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Antioxidant and anti-proliferative activities of different parts of *Cyclamen cilicium*

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Abstract

This study was designed for investigate of antioxidant, and anti-proliferative activities of acetone, methanol and water extracts of different parts (fresh and underground parts) of Cyclamen cilicium extracts. The antioxidant activities were determined using DPPH, ABTS free radical scavenging activities. Anti-proliferative activities of extracts in Caco-2 colon cancer cells were detected by XTT assay. The determination of contents was evaluated using total phenolics, flavonoid and tannin contents and found maximum values 3.69 ± 0.13 (mg GAE/g-extract), 18.48 ± 0.12 (mg QE/g- extract), 41.17 ± 0.44 (mg CE/g- extract) respectively. C. cilicium extracts exhibited anti-proliferative activity in Caco-2 colon cancer cells. IC_{50} values of methanol and water extracts of fresh part and water extract of underground part were determined as 35.3276 µg/mL, 42.1831 µg/mL and 64.6144 µg/mL at 24h, respectively. The results showed that C. cilicium is the source of natural antioxidants and may have potential use in pharmaceutical, health, biotechnology, agricultural industry.

Keywords: *Cyclamen cilicium, antioxidant activity, anti-proliferative activity, Caco-2 cells, colon cancer*

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Cyclamen cilicium'un farklı kısımlarının antioksidan ve anti-proliferatif aktiviteleri

Öz

Bu çalışma Cyclamen cilicium türünün farklı kısımlarının (toprak üstü ve toprak altı kısımları) aseton, metanol ve su ekstraklarının antioksidan ve anti-proliferatif aktivitelerini araştırılması için tasarlanmıştır. Antioksidan aktivite için DPPH, ABTS serbest radikal giderim aktiviteleri belirlenmiştir. Ekstrelerin Caco-2 kolon kanseri hücrelerindeki anti-proliferatif aktiviteleri XTT testi ile belirlenmiştir. İçeriklerin belirlenmesi için toplam fenolik, flavonoid ve tanen içerikleri belirlenmiştir ve sırasıyla 3.69 ± 0.13 (mg GAE/g-ekstrakt), 18.48 ± 0.12 (mg QE/g-ekstrakt), 41.17 ± 0.44 (mg CE/g-ekstrakt) bulunmuştur. C. cilicium ekstreleri, Caco-2 kolon kanseri hücrelerinde anti-proliferatif aktivite sergilemiştir. Toprak üstü kısmının metanol ve su ekstreleri ve toprak altı kısmının su ekstresinin IC_{50} değerleri 24 saatte sırasıyla 35.3276 µg/mL, 42.1831 µg/mL and 64.6144 µg/mL bulunmuştur. Sonuçlar, C. cilicium'un doğal antioksidanların bir kaynağı olduğu ve ilaç, sağlık, biyoteknoloji ve tarımsal endüstrilerde potansiyel kullanımlara sahip olabileceğini göstermiştir.

Anahtar kelimeler: *Cyclamen cilicium, antioksidan aktivite, anti-proliferatif aktivite, Caco-2 hücreleri, kolon kanseri*

1. Introduction

Free radicals are unstable molecules that attack and damage intracellular structures [1]. In normal metabolism, free radicals are continuously produced and these molecules are stabilized. This situation continues in balance. The increase in free radical production in metabolism and the insufficiency of antioxidants that stabilize free radicals accordingly is called oxidative stress. A cell under oxidative stress disrupts the structures of the cell with the attack of unstable free radicals, causing cell, tissue, and organ deaths, diseases such as cancer, diabetes, Parkinson, Alzheimer's disease, and accelerating aging [2-4]. To eliminate the damage caused by free radicals, antioxidant supplements are required from the outside to the metabolism. Since the chemical antioxidants used today can cause cancer, natural antioxidants and polyphenols have begun to be needed [5,6]. Polyphenols are bioactive compounds found in various plant sources and have the ability to destroy free radical damage such as cancer risk, oxidative stress and inflammation [7-9]. *Cyclamen* species belonging to the Primulaceae family is represented by 11 species and 12 taxa in Turkey [10-12]. Some species have been cultivated in Western European countries since the 18th century [13]. Bulbs of all species in the *Cyclamen* genus are toxic due to the cyclamine saponin it contains. *Cyclamen* species, which have an important place in ornamental and medicinal plants, are used as laxatives and abortives in Sardinian folk medicine and are used in the treatment of infertility in Turkish folk medicine [14]. This study aimed to determine the antioxidant activity (DPPH free radical and ABTS radical cation scavenging activity), determination of contents (total phenolics, flavonoid and tannin contents) and anti-proliferative activity of *C. cilicium* extracts on Caco-2 colon cancer cells by XTT assay. *C. cilicium* extracts were obtained from acetone, methanol and water to contribute to industrial sectors such as pharmacy and agriculture, elucidate the anti-proliferative

efficacy in colon cancer cells *in vitro*. It has an important place because it is the first study in the literature.

2. Material and methods

2.1. Chemicals

Methanol, DPPH, ABTS, Butylated hydroxyanisole (BHA), Dulbecco Modified Eagle Medium (DMEM) were purchased from Sigma-Aldrich. Potassium persulfate was purchased from Merck. Heat-inactivated Fetal Bovine Serum (FBS), Phosphate Buffered Saline (PBS) without calcium and magnesium. Water was used as ultra-distilled pure and molecular biological grade water (MultiCell, UK). Other solvents and chemicals were used the analytical grade.

2.2. Plant materials and extract preparation

Cyclamen cilicium Boiss. & Heldr. [10,11,15,16] was collected from Antalya province in Turkey at 938 m altitude in September 2018 during the flowering period. The plant was identified by Dr. Olcay Düşen and stored with voucher specimens (Herbarium No: 1004 M. Turan) at PAMUH in Pamukkale University, Denizli, Turkey. After the plant samples (fresh and underground parts) were collected from the area determined from the literature, they were cut into small pieces and dried at room temperature. 100 g of dried plant samples were weighed and the solvent (acetone, methanol or water) was added in ratio of 1:10. After 6 hours in a shaking water bath (Memmert WNB), it was filtered through Whatman paper and solvent was added again. Then the acetone and methanol solvents in the filtrate were evaporated with rotary evaporator (IKA RV10). Samples were lyophilized (Labconco FreeZone) and extracts were kept at -20 $^{\circ}$C [17].

2.3. In vitro antioxidant activity

2.3.1 DPPH free radical scavenging activity

DPPH (2,2-Diphenyl-1-picryl hydrazyl radical) free radical scavenging activity assay was performed according to the Wu et al. method [18]. 4 mL DPPH solution was added to the extract-solvent mixture of 4 different concentrations (0.2-1 mg/mL) and incubate at 23-25 $^{\circ}$C for 30 minutes. After incubation, it was measured at 517 nm in the spectrophotometer (Peak Instruments C-7200) and the IC_{50} value was calculated.

2.3.2 ABTS radical cation scavenging activity

ABTS (2,2'-azino-bis-3-ethylbenzothiazoline-6-sulphonic acid) radical cation scavenging activity assay was performed according to the Re et al. method [19]. The mixture of 7nM ABTS and 2.45 nM potassium persulfate was mixed and kept in dark 12 hours before the experiment. Then, it was diluted with ethanol until the absorbance was 0.700 ± 0.010 at 734 nm in the spectrophotometer. 4.5 mL ABTS solution was added to the extract-solvent mixture of 4 different concentrations (0.2-1 mg/mL) and incubate at 23-25 ^{o}C for 30 minutes. After incubation, it was measured at 734 nm in the spectrophotometer and the IC_{50} value was calculated.

2.3.3 Determination of total phenolic contents assay

In the determination of total phenolic contents assay, Singleton and Rossi method was used [20]. Extract, Folin-Ciocalteu's phenol reagent and dH_2O (1mg/mL: 1 mL: 46 mL) mixed. After 3 min, sodium carbonate (Na_2CO_3) solution was added. 2h incubated, then

measured at 760 nm. For results equivalent to gallic acid (mg GAE/g-extract), the formula y=0.0156x-0.0387 (R^2=0.9861) was used.

2.3.4 Determination of total flavonoid contents assay

Determination of total flavonoid contents assay was used by making some changes in the Aryal et al. method [21]. Dissolved in 0.2 mL of methanol (1M) sodium acetate was added into 1 mL of extract solution, then 10% (w/v) $AlCl_3$ dissolved in 0.2 mL of methanol and 5.6 mL of dH_2O were added. After 30 minutes of incubation at room temperature, after absorbing blind with methanol at 415 nm in the spectrophotometer, absorbance values were read. Quercetin is used as standard. Quercetin was applied at 5 concentrations (0.01-0.05 mg/mL) from the same processes and a calibration chart was created. For the quercetin equivalent (mg QE/g-extract) results, the formula y=0.0142x-0.0606 (R^2 = 0.981) was used.

2.3.5 Determination of condensed tannin contents assay

Determination of condensed tannin contents assay, the vanillic acid-H_2SO_4 method made by Bekir et al. was used [22]. 0.5 mL extract and %1 in 7 M H_2SO_4 mixed in an ice bath and was measured at 500 nm in spectrophotometer after 15 min incubation. For results equivalent to catechin (mg CE/g-extract), the formula y=0.0198x-0.0809 (R^2=0.984) was used.

2.4. Cell culture

Caco-2 human colonic adenocarcinoma cell line was used and cell passages were observed under an inverted microscope (Olympus CKX4). Cells were cultured in petri dish and 75 cm^2 cell culture flasks under suitable conditions at 37 °C in 5% CO_2 and cells were grown in Dulbecco Modified Eagle Medium (DMEM; Sigma) supplemented with 10% heat-inactivated fetal bovine serum (FBS; Biological Industries), 20 units/ml penicillin and 20 µg/mL streptomycin, 0,1 mM amino acid solution (PAN-Biotech) and 1 mM sodium pyruvate (Biological Industries). Different concentrations of extract were treated to the cells in dose-dependent manner.

2.4.1 Cell proliferation determination in Caco-2 colon cancer cells by XTT assay

Anti-proliferative activity of *C. cilicium* extracts on Caco-2 human colon cancer cells was detected by XTT (2,3-bis(2-methoxy-4 nitro-5- sulfophenyl)-2H-tetrazolium-5-carboxanilide) assay according to the manufacturer experimental protocol (Biotium cat no: 30007). Caco-2 cells were seeded into 96-well plates at as concentration of 2×10^4 cells per well. *C. cilicium* extracts were treated to the cells with different concentrations including 10 µg/mL, 25 µg/mL, 50 µg/mL, 100 µg/mL during 24h. Untreated colon cancer cells were used as control cells. After the incubation period, XTT mixture was added and then formations of formazan were determined spectrophotometrically at 450 nm (reference wavelength 630 nm) by a microplate reader (Thermo Scientific™ Multiskan™ GO Microplate Spectrophotometer). IC_{50} doses of extracts on Caco-2 cells were calculated by AAT Bioquest online IC_{50} Calculator [23]. Viability (%) of colon cancer cells was determined by using absorbance values with the following formula:

Viability (%) of Caco-2 cells = Absorbance of experiment well (Extract treated) / Absorbance of control well (untreated) × 100

2.5. Statistical analysis

All assays were performed in 3 replicates. The mean ± standard error was analyzed with Microsoft Excel and the results were analyzed with the statistical software of the Statistical Package for the Social Sciences (SPSS). Significant differences among groups were identified by One-Way analysis of variance (ANOVA) with Duncan's multiple range test, setting $p \leq 0.05$ as the level of significance. The mean ± standard error was analyzed using Microsoft Excel for XTT assay. IC_{50} doses of extracts on Caco-2 cells were calculated by AAT Bioquest online IC_{50} Calculator.

3. Results and discussion

Evaluation of the antioxidant activity of plant products cannot be performed accurately by any method due to the complex nature of phytochemicals, as there are too many types of compounds in plant structure. Several antioxidant analyses must be performed, these antioxidant analysismust be corroborated with each other and supported by analyses of total phenols, flavonoids and tannins [24]. The *in vitro* antioxidant activities with acetone, methanol, and water solvents of *C. cilicium* were determined by a series of assays including scavenging (DPPH, ABTS), determination of contents (total phenolic, flavonoid, tannin contents) and anti-proliferative activity by XTT analysis. Acetone, methanol, and water were chosen as solvents because they are the most common solvents used to extract phenolic compounds from plant materials [25].

Table 1. Antioxidant activity of *C. cilicium* extracts

Solvent\Assay	DPPH*	ABTS**
F.P. Acetone	39.07 ± 0.17 (1.26 ± 0.001)[a]	29.20 ± 0.12 (0.41 ± 0.001)[a]
F.P. Methanol	42.62 ± 0.27 (1.17 ± 0.005)[b]	32.08 ± 1.74 (0.38 ± 0.002)[b]
F.P. Water	61.92 ± 0.07 (0.68 ± 0.001)[c]	51.18 ±0.77 (0.24 ± 0.002)[c]
U.P. Acetone	24.78 ± 0.50 (2.15 ± 0.002)[d]	11.55 ± 0.12 (1.28 ± 0.001)[d]
U.P. Methanol	31.23 ± 0.27 (1.57 ± 0.003)[e]	14.29 ± 0.10 (0.89 ± 0.001)[e]
U.P. Water	18.48 ± 0.22 (3.01 ± 0.002)[f]	6.66 ± 0.99 (1.52 ± 0.001) [f]
BHA(Methanol)	55.54 ± 0.51*** (0.04 ± 0.001)[g]	92.94 ± 0.08**** (0.09 ± 0.001)[g]

[a:] If the lower cases in the line are the same, there is no statistical difference in Duncan's multiple range test ($p > 0.05$). *1 mg/mL (mg/mL, IC_{50}) **0.25 mg/mL (mg/mL, IC_{50}) ***0.05 mg / mL. ****0.15 mg/mL. F.P.: Fresh Part. U.P.: Underground Part.

In DPPH free radical scavenging activity assay of *C. cilicium*, the water extract of the fresh part showed the highest activity with the value of 61.92 ± 0.07% (0.68 ± 0.001 mg/mL, IC_{50}) and water extract of the underground part showed the lowest antioxidant activity with a value of 18.48 ± 0.22% (3.01 ± 0.002 mg/mL, IC_{50}) at 1 mg/mL. There is a statistical difference between all parts, including BHA ($p \leq 0.05$) (Table 1). In a study conducted with methanol, ethanol, acetone and petroleum ether extracts of tuber and leaves of *C. graecum*, the highest antioxidant activity was found in the ethanol extract of the leaf part with 97.3 ± 0.55 % [26]. In the study conducted by Turan and

Mammadov [27] with *Cyclamen alpinum* Dammann ex Spreng, ethanol, methanol and acetone extracts of tuber and leaf parts at 1 mg/mL concentration were used and found the best antioxidant activity in acetone extract of the tuber part with 86.73 ± 0.16 %. In a study conducted with methanol extract of tuber, root, flower and leaves of *C. cilicium* species collected from Konya province in Turkey, the highest antioxidant activity was found in the root part with 94.28 ± 1.15 mg TE/g value [28]. Sofiane and Wafa [29] found 36.85 ± 14.99 % in their study with *Cyclamen africanum* Boiss. & Reut. Species. Turan [30] studied with ethanol, methanol and acetone extracts of the above and underground parts of *C. alpinum* and *Cyclamen parviflorum* Pobed. species, and the best antioxidant activity at a concentration of 1 mg/mL was found in the methanol extract (91.39 %) of the fresh part of the *C. parviflorum*. In ABTS radical cation scavenging activity assay of *C. cilicium*, the water extract of the fresh part showed the highest activity with the value of 51.18 ± 0.7 % (0.24 ± 0.002 mg/mL, IC_{50}) and water extract of the underground part showed the lowest antioxidant activity with a value of 6.66 ± 0.99 % (1.52 ± 0.001 mg/mL, IC_{50}) at 1 mg/mL. There is a statistical difference between all parts, including BHA ($p \leq 0.05$) (Table 1). In the study conducted with *C. alpinum*, ethanol, methanol and acetone extracts of tuber and leaf parts were used and 40 mg/mL concentration found the best antioxidant activity in acetone extract of the leaf part with a value of 76.21 ± 0.32 % [27]. In the study performed with methanol extract of tuber, root, flower and leaves of *C. cilicium* species, the highest antioxidant activity was found in the root part with a value of 139.60 ± 0.11 mg TE/g [28]. Stanojevic et al. [31] found an EC_{50} value of 0.743 ± 0.003 mg/mL in their study with the tuber part of the *Cyclamen purpurascens* Mill. The positive correlation between DPPH and ABTS is in accordance with the report in Kaska et al. [32] and Zengin and Aktümsek [33]. In both DPPH and ABTS free radical scavenging activity assays, the activity rate increases in a concentration dependent manner. The high free radical scavenging activity indicates that the secondary metabolites of *C. cilicium*, especially in the water of fresh part, can give hydrogen and electrons. Since the plant samples were collected at the flowering time of the plant, it is thought that the polyphenols in its content are more in the fresh part. Our study is supported by other studies in the literature.

Table 2. Determination of Total Phenolic, Flavonoid, Tannin Contents of *C. cilicium* extracts

Solvent\Assay	**Total Phenolic (mg GAE/g-extract)**	**Total flavonoid (mg QE/g-extract)**	**Total Tannin (mg CE/g-extract)**
F.P. Acetone	2.20 ± 0.04[a]	6.60 ± 0.18[a]	41.17 ± 0.44[a]
F.P. Methanol	2.58 ± 0.07[b]	13.60 ± 0.13[b]	16.22 ± 0.20[b]
F.P. Water	3.69 ± 0.13[c]	18.48 ± 0.12[c]	1.42 ± 0.42[c]
U.P. Acetone	1.07 ± 0.13[d]	1.15 ± 0.04[d]	11.05 ± 0.04[d]
U.P. Methanol	1.13 ± 0.09[d]	2.61 ± 0.10[e]	0.59 ± 0.04[e]
U.P. Water	0.72 ± 0.04[e]	0.71 ± 0.02[f]	0.26 ± 0.03[e]

[a]: If the lower cases in the line are the same, there is no statistical difference in Duncan's multiple range test ($p > 0.05$). F.P.: Fresh Part. U.P.: Underground Part.

In the determination of the total phenolic content assay of *C. cilicium*, the water extract of the fresh part showed the highest value of 3.69 ± 0.13 mg GAE/g-extract and the water extract of the underground part showed the lowest value of 0.72 ± 0.04 mg GAE/g-extract. Statistically, there was no significant difference between acetone and methanol extract of underground part ($p > 0.05$) (Table 2). In the study of tuber and leaves of *Cyclamen graecum* Link. with methanol, ethanol, acetone and petroleum ether

extract, the highest phenolic contents were found in the ethanol extract of the leaf part with 33.73 ± 0.69 µg PEs/mg-extract and found the lowest phenolic contents in the petroleum ether extract of the tuber part with 6.18 ± 0.04 µg PEs/mg-extract [24]. In the study with *C. alpinum*, ethanol, methanol and acetone extracts of tuber and leaf parts were used and the highest phenolic contents were found in the ethanol extract of the leaf part with 8.95 ± 0.17 mg GAE/g [27]. In the study performed with ethanol extract of *C. alpinum* species, the highest phenolic component amount was found in tuber part with 16.4 mg GAE/g [34]. In the study performed with the tuber part of *C. purpurascens*, the highest phenolic contents found 8.27 ± 0.132 mg GAE/g [31]. In the determination of the total flavonoid content assay of *C. cilicium*, the water extract of the fresh part showed the highest value of 18.48 ± 0.12 mg QE/g extract and the water extract of the underground part showed the lowest value of 0.71 ± 0.02 mg QE/g extract. There is a statistical difference between all parts ($p \leq 0.05$) (Table 2). In the study with *C. alpinum*, ethanol, methanol and acetone extracts of tuber and leaf parts were used and the highest flavonoid was found in the ethanol extract of the leaf part 92.63 ± 0.45 mg QE/g [26]. Zengin et al. [28] found the highest flavonoid contents in the flower part with a value of 31.04 ± 0.26 mg RE/g with *C. cilicium*. Stanojevic et al. [31] found the highest amount of flavonoid 11.51 ± 0.254 mg RE/g with the tuber part of the *C. purpurascens*. In the determination of condensed tannin contents assay of *C. cilicium*, the acetone extract of the fresh part showed the highest value of 41.17 ± 0.44 mg CE/g-extract and the water extract of the underground part showed the lowest value of 0.26 ± 0.03 mg CE/g-extract. There is a statistical difference between all parts ($p \leq 0.05$) (Table 2). There was no statistically significant difference between the methanol and water extract of the underground part ($p > 0.05$). Jaradat et al. [35] found the highest tannin content of 11.7 ± 0.91 mg CAE/g-extract in the study of the methanol extract of above ground part of the *C. coum*. Total phenolics and flavonoids are critical in the prevention of many diseases. According to the results of this study, it can be said that the *C. cilicium* species may have a high protective potential.

Effects of *C. cilicium* extracts in different solvents on Caco-2 colon cancer cells depending on the dose concentrations were evaluated by using XTT assay which is a colorimetric based method *in vitro* cell culture model. The fresh and underground part of the water and methanol extracts of *C. cilicium* cytotoxicity in Caco-2 colon cancer cells were demonstrated in Figure 1. IC_{50} (inhibitory concentration where 50% of the cancer cells die) values of the methanol and water extracts of both the fresh and the underground parts of *C. cilicium* in colon cancer cells were calculated as 7.2 µg/mL, 20.04 µg/mL, 5,14 µg/mL and 8.83 µg/mL at 24h, respectively (Table 3). For methanol and water solvent used to extract the underground and fresh part, it was determined that the proliferation of Caco-2 cells decreased when the dose of extract was increased. These results suggest that methanol extracts have a more powerful effect with a lower dose on the cell proliferation of Caco-2 colon cancer cells than water solvent used extracts. To our knowledge, the first anti-proliferative effects of *C. cilicium* extracts on Caco-2 colon cancer cells were observed in this study.

Table 3. IC_{50} values of the groups in Caco-2 cells (µg/mL)

F.P. Methanol	F. P. Water	U. P. Methanol	U. P. Water
7.2	20.04	5,14	8.83

F.P.: Fresh Part. U.P.:Underground Part

There are different studies in the literature about anti-proliferative and anticancer activities of the genus *Cyclamen*. It was reported that *Cyclamen* species represent a source of secondary metabolites and biological active compounds with powerful therapeutic and pharmacological properties for the treatment of human disorders such as cancer [36,37]. Anti-proliferative activity of *C. coum* extract was demonstrated in HeLa cervical cancer cell line and H1299 lung cancer cell line and IC_{50} values of extract were determined as 8,61 µg/mL in HeLa cells and 9,52 µg/mL in H1299 cells. Furthermore, it was reported that *C. coum* extract induces apoptosis in cervical and lung cancer cells (38). Anti-proliferative effects of *Cyclamen persicum* Mill. ethanolic extract in MCF-7 breast cancer cells, PC-3 and LnCaP prostate cancer cells were reported in another cell culture model between 0,05-0,5 mg/mL dose range *in vitro* [39]. Karagur et al. [40] have showed that *Cyclamen pseudibericum* Hildebr. extract inhibits proliferation and invasion in A549 human non-small cell lung carcinoma cells via gene and miRNA expression regulation. They have reported that *C. pseudibericum* extract suppressed A549 cell viability in a time and dose-dependent manner and the IC_{50} value was determined as 41.64 ± 2.35 µg/mL. They have also demonstrated that amongst the 20 miRNAs assayed, miR-200c expression was strongly upregulated in A549 cells after treatment with the CP extract at 41.64 µg/mL. In addition, it has been shown that the expression of ZEB1, which is a potential target for miR-200c and plays a role in epithelial mesenchymal transition (EMT), decreases when *Cyclamen* extract is applied. Similarly, N-cadherin and vimentin expression decreased in dose group cells, while E--cadherin expression increased. This situation suggested that Cyclamen extract also showed anticancer activity in A549 cells via EMT mechanism [40].
Cyclamen trochopteranthum Hildebr. (syn: *Cyclamen alpinum*) extract has been shown in a study to exhibit cytotoxic activity in HepG2 and Caco-2 cells. The doses of *C. trochopteranthum* extract treated in the range of 1-500 mg/ml decreased cell viability in both cell lines depending on time and increasing dose. It has also been reported in the study that cyclamen extract regulates the mRNA expressions of CYP genes in these cell lines [41].Anti-proliferative effects of *C. cilicium* in cancer cells are very limited in the literature. In a study, the cytotoxic activity of *C. cilicium* in MDA-MB-231 and MCF-7 breast cancer cells, and DU-145 prostate cancer cells were determined by MTT assay under *in vitro* conditions. Anti-proliferative activity of flowers, root and tuber parts of *C. cilicium* extract in these cancer cells were showed and anticancer potential of extract was evaluated [28].

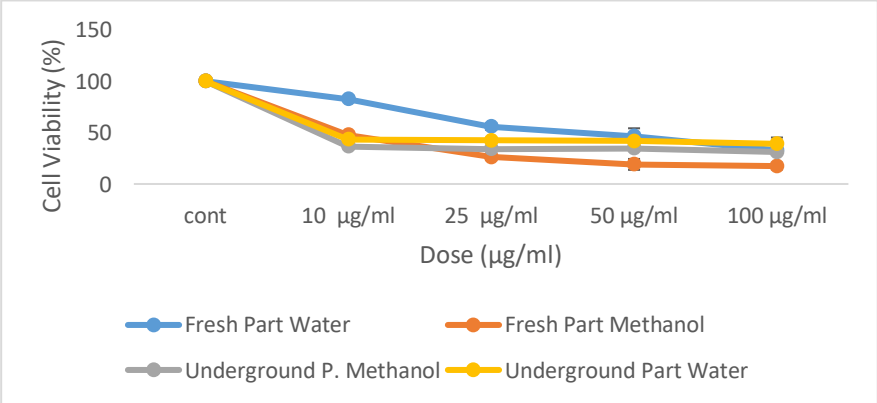

Figure 1. Cell viability (%) of Caco-2 colorectal cells after treatment with different concentration of *C. cilicium* extracts between 10 µg/mL and 100 µg/mL were measured by XTT assay

4. Conclusion

The studies conducted with extracts obtained from plants have increased their popularity in recent years and have very effective results and, accordingly, help other scientific fields. The *Cyclamen* genus is well known both as an ornamental plant and in ethnobotany, and the studies made with the ingredients of this genus attract a lot of attention. This study reveals that *C. cilicium*, especially the fresh part, has a strong biological activity in very small concentrations. Furthermore, anti-proliferative activity of *C. cilicium* extracts at low concentrations suggests that they can be an effective agent for cancer treatment studies. Therefore, these results suggest that the fresh part of *C. cilicium* could be a potential antioxidant source. This study will contribute to the literature for the next detailed molecular biological studies.

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References

[1] Guru, A., Lite, C., Freddy, A.J., Issac, P.K., Pasupuleti, M., Saraswathi, N.T., Arasu, M.V., Al-Dhabi, N.A., Arshad, A. and Arockiaraj, J., Intracellular ROS scavenging and antioxidant regulation of WL15 from cysteine and glycine-rich protein 2 demonstrated in zebrafish in vivo model., **Developmental and Comparative Immunology**, 114, 103863, (2021).

[2] Homayouni-Tabrizi, M., Asoodeh, A. and Soltani, M., Cytotoxic and antioxidant capacity of camel milk peptides: effects of isolated peptide on superoxide dismutase and catalase gene expression. **Journal of Food and Drug Analysis**, 25, 567–575, (2017).

[3] Hazra, B., Biswas, S. and Mandal, N., Antioxidant and free radical scavenging activity of *Spondias pinnata*. **BMC Complementary and Alternative Medicine**, 8, 1–10, (2008).

[4] Xu, C., Guan, S., Xu, J., Gong, W., Liu, T., Ma, X. and Sun, C., Preparation, characterization and antioxidant activity of protocatechuic acid grafted carboxymethyl chitosan and its hydrogel. **Carbohydrate Polymers**, 252, 117210, (2021).

[5] Lobo, V., Patil, A., Phatak, A. and Chandra, N., Free radicals, antioxidants and functional foods: impact on human health. **Pharmacological Reviews**, 4, 118–126, (2010).

[6] Byun, H.G., Lee, J.K., Park, H.G., Jeon, J.K. and Kim, S.K., Antioxidant peptides isolated from the marine rotifer, *Brachionus rotundiformis*. **Process Biochemistry**, 44, 842–846, (2009).

[7] Pereira, J. A., Pereira, A. P., Ferreira, I. C., Valentao, P., Andrade, P. B., Seabra, R., Estevinho, L. and Bento, A., Table olives from Portugal: Phenolic compounds, antioxidant potential, and antimicrobial activity. **Journal of Agricultural and Food Chemistry**, 54(22), 8425–843, (2006).

[8] Dong, R., Liu, S., Zheng, Y., Zhang, X., He, Z., Wang, Z., Wang, Y., Xie, J., Chen, Y. and Yu, Q., Release and metabolism of bound polyphenols from carrot dietary fiber and their potential activity under in vitro digestion and colonic fermentation. **Food & Function**, 11(7), (2020).

[9] Dong, R., Yu, Q., Liao, W., Liu, S., He, Z., Hu, X., Chen, Y., Xie, J., Nie, S. and Xie, M., Composition of bound polyphenols from carrot dietary fiber and its in vivo and in vitro antioxidant activity. **Food Chemistry**, 339, 127879, (2021).

[10] Davis, P.H., Flora of Turkey and East Aegean Islands. Vol. 6. Edinburgh. University of Edinburgh Press, Edinburgh, 128-135, (1978).

[11] Güner, A, Aslan, S, Ekim, T, Vural, M and Babaç, M.T., Türkiye Bitkileri Listesi-Damarlı Bitkiler. Nezahat Gökyiğit Vakfı Yayınları, İstanbul, (2012).

[12] Çelemli, Ö.G., Kızılpınar Temizer, I. and Altınözlü, H., Anatomy, Palynology of Endemic *Cyclamen pseud-ibericum* in Mediterranean Phytogeographic Region of Turkey and Chemical Analysis of Its Tuber Extracts. **Hacettepe Journal of Biology and Chemistry**, 43(2), 105-113, (2015).

[13] Prange, A. N. S., Bartsch, M., Serek, M. and Winkelmann, T., Regeneration of different *Cyclamen* species via somatic embryogenesis from callus, suspension cultures and protoplasts. **Scientia Horticulturae**, 125, 442–450, (2010).

[14] İzgü, T., Sevindik, B., Çürük, P., Şimşek, Ö., Aka Kaçar, Y., Teixeira da Silva, J.A. and Yalçın Mendi, Y., Development of an efficient regeneration protocol for four *Cyclamen* species endemic to Turkey. **Plant Cell, Tissue and Organ Culture,** 127, 95-113, (2016).

[15] *Cyclamen cilicium* Boiss. & Heldr. in GBIF Secretariat. GBIF Backbone Taxonomy. Checklist dataset. https://www.gbif.org/species/7127370, (2019).

[16] *Cyclamen cilicium* Boiss. & Heldr. in Plant List. The Plant List, Published on the Internet; http://www.theplantlist.org/tpl1.1/record/kew-2749712, (2012). (accessed on 2020-05-07).

[17] Yılmaz, U., Kaya, H., Turan, M., Bir, F. and Şahin, B., Investigation the Effect of *Hypericum perforatum* on Corneal Alkali Burns. **Cutaneous and Ocular Toxicology**, 38, 356-359, (2019).

[18] Wu, C., Chen, F., Wang, X., Kim, H., He, G., Haley-Zitlin, V. and Huang, G., Antioxidant Constituents in Feverfew (*Tanacetum parthenium*) Extract and Their Chromatographic Quantification. **Food Chemistry**, 96, 220-227, (2006).

[19] Re, R., Pellegrini, N., Proteggente, A., Pannala, A., Yang, M. and Rice-Evans, C. Antioxidant Activity Applying an Improved ABTS Radical Cation Decolorization Assay. **Free Radical Biology & Medicine**, 26, 1231-1237, (1999).

[20] Singleton, V.L. and Rossi, J.A., Colorimetry of Total Phenolics with Phosphomolybdic-Phoshotungstic Acid Reagents. **American Journal of Enology and Viticulture**, 16, 144-158, (1965).

[21] Aryal, S., Baniya, M.K., Danekhu, K., Kunwar, P., Gurung, R. and Koirala, N., Total Phenolic Content, Flavonoid Content and Antioxidant Potential of Wild Vegetables from Western Nepal. **Plants**, 8, 96, (2019).

[22] Bekir, J., Mars, M., Souchard, J.P. and Bouajila, J., Assessment of Antioxidant, Anti-İnflammatory, Anti-Cholinesterase and Cytotoxic Activities of Pomegranate (*Punica granatum*) Leaves. **Food and Chemical Toxicology**, 55, 470-475, (2013).

[23] AAT Bioquest, Inc. Quest Graph™ IC50 Calculator.". Retrieved from https://www.aatbio.com/tools/ic50-calculator (accessed on 2021-04-17)

[24] Kaska, A., The Phenolic Content, Antioxidant and Cytotoxic Activities of *Origanum sipyleum* from Turkey. **International Journal of Secondary Metabolite**, 5, 343-352, (2018).

[25] Fernandez-Poytaos, M., Ruiz-Medina, A., Zengin, G. and Llorent-Martinez, E.J., Phenolic Characterization, Antioxidant Activity, and Enzyme Inhibitory Properties of *Berberis thunbergii* DC. Leaves: A Valuable Source of Phenolic Acids. **Molecules**, 24, 4171, (2019).

[26] Metin, H., Aydın, C., Ozay, C. and Mammadov, R. Antioxidant Activity of the Various Extracts of *Cyclamen graecum* Link Tubers and Leaves from Turkey, **Journal of The Chemical Society of Pakistan**, 35(5), 1332-1336, (2013).

[27] Turan, M. and Mammadov, R. Antioxidant, Antimicrobial, Cytotoxic, Larvicidal and Anthelmintic Activities and Phenolic Contents of *Cyclamen alpinum*. **Pharmacology & Pharmacy**, 9, 100-116, (2018).

[28] Zengin, G., Mahomoodally, M.F., Sinan, K.I., Picot-Allain, M.C.N., Yildiztugay, E., Cziaky, Z., Jeko, J., Saleem, H. and Ahemad, N., Chemical characterization, antioxidant, enzyme inhibitory and cytotoxic properties of two geophytes*: Crocus pallasii* and *Cyclamen cilicium*. **Food Research International**, 133, (2020).

[29] Sofiane, G. and Wafa, N., Antioxidant, Antimicrobial and Anti-Inflammatory Activities Development of Methanol Extract of *Cyclamen africanum* B. Et R., Growth in Jijel – Algeria. **Journal of Drug Delivery and Therapeutics**, 10, 130-134, (2020).

[30] Turan, M., *Cyclamen alpinum* ve *Cyclamen parviflorum* Ekstraktlarının Fenolik Bileşenleri ve Bazı Biyolojik Özelliklerini Belirlenmesi (in Turkish), Master Thesis, Pamukkale Üniversity, Denizli, Turkey (2016).

[31] Stanojević, L.P., Cakić, M.D., Stanojević, J.S., Cvetković, D.J. and Danilović, B.R., Aqueous Extract of Wild *Cyclamen* Tubers (*Cyclamen purpurascens* L.) - A Potential Source of Natural Antioxidants and Antimicrobial Agents. **Quality of Life**, 9(1-2), 13-19, (2018).

[32] Kaska, A., Çiçek, M., Deniz, N. and Mammadov, R., Investigation of Phenolic Content, Antioxidant Capacities, Anthelmintic and Cytotoxic Activities of *Thymus zygioides* Griseb. **Journal of Pharmaceutical Research International**, 21, 1-13, (2018).

[33] Zengin, G. and Aktümsek, A., Investigation of Antioxidant Potentials of Solvent Extracts from Different Anatomical Parts of *Asphodeline anatolica* E. Tuzlaci: an Endemic Plant to Turkey. **African Journal of Traditional, Complementary and Alternative Medicines**, 11, 481-488, (2014).

[34] Arslan, S., Ozgun, O., Celık, G., Semız, A., Dusen, O., Mammadov, R. and Sen, A., Effects Of *Cyclamen trochopteranthum* On Hepatic Drug-Metabolizing Enzymes, **Archives of Biological Science Belgrade**, 63(3), 545-555, (2011).

[35] Jaradat, N.A., Al-Masri, M., Hussen, F., Zaid, A.N., Ali, I., Tammam, A., Odeh, D.M., Shakarneh, O.H. and Rajabi, A. Preliminary Phytochemical and Biological Screening of *Cyclamen coum* a Member of Palestinian Flora, **Pharmaceutical Sciences**, 23, 231-237, (2017).

[36] Speroni, E., Cervellati, R., Costa, S., Dall'Acqua, S., Guerra, M. C., Panizzolo, C., Utan, A., and Innocenti, G., Analgesic and antiinflammatory activity of *Cyclamen repandum* S. et S. **Phytotherapy Research**, 21(7), 684–689, (2007). https://doi.org/10.1002/ptr.2145

[37] Cornea-Cipcigan M., Pamfil D., Sisea C.R., Gavri C.P., Campos M.G. and Margaoan R., A Review On *Cyclamen* Species: Transcription Factors vs.

Pharmacological Effects. **Acta Poloniae Pharmaceutica ñ Drug Research**, 76(6), 919-938, (2019).

[38] Yildiz, M., Bozcu, H., Tokgun, O., Karagur, E. R., Akyurt, O. and Akca, H., *Cyclamen* exerts cytotoxicity in solid tumor cell lines: a step toward new anticancer agents? **Asian Pacific journal of Cancer Prevention**, 14(10), 5911–5913, (2013).

[39] Khalilia, W.M., Cytotoxic Activity of Cyclamen Persicum Ethanolic Extract on MCF-7, PC-3 and LNCaP Cancer Cell Lines. **Journal of Natural Sciences**, 10(2), 42-46, (2020).

[40] Karagur, E. R., Ozay, C., Mammadov, R., and Akca, H., Anti-invasive effect of *Cyclamen pseudibericum* extract on A549 non-small cell lung carcinoma cells via inhibition of ZEB1 mediated by miR-200c. **Journal of Natural Medicines**, 72(3), 686–693, (2018).

[41] Arslan, S., & Ozgun, O. (2012). Cyclamen Trochopteranthum: Cytotoxic activity and possible adverse interactions including drugs and carcinogens. **Chinese journal of integrative medicine**, Advance online publication. https://doi.org/10.1007/s11655-012-1253-1