimurium bakteri suşlarına karşı test edilmiştir. Analiz edilen ekstraktlar farklı konsantrasyonlarda test edilen bakteriler üzerinde antimikrobiyal etki göstermiştir. Bu çalışmada elde edilen sonuçlar, test edilen *Aronia melanocarpa* kuru ekstraktının ve sıvı ekstraktının antimikrobiyal özelliklere sahip olduğunu vurgulamaktadır.

Kromatografik, kimyasal ve sitolojik veriler, *Aronia melanocarpa* sıvı ve kuru ekstraktlarının antioksidatif ve antiproliferatif ürünler olarak sağlık alanında gıda takviyesi olarak kullanılabileceğini ortaya koymaktadır.

To my parents and

my grandmother Ayşe Gökçen YEL, in loving memory…

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ABBREVIATIONS

WHO	:World Heath Organization
A. melanocarpa	:*Aronia melanocarpa*
A. arbutifolia	:*Aronia arbutifolia*
A. prunifolia	:*Aronia prunifolia*
g	:Gram
L	:Liter
mg	:Miligram
kg	:Kilogram
FW	:Fresh Weight
DW	:Dry Weight
DNA	:Deoxyribonucleic Acid
A/H1N1	:Influenza A Virus
HCoV-OC43	:Coronavirus-1
HHV-1	:Human Herpesvirus Type 1
HAdV-5	:Human Adenovirus Type 5
SARS-CoV-2	:Severe Acute Respiratory Syndrome Coronavirus 2
COVID-19	:Coronavirus Disease 2019
ROS	:Radical Oxygen Species
DPPH	:2,2-diphenyl-1picrylhydrazyl
ABTS	:2,2'-azinobis(3 ethylbenzothiazoline-6 -sulfonic acid)
CL	:Chemiluminescence
CEACAM	:Carcinoembryonic Antigen-Associated Cell Adhesion Molecule
AMA	:*Aronia melanocarpa* Anthocyanins
CRC	:Colitis-Associated Colorectal Cancer
mTORC1	:Mammalian Target Of Rapamycin Complex 1
RT-PCR	:Reverse Transcription *Polymerase Chain Reaction*
PCR	:Polymerase Chain Reaction
NSCLC	:Non-Small Cell Lung Cancer
AMJ	:*Aronia melanocarpa* Juice
EC	:Embryonal Carcinoma

CSC.	:Cancer Stem Cell
UHRF1	:Ubiquitin Like With PHD And Ring Finger Domains 1
Cy-3-glu	:Cyanidin 3-Galactoside
hTERT	:Human Telomerase Reverse Transcriptase
MMT Assay	:3-[4,5-Dimethylthiazol-2-yl]-2,5 Diphenyl Tetrazolium Tromide
CE	:Chokeberry Extract
MDSC	:Myeloid-Derived Suppressor Cells
CCR5	:C-C Chemokine Receptor Type 5
IFN	:Interferon
ELISA	:The Enzyme-Linked Immunosorbent Assay
LPS	:Lipopolysaccharides
RC	:Red Chinese Cabbage
ml	:Mililiter
E. coli	:*Escherichia coli*
S. typhimurium	:*Salmonella typhimurium*
S. aureus	:*Staphylococcus aureus*
S. cerevisiae	:*Saccharomyces cerevisiae*
dH_2O	:Distilled Water
HPLC	:High Performance Liquid Chromatography
µm	:Micrometer
µL	:Microliter
cm	:Centimeter
mm	:Milimeter
m	:Meter
min	:Minute
LC-Q-TOF-MS	:Liquid Chromatography Quadrupole Time-Of-Flight Mass Spectrometry
C	:Centigrade
NB	:Nutrient Broth
TSB	:Tryptic Soy Broth
CFU	:Colony-Forming Unit
RPM	:Revolutions Per Minute
h	:Hour
L	:Litre

FTIR	:Fourier Transform Infrared Spectroscopy Analysis
ATR	:Attenuated Total Reflection
nm	:Nanometer
GAE	:The Equivalent Of Gallic Acid
TFC	:Total Flavonoid Content
TAC	:Total Monomeric Anthocyanin Content
A	:Absorbance
ε	:Molar Extinction Coefficient
L	:Path Length
MW	:Molecular Weight
DF	:Dilution Factor
V	:Volume
Wt	:Sample Weight
H_2O_2	:Hydrogen Peroxide
RT	:Retention Time
M	:Molar

CHAPTER 1

INTRODUCTION

People's attitudes toward natural resources have been more positive in recent years due to changes in lifestyle and growing health consciousness. In this circumstance, using plants for multiple reasons, such as food, aroma and sweetener, firewood, weaponry, medicine, and shelter building, is very efficient. Numerous ailments have been tried to be treated, especially with compounds obtained from plants for medicinal, and therefore healing has evolved as a profession (Emin and Balıkesir 2009). According to research, people utilized 250 herbs in therapies at about 5000 BC. For traditional and modern therapeutic purposes, the plant used in herbal medicine is known as a medicinal plant (Deveci et al., 2016). According to a World Health Organization (WHO) report, conventional pharmaceuticals are used for primary care by more than 80% of the world's population (Mishra, Naranje, and Mishra 2015; Zakaryan et al. 2017). Therefore, the studies focused on food components and their relationship to human health (Ghareeb et al. 2015). So much so that the nickname "superfood" has been used to indicate the importance of these food ingredients.

Due to its rich flora, Turkey is home to many plant species. There are approximately 11000 plant taxa, with about 500 employed in alternative medicine. (Türkan et al., 2006). These plants, which can be used fresh or dried, have both uses. All plant organs (leaves, flowers, seeds, tubers, bark) are used for different purposes and in different ways (Göktaş et al., 2019). These parts of the plant, individually or in mixtures, effectively treat various diseases, such as cancer, heart disease, rheumatism, and diabetes (Faydaoğlu et al., 2011).

Because of their antibacterial qualities, plant parts such as fruits, vegetables, seeds, leaves, roots, bark, and resin are utilized as food preservatives. Phytochemicals include vitamins, specific internal metabolites, nitrogenous compounds, terpenoids, carotenoids, and phenolic acids are responsible for plants' antibacterial effects. The antioxidant activities of phenolic substances are associated with the hydroxyl group in

their molecules (Ziaková et al., 2011; Demirel et al., 2019). Conversely, flavonoids constitute the most significant part of plant phenolics, with more than 8000 known compounds (Tungmunnithum et al., 2018).

Secondary metabolites have been extracted from numerous parts of plants and are a valuable source of therapeutic substances (Jouda et al. 2016). Because they are abundant in plants, their antibacterial properties make them valuable. People mostly use antimicrobial drugs to treat many diseases caused by microorganisms. However, the resistance of microorganisms to such drugs limits their use and importance because of their antimicrobial effects. People mostly use antimicrobial drugs to treat many diseases caused by microorganisms. However, the resistance of microorganisms to such drugs limits their use. Due to the resistance mechanisms of microorganisms against antibiotics in the last century, there has been an increase in the importance of herbal products with antimicrobial effects (Marini-Bettòlo 1980). Plant extracts constitute the natural source of antimicrobial compounds (Tepe et al., 2004). Secondary metabolites with antimicrobial effects can inhibit the growth of microorganisms in food. Therefore, the shelf life of foods is prolonged (Burt 2004).

The pharmaceutical institution, which gained popularity with the synthetic manufacture of active chemicals in plants in the 1800s, largely abandoned traditional methods. However, there has been an increase in interest in alternative medicine in the previous 25-30 years since synthetic pharmaceuticals used in modern medicine can not reach the required result in treatment, have many undesirable side effects, have a single sound effect, and other similar reasons. Natural plant medications are frequently more appealing than synthetic treatments since they have fewer adverse effects and more than one benefit. As a result, botanical medicine research, which has had a long medical influence, has developed into a fascinating topic of study.

According to numerous research findings, the value of plants is gradually rising. Turkey and European flora commonly contain members of the Rosaceae family (Özçelik et al., 2015). Aronia is often referred to as a superfood since many experts believe it is one of the planet's healthiest fruits due to its high antioxidant content (Şahin et al., 2022). Aronia, a member of the Rosaceae family, is a berry and a shrub native to North America. Aronia fruit has a bitter taste and is purple. Fruits and freshly squeezed juices differ chemically from other fruits due to high levels of polyphenols and sorbitol (Kulling et al., 2008; Wawer, 2006). The leaves and fruit of *Aronia melanocarpa* contain high amounts of various bioactive components such as vitamins, minerals, and polyphenolic

compounds and have very positive effects in terms of health (Szopa et al., 2017; Jurikova et al., 2017). The polyphenolic components in aronia have a high antioxidant capacity as well as anti-inflammatory, anticancer (Sharif et al., 2012), antimutagenic activity (Gąsiorowski et al., 1997), antimicrobial, antiviral, antidiabetic (P. N. Denev et al., 2012; Kulling et al., 2008), antiatherosclerotic, gastroprotective (Matsumoto et al., 2004), hypotensive, hepatoprotective (Valcheva-Kuzmanova et al., 2006), antiatherogenic (Daskalova et al., 2019) shows antiplatelet effect. Most of the studies on aronia are in the field of health. Aronia has a critical economic value in the domestic and foreign markets. Aronia is important as a functional food due to its high phenol content and therapeutic benefits.

This study aimed to investigate and evaluate *Aronia melanocarpa* dry and liquid extracts for their biological activities, such as antimicrobial and antioxidant capacities.

This investigation achieves the following objectives:

1. Investigating the chemical composition of *Aronia melanocarpa* dry and liquid extracts

2. Researching the effects of *Aronia melanocarpa* extracts on bacteria and yeast.

3. Evaluation of the antioxidant activity of *Aronia melanocarpa* dry and liquid extracts.

4. Evaluation of antiproliferative and cytotoxic activities of *Aronia melanocarpa* dry and liquid extracts.

CHAPTER 2

LITERATURE REVIEW

2.1. General Characteristics of the Rosaceae Family

The Rosaceae family consists of an angiosperm family in the order Rosales, a characteristic member of the world's temperate regions, with about 3000 species, three subfamilies, 16 tribes, and 88-100 genera (Folta and Gardiner 2009; Flora of North America Editorial Committee n.d.). The Rosaceae species are divided into three subfamilies, the two large Rosoideae and Amygdaloideae, with about 2000 and about 1000 species, respectively, and a small subfamily Dryadoideae with less than 30 species (Potter et al., 2007). The Rosaceae family has a worldwide distribution (Mimida et al., 2012). This family includes woody trees, shrubs, climbers, perennial herbaceous plants, and some evergreen species. There are about 100 genera and 3000 species in total. In contrast, most members of this family are deciduous (Rawat et al., 2015; Sevindik, Murathan 2020). The Rosaceae family, which overgrows worldwide under suitable living conditions, develops best in the temperate and subtropical regions of the northern hemisphere. Herbaceous forms can grow as undergrowth plants in temperate forests, fresh or saltwater marshes, arctic tundra, along roadsides, and in used fields. On the other hand, woody structures come to the fore in the first step of forest succession and are pioneer species. In addition, trees belonging to the family can be intermediate components of deciduous mixed-aged forests (Folta and Gardiner 2009; Mesud Hürkul, Köroğlu, and Bir 2019).

Most plants in the Rosaceae family are economically essential but are a source of income for people. Family members include ornamental plants, fruit trees and berry bushes, trees used for timber, and plants for medicinal purposes. Its fruits contain acid,

sugar, and vitamins and can be consumed raw and used for making candy, jams, beverages, and wine.

2.2. Aronia

In recent years, the popularity of aronia berry has increased in the superfood category due to its numerous health benefits. A plant native to North America, aronia is a perennial deciduous shrub with small, dark purple fruits belonging to the Rosaceae family (Wawer 2006; Kulling and Rawel 2008). It is closely related to other popular fruits such as apples, pears, and cherries. With their migration to European people, the aronia plant was transported to Russia and various parts of Europe via Germany in the 1900s. 1946 the aronia plant began growing in the former Soldiers' Union. It is common knowledge that it has recently been cultivated in Germany (Oberlausitz) and other Eastern European countries (Strigl, Leitner, and Pfannhauser 1995). One species of the aronia plant, *Aronia melanocarpa* [Michx.] Elliot (also known as the black chokeberry, Aronia noir) and the other, *Aronia arbutifolia* [L.] Elliot (also known as the red chokeberry, Aronia rouge), can be identified from one another (Hardin 1973). There is a third species, *Aronia prunifolia* is a cross between *Aronia arbutifolia* and *Aronia melanocarpa* (Suvajdžić et al., 2017). *Aronia melanocarpa* is the best-known and most widely cultivated among these species. It is known to contain higher amounts of polyphenols, phenolic acids, proanthocyanidins, anthocyanins, flavonols, and flavanones when compared to other species (Hwang and Lee 2020).

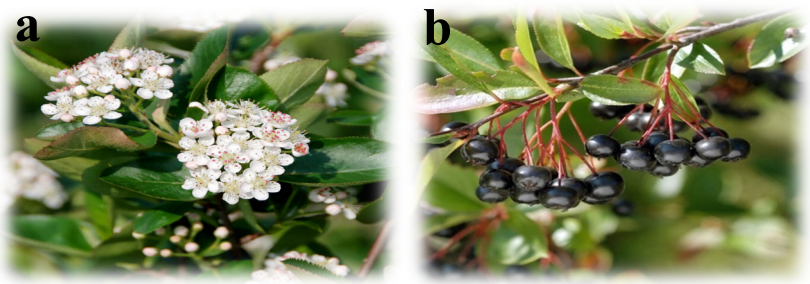

Figure 2.1. a) Aronia flower, b) Ripe Aronia berries.

Figure 2.2. Aronia field.

Aronia plant has been used in traditional medicine for various health benefits for generations. Aronia fruit shows high antioxidant activity due to its rich phytochemical compounds. It plays an active role in preventing and treating many diseases, such as heart disease, cancer, and chronic diseases (Gürer, Karadağ, and Altıntaş 2023). Aronia species are grown and consumed all over the world. The aronia plant is grown for its ornamental value and the consumption of its fruits as food. Aronia berries are also used for various purposes, such as juice, liquor, tea, vinegar, jam, and marmalade (Şahin et al., 2022).

2.3. Botanical Characteristics of the Aronia Plant

Aronia melanocarpa is a purplish-black shrub that grows in clusters of 8-14 fruits on red flower stalks and reaches 90-180 cm tall and 6 mm in diameter (Kokotkiewicz, Jaremicz, and Luczkiewicz 2010). The fruits mature early. The shiny, hairless leaves are 3-7 cm long and do not turn red in autumn (Kokotkiewicz, Jaremicz, and Luczkiewicz 2010). The plant blooms white-pink flowers in May. The taste of the fruit is bitter and astringent, so it is called "chokeberry." Although it is not typically ingested uncooked, processed items like jams, juices, and teas are frequently chosen.

Figure 2.3. *Aronia melanocarpa*, Black chokeberry.

Aronia arbutifolia has sweet fruits of bright red colour that persist until winter. The underside of the flat green leaves is composed of gray hairs and turns red in autumn. *A. melanocarpa*'s grazing area includes northern North America, the Great Lakes region, mountain marshes, and the southern Appalachian Plateau. The southeastern coastal plain and eastern North America are also home to *A. arbutifolia*. Typically found in wetlands, savannahs, and moist woodlands.

Figure 2.4. *Aronia arbutifolia,* Red chokeberry.

In addition to the two species, there is a third, contentious species known as *Aronia prunifolia* (purple chokeberry). The species resembles *Aronia melanocarpa* and *Aronia albutifolia* in appearance, having purple-black fruits and glabrous leaves when ripe. *Aronia prunifolia*'s grazing range is comparable to that of black chokeberry, but it also extends into places where red chokeberry thrives (Kokotkiewicz, Jaremicz, and Luczkiewicz 2010). Purple chokeberry is thought to be a cross between *Aronia melanocarpa* and *Aronia albutifolia* (Kokotkiewicz, Jaremicz, and Luczkiewicz 2010). The closely related rowan (*Sorbus*) is sometimes known as the black chokeberry, and this hybrid has been developed for cultivation in Russia. Some cultivars are derived from black chokeberry (*Aronia melanocarpa*), while others are hybrids (Aronia x *Sorbus*) (Jeppsson and Johansson 2015). Essential varieties include "Nero" (Czech), "Rubina" (crossbreeding from Russian and Finnish plants), "Viking" (Finland), "Kurkumäcki" (Finland), "Hugin" (Sweden), "Fertödi" (Hungary) and" Aron" (Denmark) (Strigl, Leitner, and Pfannhauser 1995; Botanicae et al. 2012; Kulling and Rawel 2008). "Viking" and "Nero" are varieties commonly found in North America (Kulling and Rawel 2008c).

Figure 2.5. *Aronia prunifolia*, Purple chokeberry.

Fruit harvesting occurs between August and September. It can withstand harsh winters and grow for 15 to 20 years (Wawer 2006). The most productive branches of the

aronia plant are between 2 and 6 years old (Wawer 2006). Harvesting is usually done with special equipment or by hand. A bunch on the shrub may produce up to 15 fruits weighing 1 to 1.5 grams apiece, and each bush can yield 20-30 pounds (Wawer 2006). When an aronia plant develops, it may produce five to twelve tons of crop per hectare in around five years (Kulling and Rawel 2008).

Shrubs are not very picky about soil type but prefer plenty of sunlight and air circulation and are typically pest and disease resistant. Aronia is famous as an ornamental plant because of its beautiful blooms, fruits, and fall leaves, which provide aesthetic value to gardens. Aronia species can quickly grow and tolerate various soil types and growing conditions. Pruning, which consists of removing dead or diseased branches and shaping the bush as desired, is usually minimal.

2.4. The History and Global Distribution of the Aronia

Native Americans have used aronia for centuries as a remedy for diseases (Moerman 1999). The natural distribution area of the plant consists of mountain slopes and swamps extending to the Great Lakes region in the northeast of North America and the high parts of the Appalachian region in the southern region (Rossell and Kesgen 2003). The wise Potawatomi clan used the names nîki'mînûn or sakwako'mînûn for aronia and used the tea of the aronia fruit in the treatment of colds (Smith 1933; Kokotkiewicz, Jaremicz, and Luczkiewicz 2010). The Potawatomi and Abnaki clans combined aronia berries with animal fat and dried meat to prepare pemmican, a nutritious long-lived food (Kokotkiewicz et al., 2010).

In the 1930s, Russian botanist Ivan Mitschurin discovered that aronia is nutritious and can be grown in cold climates (Wawer 2006). Then, Mitschurin conducted breeding studies to develop a sweet fruit that would be formed by crossing aronia, Sorbus, and Mespilus. As a result of these studies, two varieties, Likernaja and Desertnaja Michurina, were obtained (Şahin and Erdoğan 2022). Since 1946, aronia has grown all over Europe. Planting aronia on large decares has become widespread in Belarus, Moldova, Russia (Siberian Federal District), and Ukraine, and it was subsequently taken to Japan in 1976 (Kokotkiewicz, Jaremicz, and Luczkiewicz 2010). It grew in the former Soviet Bloc

countries and various Scandinavian countries in the 1980s (Şahin and Erdoğan 2022). However, in the late 1980s, Sweden started growing aronia for commercial purposes for the first time (Jeppsson and Johansson 2015). In 1988, Agropharm S.A., a Polish company, first produced the red pigment from aronia to change colour in foods in Tuszyn (Wawer 2006). Aronia S.A., situated in Leczyca, introduced aronia juice to the Polish market a few years later. The juice was in great demand. In 1996, Jan Mills Wayne brought aronia varieties from agricultural schools in Poland to the United States for commercial cultivation. Poland's share in aronia cultivation is over 90%. The United States is behind Poland with 2500 tons and Germany with 1434 tons. Aron in Denmark, Nero in the Czech Republic, Viking in Finland, Hugin in Sweden, Fertödi in Hungary, and Nero and Viking in Turkey are widely grown (Strigl, Leitner, and Pfannhauser 1995; Šnebergrová et al. 2014; Şahin and Erdoğan 2022).

Table 2.1. Aronia production areas and production amounts in the world (Şahin and Erdoğan, 2022).

Countries	**Production Areas (Total Area)**	**Production Quantities (Ton)**
Poland	6000	50000
United States of America	800	2500
Germany	853	1434
Turkey	78	130
Finland	60	4

In recent years, due to the increasing demand for aronia, a berry-like fruit in Turkey, aronia cultivation has become increasingly widespread. Aronia cultivation started with sapling production at Atatürk Horticultural Central Research Institute in 2012 and continued establishing aronia gardens in Yalova and Kırklareli in 2014. Since 2017, aronia orchards have begun to spring up in Çanakkale, Samsun, İstanbul, Antalya, and Bursa due to the rising demand. Again in 2017, two aronia varieties were registered by Atatürk Horticultural Central Research Institute. After that, accredited seedlings began to grow, and the following year saw the first aronia harvest celebration. The following years

saw the establishment of aronia orchards in the provinces of Bayburt, Kırklareli, Ankara, Bursa, İzmir, Çanakkale, Bolu, Trabzon, Giresun, Kırşehir, and Tekirdağ (Şahin and Erdoğan 2022). Our country's largest aronia production area is in Kırklareli, followed by Bursa and Manisa.

Table 2.2. Aronia production values in Turkey (Şahin and Erdoğan, 2022).

Provinces	**Number Of Saplings**	**Production Area**
Kırklareli	40.000	240
Bursa	23.500	141
Manisa	15.500	90
Kırşehir	8.000	48
Yalova	8.000	48
Çankkale	7.000	42
Samsun	6.000	36
İzmir	5.000	30
Antalya	3.000	18
İstanbul	3.000	18
Ordu	3.000	18
Ankara	2.000	12

2.5. Bioactive Compounds of *Aronia melanocarpa*

A. melanocarpa fruits are beneficial for health due to their rich bioactive content. Studies indicate that *A. melanocarpa* can capture free radicals due to its abundant procyanidin, anthocyanin, flavonoid, and phenolic acids in its fruits (Cvetanović et al. 2018). As shown in various studies, the chemical components of the leaves of *Aronia melanocarpa* vary depending on many factors such as cultivar, fertilization, fruit ripening, harvest time, habitat, and the extraction method used (Zdunić et al. 2020; Jurendi´c et al. 2021; Shahin et al. 2019; Cvetanović et al. 2018). The chemical composition of aronia

leaves was determined using mixed spectrophotometric and chromatographic methods (Lee et al. 2014; Thi and Hwang 2014; Cvetanović et al. 2018; Negreanu-Pirjol et al. 2023;Biel & Jaroszewska, 2017). Young leaves contained more chlorogenic acid, caffeoylquinic acid derivatives, quercetin, rutin, sorbitol, anthocyanins, chlorophyll, carotenoids, macro- and microelements, and dietary fiber than mature leaves when these analyses were done (Negreanu-Pirjol et al. 2023).

Figure 2.6. Potential applications of *Aronia melanocarpa* and its byproducts (Negreanu Pirjol et al. 2023).

Aronia berries and their extracts lead to antibacterial, antiviral, anti-hypertensive, anticoagulant, antilipidemic, anti-diabetic, cardio-, gastro-, hepatoprotective, anti-inflammatory, and immunomodulatory activity (Kulling and Rawel 2008; Negreanu-Pirjol et al. 2023).

2.6. Characteristical Compounds of *Aronia melanocarpa*

The reduced sugar content of aronia berries is between 16 and 18, but the fruits have a pH between 3.3 and 3.7 (Kulling and Rawel 2008; Banach et al. 2020). Researchers have yet to find sucrose despite detecting high amounts of glucose and fructose (Kulling and Rawel 2008). The sorbitol, a sugar substitute used in diet foods, was determined to be 80 g/L in freshly squeezed aronia juices. The studies concluded that this fruit exhibited the highest concentration of these compounds compared to others (Kulling and Rawel 2008). Moreover, the study revealed aronia's potential biomarker use (Kulling and Rawel 2008).

The lipid content of aronia berry seeds was determined to be 1400 mg/kg FW. Researchers identified linoleic acid as the primary fatty acid among the seed contents, with an over 19,000 mg/kg concentration (Kulling and Rawel 2008). Phospholipids and sterols, mainly phosphatidylcholine, phosphatidylinositol, and phosphatidylethanolamine are essential for aronia seeds. The primary amino acid asparagine was determined to be protein, and the amount was determined to be 7000 mg/kg in fresh fruit weight.

The macro elements in aronia are potassium, calcium, phosphorus, magnesium, and sodium, while the microelements are iron, selenium, silicon, manganese, and zinc. Aronia berries and products derived from aronia are rich sources of calcium, magnesium, iron, potassium, phosphorus, and sodium (Kulling and Rawel 2008; Jurendi´c et al. 2021). Aronia surpasses blueberries, elderberries, and cranberries in terms of antioxidant content, making it highly valued in the pharmaceutical and cosmetic industries due to its depsides. Aronia is also a significant source of vitamin C; it constitutes approximately 120 mg/kg of the fresh fruit of aronia. More than 60% of dietary fiber in aronia consists of lignin, cellulose, and hemicellulose. Aronia includes vitamins B and K, as well as tocopherols, folic acid, and carotenoids. Beta-carotene, beta-cryptoxanthin, and violaxanthin are the most prevalent carotenoids. Aronia pomace, in addition to fresh fruit and juice, provides essential nutrients such as fiber, sugar, vitamin C, and minerals. These findings suggest that pomace powder could be used as a functional element in baked goods or as a nutritional supplement (Lazăr et al., 2023).

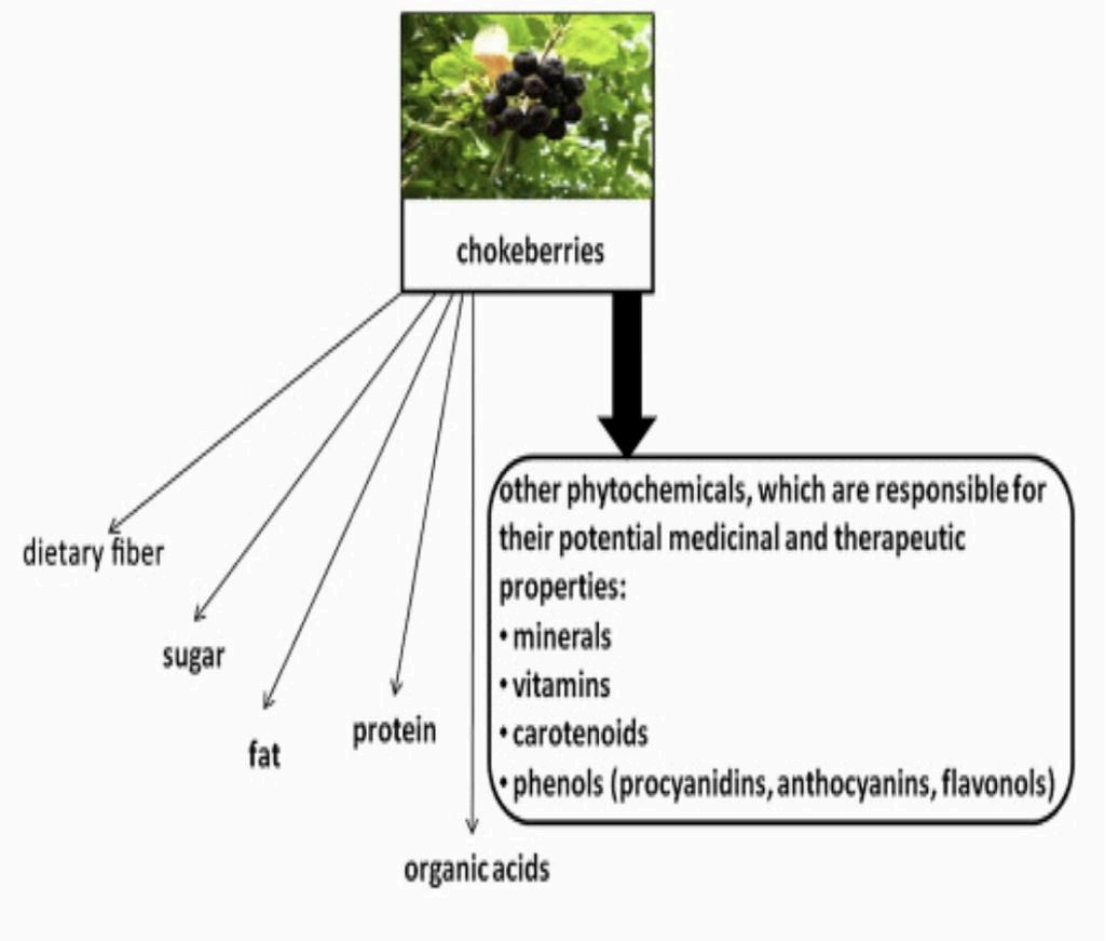

Figure 2.7. Chemical compounds of chokeberries, which are responsible for their potential medical and therapeutic properties (Olas, 2014).

2.7. Aronia Phenolic Compounds

Aronia plant is rich in phenolic compounds, essential bioactive substances with high antioxidant potential (Tolić et al. 2017). Although aronia contains incredibly high amounts of procyanidin, anthocyanidin, and phenolic acids, its flavonol content is relatively low. Despite the leaves of aronia are rich in polyphenols, flavonoids, and phenolic acids, they contain a small number of anthocyanins. The chemical components of melanocarpa differ between climatic region, harvest time, fruit ripening, pretreatment conditions, and extraction methods in studies (Zdunić et al. 2020; Jurendi´c et al. 2021; Shahin et al. 2019; Cvetanović et al. 2018). The main components of phenolic acids in

aronia are chlorogenic acid and neochlorogenic acid (Oszmiański and Wojdylo 2005; Rodríguez-Werner, Winterhalter, and Esatbeyoglu 2019). Aronia berries contain chlorogenic acid, 3-caffeoylquinic acid, 5-caffeoylquinic and 4-caffeoylquinic acid, while aronia leaves contain chlorogenic and non-chlorogenic acids (Deineka et al. 2020). Procyanidins in aronia are responsible for approximately 40% of the antioxidant activity (Gralec, Wawer, and Zawada 2019). As the fruits begin to mature, a decrease in the amount of procyanidin has begun to be observed (Gralec, Wawer, and Zawada 2019). The most common phenols in chokeberry are cyanidin-3-glycoside, epicatechin, and proanthocyanidins, accounting for 60% of the total.

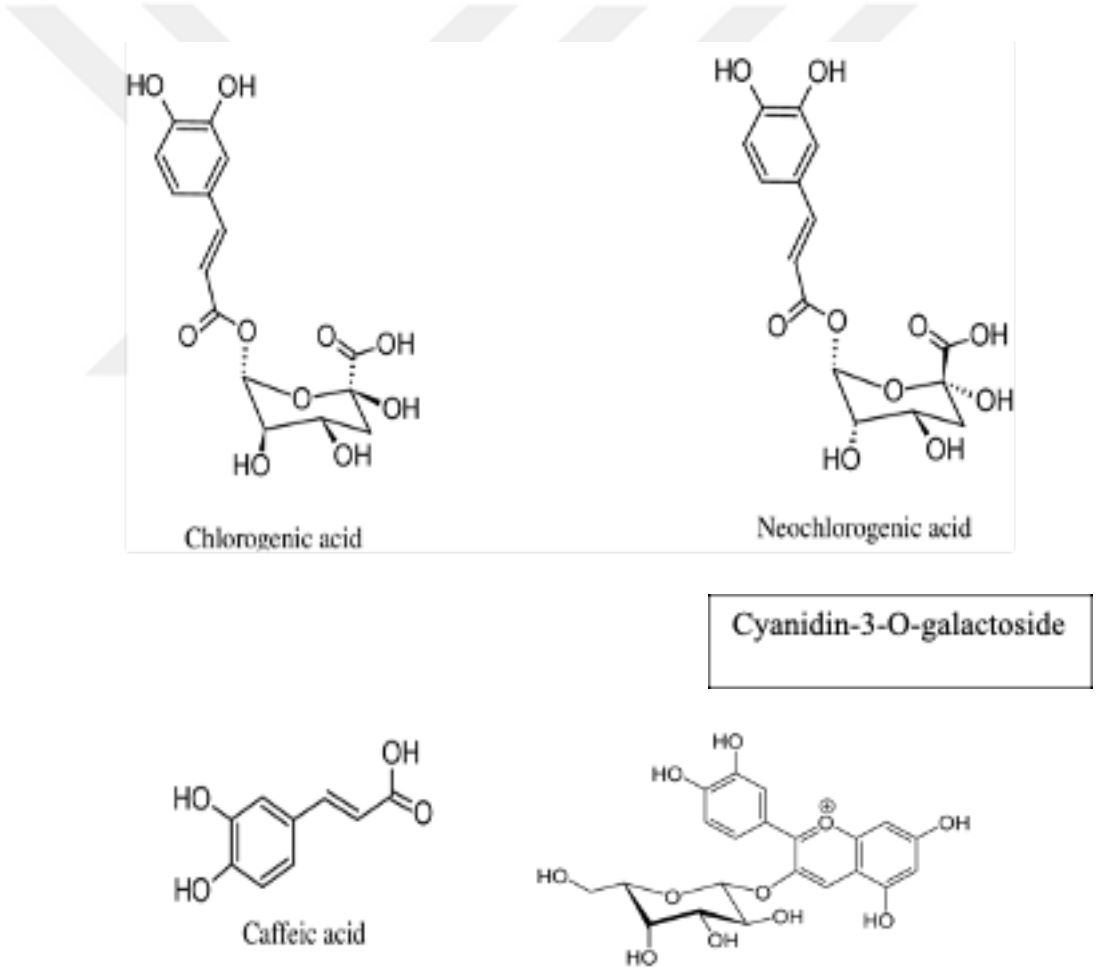

Figure 2.8. Chemical structures of cyanidin-3-O-galactoside, caffeic acid, chlorogenic acid, and neochlorogenic acid are present in *A. melanocarpa*.

According to recent research, anthocyanidins' antioxidant activity has the ability to prevent and/or lessen diabetes, cancer, and cardiovascular disorders (Miguel 2023). There are four main anthocyanins in aronia. These are 3-O-galactosides, 3-O-glucosides, 3-O-arabinosides, and 3-Oxyloside, making up about 25% of the polyphenol content in aronia (Oszmiański and Wojdylo 2005). The amount of anthocyanidins can be affected by environmental conditions. Storing the aronia fruits at 70°C for 24 hours decreased approximately 50% of the anthocyanidins, as observed in the research (Liang et al. 2021). Aronia berries also contained β-sitosterol, campesterol, triterpene, betulinic acid derivatives, 23-hydroxybetulinic acid, and 2α-hydroxy oleanolic acid. *Aronia melanocarpa* also has more than 40 volatile compounds. Chemical compounds found in aronia leaves stand out as valuable components in herbal teas, biocosmetic, food, and pharmaceutical industries (Sidor and Gramza-Michałowska 2019).

2.8. *Aronia melanocarpa* Application

The bitter and sour taste of aronia berries, which come from the *Aronia melanocarpa* plant, discourages people from eating them straight (Ren et al. 2022). However, they are extensively used in the food industry for various purposes. These berries, known for their high anthocyanin content, are used to make bread, doughnuts, cakes, yogurt, sauces, ice cream, pudding, pastries, spices, jams, marmalades, preserves, fruit juice, soft candies, chewing gum, wine, vinegar, and alcoholic beverages. Aronia berries can also be processed into fruit juice concentrate, nutritional supplements, and tea. When exposed to temperatures of 60°C for 8 hours, the anthocyanin content of aronia berries can decrease by 30%, leading to a decline of over 50% in their antioxidant properties. The co-pigmentation method is employed to address this issue.

Additionally, aronia juice is used in the food and pharmaceutical industries to enhance the color intensity, flavonoid, and anthocyanin levels of other fruit juices. Due to their high pectin content, aronia berries are often added to low-pectin fruits to produce mixed jams to improve flavor, color, and antioxidant properties. Aronia berries are considered a significant source of anthocyanin in the food industry and are utilized as a natural colorant (Rodríguez-Werner, Winterhalter, and Esatbeyoglu 2019). The pharmaceutical industry also evaluates aronia berry extract to produce syrups and dietary

supplements. Aronia berries are recognized as a functional food due to their notable antioxidant activity, and their popularity is growing worldwide.

2.9. Bioactive Properties of *Aronia melanocarpa*

Phenolic compounds found in aronia fruits and extracts show various biological activities. There is no harmful data in the literature about aronia berries or products derived from aronia (Valcheva-Kuzmanova and Belcheva 2006). According to Jurikova et al. 2017, aronia berries are known for their potential benefits, including anticancer, antiviral, antibiotic, antidiabetic, anti-inflammatory, antimutagenic, cardioprotective, gastroprotective, hepatoprotective, immunomodulatory, and radioprotective properties. These effects have been observed in laboratory and animal/human studies (Rodríguez-Werner, Winterhalter, and Esatbeyoglu 2019).

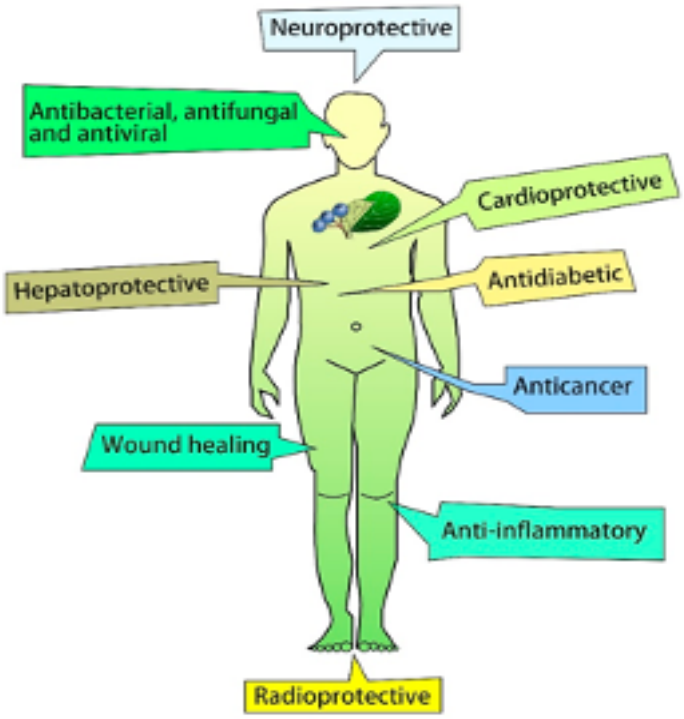

Figure 2.9. Pharmacological effects of *Aronia melanocarpa* (Negreanu-Pirjol et al. 2023).

2.9.1. Antimicrobial Activity

Aronia berries have strong antimicrobial properties, which previous studies have also documented. Extracts of aronia berries dissolved in water and ethanol show antimicrobial effects against food-borne bacteria, such as *Streptococcus pyogenes* (Daoutidou et al. 2021). The phenolic compounds found in aronia contribute to its antimicrobial effects (P. Denev et al. 2019). Previous studies have shown that aronia extract is effective against bacteria that cause meat spoilage, inhibits DNA synthesis in pathogenic bacteria, and causes morphological changes in bacterial cells (Efenberger-Szmechtyk et al. 2021). Research found that *Aronia melanocarpa* extract possesses antibacterial properties against *Proteus mirabilis*, *Proteus vulgaris*, *Bacillus subtilis*, *Staphylococcus aureus*, *Klebsiella pneumonia*, and *Escherichia coli*, and antifungal abilities against *Candida albicans* and *Aspergillus niger* (Cvetanović et al. 2018).

Furthermore, aronia fruit extracts have antibacterial consequences in opposition to *Bacillus cereus* and *Pseudomonas aeruginosa*. The antibacterial effect of this compound inhibits the formation of biofilm and bacterial homeostasis (Shahin et al. 2019). The antibacterial activity of *Aronia melanocarpa* fruits is believed to be a result of anthocyanins. A leading study found that anthocyanins from *Aronia melanocarpa* fruits destroyed *E. coli* cell walls and membranes, inhibited protein synthesis, stimulated protein degradation, and caused cell death (Deng et al. 2021).

An ethanol extract of *A. melanocarpa* was tested for antibacterial activity, and it showed complete inhibition against *Bacillus cereus*, moderate inhibition against *Staphylococcus aureus*, and no inhibition against *Cronobacter sakazakii* and *Salmonella enteritidis* (D.-H. Kim, Lim, et al. 2018). *A. melanocarpa* was more effective against gram-positive bacteria.

The researchers discovered that *A. melanocarpa* leaf extracts from various cultivars and maturity stages exhibited antibacterial activity against *Bacillus cereus*, as well as promising anti-dementia effects via inhibition of acetylcholinesterase and butyrylcholinesterase, indicating their potential as a natural resource with both antibacterial and anti-dementia properties (S. S. Kim and Shin 2020).

Investigating the impact of chokeberry pomace extracts on the growth of *Listeria monocytogenes*, *Brochothrix thermosphacta*, *Pseudomonas putida*, lactic acid bacteria,

and aerobic mesophilic bacteria (Tamkutė et al. 2021). The results revealed that extracts at 3% concentration efficiently inhibited the tested microorganisms (Tamkutė et al. 2021). After heating, the extracts were less effective against bacteria with a higher microbial load. Notably, a 2% ethanolic extract inhibited bacteria in several meat products. *A. melanocarpa* pomace extract could be a natural alternative to synthetic antimicrobials.

Anthocyanins, proanthocyanidins, flavonoids, and phenolic acids are all bioactive substances found in aronia berries that can act as antivirals against the influenza virüs (Negreanu-Pirjol et al. 2023). These compounds suppress virus surface glycoproteins. The evidence indicates that aronia berries might help in warding off and treating influenza. Ellagic acid and myricetin, compounds found in aronia berry powder, have been associated with anti-influenza effects, according to Park et al., 2013. Liquid extracts from frozen fresh fruits of *A. melanocarpa* and *Sambucus nigra* L. (elderberries) were tested against four human respiratory tract viruses, including influenza A virus (A/H1N1), coronavirus-1 (HCoV-OC43), human herpesvirus type 1 (HHV-1) and human adenovirus type 5 (HAdV-5) (Ochnik et al. 2022; Negreanu-Pirjol et al. 2023). A combination of these two fruits demonstrates anti-viral action against A/H1N1 and HCoV-OC43, but not much effectiveness against HHV-1 and HAdV-5. Using aronia berries or blackberries separately shows inhibition against A/H1N1 but not against HCoV-OC43. The outcomes demonstrate that aronia berry or blackberry effects on A/H1N1 and HCoV-OC43 may vary, and the mixed use of these fruits may have a restraining effect on HCoV-OC43.

Virus infections can also be associated with oxidative stress. In other words, viral infections trigger both the innate and adaptive immune systems, which in turn raise free radical production. Through the immune system's stimulation and the elimination of free radicals, antioxidants prevent viral infections (Hejrati et al., 2023). Aronia berry phenolic compounds exhibit strong antioxidant action (Sun et al., 2017). In addition, studies have shown that they may have immunomodulatory and anti-inflammatory activities. For example, they have been reported to inhibit nitric oxide production induced by lipopolysaccharide. Such properties suggest that aronia berries may have potential effects in preventing and treating viral infections associated with coronavirus disease 2019 (COVID-19). The effect of ursolic acid, one of the antioxidant phenolic compounds found in aronia berries, on signaling pathways to COVID-19 was investigated (Al-kuraishy et al. 2022). Clinical studies have been conducted to analyze the impact of Quercetin on severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2).

2.9.2. Antioxidant Activity

Antioxidant activities of extracts obtained from *Aronia melanocarpa* fruits have been reported in the literature in various studies. These extracts are notable for reducing oxidative stress and neutralizing free radicals. Research has linked aronia extracts to methods for evaluating antioxidant capacity.

The human body is continually producing free radicals and reactive oxygen species, and their excessive concentrations cause oxidative damage (Negreanu-Pirjol et al. 2023). Antioxidants protect nucleic acids, lipids, and proteins from free radicals, which can lead to inflammation, cancer, and other diseases (Tsao 2010; Vasantha Rupasinghe et al. 2015). Polyphenols can inhibit the generation of free radical precursors. More significantly, though, they stabilize the free radical by adding an electron to it, which ends the chain reactions that lead to lipid peroxidation (Rahman 2014). Due to the reducing nature of phenolic -OH groups and their capacity to chelate bi- and trivalent metals, flavonoids, flavonols, and phenolic acids all possess antioxidant properties (Rahman 2014). Against lipid peroxidation and free radical oxygen species (ROS), flavonoids offer protection (Rahman 2014; Shahin et al. 2019). Phenolic compounds scavenge free radicals and inhibit lipid peroxidation better than ligands.

Researchers evaluated the antioxidant activity of *Aronia melanocarpa* fruit and leaf extracts using two approaches: 2,2-diphenyl-1-picrylhydrazyl DPPH and 2,2'-azinobis (3-ethylbenzothiazoline-6 -sulfonic acid) ABTS tests. As shown by the DPPH test results, leaf extract displayed the highest antioxidant activity. For the ABTS test, on the other hand, the fruit extract showed the most powerful scavenging activity (Cvetanović et al. 2018). The differences between the two tests are probably because the DPPH and ABTS tests are based on different working mechanisms. By determining DPPH, ABTS, reducing power, and inhibition activity against lipid peroxidation in various *Aronia melanocarpa* leaf extracts, antioxidant activity was determined (Cvetanović et al., 2018; Negreanu-Pirjol et al., 2023), superoxide anion scavenging activity (Thi & Hwang, 2014), chemiluminescence method (CL), luminol-H_2O_2 system, pH 8.9 (Munteanu et al. 2020) and β-carotene fading test (Zdunić et al., 2020). As expected, antioxidant activity has been associated with various biological activities, such as anti-inflammatory activity (Zielińska et al. 2020), antitumor activity (Cvetanović et al.

2018), antimicrobial activity (Cvetanović et al. 2018), and hypoglycemic and anti-neurodegenerative activity.

In experiments with mouse models, *A. melanocarpa* extracts have been observed to reduce oxidative stress and inhibit lipid peroxidation. In particular, they were adding black aronia extract to the high-cholesterol diets of mice significantly reduced erythrocyte superoxide dismutase levels. Furthermore, lipid peroxidation induced by administering an oxidized fat mixture to mice was significantly reduced by black aronia extract supplementation. Furthermore, in an in vivo study, *A. melanocarpa* leaf extract was reported to accelerate skin epithelialization by showing antioxidant activity when applied to the damaged skin of female rabbits (Pirvu et al. 2015).

The antioxidant effects of *A. melanocarpa* extracts have also been studied in humans. Aronia extracts have been shown to improve platelet function and reduce oxidative stress in studies conducted on patients with cardiovascular risk factors. Furthermore, in patients with hypercholesterolemia, *A. melanocarpa* anthocyanin supplementation has been found to improve oxidative status and reduce levels of autoantibodies against oxidized low-density lipoproteins. Aronia extracts have also reduced oxidative stress associated with physical exercise. In studies on exercising individuals, black aronia extract supplementation inhibited lipid peroxidation and protected the antioxidant enzyme system after exercise. Another study observed that serum antioxidant capacity increased significantly in healthy individuals who consumed aronia juice daily (Nowak et al. 2016). However, another clinical trial found that it did not alter oxidative stress markers and total antioxidant activity in plasma and urine in healthy individuals consuming aronia fruit ethanolic extract daily (Xie et al. 2017). These studies suggest that the antioxidant capacity of aronia berries may depend on factors such as dose and duration. Future clinical trials may focus on optimizing the effective dose and examining the potentially toxic effects of aronia berries (Olas 2018).

A comparative study using the oxygen radical absorbing capacity assay showed that extracts from *A. melanocarpa* exhibited more potent antioxidant activity than extracts from blueberries (five times more), cranberry (eight times more) and lingonberry (four times more) (Zheng and Wang 2002). *Aronia melanocarpa* was found to be the most effective antioxidant among black currant, red currant, rosehip, and elderberry fruits evaluated based on oxygen radical absorbing capacity (Wu et al. 2004; Kokotkiewicz, Jaremicz, and Luczkiewicz 2010). Other studies based on the capacity of *A. melanocarpa* methanolic extracts to scavenge 2,2-diphenyl-1-picrylhydrazyl radicals revealed that

these extracts had stronger antioxidant potential than those of blackberry, red raspberry, and strawberry (Stojković et al. 2021; Espín et al. 2000; Kokotkiewicz, Jaremicz, and Luczkiewicz 2010). Moreover, regarding antioxidant activity, aronia extracts were found to be more potent than the synthetic antioxidants butylhydroxytoluene and butyl hydroxyanisole but weaker than α-tocopherol (Espín et al. 2000). A similar study evaluated the anti-DPPH radical activity of *A. melanocarpa* berries and extracts of other berries such as blueberry, rabbiteye blueberry, black currant and elderberry and found high antioxidant activity in all berries (Nakajima et al. 2004).

Among other berry species, such as crowberry, cloudberry, and whortleberry, *Aronia melanocarpa* berry extracts inhibit methyl linoleate autooxidation (Kähkönen et al. 1999). As compared with black currant juice, aronia juice inhibited phosphatidylcholine oxidation in peroxidizing liposomes twice as effectively. Furthermore, in this research, aronia juice demonstrated a strong synergistic impact with α-tocopherol, which was not detected with black currant. According to the findings, black aronia can be used as a colorant as well as an antioxidant to protect a-tocopherol and unsaturated fats in food products (Marek Pieszka et al. 2015).

Overall, black aronia extracts appear to have potent antioxidant properties and play a potential role in reducing oxidative stress. These extracts have positive effects on health and have the potential to be used in disease prevention.

2.9.3. Antiproliferative Activity

Numerous reports, in vitro studies and animal studies suggest that black aronia and/or black aronia extracts have antiproliferative effects (Bermúdez-Soto et al. 2007; Zhao et al. 2004; Lala et al. 2006; Malik et al. 2003).

Anthocyanin-rich *A. melanocarpa* extract was reported to inhibit the growth of human HT-29 colon cancer cells and stimulates apoptosis. However, aronia was shown to have a limited effect on the growth of NCM460 cells (Malik et al. 2003). *A. melanocarpa* extract inhibited growth more than grape and blueberry extracts (Malik et al. 2003). According to the study, prolonged exposure to the extract did not cause further changes in cell number, indicating that cell growth was inhibited cytostatically. Total

phenolic content, caffeic acid, and chlorogenic acid levels are related to the suppression of HT-29 cells by extracts of three different aronia species (Bermúdez-Soto et al. 2007). Aronia juice has also been shown to inhibit Caco-2 human colon cancer cells. *A. melanocarpa* extract has been shown to reduce HeLa cell proliferation and increase the generation of reactive oxygen species (Rugină et al. 2012).

A. melanocarpa extract inhibited the development of dysplasia, malignant transformation indicators generated by azoximazan, and the rate of colonic epithelial cell proliferation and faecal bile acid levels (Lala et al. 2006). A recent study found that 3-O-glucoside has chemopreventive properties (Lala et al. 2006). Another look investigated the efficacy of chokeberry, blueberry, and haskap berry polyphenols in suppressing the proliferation of human hepatoma cells HepG2 (Gao et al., 2018). The antiproliferative effect of polyphenols was determined to be dose-established. The most potent inhibitors of HepG2 cell growth were discovered to be berry polyphenols.

In this study, the researchers investigated the effect of a semipurified extract rich in anthocyanins from *A. melanocarpa* fruits on colon cancer cells (Malik et al. 2003). Following a 24-hour treatment, the researchers discovered a significant inhibitory effect (60% growth suppression) on HT-29 colon cancer cells, resulting in cell cycle arrest. Additionally, normal colon cells had no response to the extract, whereas colon cancer cells had lower cyclooxygenase-2 gene expression.

Another research investigation explored the effect of chokeberry juice, which is high in polyphenols, on CaCo2 colon cancer cells (Bermúdez-Soto et al. 2007). The exposure to the juice reduced cell proliferation and caused cell cycle arrest. The scientists discovered gene variations linked to colorectal cancer and in a set of genes involved in cell cycle regulation. Gene expression altered, especially for carcinoembryonic antigen-associated cell adhesion molecule (CEACAM1).

An azoxymethane/dextran sulfate sodium mice model and the Caco-2 cell line were used in this study to evaluate the therapeutic benefits and mechanisms of *A. melanocarpa* anthocyanins (AMA) in colitis-associated colorectal cancer (CRC) (Yu et al. 2021). The findings demonstrated that AMA dramatically suppressed cell growth, decreased inflammation, and downregulated the mammalian target of rapamycin complex 1 (mTORC1) signaling pathway. In addition, a reverse transcription PCR (RT-PCR) and Western blot analysis (WBT) analysis reveals that AMA dramatically decreased the levels of cytoplasmic catenin while inhibiting protein synthesis, which is linked with a pathway for WNT/beta-catenin signaling (Wei et al. 2020). The study was designed to

investigate how combinations of berry anthocyanidins affected the ability of non-small cell lung cancer (NSCLC) cells to fight cancer. Combined anthocyanidin inhibits the proliferation of her NSCLC cells, leading to cell cycle arrest, increased apoptosis, and reduced cell invasion and migration. These effects were related to regulating oncogenic pathways and their downstream targets, such as Notch and WNT (Kausar et al. 2012). Anthocyanidin has been demonstrated in animal experiments to considerably slow tumor growth. Lastly, the research revealed that some anthocyanidins found in berries may be useful for treating NSCLC and preventing metastasis and recurrence (Shi et al. 2021; Tsakiroglou et al. 2019; Kausar et al. 2012).

Aronia melanocarpa juice (AMJ) derived from *Aronia melanocarpa* was tested on mouse embryonal carcinoma (EC) stem cell line P19 to determine whether it affected cancer stem cells (CSCs). The findings showed that AMJ decreased CSC growth, caused cell cycle suppression and promoted apoptosis. It has been found that p53 and p73 tumor suppressor proteins are increased while UHRF1 anti-apoptotic protein and Oct-4 stemness factor are downregulated. The AMJ selectively targeted undifferentiated EC cells while not affecting normal cells, which suggests that it may be able to be applied as a targeted therapy for CSCs. According to these findings, AMJ has the potential as a chemopreventive agent to inhibit carcinogenesis and to suppress CSC proliferation (Sharif et al. 2013).

Scientists investigated whether phytochemicals could successfully target breast CSCs, which are highly resistant to conventional therapies. A chemical named 3-O-p-coumaroyltormentic acid was isolated from aronia extracts using rigorous testing procedures. The anticancer agent inhibits breast cancer cell growth and the formation of mammospheres, an important hallmark of CSCs. In additon, the CSC marker population was dramatically reduced, and self-renewal genes associated with CSCs were reduced as well. c-Myc, a critical protein for the survival of CSCs, was destroyed by 3-O-p-coumaryltormentic acid. By disrupting the c-Myc protein using 3-O-p-coumaroyltormentic acid, these findings provide a unique therapeutic method for breast cancer (Choi et al. 2018).

As part of the study, chokeberry juice was combined with a meal matrix to examine how stomach digestion affects chokeberry juice (Stanisavljević et al. 2015). After the juice was added to the food matrix, its polyphenol and anthocyanin content was immediately reduced, as was its DPPH scavenging activity and overall reduction power. While these characteristics increased following digestion, they remained low compared

to the original non-digested juice. Furthermore, the digested juice decreased the growth rate of Caco-2 cells. These findings demonstrate that, despite being transformed during digestion, chokeberry phenolics still have potent antioxidant and antiproliferative activities.

A recent study examined how *Aronia melanocarpa* extract affected colon cancer and non-tumorigenic umbilical vein endothelial cells (Çalışkan et al. 2023). The extract showed a toxic effect on colon cancer cells that decreased their viability. The discovery that human telomerase reverse transcriptase hTERT, a protein essential for colon cancer development, is more abundant in cancer cells than in endothelial cells is remarkable. Analysis of cancer cells revealed a substantial decrease in the presence of hTERT protein following treatment with aronia extract. This study has indicated that *Aronia melanocarpa* extract could potentially be used to treat colon cancer because of its ability to lessen cancer cell growth and change hTERT levels.

2.9.4. Cytotoxic Activity

Evaluating the cytotoxic effect of *Aronia melanocarpa* has been explored through in vitro and in vivo studies.

Examining the effect of chokeberry extract on the pancreatic cell AsPC1 and how it interacts with gemcitabine is the focus of this study (Thani et al. 2014). MTT assays and flow cytometry analyses have been used to assess the increase of cancer cells. Research has shown that chokeberry extract, either on its own or combined with gemcitabine, strongly inhibits cell growth. In addition, the extract has been demonstrated to cause apoptosis as supported by specific tests. This study suggests that chokeberry extract might be a good way to supplement gemcitabine in the treatment of pancreatic cancer.

The researchers studied the capability of *Aronia melanocarpa* fruit juice to protect human embryonal kidney cell HEK-239 from cisplatin-induced toxicity (Valcheva-Kuzmanova, Beronova, and Momekov 2013). Using MTT assays to determine the viability of cells, various cisplatin doses have been applied with or without pretreatment with *A. melanocarpa* fruit juice. According to the findings, cisplatin alone led to a

significant decrease in cell viability. The cytotoxicity of cisplatin was dramatically reduced when the cells were pretreated with *Aronia melanocarpa* fruit juice. Because oxidative stress contributes to the harmful effects of cisplatin, the observed protective effect of *Aronia melanocarpa* fruit juice is most likely due to its antioxidant activity.

The researchers examined the effects of leaf extracts from *Aronia melanocarpa*, *Cornus mas*, and *Chaenomeles superba* on the human colon cancer cell line Caco-2 (Efenberger-Szmechtyk and Nowak 2020). The extracts, which were high in bioactive components including polyphenols, showed antiproliferative action, with the *C. mas* extract being the most cytotoxic. In Caco-2 cells, the extracts caused morphological alterations and enhanced DNA damage in a concentration-dependent way. Interestingly, *A. melanocarpa* extract relatively increased cell proliferation at lower concentrations, indicating a potential defence mechanism. These data suggest that these leaf extracts may have anticancer action, but more research is needed to understand their processes and conduct in vivo experiments.

The researchers explored the effects of aronia extracts on malignant cell lines (A-549, LS-174T, and HeLa) as well as normal lung fibroblasts (MRC5) (Cvetanović et al. 2018). The extracts reduced the growth of cancerous cells, with HeLa cells being the most vulnerable. The cytotoxicity of the leaves extract was higher than that of the berries extract. Interestingly, the extracts had cytotoxic effects on normal cells as well, although with a higher selectivity than the chemotherapeutic drugs cisplatin. These data imply that aronia extracts, particularly from the leaves, have anti-cancer potential and justify further investigation for active chemicals and combination therapy with cisplatin.

In another study, chokeberry extract (CE) was tested in a B16-induced melanoma mice model (Gajić et al. 2022). CE was given to the mice both before and during the observation period. CE delayed the beginning of melanoma and increased the amount of immune cells in the tumor microenvironment, according to the findings. Although there were no significant alterations in several immunological molecules, CE-treated animals exhibited more IFN-producing cells and fewer CCR5-expressing MDSC in the tumor microenvironment. Although CE did not directly kill B16 cells in vitro, splenocytes from CE-treated animals revealed considerable cytotoxic effects on the cancer cells, mostly via IFN-. Finally, pre-treatment with CE increased the immune response to melanoma, indicating its promise as a therapeutic agent.

A recent study investigated the anti-inflammatory effects of Red Chinese cabbage (RC) and a combination of commercial Red Chinese cabbage leaves and aronia fruits on

LPS-stimulated RAW 264.7 cells (Kwak et al. 2020). The researchers assessed cytotoxicity and inflammatory markers using MTT assay and ELISA. The findings showed that pretreatment with aronia fruits extracts had no effect on cell growth or cytotoxicity. However, when compared to RC extracts, aronia fruits extracts showed significant reductions in inflammation-related indicators synthesis and gene expression. These data imply that aronia fruits has significant anti-inflammatory capabilities and could be used as a natural treatment for inflammatory diseases.

CHAPTER 3

MATERIAL AND METHOD

3.1. Microbial strains

Gram-negative and gram-positive bacteria were both used in the study's bacterial strains. *Escherichia coli* O157:H7 and *Salmonella* Typhimurium were chosen as gram-negative bacteria, whereas *Staphylococcus aureus* was chosen as a gram-positive bacteria. *Saccharomyces cerevisiae* was also selected as a yeast strain. Izmir Institute of Technology, Food Engineering Department, provided *Staphylococcus aureus*, *Escherichia coli* O157:H7, *Salmonella* Typhimurium, and *Saccharomyces cerevisiae*.

3.2. Extracts

Aronia melanocarpa dried extract and liquid extract were purchased from native producers.

Figure 3.1. *Aronia melanocarpa* dry extract and liquid extract.

3.3. Chemical Characterization

3.3.1. High Performance Liquid Chromatography (HPLC)

For identification and quantification of phenolic chemicals, high-performance liquid chromatography (HPLC) was used. The frozen plant extracts were allowed to defrost. The dissolved solutions were vortexed and filtered (0.2 μm), and 20 μL were injected into an analytical HPLC system (Agilent 1200) equipped with a Nucleosil 100-5C8 (25 cm x 4.6 mm; 5 m particle size) column. According to Fernandes et al. (2013) at a flow rate of 1 mL/min, solvent A (water/acetic acid, 98/2, v/v) and solvent B (methanol) were applied to the system: 0 to 3 min: 5-15% B linear; 3-13 minutes: 15-25% B linear; 13-25 minutes: 25-30% B linear; 25-35 minutes: 30-40% B linear; 35-40 minutes: 40-43% B linear; 40-50 minutes: 43-47% B linear; 50-54 minutes: 47-100% B linear. For detection, a diode array and Multiple Wavelength detector SL were utilized. All chromatograms were taken at the following wavelengths: 280, 320, 350, and 500 nm. All data was processed using Agilent ChemStation software (Hewlett-Packard ChemStation System). All measurements were taken three times.

3.3.2. Liquid Chromatography Quadrupole Time-Of-Flight Mass Spectrometry (LC-Q-TOF-MS)

An Agilent 1260 Binary LC system was used for the chromatographic separation. The mobile phases A and B were employed for the gradient elution, consisting of a mixture of water and 0.1% formic acid, and acetonitrile, respectively, based on the stepwise gradient shown in the table. At a temperature of 30 °C, the temperature of the column was maintained, and the volume of the injected sample was set at 2 μL, with a flow rate of 0.5 mL/min. The gradient elution proceeded as follows: 0-0.5 min, 5% B; 0.5-2 min, 25% B; 2-4 min, 50% B; 4-6 min, 75% B; 6-10 min, 95% B; and a column

conditioning step from 10 to 16 min with 5% B. The MS analysis was performed using the Agilent 6550 high-resolution Accurate Mass QTOF-MS instrument, with a sensitivity down to the femtogram level, a resolution of 40,000, and a scanning rate of 50 spectra per minute. The mass spectra were recorded within a 20-100,000 m/z mass range. Integration and data processing were carried out using the "MassHunter Workstation" software.

3.4. Antimicrobial Activity

The antibacterial activity of powder of aronia dried berries and extract of *Aronia melanocarpa* juice was tested against gram-negative *Escherichia coli* (ATCC 25922), gram-positive *Staphylococcus aureus* (RSKK 1009 strain), *Salmonella typhimurium*, and a yeast *Saccharomyces cerevisiae*. Bacterial strains were acquired from the Faculty of Food Engineering at IZTECH in İzmir, Turkey. The cultivation of bacterial cultures overnight in Nutrient Broth (NB) and Tryptic Soy Broth (TSB) is a recommended antibacterial protocol for different microorganisms (*Escherichia coli*, *Staphylococcus aureus*, *Salmonella typhimurium*, and *Saccharomyces cerevisiae*). The cultures were placed in a test tube the next day, and their optical density was adjusted to the 0.5 McFarland standard with peptone water (DEN-1 Densitometer, Riga, Latvia). The bacterial cultures were then diluted with NB and TSB to 10^5 CFU mL^{-1} for *E. coli*, *S. typhimurium*, *S. aureus*, and *S. cerevisiae*, respectively. *E. coli*, *S. typhimurium*, *S. aureus*, and *S. cerevisiae* were cultured on NB and TSB media with varying doses of *A. melanocarpa* dried extract and *A. melanocarpa* juice extract to investigate antibacterial activity. Following preliminary research, the necessary doses for these tests were determined to be 180 ml-18 ml. In addition, control samples for *E. coli*, *S. typhimurium*, *S. aureus*, and *S. cerevisiae* were created without the addition of *A. melanocarpa*. The bacterial cultures were cultured for 24 hours in an incubator set to 37 °C and 200 rpm, and three tests were performed for each sample and bacterial culture. Initially, turbidity visualization was employed to determine bacterium turbidity. The cultured bacterial cultures were diluted with peptone water in a 1:10 ratio. For *E. coli*, *S. typhimurium*, *S. aureus*, and *S. cerevisiae*, this method was repeated at the sixth dilution. Then, in that

sequence, 100 L of each stock and diluted sample was put onto the NB and TSB agar plates for *E. coli*, *S. typhimurium*, *S. aureus*, and *S. cerevisiae*. The plates were incubated for 24 hours at 37 °C while growing. After counting the viable bacteria, colony forming units (CFU mL^{-1}) values were calculated. The antibacterial activity was defined as follows:

$$\text{Antibacterial activity (\%)} = (N0\text{-}N/N0) \times 100$$

N0 denotes the number of CFU before the antibacterial agent was added, and N represents the number of CFU after it was added.

3.5. Fourier Transform Infrared Spectroscopy Analysis (FTIR)

A Perkin-Elmer spectrometer (UATR Two) was used to produce the FTIR spectra of *A. melanocarpa* dry extract and *A. melanocarpa* liquid extract. The spectra were obtained at room temperature using the attenuated total reflection (ATR) method in the 450-4000 cm^{-1} region with a resolution of 4 cm^{1}. The extracts' absorbance values were recorded. Each spectrum was subjected to 64 scans. The OMNIC software was used to analyze the FTIR spectra (Li et al. 2016).

3.6. Total Phenol Content

The total phenolic content was determined using the Folin-Ciocalteu test. Mixed samples were diluted and combined with Folin-Ciocalteu's reagent. After adding sodium carbonate, the mixture was covered in darkness for one hour. Then each solution was taken into account for a 765 nm absorbance measurement. In the same way, a calibration curve is generated using different concentrations of gallic acid. The equivalent of gallic acid (GAE) is given in mililiters to make the calculation easier (Slinkard and Singleton 1977).

3.7. Total Flavonoid Content

Total flavonoid content (TFC) of aronia extracts was evaluated using colorimetric methods described in previous investigations (Kim et al., 2003; Zhishen et al., 1999). TFC is evaluated by diluting the extract in 2 ml of distilled water and mixing it with 150 µl of 5% $NaNO_2$. The mixture was treated with 150 µl of 10% $AlCl_3$ after a 6-minute incubation time. After 6 minutes, 2 mL of 1N NaOH was added, reducing the final volume to 5 mL. The absorbance of the solution at 510 nm was measured using a spectrophotometer, and the flavonoid concentration was reported as mg of quercetin equivalents per 100 g of fresh weight (FW).

3.8. Total Monomeric Anhocyanin Content

The pH differential technique published by Giusti et al. (2007) was used to calculate the total monomeric anthocyanins content (TAC). This method capitalizes on anthocyanin pigments' propensity to change color in response to pH levels. To calculate TAC, the same sample was diluted twice: once in a potassium chloride buffer (0.025 M, pH 1.0) and once in a sodium acetate buffer (0.4 M, pH 4.5), with the pH adjusted with 0.2N HCl. After 15 minutes of equilibration at room temperature, the absorbance of both dilutions was measured at 510 nm and 700 nm with a UV-Vis Microplate Reader. After that, the total amount of monomeric anthocyanins was calculated as mg of cyanidin 3-galactoside (cy-3-glu) equivalent per 100 g of fresh weight.

TAC (mg / L) = $(A \cdot MW \cdot DF \cdot V \cdot 100) / \varepsilon \cdot L \cdot Wt$

A = $(A_{510nm} - A_{700nm})_{pH=1} - (A_{510nm} - A_{700nm})_{pH=4.5}$

The semnifications of symbols used in these relations are:

A – Absorbance

ε – Molar extinction coefficient

L – Path length

MW – Molecular weight (484.84 g/mol for cy-3-glu)

DF – Dilution factor

V – Volume

Wt – Sample weight

3.9. Scavenging Effect On DPPH And ABTS Radicals

The DPPH and ABTS radical scavenging method was used to investigate antioxidant activity. DPPH (2,2-diphenyl-1-picrylhydrazyl) is used to identify free radicals, while ABTS (2,2′-azinobis-(3-ethylbenzothiazoline-6-sulfonic acid)) is used to quantify the reduction of free radicals via an oxidation process. The samples were diluted in three different quantities, and the experiment was carried out three times. For the antioxidant analyses of ABTS and DPPH, 0.5 grams of *Aronia melanocarpa* dried extract was weighed and extracted with 10 ml of methanol.

Table 3.1. The determination of antioxidant capacity.

	Control (µl)	**Blind 1 (µl)**	**Sample (µl)**	**Blind 2 (µl)**
Methanol	100	200	-	100
DPPH/ABTS	100	-	100	-
Sample			100	100

% Antioxidant Activity = $[(A_{Control} - A_{Sample}) / (A_{Control})]x100$ was used to calculate antioxidant activity.

The EC_{50} values were derived utilizing experiments with samples at various doses. For this reason, activity studies with at least five distinct sample concentrations were carried out three times. The results were expressed as a percentage of activity compared to the control. Using linear regression formulae, the acquired percentage activity curves were evaluated to calculate the EC_{50} value, which reflects the phenol concentration that impacts 50% of the biological activity.

3.10. Cellular Antioxidant Activity Assay

The antioxidant activity test was carried out on human umbilical vein endothelial cells (HUVEC) at the cellular level. Three compounds were evaluated at four different doses to determine their antioxidant activity on HUVEC cells. The cells were seeded at a density of $1x10^5$ cells/mL in 96-well microplates. The experiment was carried out in a 37°C atmosphere with 5% CO_2 and 95% humidity. After a 24-hour incubation period, the cells were exposed for one hour to DMEM High Glucose solution containing 1mM hydrogen peroxide. This exposure caused oxidative stress in the cells, allowing the samples' protective effects to be assessed. Cell viability was determined after incubation by measuring absorbance at 570 nm using the MTT assay.

3.11. Cytotoxicity MTT Assay

A colorectal adenocarcinoma cell line, Caco-2, was used for cytotoxicity analysis in this study. In 96-well microplates, cells were seeded at a density of $1x10^5$ cells/mL. The cells were exposed to the chemical at four different concentrations for 24 hours (at 37°C, 5% CO_2, and 95% humidity). To investigate the cytotoxicity of the substance, the absorbance at 570 nm was determined using the MTT test and a spectrophotometer.

3.12. Scratch Assay

HUVEC (Human Umbilical Vein Endothelial Cells) and HaCat cell line, a spontaneously immortalized human keratinocyte cell line were used in the wound healing assay. The cells were planted in 24-well plates at 5x105 cells/mL density and incubated at 37°C with 5.0% CO_2 and 95% humidity until confluent. When the cells had reached confluence, a scratch line was drawn with a yellow pipette tip and the media was refilled.

After applying the test substance, the cells were incubated at 37°C with 5.0% CO_2 and 95% humidity. Furthermore, cells without any test material were used as the study's control group. Images of the cells were acquired at 10X magnification using an inverted microscope (Axio, Zeiss, Germany).

CHAPTER 4

RESULTS AND DISCUSSION

4.1. Chemical Characterization

4.1.1 HPLC

HPLC was used in the study to conduct quantitative analyses on the dry and liquid extracts of *A. melanocarpa*. Compound retention times, peak areas, and peak widths were measured as part of the investigation to determine the HPLC system's separation time, compound quantity, and separation quality. One mililiter per minute flow rate was used for all analyses.

Figure 4.1. exhibits the observations of *Aronia melanocarpa* liquid extract at various wavelengths. The fractions observed at a wavelength of 280 nm correspond to the peaks A, B, C, D, and E for the liquid extract of *Aronia melanocarpa.* Peaks F, G, and H, on the other hand, matched fractions found at 320 nm, and peak I, fractions found at 350 nm.

Compound A's peak area, peak width, and retention time for the liquid extract of *Aronia melanocarpa* were 1415 units, 4.749 minutes, and 0.1 seconds, respectively. Compound B had a retention time of 6.515 minutes, a peak area of 1612 units, and a peak width of 0.2 seconds. Compound C had a retention time of 9.078 minutes, a peak area of 4061 units, and a peak width of 0.2 seconds. Compound D had a retention time of 10.644 minutes, a peak area of 2426 units, and a peak width of 0.2 seconds. Compound E had a retention time of 19.766 minutes, a peak area of 2255 units, and a peak width of 0.4 seconds.

Compound F had a retention time of 10.645 minutes, a peak area of 4374 units, and a peak width of 0.2 seconds. Compound G had a retention time of 19.752 minutes, a peak area of 4938 units, and a peak width of 0.3 seconds. Compound H had a retention time of 15.001 minutes, a peak area of 1951 units, and a peak width of 0.3 seconds.

Compound I had a retention time of 15.004 minutes, a peak area of 693 units, and a peak width of 0.3 seconds.

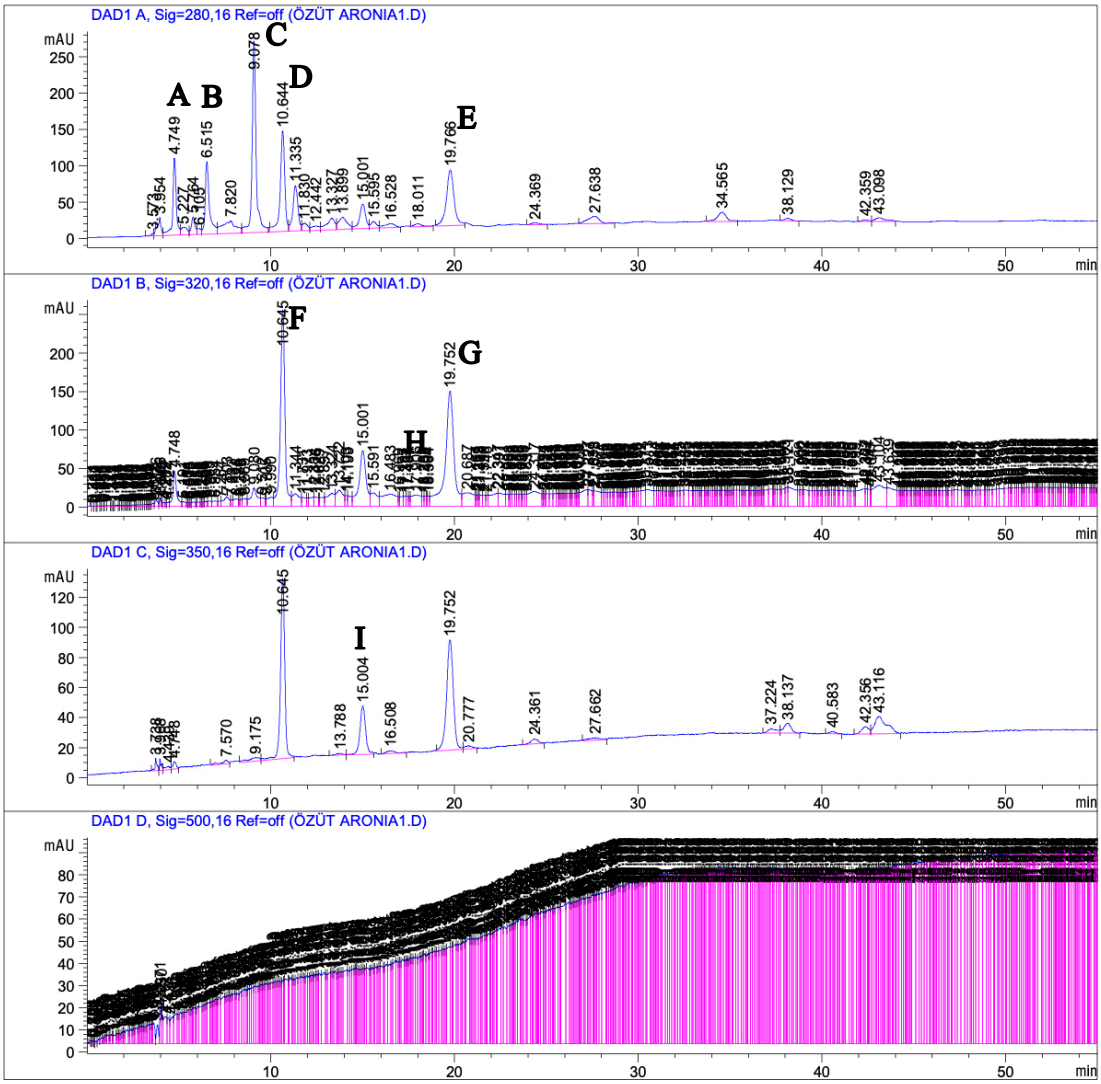

Figure 4.1. Chromatogram of *Aronia melanocarpa* liquid extract.

Figure 4.2. exhibits the observations of *Aronia melanocarpa* liquid extract at various wavelengths. For the dry extract of *Aronia melanocarpa*, peaks A, B, and C

corresponded to fractions detected at 280 nm. In comparison, peaks D and E corresponded to fractions detected at 350 nm, and peak F corresponded to fractions detected at 500 nm.

For the dry extract of *Aronia melanocarpa*, compound A had a retention time of 1.223 minutes, a peak area of 7388 units, and a peak width of 0.2 seconds. Compound B had a retention time of 1.923 minutes, a peak area of 8.863 units, and a peak width of 0.3 seconds. Compound C had a retention time of 3.918 minutes, a peak area of 2.115 units, and a peak width of 0.1 seconds.

Compound D had a retention time of 3.922 minutes, a peak area of 808.66 units, and a peak width of 0.1 seconds. Compound E had a retention time of 19.246 minutes, a peak area of 20.776 units, and a peak width of 0.2 seconds.

Compound F had a retention time of 3.917 minutes, a peak area of 6910 units, and a peak width of 0.1 seconds.

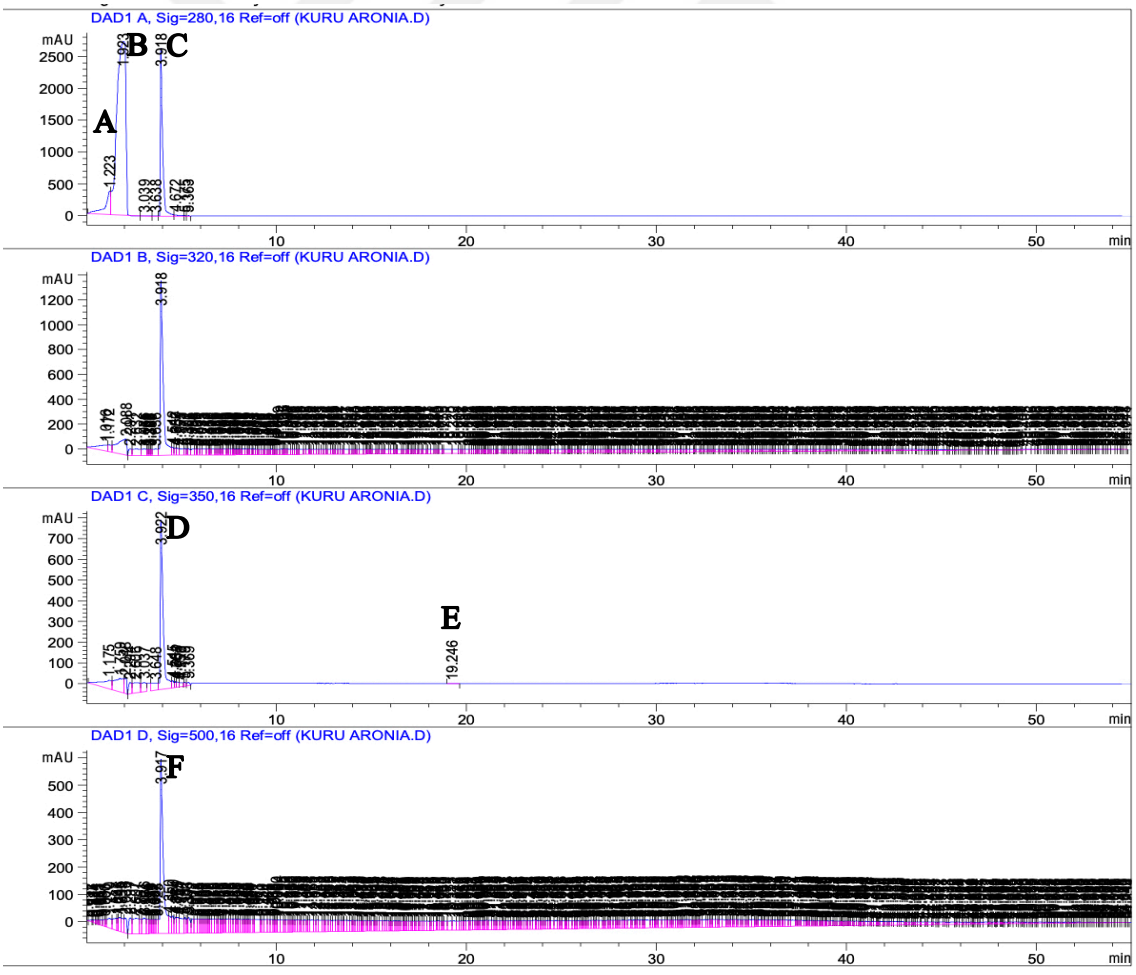

Figure 4.2. Chromatogram of *Aronia melanocarpa* dry extract.

Black aronia fruits were studied by Jakobek et al., 2007 in order to determine their polyphenol content, anthocyanin content, and antioxidant properties. According to the study, these fruits contain large amounts of anthocyanins and polyphenols. Using HPLC analysis, the flavonols (quercetin, chemferol, and myricetin) and anthocyanins in the aronia fruit composition were identified. The cyanidin derivatives cyanidin-3-galactoside (68.9%) and cyanidin-3-arabinoside (68.1%) have the highest anthocyanin content. In addition, myricetin was absent from the fruit's composition, chemferol levels were low (6.93%), and quercetin made up 93.17% of the fruit's total flavonol content (Jakobek et al. 2007).

In another HPLC study, chlorogenic acid was found to be highly concentrated in the fruit extract from green aronia (Zielińska et al. 2020). In a different study focused on extracts from *Aronia melanocarpa* fruits cultivated in the Kırklareli region, revealed the presence of various phenolic compounds such as ferulic acid, caffeic acid, quercetin, and p-coumaric acid (Gürer, Karadağ, and Altıntaş 2023). The ethyl acetate extract showed the highest concentration of phenolic substances, while the malvin anthocyanin compound was detected only in the methanol extract.

4.1.2. LC-Q-TOF-MS

High-resolution mass spectrometry (Q-TOF-MS) is an analytical method used to find substances' atomic composition and arrangement based on their relative masses. *Aronia melanocarpa* dried and liquid extracts provided efficient data for Q-TOF-MS analysis. The total ion chromatograms of the samples are presented in Figure 4.3. and Figure 4.4. The individual Q-TOF-MS chromatograms of the extracts with mass ratios and retention time (RT) information showing detailed fragmentations are also included below in Table 4.1. and Table 4.2. The major components of *Aronia melanocarpa* dried and liquid investigated in this study are presented in Figure 4.5.

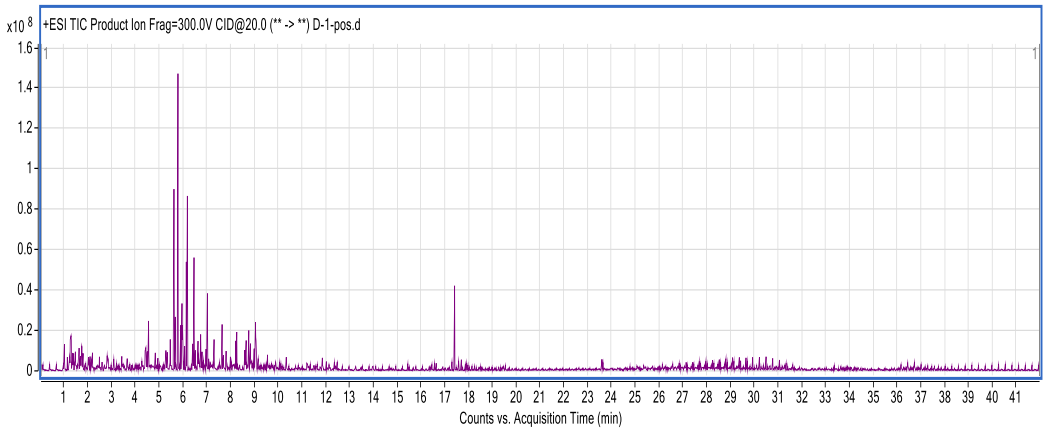

Figure 4.3. LC-MS chromatogram of *A. melanocarpa* dry extract.

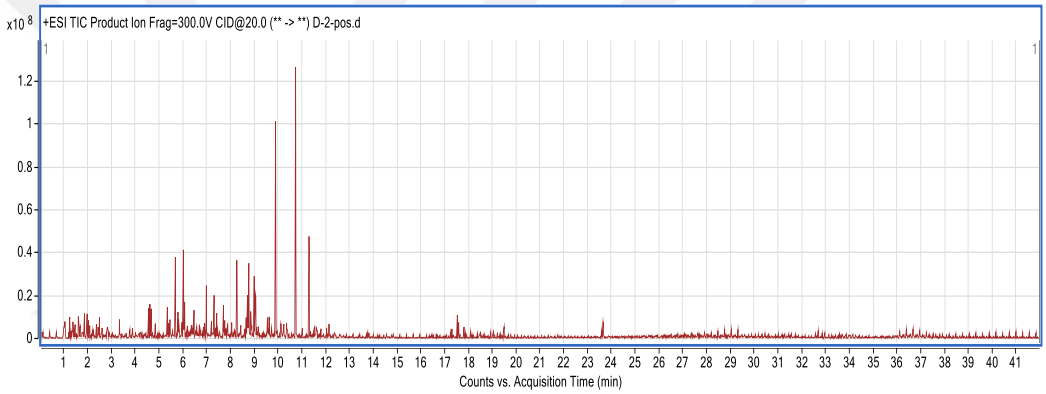

Figure 4.4. LC-MS chromatogram of *A. melanocarpa* liquid extract.

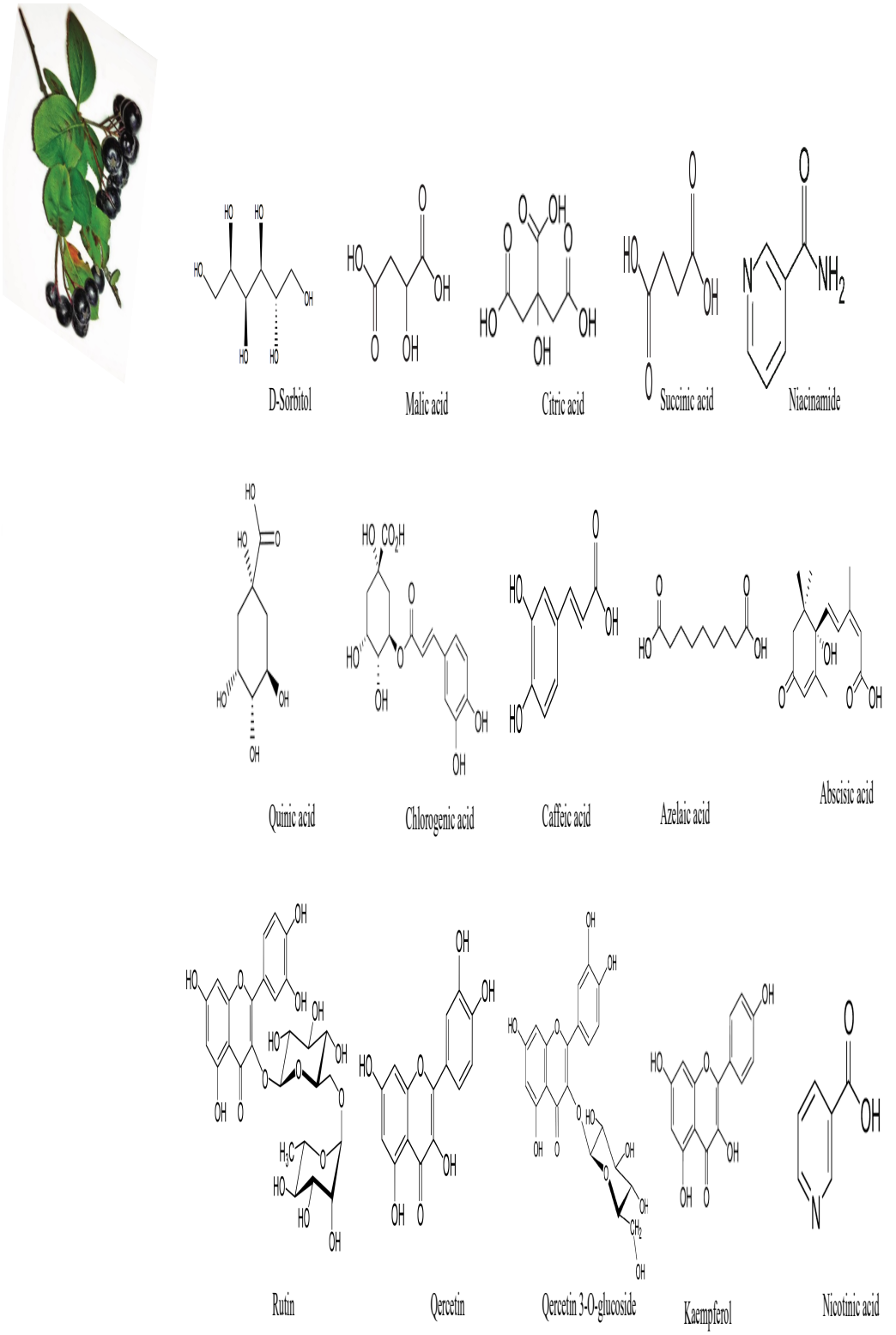

Figure 4.5. Chemical formulas of compounds in *A. melanocarpa* dry and liquid extract.

Table 4.1. Retention time, mass and formula of *A. melanocarpa* dry extract.

Compound Name	**Retention Time**	**Mass**	**Formula**
Gulonic acid	1.196	196.0614	C6H1207
Galactaric acid	1.263	210.0385	C6H10O8
Malic acid	1.365	134.0233	C4H6O5
D-Sorbitol	1.499	182.0798	C6H14O6
Citric acid	1.533	192.0308	C6H8O7
Succinic acid	1.937	118.027	C4H6O4
L-Iditol	2.06	182.0798	C6H14O6
L-Phenylalanine	2.902	165.0799	C9H11NO2
4-Formyl Indole	3.397	145.0532	C9H7NO
Pyrocatechol	4.07	110.037	C6H6O2
3,4-Dihydroxybenzoic acid	4.087	154.0289	C7H6O4
Quinic acid	4.564	192.0643	C7H12O6
Phloracetophenone	5.968	168.0425	C8H8O4
Chlorogenic acid	6.046	354.0951	C16H18O9
Amygdalin	6.091	457.1575	C20H27NO11
Caffeic acid	6.585	180.043	C9H8O4
Rutin	8.684	610.1517	C27H30O16
D-Tryptophan	8.943	204.0904	C11H12N2O2
Hyperoside	9.28	464.0945	C21H20O12
N-Acetyl-DL-tryptophan	9.302	246.101	C13H14N2O3
Kaempferol 7-O-glucoside	10.032	448.1002	C21H20O11
Azelaic acid	10.346	188.1053	C9H16O4
4-Hydroxybenzoic acid	10.571	138.0322	C7H6O3
Phlorhizin	10.739	436.1358	C21H24O10
Purpurin	10.874	256.0373	C14H8O5
(+)-Abscisic Acid	12.087	264.136	C15H20O4
Sebacic acid	12.389	202.1208	C10H18O4
Quercetin	12.603	302.0425	C15H10O7

(cont. on next page)

Table 4.1. (cont.)

Glycerophospho-N-Oleoyl Ethanolamine	15.14	479.3003	C23H46NO7P
1-Octadecyl Lysophosphatidic Acid	17.161	424.294	C21H45O6P
(±)12,13-DiHOME	19.384	314.2449	C18H34O4
Lauryl hydrogen sulfate	25.166	266.1552	C12H26O4S
1-Hexadecyl Lysophosphatidic Acid	34.944	396.2631	C19H41O6P
Trigonelline	1.273	138.0562	C7H8NO2
DL-pipecolic acid	1.386	129.0795	C6H11NO2
Guanine	1.442	151.05	C5H5N5O
Nicotinic acid	1.667	123.0321	C6H5NO2
L-Tyrosine	1.807	181.0747	C9H11NO3
Adenosine	1.813	267.0974	C10H13N5O4
L-Phenylalanine	2.88	165.0799	C9H11NO2
Luteolin 4'-O-glucoside	4.014	448.101	C21H20O11
Kaempferol 7-O-glucoside	4.295	448.101	C21H20O11
Larixinic Acid	4.396	126.0318	C6H6O3
Fisetin	4.474	286.0488	C15H10O6
L-Tryptophan	4.519	204.0909	C11H12N2O2
Chlorogenic acid	4.575	354.0955	C16H18O9
Indole	4.631	117.058	C8H7N
Astragalin	4.677	448.101	C21H20O11
Rustoside	5.653	580.1432	C26H28O15
Amygdalin	6.035	457.1585	C20H27NO11
Apigenin 7-glucoside	6.316	432.1059	C21H20O10
Procyanidin B2	6.417	578.1422	C30H26O12
(-)-Epicatechin	6.866	290.0796	C15H14O6
Quercetin	7.652	302.0435	C15H10O7

(cont. on next page)

Table 4.1. (cont.)

2,5-Dihydroxybenzaldehyde	8.011	138.0324	C7H6O3
Peltatoside	8.27	596.1387	C26H28O16
Rutin	8.663	610.1536	C27H30O16
Quercetin 3-O-glucoside	8.786	464.0962	C21H20O12
Hyperoside	9.056	464.0962	C21H20O12
3-[(2S,3R,4S,5S,6R)-4,5Dihydroxy-6-(hydroxymethyl)-3-[(2S,3R,4S,5R)-3,4,5trihydroxyoxan-2-yl]oxyoxan-2-yl]oxy-2-(3,4-dihydroxyphenyl)-5-hydroxy-7-methoxychromen-4-one	9.348	610.1536	C27H30O16
3-O-Methylquercetin	9.674	316.0592	C16H12O7
5,7-Dihydroxy-2-(4-hydroxy-3-methoxyphenyl)-3-[3,4,5-trihydroxy-6-[[(2R,3R,4R,5R,6S)-3,4,5-trihydroxy-6- C28H32O16 -0.23 1 methyloxan-2-yl]oxymethyl]oxan-2-yl]oxychromen-4-one	9.786	624.1692	C28H32O16
Luteolin	10.011	286.0488	C15H10O6
Fungichromin	14.559	670.3926	C35H58O12
Stearidonic Acid	14.861	276.2098	C18H28O2
3-methoxy Prostaglandin F1α	16.928	386.2674	C21H38O6

(cont. on next page)

Table 4.1. (cont.)

N-tert-Butyl-α-phenylnitrone	17.085	177.1161	C11H15NO
Tributyl phosphate	21.6	266.1657	C12H27O4P
MG(18:2(9Z,12Z)/0:0/0:0)[rac]	22.105	354.2775	C21H38O4
Dibutyl phthalate	23.621	278.1528	C16H22O4
Acetyl tributyl citrate	25.564	402.2264	C20H34O8
1,2-Dioleoyl PC	33.615	786.6015	C44H85NO8P
1-Hexadecyl Lysophosphatidic Acid	35.738	396.2646	C19H41O6P

Table 4.2. Retention time, mass and formula of *A. melanocarpa* liquid extract.

Compound Name	**Retention Time**	**Mass**	**Formula**
D-Sorbitol	0.544	182.0796	C6H14O6
Gulonic acid	1.196	196.0601	C6H12O7
Maltitol	1.218	344.131	C12H24O11
Hydroxycitric acid	1.319	208.023	C6H8O8
Malic acid	1.375	134.0216	C4H6O5
Pyrocatechol	1.869	110.0369	C6H6O2
Succinic acid	1.937	118.027	C4H6O4
(S)-Citramalic acid	1.948	148.0375	C5H8O5
Citric acid	2.105	192.0275	C6H8O7
Gallic acid	2.554	170.0221	C7H6O5
3,4-Dihydroxybenzoic acid	4.059	154.027	C7H6O4
Esculetin	5.147	178.0271	C9H6O4
4-Formyl Indole	5.529	145.0531	C9H7NO

(cont. on next page)

Table 4.2. (cont.)

4-Hydroxy-3-methoxycinnamaldehyde	5.821	178.0635	C10H10O3
m-Coumaric acid	5.922	164.0478	C9H8O3
Phloracetophenone	5.989	168.0425	C8H8O4
Chlorogenic acid	6.046	354.0955	C16H18O9
Quinic acid	6.141	192.0661	C7H12O6
Amygdalin	6.147	457.1568	C20H27NO11
L-Iditol	6.203	182.0796	C6H14O6
Caffeic acid	6.596	180.0428	C9H8O4
N-Acetyl-L-phenylalanine	8.01	207.0899	C11H13NO3
Rutin	8.718	610.1526	C27H30O16
N-Acetyl-DL-tryptophan	8.943	246.1002	C13H14N2O3
Hyperoside	9.291	464.0948	C21H20O12
Azelaic acid	10.346	188.1053	C9H16O4
4-Hydroxybenzoic acid	10.571	138.0323	C7H6O3
Phlorhizin	10.705	436.1359	C21H24O10
Abscisic acid (cis,trans)	12.086	264.136	C15H20O4
Quercetin	12.602	302.0424	C15H10O7
1-Octadecyl Lysophosphatidic Acid	15.308	424.2939	C21H45O6P
Lauryl hydrogen sulfate	25.233	266.1549	C12H26O4S
1-Hexadecyl Lysophosphatidic Acid	35.123	396.2628	C19H41O6P
Glycerophosphocholine	1.32	258.1115	C8H21NO6P
DL-pipecolic acid	1.432	129.0797	C6H11NO2
Guanine	1.489	151.0502	C5H5N5O
Niacinamide	1.713	122.0484	C6H6N2O
Adenosine	1.87	267.0983	C10H13N5O4
L-Phenylalanine	2.847	165.0799	C9H11NO2
Larixinic Acid	3.162	126.032	C6H6O3

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Table 4.2. (cont.)

Pro Leu	3.51	228.1486	C11H20N2O3
Ile Val	3.577	230.1642	C11H22N2O3
Pro Leu	3.914	228.1486	C11H20N2O3
Ile Asp	3.948	246.1227	C10H18N2O5
Val Ile	4.026	230.1642	C11H22N2O3
Pseudoephedrine	4.296	165.1159	C10H15NO
Larixinic Acid	4.363	126.032	C6H6O3
Val Ile	4.7	230.1642	C11H22N2O3
Phe Val	5.093	264.1482	C14H20N2O3
Astragalin	5.655	448.1011	C21H20O11
Ile Leu	5.924	244.1796	C12H24N2O3
Chlorogenic acid	6.037	354.0959	C16H18O9
Leu Ile	6.497	244.1796	C12H24N2O3
(R)-(-)-Mellein	7.092	178.0641	C10H10O3
Irigenol	7.451	318.0382	C15H10O8
Phe Ile	7.665	278.1637	C15H22N2O3
Quercetin	7.71	302.0436	C15H10O7
Quercetin3-Oglucoside	7.777	464.0962	C21H20O12
Peltatoside	8.282	596.1381	C26H28O16
Rutin	8.732	610.1534	C27H30O16
Hyperoside	9.058	464.0962	C21H20O12
3-O-Methylquercetin	9.338	316.0589	C16H12O7
3-[(2S,3R,4S,5S,6R)-4,5-Dihydroxy-6-(hydroxymethyl)-3-[(2S,3R,4S,5R)- 3,4,5-trihydroxyoxan-2-yl]oxyoxan-2-yl]oxy-2-(3,4-dihydroxyphenyl)-5- hydroxy-7-methoxychromen-4-one	9.349	610.1534	C27H30O16

(cont. on next page)

Table 4.2. (cont.)

Dextromethorphan	11.528	271.1945	C18H25NO
2,3-dinor Prostaglandin E1	15.189	326.2099	C18H30O5
Diethyltoluamide	15.47	191.1322	C12H17NO
N-tert-Butyl-α-phenylnitrone	16.862	177.1165	C11H15NO
Dibutyl phthalate	23.622	278.153	C16H22O4
Acetyl tributyl citrate	25.566	402.2267	C20H34O8
1,2-Dioleoyl PC	26.374	786.6025	C44H85NO8P
1-Monopalmitin	28.171	330.2783	C19H38O4
1-Hexadecyl Lysophosphatidic Acid	35.245	396.2651	C19H41O6P

Compounds were determined by LC/MS analysis of *Aronia melanocarpa* dry extract and liquid extract. The type and content of plant polyphenols can be affected by maturity. In their study, Lee et al., 2014 extracted *Aronia melanocarpa* leaves at three maturities (young, mature, and old) with 70% aqueous methanol. The characterisation of five flavonoids and a dicaffeoylquinic acid derivative in aronia leaves was conducted for the first time in their study.

Anthocyanins, flavonols, and phenolic acids were measured in methanolic extracts of *Aronia melanocarpa*, *Aronia arbutifolia*, and *Aronia prunifolia* fruits and leaves (Szopa et al. 2017b). The fruits contained significant amounts of chlorogenic acid. Leaf extracts contained high rutin and chlorogenic acid (Szopa et al. 2017b).

In this study, fractions with qualitative and quantitative composition were obtained.

4.2. Antimicrobial Activity

The aim was to determine the effectiveness of aronia dry and liquid extracts in inhibiting the growth of the bacteria.

The antibacterial properties of four different concentrations (180, 90, 36, 18 µg/mL) of aronia dry and liquid extract were studied by testing on two gram-positive bacteria (*S. aureus*, *S. typhimurium*), one gram-negative bacteria (*E. coli*) and one yeast strain (*S. cerevisiae*). The antifungal activity of aronia liquid extract and dry extract at 180 µg/mL concentration was determined on the fungal species (*S. cerevisiae*) (Figure 4.6.). In the tests against *S. cerevisiae* yeast, a 99% bacterial reduction was obtained. According to the results, no antibacterial activity was found in aronia dry extract for *E. coli* bacteria (Figure 4.7.). At the same time, 99% bacterial reduction was observed at a concentration of 180 µg / ml of the liquid extract. When *S.* Typhimurium bacteria were examined at 180 µg/mL concentration of aronia liquid extract, 100% inhibition was detected in the growth of bacteria (Figure 4.8.). Aronia dry extract showed no antibacterial effect when *S.* Typhimurium bacteria were examined. The antibacterial activity of liquid and dry extracts of aronia was investigated at different concentrations (180 µg/mL and 90 µg/mL) against *S. aureus*. In the tests against *S. aureus* bacteria, 100% bacterial reduction was determined at 180 µg/mL and 99.9% at a 90 µg/mL concentration in aronia liquid extract (Figure 4.9.). In dry aronia extract for *S. aureus*, 99.9% bacterial inhibition was determined at a 180 µg/mL concentration.

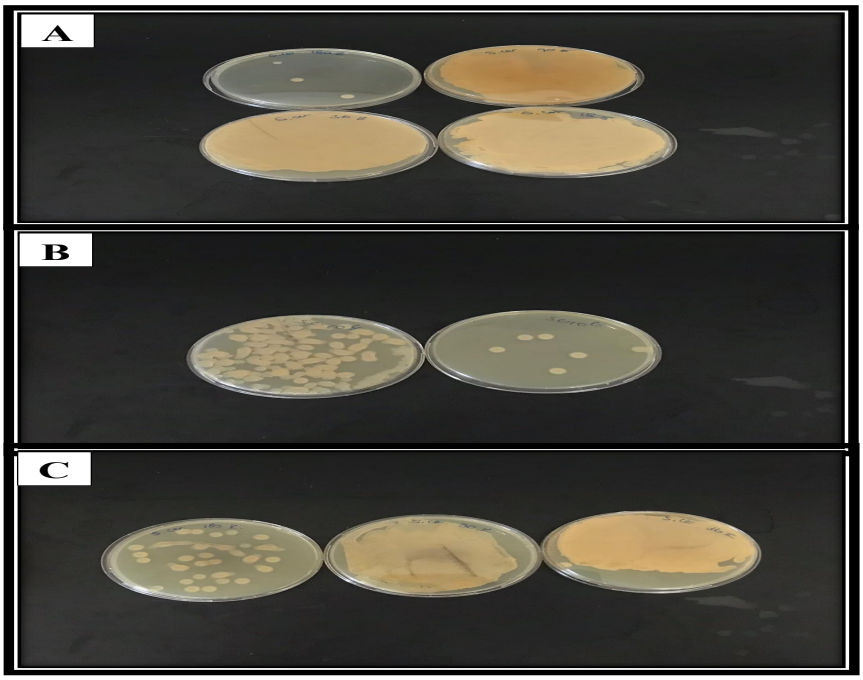

Figure 4.6. *S. cerevisiae* on exposure to *Aronia melanocarpa* dry and liquid extract.

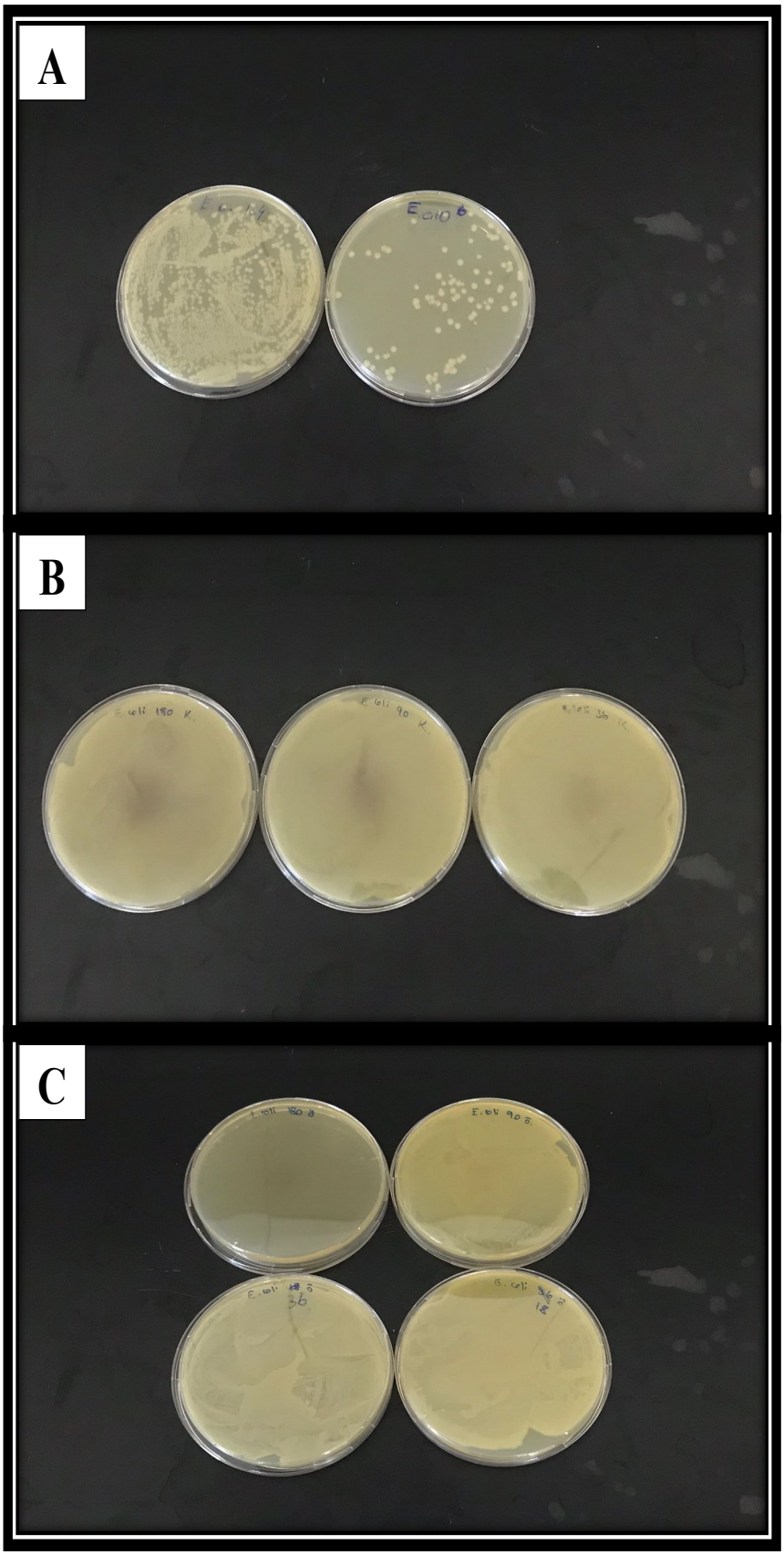

Figure 4.7. *E. coli* on exposure to *Aronia melanocarpa* dry and liquid extract.

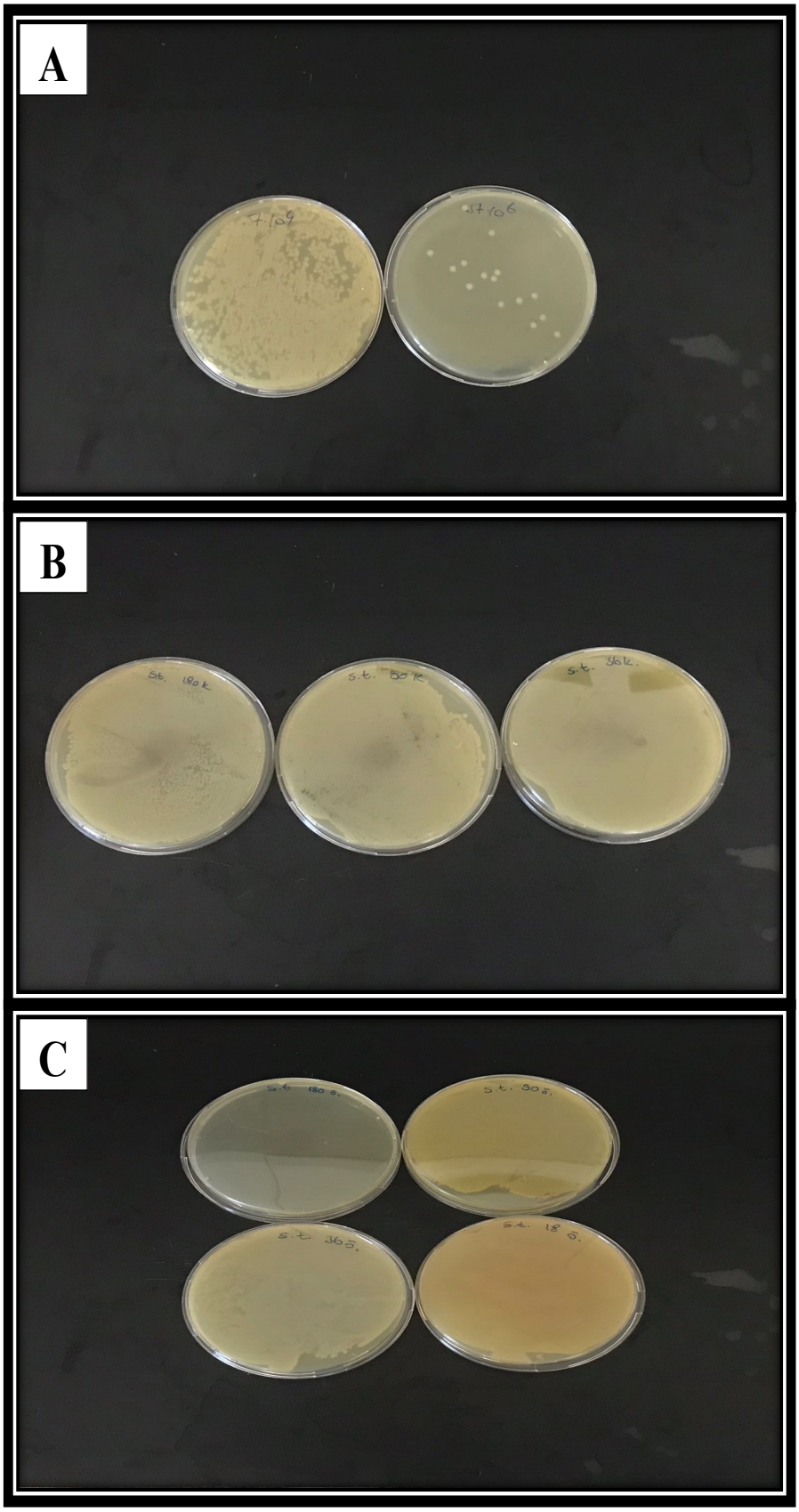

Figure 4.8. *S.* Typhimurium on exposure to *Aronia melanocarpa* dry and liquid extract.

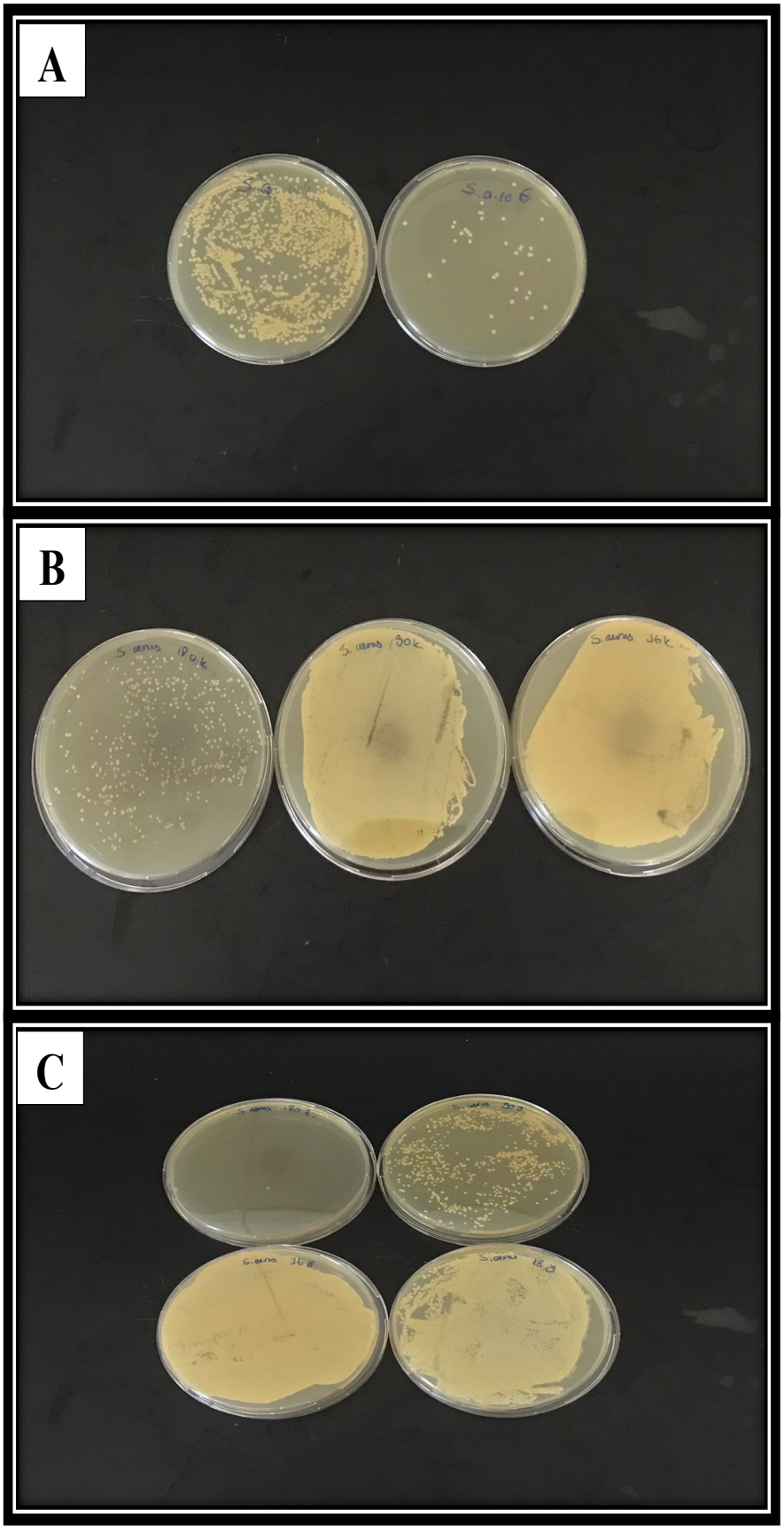

Figure 4.9. *S. aureus* on exposure to *Aronia melanocarpa* dry and liquid extract.

Aronia melanocarpa extracts may include a variety of bioactive chemicals that are beneficial to human health, according to research by Banjari et al., 2017. The effectiveness of the extracts was evaluated against solvent extraction (methanol, ethanol, water) (Borowska et al. 2017; Jurikova et al. 2017; Kim, et al. 2018).

In the investigation conducted by H.-M. Park & Hong, 2014, the combined anthocyanin quantity across the three contrasting extracts examined was reported as 395.10 mg/100 g for the methanol extract, 318.61 mg/100 g for the ethanol extract, and 252.82 mg/100g for the hot water extract.

According to Liepiņa et al., 2013, previous studies have revealed that aronia extracts possess inhibitory properties against the growth of gram-negative bacteria, specifically *Pseudomonas aeruginosa*. Nonetheless, no discernible inhibitory activities were detected in relation to *E. coli* (Liepiņa, Nikolajeva, and Jākobsone 2013). Liepiņa et al., 2013 indicated that ethanolic and liquid extracts derived from *A. melanocarpa* and *Sorbus aucuparia* showed antimicrobial effects against gram-positive bacteria such as *Bacillus cereus* and *S. aureus*. However, no antifungal properties were observed in these extracts. In this study, both liquid and dry extracts of aronia were effective against *S. aureus*.

The present study's results indicated that the liquid extract derived from *Aronia melanocarpa* demonstrated inhibitory properties towards the proliferation of *S. aureus*, *S. typhimurium*, *E. coli*, and *S. cerevisiae*. This investigation additionally exhibited that aronia dry extract displays inhibitory properties against the proliferation of *S. aureus* and *S. cerevisiae*. The findings of the study indicate that the *A. melanocarpa* extract possesses a wide-ranging antimicrobial efficacy, impeding the proliferation of both gram-positive and gram-negative bacterial species, as well as yeast. These findings highlight the potential of *A. melanocarpa* as a natural antimicrobial agent with diverse applications in food preservation, healthcare, and other related industries.

4.3. FTIR

The Fourier Transform Infrared Spectrophotometer (FTIR) is widely recognized as a highly effective instrument for determining the presence of functional groups in

compounds. Mihaela Topală & Rusea, 2018 explored the distinctive vibration patterns of different functional groups in the MIR spectrum of plants, leading to specific absorptions in the infrared range that can be used to differentiate various substances, as shown in Table 4.3.

Table 4.3. Some general bands assignments of MIR spectrum of plants based on literature (Mihaela Topală & Rusea, 2018).

Frequency (cm-1)	**Spectral Assignments**
3500- 3200	O-H, N-H stretch, carbohydrates, proteins, alcohols, phenolic compounds
2960-2950	CH3 asym stretching, mainly lipid with a little contribution from protein, carbohydrate, and nucleic acid
2930-2920	CH2 asym stretching, mainly lipid with a little contribution from protein, carbohydrate, and nucleic acid
2875-2870	CH3 sym stretch, mainly protein with a little contribution from lipid, carbohydrate, and nucleic acid
2860-2840	CH2 sym stretching, mainly lipid with a little contribution from protein, carbohydrate, and nucleic acid
1745-1730	C=O stretch from saturated ester, phospholipid, cholesterol ester, hemicellulose, pectin, lignin, suberin/cutin esters
1650-1630	C=O stretch (Amide I) from protein, pectin
1560-1540	C=N and N-H stretch (Amide II), mainly protein
1455-1440	C-H asym bending of CH_2 and CH_3, cell wall polysaccharide, lipid and protein
1250-1240	C=O stretch from pectic substances, lignin, hemicellulose
1235-1230	C-O stretch, lignin, xylan
1170-1105	C-O-C asym and sym stretch of various groups:cutin, cellulose, cell wall polysaccharide
1045-1020	O–H and C–OH stretch: cell wall polysaccarides (arabinan, cellulose)

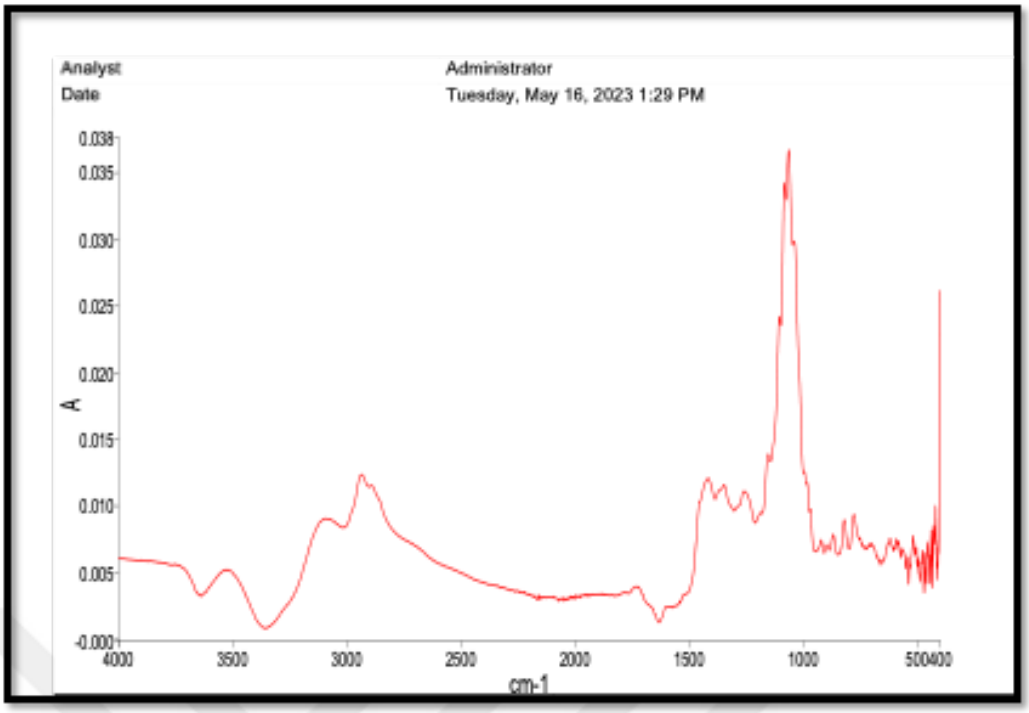

Figure 4.10. FTIR Spectra for *Aronia melanocarpa* liquid extract.

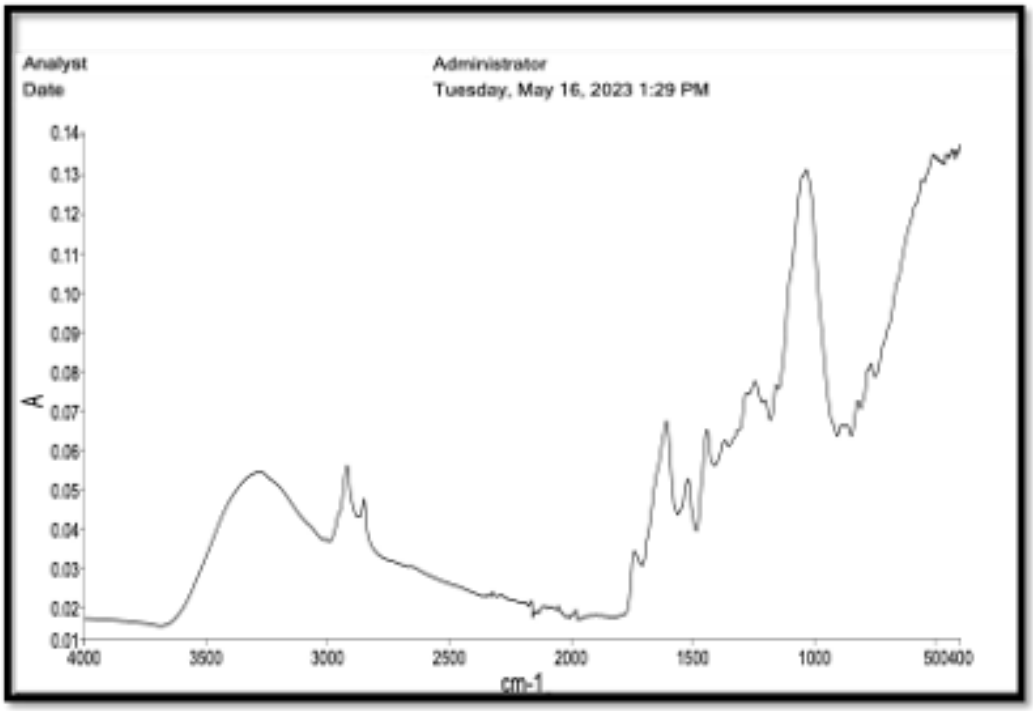

Figure 4.11. FTIR Spectra for *Aronia melanocarpa* dry extract.

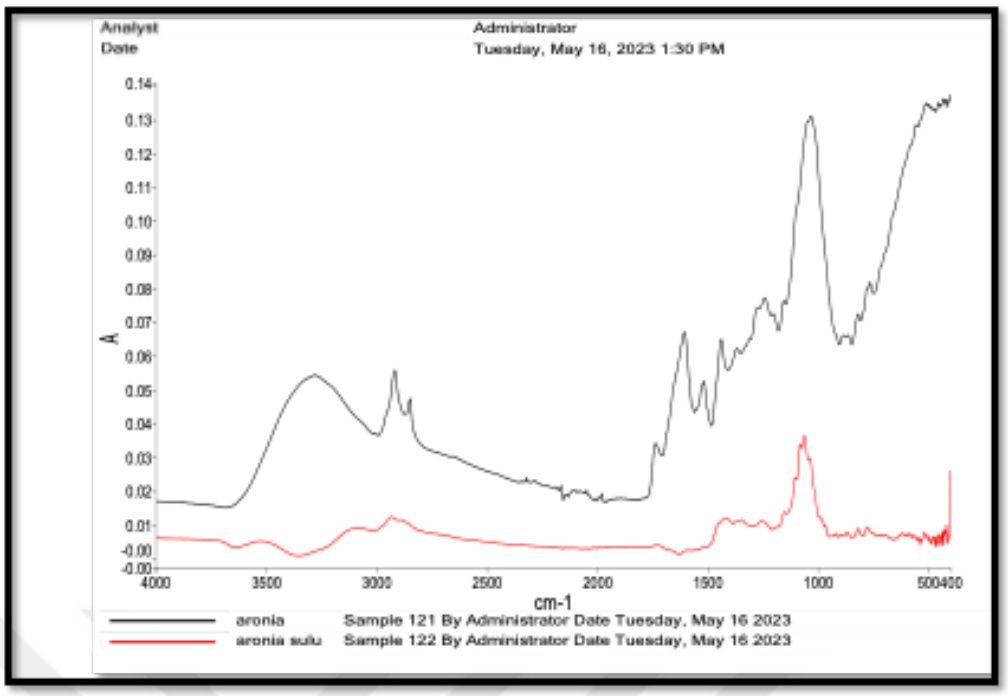

Figure 4.12. FTIR Spectra for *Aronia melanocarpa* dry and liquid extract.

The FTIR spectrum of the dried extract of *A. melanocarpa* and the liquid extract of *A. melanocarpa* are expected to exhibit distinctive features in the transmittance mode in the range of 450-4000 cm^{-1}, and this spectrum displays absorption bands from functional groups in the dried and liquid extract of A. *melanocarpa*.

Aromatic C-H in-plane band vibrations of the liquid extract of *A. melanocarpa* are expected to be found in the region of 1200-950 cm^{-1}.

Aromatic C-H in-plane band vibrations should be detectable in the dried extract's 1200-1000 cm−1 region.

Polysaccharides, which are usually present in *A. melanocarpa*, have a feature at about 1100-1000 cm^{-1} (Janković et al., 2016). Moreover, the wavenumber value of 1100 cm^{-1}, which is related to catechins, is attributed to -C-O alcohols.

The C-C-O ring deformation in the dried extract of *A. melanocarpa* is demonstrated at around 450 cm^{-1}, and C-O-C glycosidic bond deformation can be observed in the 500-550 cm−1 range.

The Amide I (~1550-1700 cm^{-1}) band is the most noticeable when looking at the *A. melanocarpa* extract in dried form caused by the protein backbones vibrating.

In addition, the area of 2000-1500 cm^{-1} in the dried extract of *A. melanocarpa* is caused by the existence of aromatic phenolic compounds.

The dried extract of *A. melanocarpa* shows aliphatic features in the spectrum for CH_2 bands from 2900 cm to 2800 cm^{-1}.

The broad feature at approximately 3500-3300 cm^{-1} is due to intermolecular H-bonded and O-H stretching modes (Jankovic, Marinovic-Cincovic, and Jankovic 2016), resulting from a wide variety of hydrogen bonding between OH groups (Janković et al., 2016).

The 3000-3700 cm^{-1} peak corresponds to the vibration of the H bond produced between the H and OH groups in carboxylic acids and carbohydrates, according to the FTIR spectrum of *Aronia melanocarpa* extract. This outcome is consistent with earlier research (He, Rodriguez-Saona, and Giusti 2007).

Furthermore, phenolic acids such as gallic acids, caffeic acids, and tannic acids can be found in the spectra of *Aronia melanocarpa*, notably in the 2000-2450 cm^{-1} range (Jankovic, Marinovic-Cincovic, and Jankovic 2016).

The C=O group of carboxylic acids is responsible for the peaks about 1700 cm^{-1}. Furthermore, the vibration of phenolic cyclo ether has been detected in the 1044-1086 cm^{-1} range (He, Rodriguez-Saona, and Giusti 2007; Pereira, de Arruda, and Stefani 2015).

At 2920 cm^{-1}, symmetrical and asymmetrical carbon-hydrogen vibrations can be observed. The glycosidic bond peaks can be seen at 995 cm^{-1}, whereas the pyranose ring vibrations can be seen below 800 cm^{-1} (Huang et al. 2006).

4.4. Total Phenol Content, Total Flavonoid Content And Total Monomeric Anthocyanin Content

The total phenolic content, total flavonoid content, and total monomeric anthocyanin content of *Aronia melanocarpa* liquid extract were all determined in this study.

Table 4.4. displays the total phenol content, total flavonoid content, and total monomeric anthocyanin content in the liquid extract of *Aronia melanocarpa*. The total phenolic content of *A. melanocarpa* liquid extract was determined as 1.8± 0.03 (mg

GAE/g). The total flavonoid content of *A. melanocarpa* liquid extract was determined as 0.2± 0.05 (mg QE/g). Total monomeric anthocyanin amounts were determined as 5.22 mg/L for *Aronia melanocarpa* liquid extract.

Table 4.4. Total phenol content, total flavonoid content and total monomeric anthocyanin content in liquid extract of *Aronia melanocarpa.*

	Liquid extracts of *Aronia melanocarpa*
Total Phenol Content (mg GAE/g)	1.8± 0.03
Total Flavonoid Content (mg QE/g)	0.2± 0.05
Total Monomeric Anthocyanin Content (mg/L)	5.22

In a study carried out in Korea, researchers discovered that aronia harvested from mountainous regions had a total phenolic content ranging from 78.3 to 8 mg GAE/g, slightly greater than our investigation results (Lee et al., 2014).

Chung, 2014 reported a total flavonoid content of 32.5 mg RE/g for aronia cultivated in Korea, which differed considerably from our data.

Another study discovered that aronia fruit has a total phenolic content of 135.5 mg GAE/g dw, a total flavonoid content of 8.50 mg RE/g dw, and a total anthocyanin content of 16.4 mg CYE/g dw (D. W. Kim et al. 2021). It is generally accepted that anthocyanin content varies depending on the color of the fruit. Differences in phenolic concentration are also expected as a result of factors such as fruit variety, climate, and maturity stages.

According to Jakobek et al., 2012 and other studies, aronia juice has a greater total anthocyanin concentration. Horszwald et al., 2013 also found a high total anthocyanin concentration in aronia powders. Aronia contains more anthocyanins than other fruits such as blueberry, blackberry, raspberry, grape, and cherry and is known as a rich source of anthocyanins (P. N. Denev et al. 2012). According to Jakobek et al., 2007 the proportion of anthocyanins in aronia is 41%, which is significantly greater than the

fractions in red raspberry (19%) and strawberry (23%). Similar variations in total phenolic content were observed, with Jurgoński et al., 2008 reporting much greater anthocyanin contents.

Total anthocyanin levels in aronia products are reduced due to factors such as pH, chemical composition, temperature, light, and oxygen. These variables are easily influenced by the processing of aronia into juice and other products. Anthocyanins have been shown to be impacted at various stages of juice processing, including pressing and pasteurization (Technologia, 2007).

4.5. Scavenging Effect On DPPH And ABTS Radicals

Various approaches have been used to assess the antioxidant capacity of fruit-derived products. It is crucial to remember that the test method can alter the assessed antioxidant activity, and a single approach may not yield precise numbers. In this work, the antioxidant properties of *Aronia melanocarpa* extracts were assessed using two extensively used methods: DPPH radical scavenging activity and ABTS radical scavenging activity.

Table 4.5. DPPH and ABTS antioxidant activity of liquid extract of *Aronia melanocarpa.*

	Liquid Sample	**Powdered Sample (Methanol Extraction)**
DPPH % Reduction	21.2±0.003 (at 50x concentration)	55.5±0.03 (at 250x concentration)
Ascorbic Acid Equivalent	1.01	13.88
ABTS % Reduction	90.6 (at 50x concentration)	64.4 (at 250x concentration)
Trolox Equivalent	1.5±0.006	5.7±0.02

Table 4.5. presents the results of the DPPH and ABTS antioxidant activity determination of the liquid extract of *Aronia melanocarpa*. The liquid extract had a DPPH radical scavenging activity of 21.2±0.003 when diluted 50 times, whereas the dried extract had a value of 55.5±0.03 when diluted 250 times. The ascorbic acid value of the liquid extract was 1.01, while the value of the dry extract was 13.88.

The liquid extract of *Aronia melanocarpa* had an ABTS antioxidant value of 90.6 at a 50 times dilution, while the dry extract had a value of 64.4 at a 250 times dilution. The Trolox equivalents for the liquid extract were 1.5±0.006 and 5.7±0.02 for the dried extract.

Chokeberry fruits were found to have higher antioxidative activity than eight other fruits in studies conducted by Jakobek et al., 2012. With a few exceptions, Jakobek et al., 2012 discovered that wild chokeberries showed higher antiradical activity than cultivars. Furthermore, Strugala & Gabrielska, 2014 found that chokeberry extracts had significantly stronger antioxidant activity than hawthorn and quince extracts at the start of storage.

Tolić et al., 2015 found that the antioxidant activity of dried *A. melanocarpa* fruits ranged from 183.52 to 191.31 mg $TE.L^{-1}$ when tested using the DPPH technique. According to Wangensteen et al., 2014, methanol extracts of *A. melanocarpa* cultivars and *Aronia prunifolia* have stronger DPPH antioxidant activity than ethanol extracts.

Pieszka, Gogol, Pietras, & Pieszka, 2015 investigated the antioxidant activity of numerous fruit kinds, including chokeberries, raspberries, and elderberries, and discovered that aronia extracts had the highest antioxidant potential. Furthermore, Pieszka, Gogol, Pietras, Science, et al., 2015 discovered that dried chokeberry pomace outperformed apple, strawberry, and carrot pomaces in terms of antioxidative characteristics (TRAP).

4.6. Cellular Antioxidant Activity Assay

The effect of different concentrations of the samples on the cell viability of HUVEC cells damaged by H_2O_2 is presented in Figure 4.13. It was observed that the viability of HUVEC cells exposed to H_2O_2 (1 mM) for one hour decreased to 67.4% in

the control group. However, when HUVEC cells were treated with various dilutions of the sample (1000x, 100x, 50x, and 10x), their cell viability was measured at 96.7%, 90.1%, 73.6%, and 71.6% respectively. These results indicate a higher cellular antioxidant capacity compared to the control group.

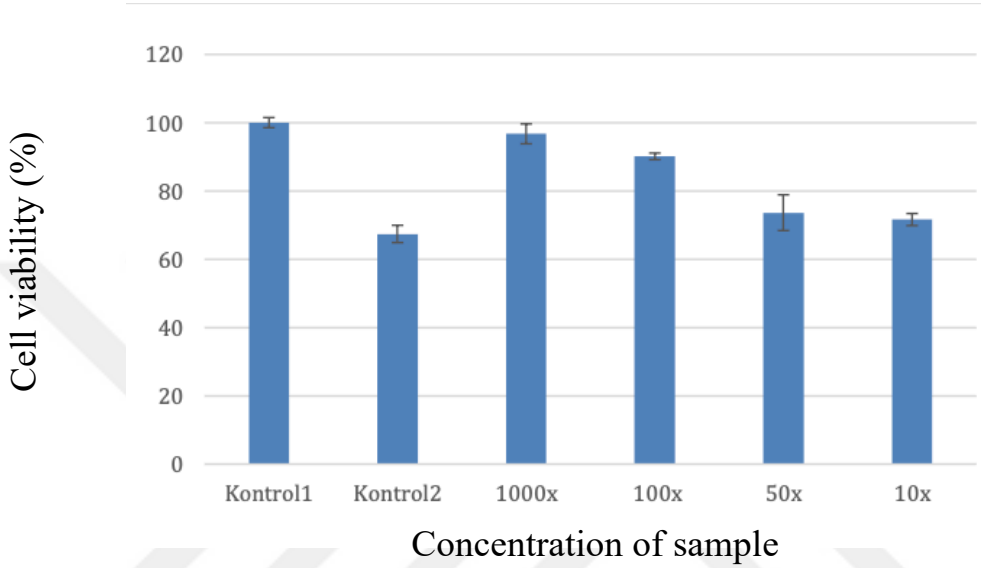

Figure 4.13. Viability of HUVEC cells treated with liquid extract of *A. melanocarpa.*

4.7. Cytotoxicity MTT Assay

The MTT assay (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide) is an effective technique for evaluating cytotoxic levels in cells.

In Figure 4.14, the cytotoxicity of *Aronia melanocarpa* on CaCo2 cells is shown. According to the MTT results of the test material; All concentrations of *Aronia melanocarpa* liquid extract did not show cytotoxic effect on CaCo2 cell.

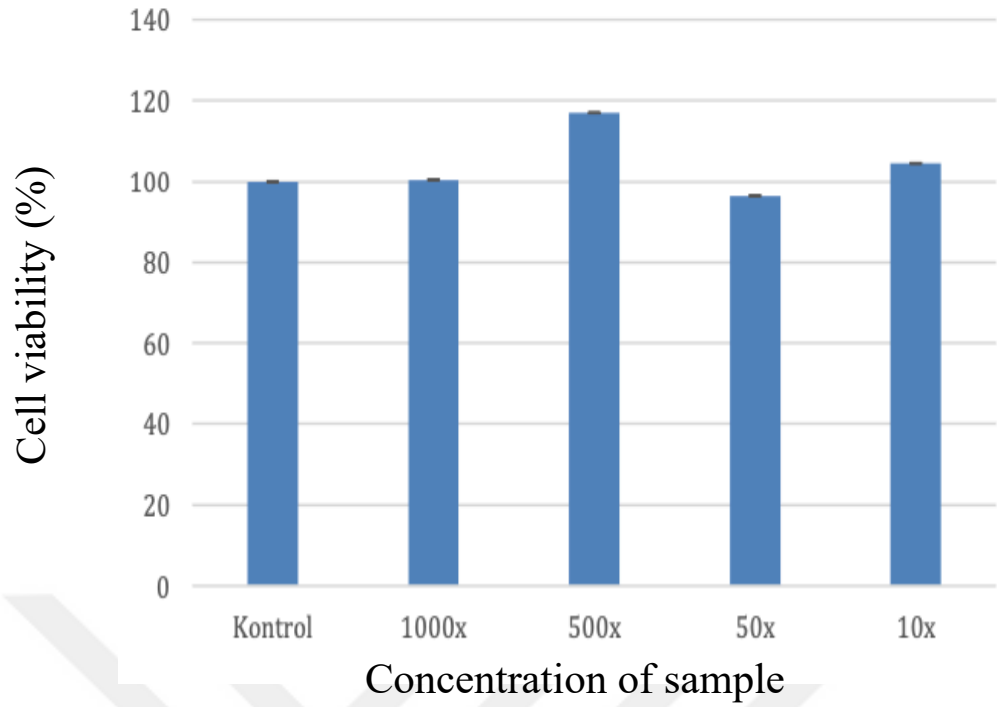

Figure 4.14. Cytotoxicity of the *Aronia melanocarpa* on CaCo2 cell.

The MTT assay was employed in a study to assess the possible toxicity of aronia extract on human epithelial keratinocytes (HaCaT cells), human skin tissues, and the effective dose of the solvent utilized (Banach et al. 2020). The HaCaT samples were diluted to different concentrations (6.25, 12.5, 25, 50, 100, and 200 g/mL) and treated for 24 hours before measuring cell viability. Certain concentrations were found to be more harmful to keratinocytes than others. Although there was no convincing evidence of toxicity against keratinocytes in human skin tissues at a specific dosage of aronia liposome, excessive quantities may cause skin irritation.

A different investigation discovered that aronia extract, at a 186 g/mL concentration, produced 50% cell death in the HT-29 cell line after 48 hours of treatment (Çalışkan et al. 2023). The cytotoxicity data revealed a dose-dependent reduction in cell viability in the HT-29 cell line. Increasing amounts of aronia extract, on the other hand, had no effect on the HUVEC cell line. Furthermore, (Banach et al. 2020) demonstrated the safety of aronia dry extract for RAW264 cells at all investigated concentration levels in a separate investigation.

4.8. Sctrach Assay

The scratch test, commonly known as the wound healing assay, is a popular in vitro tool for studying cell migration and wound closure. An artificial "scratch" or wound is generated in a monolayer of cells in this test, and the flow of cells into the wound area over time is recorded. This assay reveals cell migration and proliferation characteristics.

The effect of *A. melanocarpa* (Michx.) Elliott extract and black chokeberry-loaded silica-type matrices (Ar@MCM-41E and Ar@ZnMCM-41) on cell migration was studied in a study on A375 human melanoma cells (Buda et al. 2020). At a concentration of 100 g/mL, the black chokeberry-loaded matrices drastically decreased cell migration. Similarly, the black chokeberry extract inhibited the migration of cancer cells as compared to the control group. The matrices treatment produced results similar to the control group.

Another study found that 3-O-trans-p-coumaroyltormentic acid is an effective inhibitor based on MDA-MB-231 cell treatments (Choi et al., 2018). 3-O-trans-p-coumaroyltormentic acid efficiently inhibits MDA-MB-231 cell movement and colony formation. These findings show that this acid can inhibit the formation of mammospheres, proliferation, migration, and colony formation in cancer cells.

The metastatic detection of aronia leaf extract on SK-Hep1 cells was determined using a wound migration test (Thi and Hwang 2018). When 50-200 g/mL of aronia leaf extract was administered for 24 hours in the migration assay, wound size inhibition ranged from 61.3% to 96.3%. The study found that when the concentration of aronia leaf extract increased and the incubation duration grew, cancer cell movement distance decreased.

After determining the appropriate concentrations of non-cytotoxic *Aronia melanocarpa* liquid extract for HUVEC and HaCat cell lines, cell migration assay was performed to evaluate the effect of the extract.

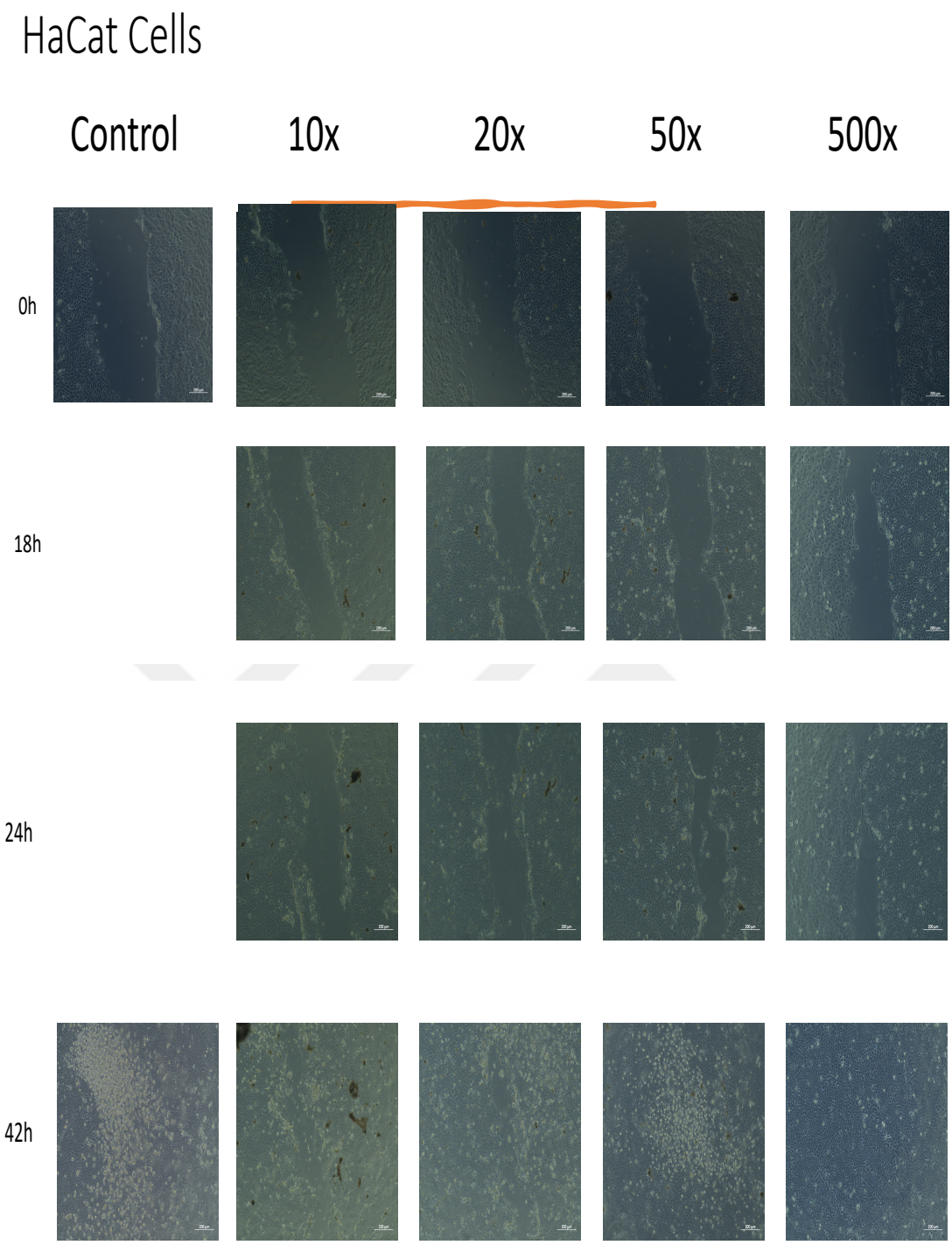

Figure 4.15. Scratch assay in HaCaT cells treated with liquid extract of *A. melanocarpa*.

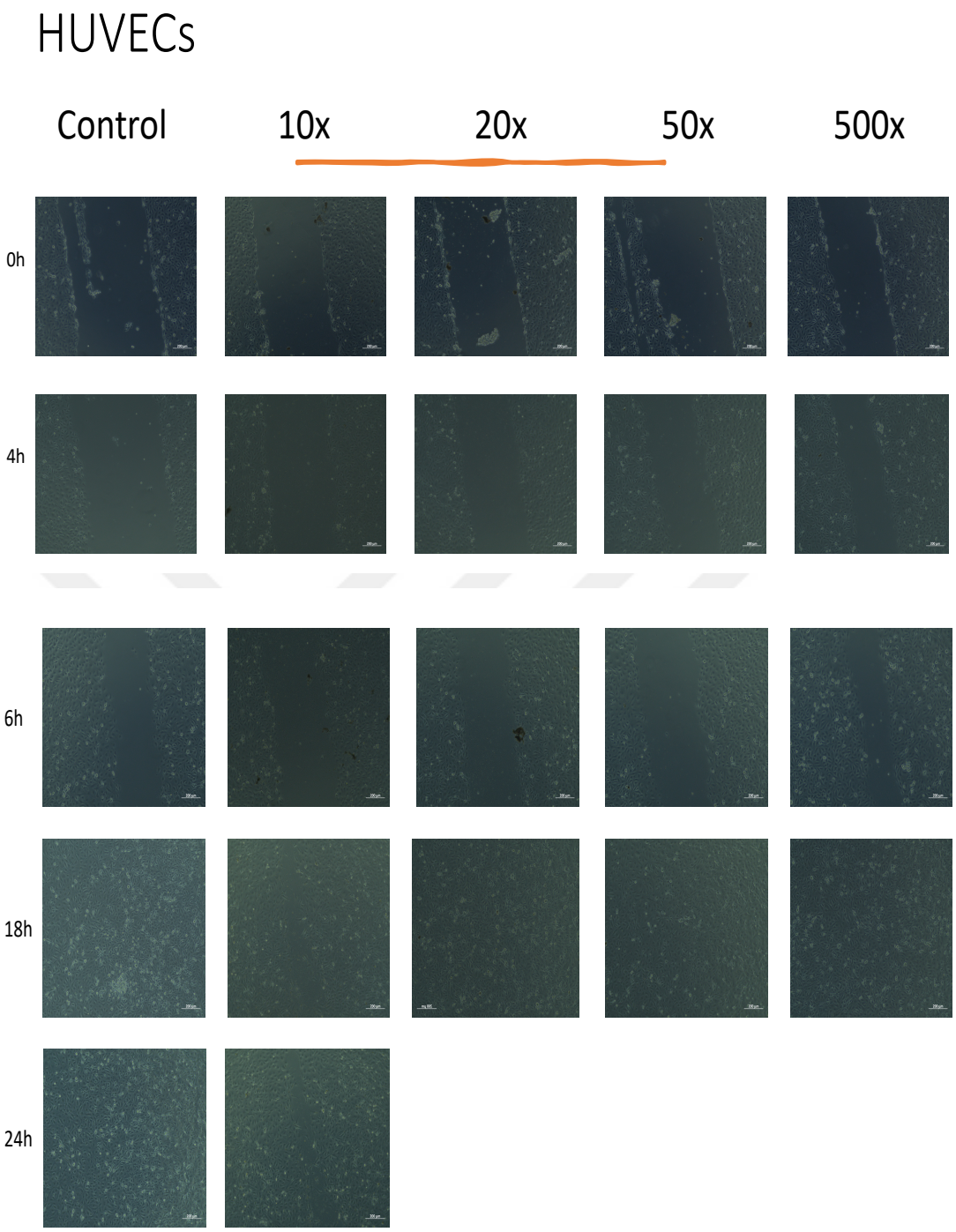

Figure 4.16. Scratch assay in HUVECs treated with liquid extract of *A. melanocarpa.*

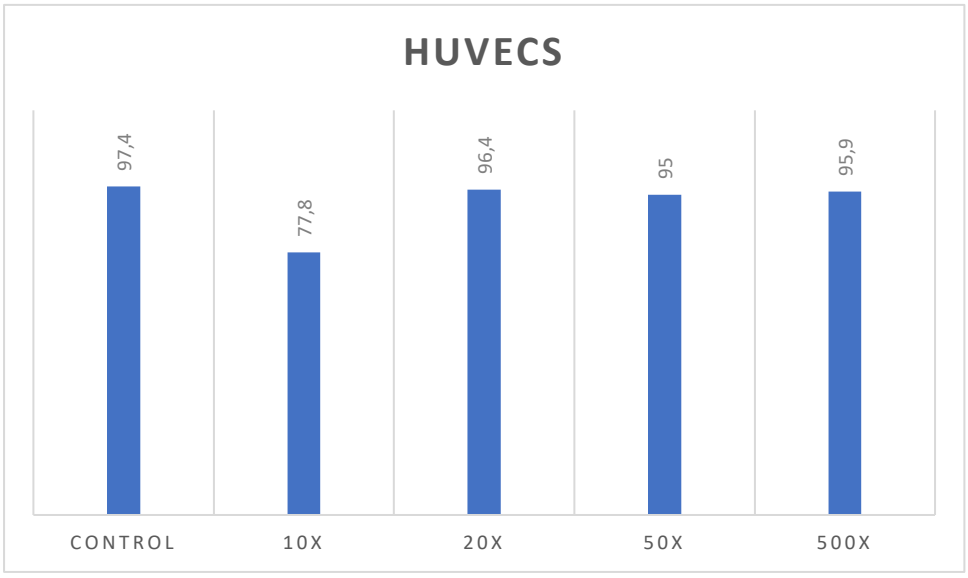

Figure 4.17. Presenting the effect of liquid extract of *A. melanocarpa* on HUVEC cells.

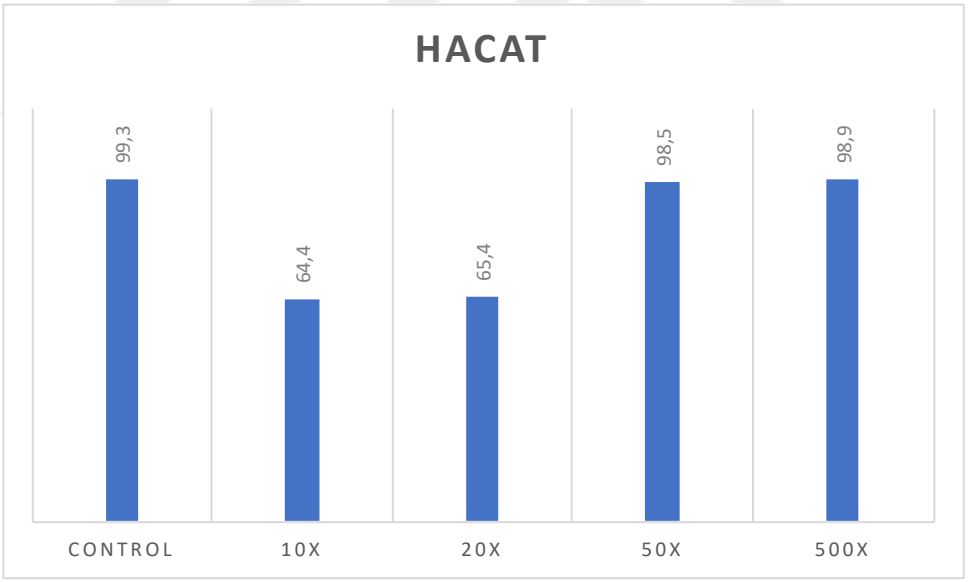

Figure 4.18. Presenting the effect of liquid extract of *A. melanocarpa* on HaCat cells.

In Figure 4.15, the results of the scratch assay in HaCat cells treated with the liquid extract of *A. melanocarpa* are shown, and in Figure 4.18, the cell migration rates of HaCat cells treated with the liquid extract of *A. melanocarpa* are presented. In Figure 4.16, the

results of the scratch assay in HUVECs treated with the liquid extract of *A. melanocarpa* are shown, and in Figure 4.17, the cell migration rates of HUVECs treated with the liquid extract of *A. melanocarpa* are presented.Visual depictions of cell migration from all treatments of *Aronia melanocarpa* liquid extract were observed with time-lapse video frames obtained from exposure of HUVEC and HaCat cell lines at different time points (0 h, 4 h, 6 h, 18 h, 24 h and 42 h). In this montage, images were taken from the time-lapse video at the time points mentioned above and it was possible to clearly observe the progression of cell migration towards the empty space.

In conclusion, the effect of liquid extract of *Aronia melanocarpa* on cell migration was better in HUVECs than in HaCat cells.

The ability of cells to close wounds can be influenced by their phenotypes. The wound healing assay is used to examine and comprehend these distinctions, which come from the unique biological features of cell types. Because of their distinct properties, the closure rates of HUVEC and HaCaT cell lines differ. Endothelial cells, due to their great migratory capacity, are critical for the formation of new blood arteries. Keratinocytes, on the other hand, are in charge of giving structural support and safeguarding the skin. Endothelial cells often migrate and multiply more quickly, whereas keratinocytes may migrate more slowly. Cell lines can also differ in their interactions with the extracellular matrix (ECM) and their responses to signaling pathways. These variables have an impact on the differences in closure rates between HUVEC and HaCaT cells.

CHAPTER 5

CONCLUSION

Aronia melanocarpa dry and liquid extract can be said to have a favorable image, which is associated with the general acceptance that it is an essential part of the diet and the general trend towards a healthier lifestyle. In addition, it has been praised for its beneficial health effects as a natural antioxidant in recent years.

The total phenolic matter contents, total monomeric anthocyanin amounts, total flavonoid matter contents, antioxidant activity levels, and detailed phenolic bioactive profiles of *Aronia melanocarpa* dry and liquid extracts were determined by FTIR, High-Pressure Liquid Chromatography (HPLC), and High-Resolution Mass Spectrometry (Quadrupole Time of Flight Mass Spectrometry) (Q-TOFF-MS). In addition, the cytotoxic effects in the CaCo2 cell line and antiproliferative effects in HUVECs and HaCat cell lines were tried to be determined. While CaCo2 does not show cytotoxic effect in cell line, it has a positive effect on cell migration in HUVECs and HaCat cell lines. Our research shows that *Aronia melanocarpa* dry and liquid extracts are high in polyphenols such as anthocyanins, flavonoids, and phenolic acids. DPPH and ABTS tests were used to assess antioxidant activity. As a result, the components of *Aronia melanocarpa* dry and liquid extracts were found to have strong antioxidant activities. In the following parts of the study, *Aronia melanocarpa* dry and liquid extracts were evaluated against three bacteria and one yeast strain regarding the antimicrobial mode of action.

Aronia melanocarpa is a nutritionally rich source of various bioactive phytochemicals. In this work, we intended to promote *Aronia melanocarpa* extracts as a beneficial and important source of bioactive chemicals, a useful source of antioxidants in the functional food company, and a crucial source of antioxidants as nutraceuticals for human health.

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