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INVESTIGATION OF BIOCHEMICAL PROPERTIES, ANTIMICROBIAL, ANTIOXIDANT AND ANTIPROLIFERATIVE ACTIVITY OF THE *RHAMNUS CATHARTICA* L. EXTRACTS COLLECTED FROM KAZAKHSTAN

KAZAKİSTAN'DAN TOPLANAN RHAMNUS CATHARTİCA L. EKSTRAKTLARININ BİYOKİMYASAL ÖZELLİKLERİ, ANTİMİKROBİYAL, ANTİOKSİDAN VE ANTİPROLİFERATİF AKTİVİTELERİNİN ARAŞTIRILMASI

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ABSTRACT

Objectives: *This research evaluated the biochemical composition of hexane, ethyl acetate, and ethanol extracts and analyzed the antimicrobial, antioxidant and antiproliferative activities of the cortex, leaf, and fruit of the Rhamnus cathartica L. plant from Almaty, Kazakhstan.*
Material and Method: *The GC-MS method was used to evaluate the chemicals of Rhamnus cathartica L. cortex, leaf, and fruit extracts. The microdilution technique was used to evaluate the MIC values of plant extracts against pathogenic microorganisms. DPPH, FRAP, and Total Flavonoid Content were evaluated for antioxidant activity. The antiproliferative effect of the extracts on MCF-7, A549, C6 and WI-38 cells was measured by XTT assay.*
Result and Discussion: *According to GC-MS results, Trans-2-Decenal had the highest proportion at 2.88%. Eicosane is second at 1.71%. DPPH, FRAP and flavonoid values showed that the fruit ethyl acetate extract (F-EtAc) had the strongest antioxidant activity. F-EtAc extract had the strongest anticancer activity according to the selectivity index table (WI-38/MCF-7: 12.67, WI-38/C6: 31.38, WI-38/A549: 8.55). All plant extracts had high MIC values against Staphylococcus aureus based on reference source values. The MIC value of cortex and leaf in ethyl acetate was 0.078 mg/ml, cortex and leaf extracts had strong antimicrobial activity (0.312 mg/ml) for Bacillus cereus, while fruits had weakly effective MIC values (Significant=MIC <0.1 mg/ml). According to the results of this study, it can be said that Rhamnus cathartica L. plant extracts have an active content in terms of biological properties.*
Keywords: *Antimicrobial, antioxidant, antiproliferative, Rhamnus cathartica*

ÖZ

Amaç: *Bu araştırmada, Kazakistan'ın Almatı kentinden elde edilen Rhamnus cathartica L. bitkisinin*

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korteks, yaprak ve meyvesinin hekzan, etil asetat ve etanol özütlerinin biyokimyasal bileşimi değerlendirilmiş ve antimikrobiyal, antioksidan ve antiproliferatif aktiviteleri analiz edilmiştir.

Gereç ve Yöntem: *Rhamnus cathartica L. korteks, yaprak ve meyve özütü kimyasallarını belirlemek için GC-MS yöntemi kullanıldı. Bitki ekstraktlarının patojen mikroorganizmalara karşı MİK değerlerini değerlendirmek için mikrodilüsyon yöntemi kullanılmıştır. Antioksidan aktivite tayini için DPPH, FRAP ve Toplam flavonoid içeriği belirlenmiştir. Ekstraktların MCF-7, A549, C6 ve WI-38 hücreleri üzerine antiproliferatif etkisi XTT testi ile ölçülmüştür.*

Sonuç ve Tartışma: *GC-MS sonuçlarına göre; Trans-2-Decenal %2.88 ile en yüksek orana sahiptir. Eicosane %1.71 ile ikinci sıradadır. DPPH, FRAP ve flavonoid değerleri meyve etil asetat ekstresinin (F-EtAc) en güçlü antioksidan aktiviteye sahip olduğunu göstermiştir. F-EtAc ekstresi, seçicilik indeksi tablosuna göre en güçlü antikanser aktivitesine sahiptir (WI-38/MCF-7: 12.67, WI-38/C6: 31.38, WI-38/A549: 8.55). Tüm bitki ekstraktları, referans kaynak değerlerine göre Staphylococcus aureus'a karşı anlamlı MİK değerlerine ulaşmıştır. Korteks ve yaprağın etil asetat içindeki MİK değeri 0.078 mg/ml, korteks ve yaprak ekstraktları Bacillus cereus için anlamlı antimikrobiyal aktiviteye (0.312 mg/ml) sahipken, meyveler zayıf etkili MİK değerlerine ulaşmıştır (Anlamlı=MİK <0.1 mg/ml). Bu çalışmanın sonuçlarna göre, Rhamnus cathartica L. bitki ekstraktlarının biyolojik özellikler açısından aktif bir içeriğe sahip olduğu söylenebilir.*

Anahtar Kelimeler: *Antimikrobiyal, antioksidan, antiproliferatif, Rhamnus cathartica*

INTRODUCTION

Rhamnaceae is a worldwide family with 50 genera and 900 species. Simple leaves, small flowers with four or five sepals that are valvate in bud, four or five stamens alternating with the sepals (obhaplostemony), anthers that are frequently enfolded by the hooded petal apices, ovaries that are usually two to three (four to five)-locular, an intrastaminal, nectariferous disc, and a tendency towards xeromorphism characterize them. On the inner side of the sepals, there is normally a fleshy layer that creates a keel and ends with a tubercle. This layer is histologically comparable to the nectariferous intrastaminal layer [1].

Genus *Rhamnus*; is one of the genera that is a member of the Rhamnaceae family, adapted to living in temperate and subtropical climates in the northern hemisphere [2].

Rhamnus cathartica is native to Europe, Northwest Africa, and Western Asia. It is a deciduous shrub or small tree; its seeds and leaves are considered toxic to humans and animals. It has been used as a folk remedy because of its purgative effect and good remedy against stomach cramps. It has also been used against various cancer diseases and as a diuretic in history [3].

In studies, antioxidant [4], antimicrobial [3,5], and anticancer [6,7] activities were determined in plant extracts of some *Rhamnus* species.

Numerous novel herbal medications have advanced to clinical trials, and medicinal plants are acknowledged as one of the possible sources for therapeutic development. To create novel antimicrobials from natural products, antibiofilm agents against different illnesses, antioxidant chemicals, and new medications against various diseases, including cancer, researchers are currently looking into the qualities of medicinal plants [8].

Due to the excessive use of antibiotics in human medicine, microbial antibiotic resistance is increasing. The problem of antibiotic resistance is not limited to just one continent, it is a global problem. Antimicrobial resistance is a hidden epidemic among us [9].

Infections caused by microorganisms with multi-drug resistance are a major problem, especially in intensive care units. In addition to being inexpensive to produce, using herbal medicines to treat human illnesses has several benefits, such as being easily accessible and biodegradable. Effective plant extracts can combat harmful germs in humans without causing harmful side effects or environmental risks [3].

The plant kingdom produces a wide variety of compounds with antioxidant properties that are thought to be able to protect against oxidative damage in the human organism. Multipotent antioxidants are molecules that have an additional pharmacological activity as well as antioxidant activity. Numerous examples of extremely potent antioxidants can be found in natural products. In addition to their antioxidant qualities, certain natural antioxidants have anti-inflammatory, anti-tumor, and plateletaggregation-inhibiting effects. However, the antibacterial action of many natural phenolic antioxidants is one of their most frequently mentioned characteristics. Because both effects are very desirable to keep food as fresh as possible, this is very significant to the food business [10,11].

Chemotherapeutic cytotoxic drugs are used to treat cancer. However, these drugs can cause serious side effects that continue to be a major dilemma for cancer patients [12]. In recent years, there has been an increasing interest in new medicinal plant-derived bioactive molecules for cancer drug discovery, due to their abundant and easy availability, their safety due to low toxicity, and fewer side effects [13].

Our study; It was aimed to determine the biochemical content of the extracts prepared with hexane, ethyl acetate, and ethanol of the bark, leaf, and fruit parts of the *Rhamnus cathartica* L. plant collected from the region of Kazakhstan and to examine the antioxidant, antimicrobial and antiproliferative effects.

MATERIAL AND METHOD

Plant Material

Rhamnus cathartica L. samples were collected from an altitude of 2000 m on the banks of the Talgar River in Ile Alatau, Almaty region, Republic of Kazakhstan.

Preparation of the Extract

Collected leaf, cortex and fruit samples were washed with tap water and distilled water, respectively, and dried under suitable conditions. After the dried samples were powdered in the grinder, 100 g of each sample was weighed. 250 ml of ethyl alcohol, hexane, and ethyl acetate solvents, each with a different polarity, were added to the weighed samples. The samples, which were shaken for 24 hours at 125 rpm at room temperature, were filtered through filter paper at the end of the period. Thus, plant particles are separated. The solvent was removed under vacuum with the help of an evaporator (Buchi R-100 equipped with Vacuum Pump V-300 and Control unit I-300) at a temperature not exceeding 37º C and 9 dry extracts obtained were kept at -20º C for use in analysis [14].

GC-MS Analysis

Agilent Technologies GC 7890A, equipped with a 5975 Triple Axis Detector mass spectrometer was employed to perform GC-MS analyses. DBWAXETR column (320 m x 60 m x 0.25 m), electron ionization system, and ionization energy of 70 eV were used for GC-MS detection. Helium was the carrier gas at a flow rate of 1 ml/min. The column temperate was operated under the same conditions as described above. After the oven temperature is kept at the initial temperature of 50 °C for 2 minutes, the temperature rise rate at all stages is 5 °C/minute, respectively, to 80 °C (waiting 2 minutes at this temperature), 100 °C (at this temperature 1 minute), 150 °C (holding 1 minute at this temperature), 240 °C (holding 1 minute at this temperature), and 270 °C (7 minutes standing at this temperature).

GC-MS analyses were performed by Giresun University Central Research Laboratory Application and Research Center (GRÜMLAB).

Antioxidant Assay

DPPH

2,2-Diphenyl-1-picrylhydrazyl radical scavenging activity of the resulting extract was performed as the modified method developed by Ou *et al.* [15]. Different concentrations of the extracts were prepared. Equal volumes (1000 μL) of DPPH and sample solutions were incubated with stirring for 30 min. After incubation, absorbance was measured by a spectrophotometer at 517 nm. The ascorbic acid as an antioxidant was used to compare the results. Samples and radical scavenging activity of the standard were calculated.

FRAP

The amount of FRAP antioxidant power in the analysis of the extracts of different solvents belonging to the plant *R. cathartica* L. according to the Benzie and Strain method, the amounts of the FRAP antioxidant power was calculated as the trolox equivalent. Trolox was used as a standard.

Total Flavonoid Content

Total flavonoid substance amount in the analysis of extracts of different solvents belonging to *Rhamnus cathartica* L. plant according to Chang et al method, total phenolic substance amounts were calculated as the equivalent of quercetin. Quercetin was used as a standard [16].

Antimicrobial Activity

The Broth Microdilution method was used to determine as the Minimum Inhibition Concentration (MIC) of *Rhamnus cathartica* L. plant cortex, leaf, and fruit extracts against microorganisms according to CLSI and EUCAST standards and Eloff's method [17-20]. In the study, *Staphylococcus aureus* ATCC 29213, *Enterococcus faecalis* ATCC 29212, *Pseudomonas aeruginosa* ATCC 27853, *Escherichia coli* ATCC 25922, *Bacillus cereus* ATCC 11778, *Candida albicans* ATCC 10233 and *Candida albicans* ATCC 10231 were used as test microorganisms.

The extracts were dissolved in 40% Dimethyl sulfoxide (DMSO), and concentrated solutions were prepared. Cation Adjusted Mueller Hinton Broth (CAMHB, Becton Dickenson BBL, Sparks, MD, USA) for bacteria; RPMI 1640 medium (Sigma-Aldrich R8758, Germany) was used for yeasts [18-20]. Plate wells in the 12th row were used as breeding controls. 10 µL of extract was added to the wells in the first row and serial dilutions were made. After two-fold serial dilution, bacteria and yeast were added to the wells and final concentrations of 5×10^5 cfu/ml for bacteria and $0.5\text{-}2.5\times10^3$ cfu/ml for yeast were reached in each well. Bacterial plates were incubated at 37°C and yeast plates were incubated at 25°C for 24-48 hours. Fluconazole was used as reference antifungal. After incubation, the lowest concentration at which visible growth was inhibited was accepted as the Minimum Inhibitory Concentration value (MIC) (µg/ml) [19-21]. Experiments were performed in triplicate.

Antiproliferative Activity

The antiproliferative effects of *Rhamnus cathartica* L. plant extracts were tested on Human lung cancer cell line (A549, ATCC-CCL-185), human breast cancer cell line (MCF-7, ATCC-HTB-22), mouse glioma cell line (C6, ATCC-CCL-107) and human normal lung fibroblast (WI-38, ATCC-CCL-75) cell lines using the XTT Assay method. After being seeded in growth media in a 96-well plate, the cells were treated with varying doses of test substances and incubated for 24 hours at 37°C in a humidified CO_2 atmosphere. After the incubation, each well received 100 µl of XTT solution, which was then incubated for an additional two hours. A microplate reader was used to measure the optical density values at 475 nm [22,23].

Statistical Analysis

One Way Anova test as well as the Tukey test were used for statistical analysis of the findings. For this purpose, the SPSS 16.0 (SPSS, Chicago, IL, USA) statistical program was used and a $p<0.05$ value at a 95% confidence interval was considered significant between groups.

RESULT AND DISCUSSION

Determining the Chemical Composition of Different Parts of *Rhamnus cathartica* L. Plant Extracts

The items obtained according to the results of the GC-MS analysis are shown in Table 1. According to this table, Trans-2-Decenal is the substance with the highest percentage with 2.88%. Eicosane is in the second place with a rate of 1.71%.

Table 1. The phytochemical composition of the fruit's ethyl acetate extract was analyzed by GC-MS.

Fruit's ethyl acetate	RT	Ethyl acetate (%)
Propanoic acid	3.280	1.63
3-Pentanone, 2-methyl-	3.732	0.58
Acetic acid,1-methylpropyl ester	3.830	1.06
Octane	4.448	1.49
Nonanal	13.202	0.94
Trans-2-Decenal	18.775	2.88
2-Tetradecene	22.644	0.63
1-Hexadecene	28.022	0.50
1-Heptadecene	28.022	0.50
1-Nonadecene	28.022	0.50
Pyridine-3-carbonitrile (3-cyanopyridine)	32.852	1.30
Hexadecanoic acid	45.932	0.67
9-Octadecenamide	50.281	1.54
Eicosane	56.003	1.71

Acetic acid, which is among the substances we detected as a result of GC-MS analysis (Table 1), has been used as an antiseptic agent in the disinfection of wounds for more than 6000 years in medicine [24]. Again, Pyridine-3-carbonitrile, which we determined as a result of this analysis, is a compound recently emphasized due to its extraordinary *in vitro* anticancer activity against a wide variety of cell lines. Recent studies suggest that pyridine derivatives show a range of pharmacological activities such as antibacterial, antitumor and analgesic activity [25].

It has been stated that 1-Nanodecene, which is among the substances in Table 1, shows antituberculosis, anticancer, antioxidant, and antimicrobial activity in studies [26].

DPPH Radical Sweeping Activity

According to Table 2, the fruit's ethyl acetate extract was found to have the best radical sweeping activity.

Ascorbic acid is one of the substances with the best-known antioxidant scavenging effect. Ascorbic acid was used as a positive control in the study. According to the DPPH result, the best antioxidant scavenging effect in the extracts of *R. cathartica* L. plant is observed to be 38.65±1.33 in fruit ethyl acetate when compared with ascorbic acid. While the second effect was observed in Cortex Ethyl acetate 43.87±1.74; the third effect was found to be Leaf Hexane 61.32±1.49.

Table 2. DPPH IC_{50} values

***Rhamnus cathartica* L.**	**IC_{50} (µg/ml)**
Leaf Ethyl Acetate	80.52 ± 1.23
Leaf Ethanol	135.6 ± 1.65
Leaf Hexane	61.32 ± 1.49
Fruit Ethyl Acetate	38.65 ± 1.33
Fruit Ethanol	105.4 ± 1.78
Fruit Hexane	77.35 ± 1.58
Cortex Ethyl Acetate	43.87 ± 1.74
CortexEthanol	82.74 ± 1.45
Cortex Hexane	88.96 ± 1.69
Ascorbic acid	11.07 ± 1.38

FRAP Antioxidant Power

The amount of FRAP antioxidant power in the analysis of the extracts of different solvents belonging to the plant *R. cathartica* L. according to the Benzie and Strain method, the amounts of the FRAP antioxidant power were calculated as the trolox equivalent. Trolox was used as standard (Figure 1).

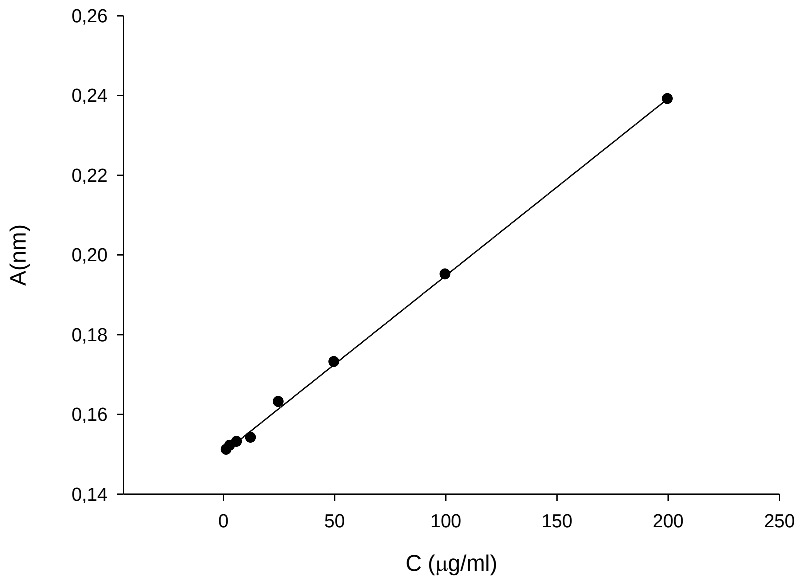

Figure 1. Trolox standard chart y = 0.1511 + 0.0005x (R^2 = 0.8772)

According to Table 3, leaf ethyl acetate, fruit ethyl acetate, and cortex ethyl acetate extracts were found to have stronger FRAP antioxidant activity than others.

In the study, fruit ethyl acetate is in first place with a value of 371.52 μg/ml, cortex ethyl acetate is in second place with a value of 349.81 μg/ml, and Leaf EA is in third place with a value of 341.58 μg/ml. Trolox was used as a positive control, and Fruit EA showed the best antioxidant results according to the FRAP results.

Table 3. FRAP Values

***Rhamnus cathartica* L.**	**(μg/g)**
Leaf Ethyl Acetate	341.58 ± 1.32
Leaf Ethanol	47.58 ± 1.12
Leaf Hexane	65.25 ± 1.18
Fruit Ethyl Acetate	371.52 ± 1.38
Fruit Ethanol	91.81 ± 1.26
Fruit Hexane	87.80 ± 1.22
Cortex Ethyl Acetate	349.81 ± 1.36
Cortex Ethanol	55.36 ± 1.08
Cortex Hexane	34.15 ± 1.04

Total Flavonoid Content

Total flavonoid substance amount in the analysis of extracts of different solvents belonging to *R. cathartica L.* plant, according to Chang et al method, total phenolic substance amounts were calculated as the equivalent of quercetin. Quercetin was used as a standard (Figure 2).

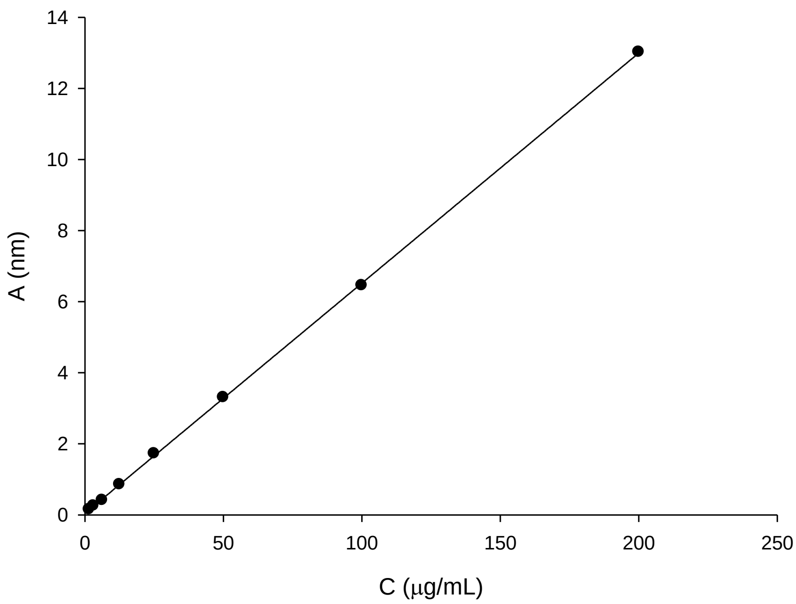

Figure 2. Quercetin standard graphic = 0.0433 + 0.0648x (R2 = 0.9999)

Table 4 shows the total flavonoid substance amount of *R. cathartica* L. ethanol, ethyl acetate, and hexane extracts.

Table 4. Flavonoid values

Rhamnus cathartica L.	**(μg/g)**
Leaf ethyl acetate	32.69 ± 0.98
Leaf ethanol	1.19 ± 0.08
Leaf hexane	0.98 ± 0.04
Fruit ethyl acetate	38.20 ± 0.93
Fruit ethanol	0.92 ± 0.03
Fruit hexane	0.87 ± 0.02
Cortex ethyl acetate	37.01 ± 0.93
Cortex ethanol	0.60 ± 0.01
Cortex hexane	0.43 ± 0.01

According to Table 4, the most flavored leaf extracts are ethyl acetate, fruit hexane, and cortex ethyl acetate extracts. The high flavonoid values indicate that they may have anticancer and antioxidant properties.

Based on the results of total flavonoid content, 38.20±0.93 flavonoid content of Fruit ethyl acetate was found to be the highest; secondly, Cortex ethyl acetate 37.01; third, Leaf ethyl acetate was found to be 32.69±0.98.

In many studies on the genus *Rhamnus*, phenolic compounds such as flavonoids, anthraquinones, and tannins, which are defined as antioxidant molecules, are high [27-30]. However, antioxidant activity studies with *Rhamnus cathartica* species are not sufficient. According to our study, it was determined that the fruit ethyl acetate extract of this species showed higher antioxidant activity than the other extracts (Table 2, Table 3 and Table 4).

Antimicrobial Activity

MIC results were evaluated according to reference sources (Highly Effective/Significant MIC

<0.1 mg/ml, Medium Effective/Moderate = 0.1 <MIC ≤ 0.625 mg/ml, Weak Effective/Weak = MIC> 0.625 μg/ml) [31].

Table 5. MIC results of *R. cathartica* L. cortex extract (mg/ml)

No	Microorganisms	Cortex (Ethyl acetate)	Cortex (Ethanol)	Cortex (Hexane)
1	*E. coli*	>5	>5	2.5
2	*P. aeruginosa*	>5	>5	>5
3	*S. aureus*	0.156	0.078	0.156
4	*B. cereus*	0.312	0.312	5
5	*C. albicans*	1.25	2.5	2.5
6	*C. tropicalis*	5	5	>5

Table 6. MIC results of *R. cathartica* L. leaf extract (mg/ml)

No	Microorganisms	Leaf (Ethyl acetate)	Leaf (Ethanol)	Leaf (Hexane)
1	*E. coli*	>5	>5	2.5
2	*P. aeruginosa*	>5	>5	>5
3	*S. aureus*	0.078	0.156	0.078
4	*B. cereus*	1.25	1.25	0.312
5	*C. albicans*	>5	5	2.5
6	*C. tropicalis*	5	2.5	2.5

Table 7. MIC results of *R. cathartica* L. fruit extract (mg/ml)

No	Microorganisms	Fruit (Ethyl acetate)	Fruit (Ethanol)	Fruit (Hexane)
1	*E. coli*	>5	2.5	>5
2	*P. aeruginosa*	>5	5	>5
3	*S. aureus*	0.156	0.312	0.312
4	*B. cereus*	>5	5	5
5	*C. albicans*	5	2.5	2.5
6	*C. tropicalis*	2.5	2.5	>5

MIC values were determined at different concentrations against microorganisms. The findings regarding the MIC values of the extracts obtained from the cortex, leaf and fruit parts of the plant *R. cathartica* L. can be seen in Table 5, Table 6 and Table 7.

The antimicrobial activity of plant extracts can be classified as (Highly Effective/Significant MIC <0.1 mg/ml, Medium Effective/Moderate = 0.1 <MIC ≤ 0.625 mg / ml, Weak Effective/Weak = MIC> 0.625 μg/ml) [31]. According to this classification, the cortex, leaf and fruit extracts of *R. cathartica* L. had MIC values of 2.5 mg/ml and 5 mg/ml for *E. coli*, *P. aeruginosa*, *C. albicans* and *C. tropicalis*. When the MIC values of the *R. cathartica* L. extracts were compared with the reference antifungal Flucanazole (MIC=0.25 μg/ml), none of them reached effective values.

It was shown to be effective against *S. aureus* even at low concentrations in different solvents and all extracts of the plant (0.078-0.156 mg/ml). The MIC value for *S. aureus* was found to be effective with a value of 0.078 mg/ml, especially in cortex and leaf ethyl acetate solvents. For *B. cereus*, cortex and leaf extracts had antimicrobial activity (0.312 mg/ml), whereas fruit showed weak MIC values (Table 5, Table 6 and Table 7) [31].

Ibrahim et al. found that *R. cathartica* L. extract had high antimicrobial activity against gram-positive bacteria *B. cereus* and *E. coli* among gram-negative strains [32]. Antimicrobial activity evaluation was performed by the disk diffusion method. It is compatible with our results.

In a study conducted by Nayyeri et al. on acne treatment, it was found that *R. cathartica* L. bark extract had antimicrobial activity against *Cutibacterium acnes, Staphylococcus epidermidis,* and *Staphylococcus aureus* [5]. The MIC value against *S. aureus* was found to be 97.65 µg/ml. It is compatible with our results.

In another study, the antimicrobial effect of some components of *R. cathartica* L. was investigated. Three components had antibacterial activity against *E. coli* and *S. aureus* bacteria, and two components had antifungal activity against *C. albicans* [3]. Antimicrobial activity evaluation was performed by the disk diffusion method. Although our study is compatible with bacteria, it is not compatible with yeasts.

Locatelli et al. investigated the antimicrobial effects of *Rhamnus cathartica* and *Rhamnus orbiculatus* plant extracts. Both methanol extracts showed antimicrobial activity against all microbial species tested (*Staphylococcus aureus, Pseudomonas aeruginosa*, *Escherichia coli, Candida albicans, Aspergillus niger, Microsporum gypseum*) with MIC values either equal to or lower than 2.50 mg/ml [33]. Compared to our study, different MIC values were obtained on test bacteria and yeasts.

In a study performed by Kosalec et al. on 4 different *Rhamnus* species extracts, the results of antimicrobial activity can be listed as follows: *E. coli, P. aeruginosa*, *S. aureus* bacteria have MIC values (1.25-2.5 mg/ml), while *C. albicans* have MIC values (0.625 mg/ml) [10]. Compared to our study, although similar values were obtained on test bacteria and yeasts, mostly different MIC values were determined.

Antiproliferative Activity

In vitro cytotoxicity of *R. cathartica* L. plant extracts was evaluated in MCF-7, A549 and C6 cancer cell lines and WI-38 fibroblast cell line by XTT method. The percentage of cell viability and cell inhibition was determined by dividing the number of unstained cells by the total number of cells, and multiplying the ratio by 100.

IC_{50} values were generated from the dose-response curve and optical density. The dose-response results of the extracts are shown in Table 8.

Table 8. IC_{50} values

Rhamnus cathartica L.	*IC_{50} (µg/ml)*			
	MCF-7	**C6**	**A549**	**WI-38**
Leaf ethyl acetate	39.19 ± 0.32 a	3.55 ± 0.32 a	10.38 ± 0.18 a	44.74 ± 1.63 a
Leaf ethanol	42.85 ± 0.63 b	4.7 ± 0.18 b	13.35 ± 0.34 b	21.44 ± 1.32 b
Leaf hexane	4.79 ± 0.54 c	3.1 ± 0.21 a	10.28 ± 0.45 a	40.65 ± 1.45 c
Fruit ethyl acetate	10.28 ± 0.12 d	4.15 ± 0.10 b	15.24 ± 0.22 c	130.23 ± 1.93 d
Fruit ethanol	23.79 ± 0.28 e	9.04 ± 0.17 c	14.02 ± 0.62 d	15.24 ± 1.12 e
Fruit hexane	9.04 ± 0.16 f	6.62 ± 0.12 d	12.17 ± 0.24 e	41.67 ± 1.78 f
Cortex ethyl acetate	18.89 ± 0.41 g	8.45 ± 0.24 e	6.62 ± 0.14 f	18.89 ± 1.33 g
Cortex ethanol	13.35 ± 0.25 h	17.06 ± 0.13 f	17.06 ± 0.32 g	44.09 ± 1.65 a
Cortex hexane	25.68 ± 0.19 i	13.35 ± 0.27 g	13.35 ± 0.48 b	21.97 ± 1.48 b

Significant $p \leq 0.05$ level of analysis of variance (One Way ANOVA Tukey)

The difference between the IC_{50} values of the leaf, fruit and bark samples on the MCF-7 cell line in all solvents was found to be significant. In C6 and A549 cell lines, when the IC_{50} values are evaluated, the differences between the values obtained from all other samples and all solvents are statistically significant, except for the leaf ethylacetate and leaf hexane values.

In the WI-38 cell line, while the difference between leaf ethyl acetate and cortex ethanol was not significant, the difference between all other samples and solvents was statistically significant.

According to the SI table, F-EtAc extract was found to have the highest anticancer activity in cancer cell groups with WI-38/MCF-7= 12.67, WI-38/C6= 31.38, WI-38/A549= 8.55 values. L-EtAc extract with WI-38/C6= 12.60, WI-38/A549= 4.31 values and F-Hex extract with WI-38/MCF-7= 4.61 values take the second place (Table 9).

Table 9. SI values

Rhamnus cathartica L.	*Selectivity index (SI)*		
	WI-38 / MCF-7	**WI-38 / C6**	**WI-38 / A549**
Leaf ethyl acetate	1.14	12.60	4.31
Leaf ethanol	0.50	4.56	1.61
Leaf hexane	8.49	13.11	3.95
Fruit ethyl acetate	12.67	31.38	8.55
Fruit ethanol	0.64	1.69	1.09
Fruit hexane	4.61	6.29	3.42
Cortex ethyl acetate	1	2.24	2.86
Cortex ethanol	3.30	2.58	2.58
Cortex hexane	0.86	1.65	1.65

A significant difference was found between the IC_{50} values of the *R.cathartica* L. plant leaf, fruit and bark samples on the MCF-7 cell line in all solvents. In C6 and A549 cell lines, when the IC_{50} values are evaluated, the differences between the values obtained from all other samples and all solvents are statistically significant, except for the leaf ethyl acetate and leaf hexane values.

In the WI-38 cell line, while the difference between leaf ethyl acetate and bark ethanol was not significant, the difference between all other samples and solvents was statistically significant.

Shokoohian et al., in their study on the MCF-7 cell line, observed that different doses of *Rhamnus frangula* extracts (0.01 mg/ml, 0.1mg/ml, 1 mg/ml and 10 mg/ml) decreased cell viability as the dose increased [34].

A different study determined anticancer activity for the methanolic extract of *Rhamnus aternus* bark on U937 cells. The extract's IC_{50} value was discovered to be less than the NCI-recommended threshold for classification as an anticancer agent. In preliminary analyses, the US National Cancer Institute (NCI) established the following cytotoxicity criteria for the crude extract: $10 \leq IC_{50} < 20$ μg/ml of total/crude extract was considered cytotoxic, $20 \leq IC_{50} < 50$ μg/ml was considered moderately cytotoxic, and < 10 μg/ml was considered strongly cytotoxic. Furthermore, it was discovered that both conventional and aqueous extracts were only mildly harmful to healthy PBMCs; the methanolic extract exhibited cytotoxic activity exclusively on cancer cells (U937) [35].

Investigated the antiproliferative activity of components of *Rhamnus dysperma* roots on MCF-7 (human breast adenocarcinoma), A2780 (human ovarian adenocarcinoma), HT29 (human colon adenocarcinoma), and MRC5 (normal human fetal lung fibroblast) cells. The penduletine component exhibited the highest cytotoxic activity against the three cancer cell lines. MCF-7, A2780, and HT29 have IC_{50} values between 0.53-2.17 μM. It was also found to be 2-9 times more selective against the tested cancer cell lines than normal fibroblast cells (MRC5) [7].

The methanol extract of *R. cathartica bark* from Iran affected the Caucasian colon adenocarcinoma (HT-29) and human Caucasian gastric adenocarcinoma (AGS) cell lines. Compared to the isolated components and the whole extract, the methanol extract demonstrated a noticeably decreased cytotoxic impact at doses of 100, 200, and 400 mg/ml after 48 hours [36].

The antiproliferative effects of *R. cathartica* L. extracts in C6, A549 and MCF-7 cancer lines have not been investigated before. The extracts have antiproliferative effects in the studies discussing the genus *Rhamnus.*

Conclusion

In recent years, the rapid development of drug resistance of many infectious agents, the high side effects of synthetic chemotherapeutics, and their metabolically highly toxic and expensive nature have led researchers to search for new herbal drug active ingredients. Nowadays, drugs obtained from plants are preferred over synthetic chemotherapeutics due to their easy accessibility and broad spectrum of action. In addition, it should not be forgotten that herbal drugs can cause many harms due to their unconscious and unlimited use. In this case, a lot of work should be done by researchers about the concentration and effect spectrum of herbal drugs, and public awareness should be raised.

In our study, the biochemical content and antioxidant, antimicrobial, and antiproliferative effects of the extracts prepared with hexane, ethyl acetate, and ethanol of the cortex, leaf, and fruit parts of the *R. cathartica* L. collected from the Kazakhstan region were investigated. As a result of GC-MS analysis, trans-2-Decenal is the substance with the highest percentage, with 2.88%. According to DPPH IC_{50}, FRAP, and flavonoid values, the highest antioxidant activity was determined in the fruit ethyl acetate extract. Fruit ethyl acetate extract was found to have the highest anticancer activity in cancer cell groups, with the values in the Selectivity Index table prepared according to the IC_{50} values of WI-38/MCF-7: 12.67, WI-38/C6: 31.38, and WI-38/A549: 8.55. All plant extracts have reached high effective MIC values for *S. aureus*. Especially in cortex and leaf ethyl acetate solvents, the MIC value was found to be 0.078 mg/ml. for *B. cereus*, cortex and leaf extracts were found to have high antimicrobial efficacy (0.312 mg/ml), while the fruit reached weak effective MIC values.

The results of this study revealed that the plant extracts obtained from *Rhamnus cathartica L.* established high biological effects. Therefore, more studies are needed in this area.

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AUTHOR OF CONTRIBUTIONS

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CONFLICT OF INTEREST

The authors declare that there are no real, potential, or perceived conflicts of interest for this article.

ETHICS COMMITTEE APPROVAL

The authors declare that ethics committee approval is not required for this study.

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