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Antiproliferative Properties and Evaluation of Antioxidant of Different Cornelian Cherry Genotypes and Analysis of Phenolic and Sugar Compounds by HPLC,

Farklı Kızılcık Meyvelerinin Genotiplerinin Antiproliferatif Özellikleri ve Antioksidanlarının Değerlendirilmesi ve HPLC ile Fenolik ve Şeker Bileşiklerinin analizi

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ABSTRACT

The aim of this study was to investigate the chemical composition (vitamin C, phenolic, and sugar compounds), the cytotoxic effect on healthy (L-929) and lung cancer (A-549) cells, and the antioxidant capacity of fruits belong to thirteen cornelian cherry (Cornus mas L.) genotypes grown under the same conditions in Turkey. Fruit samples were extracted by the ASE technique. The chemical composition was analyzed by HPLC-DAD-RID. A reversed-phase Clipeus C18 reversed-phase column (250 mm × 4.6 mm, 5 µm) were used. For gradient elution mobile phase A contained 4.5 % acetic acid in water; solution B acetonitrile were used as mobile phase with flow rate 1.0 mL/min. Antioxidant capacity, total phenolic, and total anthocyanin content were determined using spectrophotometric methods. Cytotoxic effects were evaluated by MTT assay in L-929 and A-549 cell lines for 48 h. No toxic effect of the fruit extracts was observed on L-929 healthy mouse fibroblast cells, while it was determined to reduce cell proliferation (approximately 50%) on A-549 lung cancer cells. The featured genotypes were 44-03, 44-20, 44-21, 77-09, and 44-21, 44-16, 77-05, respectively. The featured genotypes for antioxidant capacity and cytotoxic effects on A-549 cells were 44-03, 44-20, 44-21, 77-09, and 44-21, 44-16, 77-05, respectively. The results have brought out that there are significant differences between the genotypes ($p \leq 0.05$) and cornelian cherry fruits have a significant antioxidant capacity and potential for antiproliferative effects.

Key Words

Cornus mas L., phenolic compounds, vitamin C, antioxidant capacity, antiproliferative effect, lung cancer.

ÖZ

Bu çalışmanın amacı, kızılcık meyvelerinin kimyasal bileşimi (C vitamini, bireysel fenolik ve şeker bileşikleri) ve antioksidan kapasitesinin yanında, meyve ekstrelerinin sağlıklı (L-929) ve akciğer kanseri (A-549) hücreleri üzerindeki sitotoksik etkisini araştırmaktır. Türkiye'de aynı koşullarda yetiştirilen on üç kızılcık (Cornus mas L.) genotipi meyve örnekleri ASE tekniği ile optimum koşullarda ekstrakte edildikten sonra kimyasal bileşim, HPLC-DAD-RID ile analiz edildi. Clipeus C18 ters faz kolonu (250 mm x 4.6 mm, 5 um) kullanıldı. Gradiyen elüsyon uygulanarak yapılan belirlemede dakikada 1 ml akış oranında, mobil faz olarak solvent A: %4,5 asetik asit solüsyonu ve solvent B: asetonitril kullanıldı. Antioksidan kapasitesi, toplam fenolik ve toplam antosiyanin içeriği spektrofotometrik yöntemler kullanılarak belirlendi. Sitotoksik etkiler, 48 saat boyunca L-929 ve A-549 hücre hatlarında MTT testi ile değerlendirildi. Meyve ekstraktlarının L-929 sağlıklı fare fibroblast hücreleri üzerinde toksik etkisi gözlenmezken, A-549 akciğer kanseri hücrelerinde hücre proliferasyonunu (yaklaşık %50) azalttığı belirlendi. A-549 hücreleri üzerindeki antioksidan kapasite ve sitotoksik etkiler için öne çıkan genotipler sırasıyla 44-03, 44-20, 44-21, 77-09 ve 44-21, 44-16, 77-05 olduğu belirlendi. Elde edilen sonuçlar, genotipler ($p \leq 0.05$) arasında önemli farklılıklar olduğunu ve kızılcık meyvelerinin önemli bir antioksidan kapasiteye ve antiproliferatif etki potansiyeline sahip olduğunu ortaya koymuştur.

Anahtar Kelimeler

Kızılcık (Cornus mas L.), fenolikler, C vitamin, antioksidan kapasite, antiproliferative effect, akciğer kanseri.

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INTRODUCTION

Fruits and vegetables are sources of natural antioxidants thanks to their secondary metabolites as well as being a food source. Antioxidants that have a preventive effect against harmful free radicals causing cancer, heart diseases, and many other diseases have been drawn attention due to their beneficial effects on human health [1]. Free radicals may comprise from the oxidation and reduction reactions that normally occur in the organisms, as well as harmful sources such as toxic products of cell metabolism, radiation, viruses, ultraviolet beams, and cigarette smoke. These reactive species cause oxidative damage in lipids, proteins,nucleic acids, and various pathological events. It is known that the relevant reactive species cause molecular changes and gene mutations in cells, and play a role in aging, cellular damage, and tissue destruction.

Antioxidants that neutralize free radicals and prevent their formation by giving their electrons serve a protective function in the human body. They are found in foods and the body in lower concentrations than oxidizable substrates and delay or prevent the oxidation of the substrate that causes oxidative damage [2]. Vitamins, carotenoids, and polyphenols are among the natural sources of antioxidants. Polyphenols are compounds found in many vegetables, fruits, and beverages such as tea and red wine and contain mainly flavonoids and phenolic acids. These compounds have the radical scavenging effect either by breaking free radicals chain reactions or directly by suppressing free radicals [3]. Polyphenols, commonly found in most plants, have pharmacological activity due to their ability to remove/prevent the reactive oxygen species causing destructive damage to the cells [4].

There are some problems with the use of synthetic anticancer agents following: Effecting without distinction between the normal tissue and tumor, their sensitivity to induced drug resistance, the severe side effects, etc. Such issues raise the interest in using natural bioactive ingredients as cancer preventive or healing agents [4].

Cornelian cherry is a tree plant growing naturally. Its fruits are generally consumed freshly or as jam, marmalade, syrup, jelly, compote, dried layers of fruit pulp, tarhana, and juice [5-6]. Besides, its fruit, leaf, root, and shell are used in folk medicine as an antipyretic, diarrhea preventive, and removing kidney stones. Cornelian cherry fruits have a rich content of anthocyanin, flavonoid, and phenolic compounds. Many studies have revealed the antiseptic, antioxidant, antidiabetic, and antimicrobial properties of the cornelian cherry [4-5, 7-13]. Based on a scientific basis use of herbal medicine for the prevention of diseases such as cancer, obesity, diabetes, and cardiovascular, the studies to reveal the therapeutic properties of plants are increasing, and cornelian cherry attracts attention from this aspect as well. In the genotypes used in the current study, there is no study on the individual phenolic compounds and cytotoxic activity. The aim of this study is to determine the phenolic content and antioxidant capacity properties of fruits of different cornelian cherry genotypes from the Malatya Province of Turkey and investigate the in-vitro cytotoxic effects of fruit extracts on lung cancer cells.

MATERIALS and METHODS

The fruit material used in the study was obtained from the cornelian cherry genetic resources collection orchard belonging to Apricot Research Institute, located in Malatya Province of Turkey. Fruit samples were taken from fourteen cornelian cherry genotypes. The fruit samples removing the kernel were pulped with a blender. Fruit puree was dried with lyophilizer (Christ Alpha 1-2 LD plus, Germany) and powdered. The obtained fruit powder was used in all analyzes.

Extraction Procedure and Spectrophotometric Analyses

Analyses Accelerated Solvent Extraction (ASE) 200 system (Dionex, Sunnyvale, CA, USA) with 11-60 mL stainless steel ASE vessels used for the pressurized liquid extraction was used for the extraction of polyphenol from the fruit samples. The fruit samples were extracted in the solvent mixture of methanol:water:HCl (70:29.9:0.1 v/v/v) at 25°C, 1500 psi for 60 min [14]. The obtained extract evaporated to dryness by rotavapor under reduced pressure. The dry residue was redissolved in a 4 mL solvent mixture of methanol:water (1:1 v/v), and then was filtered through a 0.45 µm PVDF (polyvinylidene difluoride) filter. The filtrate was used in all analyzes except sugar and vitamin C analyzes. The total phenolic content (TPC) was determined by the Folin-Ciocalteu method [15], and the results were expressed as mg gallic acid/100 gDW (dry weight). UV/VIS Spectrophotometer (Shimadzu 2000S Model, Japan) was used for detection of TPC in the cornelian cherry samp-

les. To determine the antioxidant capacity, DPPH radical scavenging and reducing power tests were performed. DPPH radical scavenging test was performed by using the method of Yen and Hung [16]. The reducing power test was performed by using the method of Hwang et al. [15]. Results were compared with a standard curve prepared with Trolox, and expressed as mg Trolox (TE)/100 gDW. Total anthocyanin content (TAN) was determined using the pH-differential method [17] and expressed as mg cyanidin-3-O-glucoside (c3g)/100 gDW.

Analysis of Phenolic Compounds

The qualitative and quantitative determination of polyphenols was carried out using a Shimadzu HPLC, equipped with Shimadzu DGU-20A5 model vacuum degasser and Shimadzu 20 ADXR solvent pump. Separations were performed using a Clipeus C18 5 µm reversed-phase column (250 mm × 4.6 mm). Detection was performed with a Shimadzu SPD-M20A photodiode array detector. To prepare analytically pure polyphenol standards, 1000 mg/L stock solutions were prepared by dissolving 0.010 g polyphenol into deionized pure water and completed with 10 mL methanol and water (1:1 v/v). By using these stock solutions standard solutions of each polyphenol were prepared daily. The determination of phenolic compounds was carried out by the HPLC-DAD system. In the determination by applying gradient elution, Solvent A: 4.5% acetic acid solution and Solvent B: acetonitrile were used as mobile phases. The elution conditions are given in Table 1. The content of the phenolic compounds was determined according to its calibration curve and expressed as mg/100 gDW.

Validation Studies

Limit of detection (LOD) and limit of quantification (LOQ) was calculated by multiplying 3.3 and 10 by the standard deviation of the blank solution(s) respectively. To determine the % recovery, the sample was spiked a known amount of phenolic compound standard, and then both spiked sample and unspiked sample were measured to determine amount of phenolic compound by HPLC.

Table 1. The gradient program applied by HPLC.

Analysis time (min)	Solvent B (%)	Flow rate (mL/min)	Column temp. (°C)	Injection vol. (µL)	Wavelength (nm)
0.01	0				271, 273
7	5				
12	15				278, 279
20	40	1	30	20	
25	100				325, 354
30	100				
40	5				

Table 2. The validation parameter values from HPLC for phenolic compounds.

Phenolic compounds	Retention Time (min)	LOD*	LOQ*	Calibration Curve	R2	Linear Range*	Recovery (%)	Precision intra-day RSD %	Precision inter-day RSD %
Gallic acid	5.44	0.033	0.048	y=605.33x+34.12	0.9999	0.05-10	90.39	4.84	7.95
Catechin	14.37	0.034	0.060	y=111.68x+3.29	0.9999	0.06-5.0	77.47	6.92	9.50
Chlorogenic acid	15.15	0.005	0.010	y=559.62x+12.41	0.9999	0.025-5.0	77.00	8.91	10.07
Epigal**	16.61	0.044	0.060	y=20.244x-2.04	0.9999	0.25-25.0	80.00	4.40	6.72
Epicatechin	16.93	0.026	0.043	y=13.296x+4.29	0.9998	0.05-5.0	77.40	3.22	8.03
Rutin	18.94	0.012	0.019	y=308.2x+5.50	0.9999	0.025-2.5	98.44	2.37	6.73

*mg/100 mL, **Epigallocatechin gallate

The percentage of recovery was calculated using Equation (1):

$$\%\ Recovery = \frac{C1 - C0}{C(spike)} x100 \quad (1)$$

where C1 is the measured concentration of the spiked sample, C0 is the measured concentration of the unspiked sample and C(spike) is the added concentration of phenolic compound standard. The validation parameter values calculated for the phenolic compounds are given in Table 2.

Analyses of Sugar Compounds and Vitamin C

Sugar analysis in fruit samples was carried out by HPLC-RID (refractive index detector) (Shimadzu RID-10A). Separations were performed using a Carbohydrate 5µm column (250 mm × 4.6 mm). The solvent mixture of acetonitrile:water (77:23 v/v) was used as mobile phase. 0.50 g fruit sample was weighed into a centrifuge tube, and then 10 mL ultrapure water was added on. Tubes were mixed well by vortex and then centrifuged at 6000 rpm for 5 min. The upper phase was taken and filtered through a 0.45 µm PVDF filter. Sugar content was determined by injecting to HPLC the filtrate.

The determination of vitamin C was carried out by the HPLC-DAD (Diode-Array Detection) system (Shimadzu SPD-M20A). Separations were performed using a Phenomenex C18 5µm column (250 mm × 4.6 mm). The ultrapure water (pH: 2.2) was used as mobile phase. 2.50 g fruit sample was weighed into a centrifuge tube wrapped with aluminium foil, and then 10 mL 6% metaphosphoric acid was added on. Tubes were mixed well by vortex and then centrifuged at 6000 rpm for 2 min. The upper phase was taken and filtered through a 0.45 µm PVDF filter. Vitamin C content was determined by injecting to HPLC the filtrate, at 254 nm [18].

In-vitro Cytotoxic Activity

L-929 (normal mouse fibroblast cell line, ATCC, CCL-1) and A-549 (human lung cancer cell line, ATCC, CCL-185) cells were grown and maintained in Dulbecco's Modified Eagle's Medium (DMEM) containing 10% fetal bovine serum (FBS), 1% penicillin and streptomycin and were incubated at 37 °C in a humidified atmosphere of 5% CO2. Stock cultures were passaged at 2 to 3-day intervals.

The MTT [2, 5- diphenyl - tetrazolium bromide, and 3 - (4, 5-dimethyl thiazolyl)] test was used to evaluate the cell viability. The test is based on producing a blue formazan product from mitochondrial dehydrogenase that indicates the normal functions of mitochondria and as a result of the measurement method of the resulting cell viability and cytotoxicity [19]. L-929 and A-549 cells at a density of 1x104 cells/well were seeded into 96- well plates in DMEM containing 10% FBS. Then, the cells were treated with different concentrations (100-200 µg/mL) of the extract. 48 h after the treatment, the medium of each plate was changed with a fresh medium containing MTT solution. After 4 h of incubation, the incubated media was added to the solubilization buffer. Then, the absorbance of each well was read at a wavelength of 570 nm using Elisa Reader (Epoch, Biotek, USA). Cell proliferation was obtained by proportioning the absorbance values obtained from the negative control to the absorbance values of the experimental groups. The absorption value from controls (cells not treated with extracts) was considered having 100% cell viability [20, 21].

Statistical analysis

Analyses were performed with three replicates. All data were subjected to variance analyses. Significant differences among applications were determined according to LSD multiple comparison test at $p < 0.05$. In addition, correlation coefficients between results of the analysis were calculated. The chemical compositions and cytotoxic effects on L929 and A549 cell lines of the tested fruit samples were compared using principal components analysis (PCA). Besides, Pearson's Correlation Test ($p < 0.01$ and $p < 0.05$) was applied to evaluate the relations among the assessed fruit characteristics. All of the statistical analyzes were performed using IBM SPSS Statistics 22 software.

RESULTS and DISCUSSION

TPC, total anthocyanin and antioxidant capacity

TPC values of fruit extracts belonging to cornelian cherry genotypes were measured using the calibration graph prepared in the spectrophotometer with concentrations of 1.00, 2.50, 10.00, 25.00, and 50.00 mg/100 mL of gallic acid standard. Results expressed as gallic acid equivalent (GAE) are given in Figure 1A. The TPC values of the fruits ranged from 201.45-

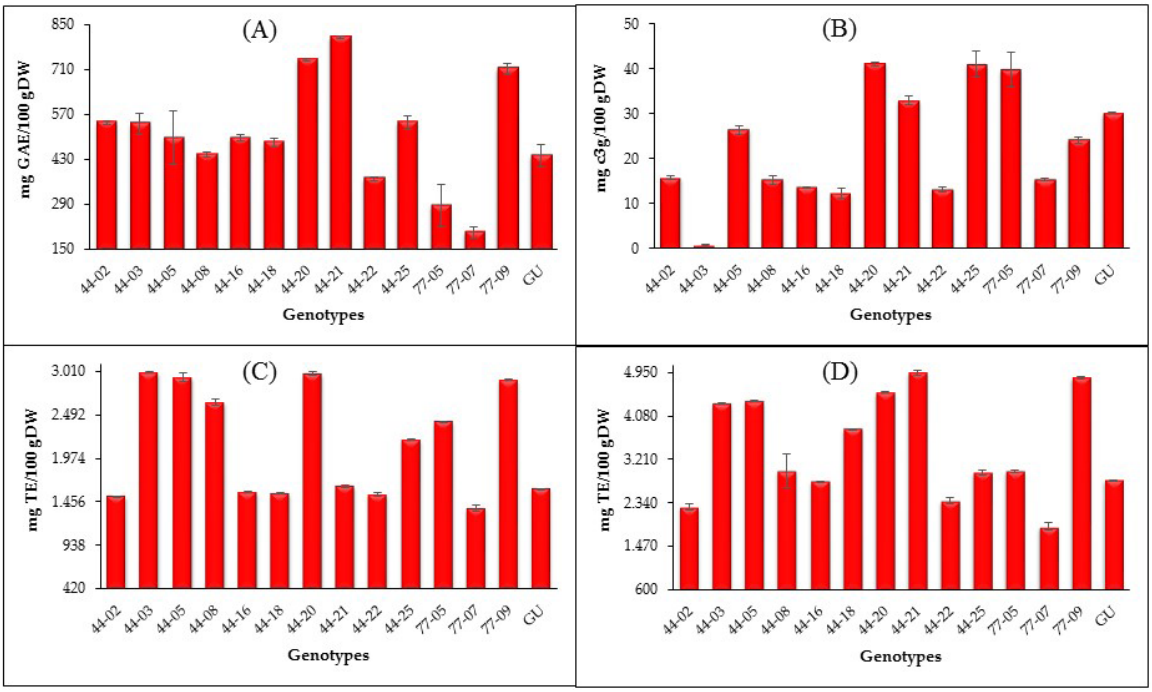

Figure 1. Total phenol (A) and total anthocyanin (B) contents, antioxidant capacity to DPPH radical scavenging (C), and reducing power (D) tests of cornelian cherry fruit extracts (GU: Güney Uzun).

808.73 mg GAE/100 gDW, and the genotypes with the highest phenolic content were 44-21 (808.73±7.27 mg GAE/100DW), 44-20 (739.64±10.91 mg GAE/100 gDW) and 77-09 (710.55±32.73 mg GAE/100 gDW), respectively. Genotype 77-07 contained the lowest phenolic content (201.45±32.73 mg GAE/100gDW). These results are in agreement with those obtained by Stiropoulos et al. (280-560 mg GAE/100 g), Tural and Koca (281-579 mg GAE/100 g), De Biaggi et al. (196.68 mg GAE/100 g), Cosmulescu et al. (163.69-359.28 mg GAE/100 g), Kucharska and Letowska (261.70-464.12 mg GAE/100 g) and Cetkovska et al. (217.4-614.3 mg GAE/100 g) [7-9, 22-24]. However, there are also higher and lower values in the literature compared to the results of our study. For instance, Yousefi et al. [25] and Pantedis et al. [10] reported that TPC is 1310.2 and 2611mg GAE/100 g (higher than the results of our study) in cornelian cherry fruit, respectively, while Klymenko et al. [26] within the range of 91.34-200.00 mg GAE/100 g (lower than the results of our study).

TAN results of fruit extracts belonging to cornelian cherry genotypes are given in Figure 1B. TAN values ranged from 0.67 to 41.08 mg c3g/100 gDW. The genotype 44-20 (41.08±0.73 mg c3g/100 gDW) came into prominence with the highest total anthocyanin content, while the genotype 44-03 (0.67±0.27 mg c3g/100 gDW) had the lowest.

Anthocyanins are pigments that give pink, red, blue, and purple colors to fruits and vegetables [17]. The results of our study are in agreement with those reported by Karaaslan (12.0-23.3 mg/100 g), Kantar (32-36 mg/100 g), and Cetkovska (6.1-34.7 mg/100 g) [17, 24, 27]. Compared to our results, either higher or lower results were reported in the previous studies. For example, De Biaggi et al. (134.71 mg/100 g), Moldovan et al. (92.23 mg/100 g), Pantelidis et al. (223 mg/100 g) and Popovic et al. (0.00058-0.03 mg/100 g) etc. [5, 8, 10, 11].

Fruit extracts were analyzed for their antioxidant capacity using DPPH radical scavenging and reducing power assays. Results are given in Figure 1C (DPPH) and 1D (reducing power). According to the DPPH assay, antioxidant capacity values varied between 1377.22-3003.91 as mg TE/100 gDW, and 75.22-88.89 as % inhibition. Genotypes 44-03 (3003.91±7.77), 44-20 (2991.82±25.24), 44-05 (2945.76±49.75), and 77-09 (2910.25±7.42 mg TE/100 gDW) showed the highest antioxidant capacity. According to the reducing power assay, antioxi-

dant capacity values varied between 1869.16-4943.36 mg TE/100 gDW. Genotypes 44-21 (4943.76±59.97), 77-09 (4847.65±21.58), 44-20 (4559.67±19.02), 44-05 (4375.19±14.43) and 44-03 (1339.88±14.68 mg TE/100 gDW) showed the highest antioxidant capacity according to reducing power assay. In a study conducted on six cornelian cherry genotypes, antioxidant capacity (DPPH) values of three genotypes were 76.32-82.37% (in agreement with our results), while the other three genotypes were reported to be between 38.98-60.86% (lower than our results) [12]. In another study in which different solvents were used, the antioxidant capacities (reducing power) of extracts obtained from ethanol and methanol solvents were 3531.91 and 2734.56, respectively, while it was 5894.99 for acetone, 1207.73 for acetonitrile, and 920.65 mg TE/100 g for water [17]. The use of different extraction solvents may be the reason for the difference in results.

Phenolic compounds

Phenolic component determinations of extracts obtained after extraction from fruits belonging to fourteen cornelian cherry genotypes were made by the HPLC-DAD system. In all genotypes, gallic acid, catechin, epicatechin, chlorogenic acid, epigallocatechin gallate (Epigal), and rutin compounds were determined in different concentrations. 44-20 and 44-21 with their gallic acid, chlorogenic acid, Epigal, and epicatechin contents, 44-20 and 44-02 with their catechin content, and 44-25 with its rutin content were determined as the prominent genotypes (Table 3). There are many previous reports on phenolic compounds of cornelian cherry fruits. The gallic acid, catechin, chlorogenic acid, epicatechin and rutin contents of the examined samples were between 21.91-55.07, 10.35-30.41, 4.09-33.98, 6.83-17.58, 7.60-36.33 and 3.69-15.68 mg/100 gDW respectively, in agreement with previous studies (gallic acid: from 0.05 to 166; catechin: from 0.39 to 395; chlorogenic acid: from 1.29 to 15; epicatechin: from 0.40 to 211; rutin: from 0.29 to 81 mg/100 g) [8, 9, 11, 13, 28-39]. In this current study, Epigal content of the examined samples was between 6.83-17.58 mg/100 gDW, lower than the result (19 mg/100 g) of Harnly et al. [40], who studied in unripped cornelian cherry fruits. As a matter of fact, the contents of some phenolic compounds unripe fruits are higher than those of ripe fruits [41]. Data from both previous studies and the present study have appeared on a wide scale. The different polarities of the phenolic compounds may affect those extraction yields depending on the polarity of the extraction solvents. Many factors, such as the solvent used in extraction, the extraction technique, genotype/variety feature, growing location, altitude, and plant nutrition status, may be the reason for the variation in the results.

Table 3. Phenolic compounds in cornelian cherry fruit extracts (mg/100 gDW); data are expressed as mean±SD (n = 3); means in the same column bearing different letters are significantly different ($p < 0.05$).

Genotype Name	Gallic Acid	Catechin	Chlorogenic Acid	Epigal	Epicatechin	Rutin
44-02	23.08±4.61gh	30.12±1.33a	24.49±0.27c	7.61±0.67ef	10.06±1.54def	9.60±0.32cd
44-03	27.19±1.65efg	20.05±1.80bc	10.92±0.36e	6.83±0.60f	14.54±1.11c	10.57±0.81c
44-05	30.42±2.33def	17.14±1.83cd	17.58±1.66d	12.83±0.42c	11.32±1.66cdef	7.88±0.65ef
44-08	22.95±0.85gh	10.35±0.13g	4.09±0.11g	6.96±0.03f	8.26±0.64ef	3.69±0.09g
44-16	38.48±0.61bc	13.43±0.75efg	8.81±0.18f	6.85±0.86f	9.65±0.41def	6.54±0.48f
44-18	39.31±5.36b	19.94±3.13bc	11.03±2.03e	13.46±2.37bc	12.48±3.41cd	13.77±2.81b
44-20	55.07±3.61a	30.41±0.22a	32.35±0.83a	17.45±1.48a	35.14±5.59a	8.45±0.80de
44-21	54.75±2.16a	21.41±5.05b	33.98±0.17a	17.58±1.64a	36.33±0.16a	10.90±0.19c
44-22	38.61±3.57bc	21.15±2.41b	27.04±2.04b	10.74±0.36d	18.95±3.05b	7.47±0.87ef
44-25	25.66±1.58fgh	21.99±2.11b	27.35±1.43b	13.36±0.94bc	11.10±3.41cdef	15.68±1.08a
77-05	21.91±3.07h	12.91±2.98fg	17.34±2.45d	14.69±0.56b	10.06±2.03def	6.45±0.91f
77-07	30.97±0.99de	18.64±0.09bc	16.59±0.11d	6.94±0.18f	10.18±1.47def	8.34±0.45de
77-09	41.24±4.83b	16.61±1.46cde	16.34±0.00d	9.22±0.47de	11.70±0.01cde	7.68±0.28ef
GU	34.01±2.02cd	14.65±0.34def	10.30±0.14ef	7.89±0.05ef	7.60±0.70f	8.91±0.40de

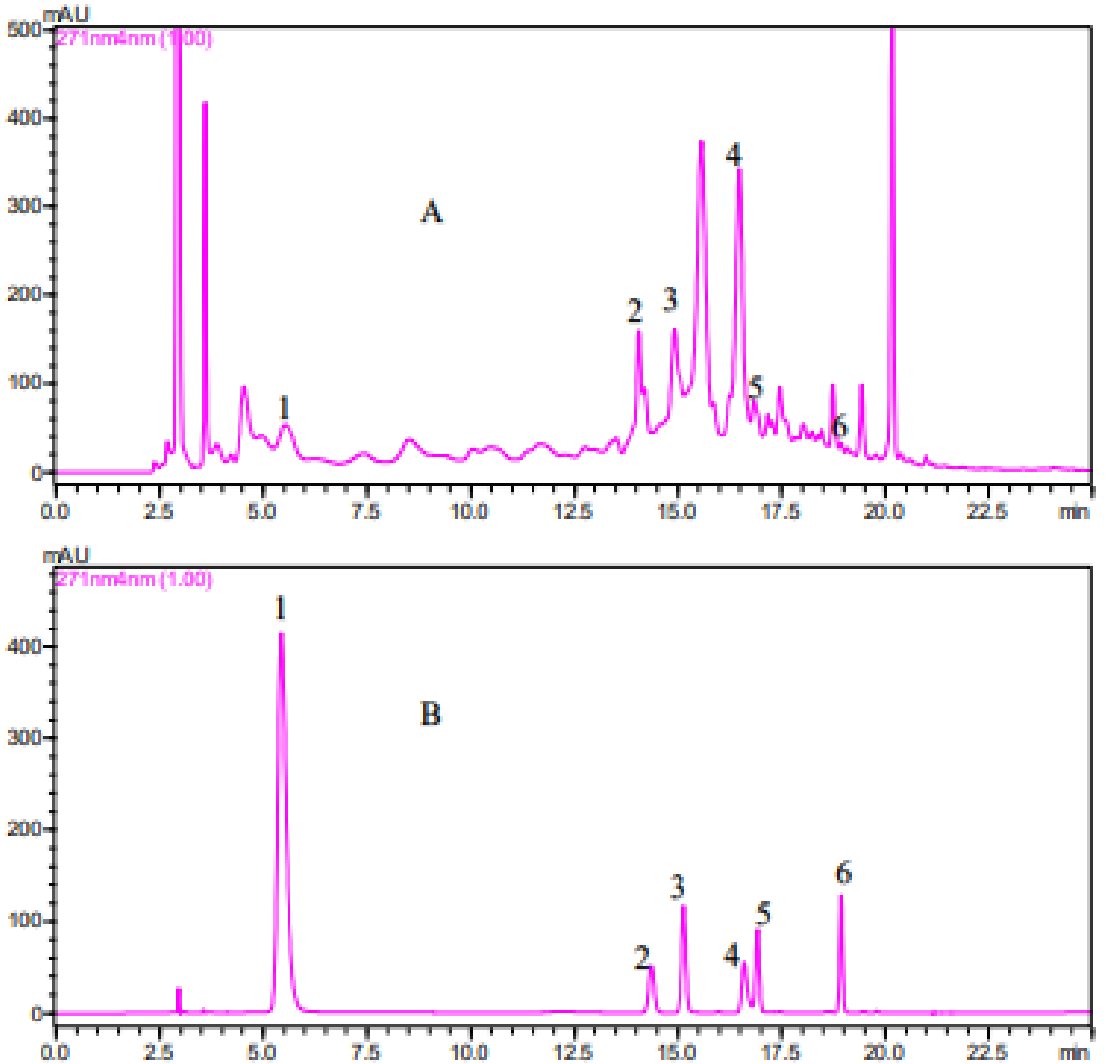

Figure 2. HPLC chromatograms of sample genotype 44-18 (A) and standards mix solution (B).
1: Gallic acid, 2: Catechin, 3: Chlorogenic acid, 4: Epigallocatechin gallate, 5: Epicatechin, 6: Rutin
Standards mix solution (1, 3, 4, and 6: 0.025 mg/100 mL; 2 and 5: 0.05 mg/100 mL).

Sugar Compounds and Vitamin C

Sugar compounds of cornelian cherry fruits was determined with the HPLC-RID, and the results are presented in Table 4. The differences in glucose contents among cornelian cherry genotypes were statistically insignificant ($p > 0.05$). Amounts of fructose, sucrose, and glucose ranged from 1.86-2.94, 0.03-0.12 and 3.05-4.59 % DW, respectively. Perova et al. [42] reported that fructose and glucose contents in cornelian cherry fruits were in the range of 2.2-3.8% and 2.5-7.0%, respectively. In a study of fresh and dried cornelian cherry fruits, fructose was found to be 1.90%, glucose 12.26% and sucrose 0.17% in fresh fruit samples. In a study [43] of fresh and dried cornelian cherry fruits, fructose was found to be 1.90%, glucose 12.26% and sucrose 0.17% in fresh fruit samples. In dried fruit samples fructose was found to be 2.63%, glucose 7.45% and sucrose 0.25%. In another study [44], it was reported that fructose and glucose contents in cornelian cherry fruits were 3.7% and 5.4%, respectively. Antolak et al. [45] reported that cornelian cherry juice contained 5.56g/100 mL fructose and 2.97g/100 mL glucose. The results of fructose and glucose reported by us were near to these literature data, while the sucrose results were lower than.

Table 3. Phenolic compounds in cornelian cherry fruit extracts (mg/100 gDW); data are expressed as mean±SD (n = 3); means in the same column bearing different letters are significantly different (p < 0.05).

Genotype Name	Vitamin C	Glucose*	Fructose	Sucrose
44-02	69.86±1.35j	4.35±0.66	2.69±0.42ab	0.10±0.02c
44-03	126.76±2.39ı	4.32±0.44	2.43±0.30abc	0.05±0.01g
44-05	198.35±1.29e	3.60±0.70	2.32±0.34abc	0.08±0.01d
44-08	254.38±0.08b	4.59±0.71	2.94±0.47a	0.08±0.01d
44-16	258.42±3.40b	4.06±0.85	2.90±0.63a	0.07±0.01de
44-18	264.68±3.12a	4.42±0.39	2.36±0.33abc	0.11±0.01bc
44-20	247.41±2.80c	3.46±0.64	2.02±0.40bc	0.07±0.02ef
44-21	216.17±5.64d	3.05±0.59	2.01±0.39bc	0.05±0.01fg
44-22	139.53±1.27g	3.71±0.51	2.81±0.62a	0.08±0.01de
44-25	8.48±0.45l	4.52±0.61	1.90±0.05c	0.12±0.01ab
77-05	132.36±0.04h	3.20±0.46	2.07±0.61bc	0.03±0.01h
77-07	213.28±8.20d	3.97±0.65	2.54±0.54abc	0.08±0.01d
77-09	21.96±0.72k	4.40±0.81	1.86±0.02c	0.12±0.01a
GU	146.16±2.95f	3.86±0.36	2.09±0.27bc	0.12±0.01ab

The vitamin C results of cornelian cherry fruits ranged from 8.48 to 264.68 mg/100 gDW (Table 4). The genotype 44-18 had the highest vitamin C content in its fruits (264.68±3.12 mg/100 gDW). The vitamin C contents of cornelian cherry fruits were reported as being between 16.0 and 299.5 mg/100 g in previous studies [7, 10, 12, 23, 24]. Vitamin C results for other genotypes except the 44-25 genotype (8.48±0.45 mg/100 gDW) are consistent with the literature data.

In-vitro Cytotoxic Activity

The cytotoxic effects of the fruit extracts on healthy cells (L-929) are presented in Figure 2. 100 and 200 µg/mL extract doses of the studied cornelian cherry genotypes showed no toxic effects on L-929 cells. The cytotoxic effects of the extracts on lung cancer cells (A-549) are given in Figure 3. The significant cytotoxic activity was observed against lung cancer cells in both extract doses of the studied genotypes, especially 44-16 and 44-21. The extracts of other genotypes were observed to reduce the proliferation of lung cancer cells, too ($p \leq 0.05$). In some genotypes (44-05, 44-08, 44-22, GU), the cytotoxic activity on A-549 (lung) cells increased by increasing the extract dose.

Yousefi et al. [25] reported that cornelian cherry fruit extracts showed anticancer potential and increased the apoptosis in SKOV3 (ovarian), MCF-7 (breast), PC-3 (prostate), and A-549 (lung) carcinogenic cell lines. Also, cornelian cherry leaves [4] and fruit juice [46] were stated to reduce cancer cell proliferation. Hosseini et al. [47] reported that the cornelian cherry extracts significantly reduced cell proliferation in the gastric carcinoma cell lines and thus could be used as a potent inhibitor of cancer cell proliferation. However, they found that when administered a 10 mg/mL extract dose in L-929 cells, the cell proliferation was reduced by approximately 80%. Depending on increasing doses, it was specified that cornelian cherry fruit extracts shown a high level of cytotoxic effect on healthy cells.

The results of correlation analysis between biochemical fruit characteristics and cytotoxic properties of cornelian cherry genotypes examined within the scope of the study are presented in Table 5. Accordingly, different correlation levels were determined among most of the evaluated fruit characteristics, which are statistically significant (at 0.01 and 0.05 significance levels).

TPC showed positive correlations in a low level with DPPH, moderate level with CAT, CA and Epigal, and a high level with RP, GA and Epicat. In addition to TPC, DPPH was found correlated with RP but in a high level ($r = 0.62$). A moderate positive correlation was found between RP and GA, Epigal and Epicat, and a low-level negative correlation ($r = -0.39$) with Fructose. TAN highly

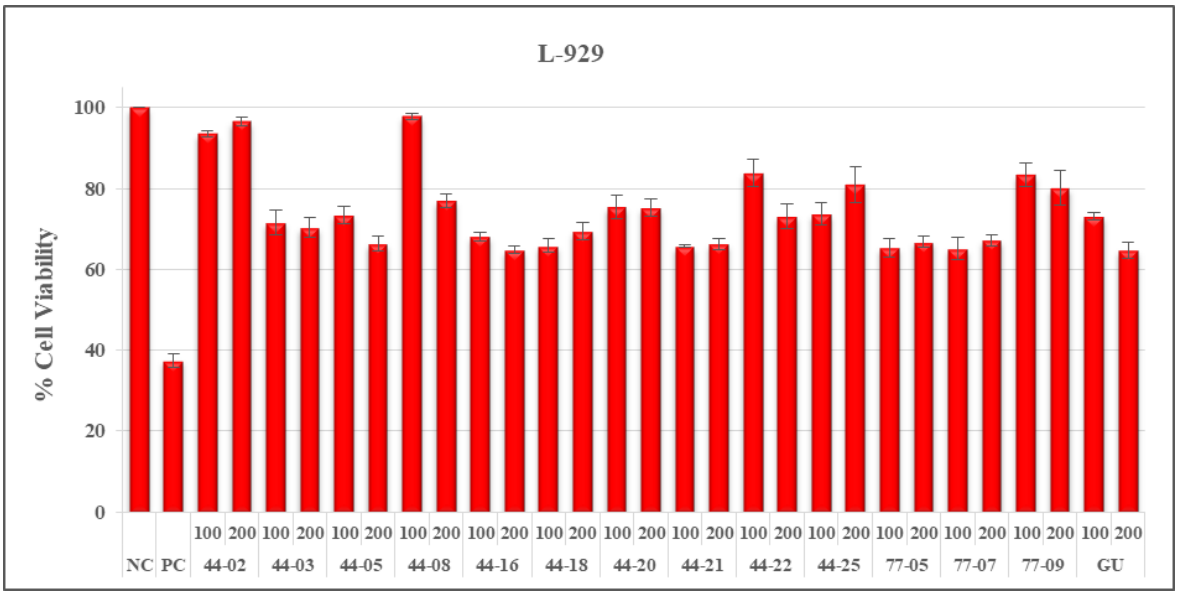

Figure 3. Cell proliferation inhibitory activities of cornelian cherry fruit extracts (100 and 200 µg/mL) against L929 healthy cell lines. The vertical bars represent mean±SD of three individual experiments (NC: Negative Control; PC: Positive Control).

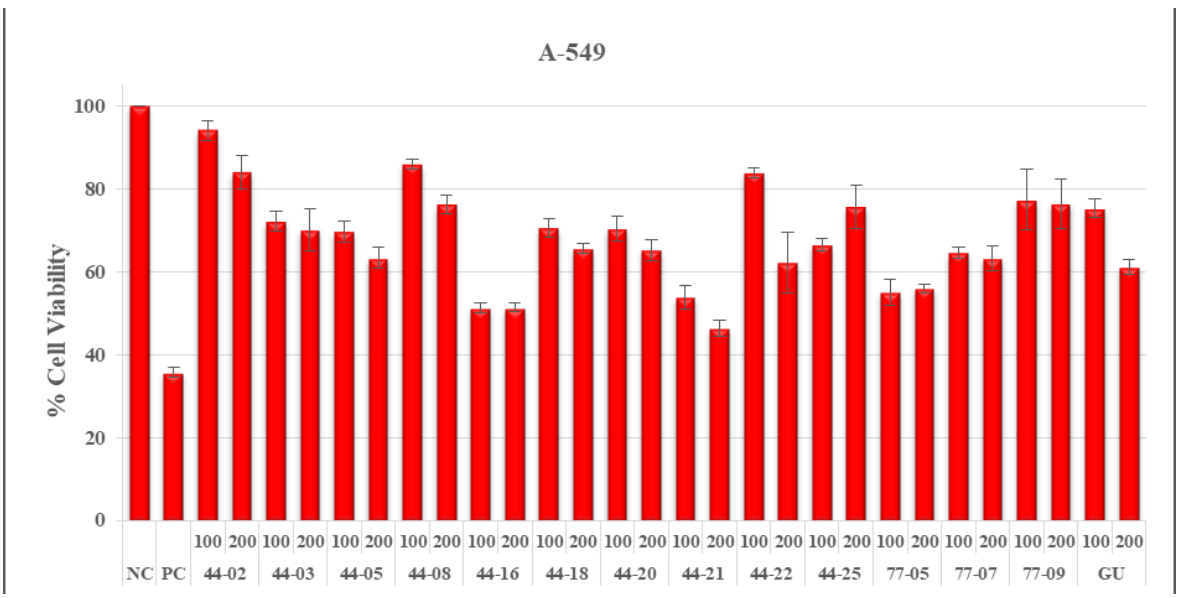

Figure 4. Cell proliferation inhibitory activities of cornelian cherry fruit extracts (100 and 200 µg/mL) against A549 lung cancer cell lines. The vertical bars represent mean±SD of three individual experiments (NC: Negative Control; PC: Positive Control).

correlated with Epigal, moderately with CA and Fructo se and in a low level with Epicat, Glucose and A-100, but the directions of the correlations were different. Low level of correlations were found for GA with CAT, VitC, Glucose, medium level with CA, Epigal and A-200, high level but different directions with Epicat. CAT showed a low level of positive correlation with Epigal (r = 0.38), a moderate level of positive correlation with Epicat, Rutin and L-200, and a high level of positive cor relation with CA (r = 0.72). CA showed positive correlation with Rutin (r = 0.37), negative and low-level correlation with Glucose (r = -0.35), and a high level of positive correlation with Epigal and Epicat. Similarly, Epigal also showed a high level of positive correlation with Epicat, besides it showed a positive low level of correlation with Rutin, a low level of negative correlation with A-100 and A-200,

Table 5. Correlation coefficients among the assessed variables obtained from Pearson's Correlation Test.

	DPPH	RP	TAN	GA	CAT	CA	Epigal	Epicat	Rutin	VitC	Glucose	Fructose	Sucrose	L-100	L-200	A-100	A-200
TPC	0.33*	0.73**	0.28	0.62**	0.43**	0.45**	0.43**	0.62**	0.26	-0.09	-0.06	-0.30	0.09	0.10	0.23	-0.02	0.00
DPPH		0.62**	0.19	-0.04	-0.05	-0.06	0.16	0.10	-0.20	-0.15	0.02	-0.24	-0.21	0.17	0.06	0.07	0.24
RP			0.20	0.55**	0.11	0.21	0.50**	0.54**	0.15	0.06	-0.16	-0.39**	-0.12	-0.16	-0.15	-0.20	-0.12
TAN				0.24	0.16	0.54**	0.70**	0.35*	0.17	-0.19	-0.33*	-0.50**	-0.01	-0.17	-0.01	-0.32*	-0.14
GA					0.35*	0.50**	0.54**	0.82**	0.15	0.35*	-0.31*	-0.22	-0.06	-0.28	-0.27	-0.30	-0.40**
CAT						0.72**	0.38*	0.55**	0.48**	-0.17	-0.05	-0.11	0.10	0.11	0.49**	0.24	0.24
CA							0.69**	0.75**	0.37*	-0.24	-0.35*	-0.30	-0.11	-0.07	0.24	-0.07	-0.09
Epigal								0.72**	0.37*	0.12	-0.42**	-0.42**	-0.23	-0.34*	-0.15	-0.37*	-0.32*
Epicat									0.18	0.26	-0.40**	-0.22	-0.34*	-0.17	-0.10	-0.22	-0.31*
Rutin										-0.31*	0.14	-0.32*	0.34*	-0.34*	0.11	-0.12	0.11
VitC											-0.19	0.30	-0.36*	-0.24	-0.51**	-0.28	-0.46**
Glucose												-0.27	0.43**	0.37*	0.27	0.34*	0.43**
Fructose													-0.10	0.22	0.06	0.15	0.07
Sucrose														0.30	0.36*	0.44**	0.47**
L-100															0.68**	0.80**	0.63**
L-200																0.68**	0.68**
A-100																	0.71**

TPC: Total Phenolics, RP: Reducing Power, TAN: Total Anthocyanin, GA: Gallic Acid, CAT: Catechin, CA: Chlorogenic Acid, Epigal: Epigallocatechin gallate, Epicat: Epicatechin, VitC: Vitamin C

and a moderate negative correlation with Glucose and Fructose. Epicat was also found negatively correlated with Sucrose and A-200 in a low level and moderate level with Glucose. Rutin showed negative correlations with VitC, Fructose and L-100, and positive but low correlations with Sucrose. VitC also showed correlations with Sucrose (r = -0.36) and a moderate negative correlation with L-200 and A-200 (r = -0.51 and r= -0.46, respectively). Glucose showed positive correlations with Sucrose, L-100, A-100 and A-200, whereas Sucrose with L-200, A-100 and A-200. All cytotoxic properties were found significant and positively correlated with each other in high and very high levels.

The eigen and variance values of the first four components out of a total of eighteen components obtained as a result of the principal component analysis of the fruit characteristics of the cornelian cherry genotypes examined within the scope of the study are summarized in Table 6.

As a result of the analysis, it was seen that the first two components expressed 52.02% of the total variance, with the first and second components being 30.34% and 21.69%, respectively. This was followed by the third and fourth components with 10.68% and 10.09%, and the cumulative variance expressed with the fourth component reached 72.79%. As a result of their study on 21 cornelian cherry varieties grown in New Zealand, Lu et al. [48], reported that the cumulative variance of the first two components was 67.9% in the results of the principal components analysis performed with 6 different physical fruit characteristics. The higher value obtained in this current study would be due to the relatively lower variance in the population in terms of the characteristics examined, as well as the lower number of biochemical properties examined.

The component score results of the characters and the genotypes of the first two components, which represent the majority of the obtained variance and express almost all of the characters examined as a result of the principal component analysis, are presen ted in the bi-plot charts given in Figure 4. When the figure is examined, similar to the results reported by Brown et al. [49], it was observed that the genotypes were distributed around the chart based on their distinguishing characteristics instead of forming clusters whose boundaries could be sharply determined.

Table 3. Phenolic compounds in cornelian cherry fruit extracts (mg/100 gDW); data are expressed as mean±SD (n = 3); means in the same column bearing different letters are significantly different ($p < 0.05$).

Variable	Vitamin C	Glucose*	Fructose	Sucrose
TPC	0.58	0.52	0.34	-0.02
DPPH	0.14	0.22	0.76	-0.40
RP	0.63	0.20	0.56	-0.30
TAN	0.58	0.21	-0.15	-0.28
GA	0.78	0.05	0.10	0.23
CAT	0.37	0.67	-0.24	0.40
CA	0.68	0.49	-0.27	0.29
Epigal	0.87	0.16	-0.07	-0.04
Epicat	0.86	0.20	0.15	0.35
Rutin	0.31	0.40	-0.61	-0.24
VitC	0.21	-0.59	0.24	0.38
Glucose	-0.51	0.31	-0.03	-0.39
Fructose	-0.41	-0.29	0.09	0.68
Sucrose	-0.35	0.50	-0.31	-0.22
L-100	-0.53	-0.56	0.39	0.28
L-200	-0.34	-0.81	0.00	0.23
A-100	-0.56	-0.62	0.19	0.27
A-200	-0.54	-0.68	0.11	-0.03
Eigen values	5.46	3.90	1.92	1.82
Variance (%)	30.34	21.69	10.68	10.09
Cumulative variance (%)	30.34	52.02	62.70	72.79

TPC: Total Phenolics, RP: Reducing Power, TAN: Total Anthocyanin, GA: Gallic Acid, CAT: Catechin, CA: Chlorogenic Acid, Epigal: Epigallocatechin gallate, Epicat: Epicatechin, VitC: Vitamin C

As a result of the analysis, most of the phytochemical fruit characteristics were represented by the first component. The most important characters affecting the first component were respectively; Epigal (0.87), Epicat (0.86), GA (0.78), RP (0.63), TAN (0.58), TPC (0.58), and Glucose (-0.51).

Positive PC1 values points the genotypes with higher values in terms of these traits, except for Glucose, which gives negative value. In support the genotypes '44-20' and '44-21' were with their higher RP, GA, Epicat and Epigal contents. All of the cytotoxic parameters were represented by the second component as well as CAT (0.67), VitC (-0.59), and Sucrose (0.50). For example, the genotype '44-02' was distinguished with higher cytotoxic properties. DPPH (0.76) and Rutin (-0.61) affected the third component and Fructose (0.68) the fourth of which effect on the distribution of the genotypes examined in the first two components was weak.

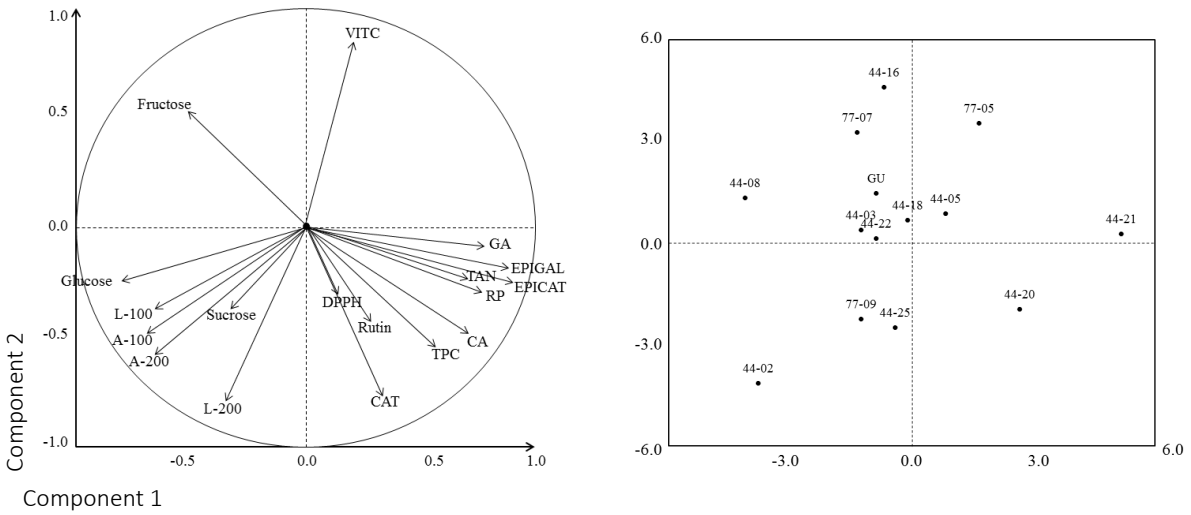

Figure 5. Segregation of apricot cultivars according to pomological and phytochemical characteristics determined by principal component analysis (TPC: Total Phenolics, RP: Reducing Power, TAN: Total Anthocyanin, GA: Gallic Acid, CAT: Catechin, CA: Chlorogenic Acid, EPIGAL: Epigallocatechin gallate, EPICAT: Epicatechin, VITC: Vitamin C).

Studies show that using cornelian cherry fruits as a functional food gains importance due to their high phenolic content and potential therapeutic benefits. Phenolic compounds are the main compounds responsible for pharmacological activity. Its health-supporting effects arising from its potent antioxidant activities were stressed in many studies. In this study, the antioxidant potential of cornelian cherry fruit extracts and their cytotoxic effects on A-549 (lung) cancer cells were investigated. Results of the present study showed that the fruit extract inhibited cell proliferation by showing the cytotoxic effect on A-549 cells. The phytochemical contents of the fruits used in the study were also determined. 44-21, 44-16, and 77-05 genotypes were the genotypes with the highest cytotoxic effects (approximately 50%) on A-549 cells. Many mechanisms may be responsible for these beneficial effects. Singlet oxygen and free radical scavenging antioxidant activity are possible action mechanisms of cornelian cherry extracts, and this activity was observed in vitro. Cornelian cherry extracts have the potent to reduce the riskof various cancer diseases. Well-designed long-term clinical studies are needed to confirm these results.

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