KSÜ Tarım ve Doğa Derg 22(Ek Sayı 2): 365-373, 2019
KSU J. Agric Nat 22(Suppl 2): 365-373, 2019
DOI:10.18016/ksutarimdoga.vi.579938

Phytochemical Analysis of *Dorycnium pentaphyllum* and Its Antiproliferative Effect on Cervix Cancer Cells

Yuksel ALİYAZİCİOGLU[1], Selim DEMİR[2]✉, Serap Ozer YAMAN[3], Sila Ozlem SENER[4], Elif AYAZOGLU DEMİR[5], Rezzan ALİYAZİCİOGLU[6], Ibrahim TURAN[7]

[1,3]Department of Medical Biochemistry, Faculty of Medicine, Karadeniz Technical University, 61080 Trabzon, Turkey. [2]Department of Nutrition and Dietetics, Faculty of Health Sciences, Karadeniz Technical University, 61080 Trabzon, Turkey. [4,6]Department of Biochemistry, Faculty of Pharmacy, Karadeniz Technical University, 61080 Trabzon, Turkey. [5]Department of Chemistry, Faculty of Science, Karadeniz Technical University, 61080 Trabzon, Turkey, [7]Department of Genetic and Bioengineering, Faculty of Engineering and Natural Sciences, Gumushane University, 29100 Gumushane, Turkey.

https://orcid.org/0000-0001-9474-4307, https://orcid.org/0000-0002-1863-6280, https://orcid.org/0000-0002-5089-0836, https://orcid.org/0000-0003-2811-4707, https://orcid.org/0000-0001-9027-7633, https://orcid.org/0000-0003-2913-2528, https://orcid.org/0000-0003-3400-5494

✉: selim-demir@hotmail.com

ABSTRACT

The purpose of this study was to investigate the phytochemical analysis of *D. pentaphyllum* and its cytotoxic activity on human cervical cancer (HeLa) cells with possible mechanisms. Total phenolic contents (TPC) and phytochemical analysis of the extract were evaluated using spectrophotometric method and RP-HPLC, respectively. The cytotoxic effect of the extract was evaluated using the MTT assay. The mechanism of the cytotoxic effect of the extract was also evaluated in terms of apoptosis, cell cycle, and mitochondrial membrane potential (MMP) using the fluorometric methods. The TPC of the extract was calculated as 68.9±2.3 mg gallic acid equivalent per g sample and quercetin was determined as the major phenolic in the extract. The extract showed a selective cytotoxic effect (6.5-fold) on HeLa cells compared to normal fibroblast cells. The cytotoxic effect of the extract was found to be due to the arresting of the cell cycle in the S phase and increasing apoptosis through decreased MMP. Further studies are needed for the comprehensive effects of the extract on cell signaling.

Research Article

Article History
Received : 19.06.2019
Accepted : 01.08.2019

Keywords
Apoptosis
Cell cycle
Cervix cancer
Cytotoxicity
Dorycnium pentaphyllum

Dorycnium pentaphyllum'un Fitokimyasal Analizi ve Serviks Kanseri Hücreleri Üzerindeki Antiproliferatif Etkisi

ÖZET

Bu çalışmanın amacı, *D. pentaphyllum*'un fitokimyasal analizini gerçekleştirmek ve bitkinin insan serviks kanseri (HeLa) hücreleri üzerindeki sitotoksik aktivitesini olası mekanizmalarla birlikte araştırmaktır. Ekstraktın toplam fenolik içerik (TPC) ve fitokimyasal analizi, sırasıyla spektrofotometrik yöntem ve RP-HPLC ile değerlendirildi. Ekstraktın sitotoksik etkisi MTT metodu kullanılarak belirlendi. Ekstraktın sitotoksik etkisinin mekanizması ise apoptoz, hücre döngüsü ve mitokondriyal membran potansiyeli (MMP) açısından florometrik yöntemler kullanılarak araştırıldı. Ekstraktın TPC değeri g örnek başına 68.9±2.3 mg gallik asit eşdeğeri olarak hesaplandı ve kuersetin ekstrakt içindeki ana fenolik bileşik olarak belirlendi. Ekstrakt normal fibroblast hücrelerine kıyasla, HeLa hücrelerinde seçici bir sitotoksik etki (6.5 kat) gösterdi. Ekstraktın sitotoksik etkisinin, hücre döngüsünü S fazında durdurmasından ve azalmış MMP nedeniyle artmış apoptozdan kaynaklandığı bulundu. Ekstraktın hücre sinyalizasyonu üzerindeki detaylı etkilerinin belirlenmesi için daha ileri çalışmalar gerekmektedir.

Araştırma Makalesi

Makale Tarihçesi
Geliş Tarihi : 19.06.2019
Kabul Tarihi : 01.08.2019

Anahtar Kelimeler
Apoptoz
Dorycnium pentaphyllum
Hücre döngüsü
Serviks kanseri
Sitotoksisite

To Cite : Aliyazıcıoğlu Y, Demir S, Yaman SÖ, Şener SÖ, Ayvazoğlu Demir E, Aliyazıcıoğlu R, Turan İ 2019. Phytochemical Analysis of *Dorycnium pentaphyllum* and Its Antiproliferative Effect on Cervix Cancer Cells. KSU J. Agric Nat 22 (Suppl 2): 365-373. DOI: 10.18016/ ksutarimdoga.vi.579938.

INTRODUCTION

Cancer is a disease characterized by uncontrolled and non-stopped cell growth (Demir et al., 2016a). International Agency for Research on Cancer reported 14.1 million new cancer cases and 8.2 million deaths worldwide in 2012, and predicted 21.7 million cancer incidents and 13 million deaths in 2030. Lung, breast, colorectal, gastric, prostate, cervix, and liver cancers are the most seen cancer types in humans. These cancers have represented 55% of the global cancer incidence in 2012 (Kim and Kim, 2018). Cervical cancer is the second most common type of cancer in women worldwide (Di Domenico et al., 2012). The main treatment methods for cervical cancer in humans are surgery, radiotherapy, and chemotherapy (Chaddha et al., 2018). Although chemotherapy is a frequently used method in the treatment of cervix cancer, it has some disadvantages, such as nausea, headache, gastritis, anorexia, oral ulceration, constipation, diarrhea, alopecia, and neuropathy (Oyenihi and Smith, 2019). In addition, the development of resistance to chemotherapeutic drugs in cancer cells and their undesirable effect on healthy cells are other serious disadvantages of chemotherapy (Turan et al., 2017a). Natural products represent significant potential for drug development studies for many years and many new generation chemotherapeutic agents are derived from natural products (Demir et al., 2017a).

The *Fabaceae* (*Leguminosae*) family is one of the largest families of flowering plants consisting of 730 genera and more than 19400 species (Saha et al., 2013). The genus *Dorycnium* belongs to the family *Fabaceae*, and is widely distributed in Europe and Asia (Stefanović et al., 2015). Members of *Fabaceae* family have been used for many years in traditional medicine to treat rheumatism, arthritis, inflammation, neoplasm, hemorrhoid, bronchitis, asthma, urinary tract infections, and liver diseases (Bremner et al., 2009; Lacerda et al., 2014; Kumar et al., 2017). *Dorycnium* genus has many biological activities, such as anti-inflammatory, antimicrobial, cytotoxic, and antioxidant (Bremner et al., 2009; Usta et al., 2014; Demir et al., 2019a). Many studies have reported the cytotoxic effects of various extracts of different *Fabaceae* family species. Soni *et al.* (2013) demonstrated that *Mucuna pruriens* Linn. extracts exhibit a cytotoxic effect on human prostate and breast cancer cells, while Zingue *et al.* (2016) reported that phenolic fraction of *Millettia macrophylla* Benth exhibits a cytotoxic effect on human breast cancer cells. Recently, it is reported that *D. pentaphyllum* has a cytotoxic effect on human breast, liver and lung cancer cells (Demir et al., 2019a). However, no study in the literature has investigated the cytotoxic effect of *D. pentaphyllum* extract on human cervical cancer (HeLa) cells. The purpose of this study was to investigate the phytochemical content of *D. pentaphyllum* and to evaluate the probable cytotoxic effect in HeLa cells together with the mechanism involved.

MATERIALS and METHOD

Chemicals

All the chemicals (ACS grade or higher) used in the phytochemical analysis were purchased from Sigma-Aldrich (St. Louis, MO, USA). All chemicals used in cell culture experiments were purchased from Lonza (Verviers, Belgium) and Biological Industries (Kibbutz Beit Haemek, Israel).

Extract Preparation

D. pentaphyllum samples were collected from Sinop, Turkey. Plant specimen was deposited in the herbarium at the herbarium of the Faculty of Pharmacy, Ankara University, Turkey (voucher specimen number AEF-26707). The leaf portions of the plant were dried at room temperature and converted to a fine powder using a blender and grinding procedure. 0.5 g of the powdered sample was then mixed with 10 mL of dimethyl sulfoxide (DMSO) and the resulting mixture was stirred at 150 rpm at 45°C for 24-h. At the end of the time, the mixture was passed through filter paper and then through 0.2 µm filters, and the extract was deposited at -20°C (Demir et al., 2019a).

Determination of Total Phenolic Content (TPC)

The TPC of the extract was determined using the previously described colorimetric method (Slinkard and Singleton, 1977). Gallic acid was used as a reference compound and the TPC value was calculated as mg gallic acid equivalent (GAE)/g sample.

Determination of Total Flavonoid Content (TFC)

The TFC of the extract was determined using the previously described colorimetric method (Moreno et al., 2000). Quercetin was used as a reference compound and the TFC value was calculated as mg quercetin equivalent (QE)/g sample.

Determination of Phenolic Compounds by RP-HPLC

HPLC analysis was performed on a Shimadzu Corporation LC 20 AT (Kyoto, Japan) system equipped with a UV-Vis detector. The analysis was performed using a reverse phase C_{18} column (150 x 4.6 mm, 5 µm; Waters Spherisorb, Milfort, MA, USA) in a gradient program with two solvent systems; methanol and 2% acetic acid in water at a constant solvent flow rate of 1.5 mL/min. The injection volume was 20 µL. Seven standards were used for HPLC analysis; *p*-OH benzoic acid, vanillic acid, syringaldehyde, *p*-coumaric acid, sinapic acid, benzoic acid, and quercetin (Aliyazicioglu et al., 2017; Ozkan et al., 2017).

Cell Culture

Human cervix adenocarcinoma (HeLa, ATCC-CCL-2) cancer and human normal foreskin fibroblast (ATCC-CRL-2522) cells were supplied by the American Type Culture Collection (Manassas, VA, USA). Both cells were cultured in Eagle's minimum essential medium supplemented with 10% fetal bovine serum and 1% antibiotic solution.

Cytotoxicity Experiments

The cytotoxic effects of *D. pentaphyllum* extract and cisplatin [used as a chemotherapeutic drug in the treatment of cervical cancer (Demir et al., 2018a)] on HeLa and fibroblast cells were evaluated by MTT test after 72-h treatment (Mosmann, 1983). Briefly, cells were seeded into a flat-bottomed 96-well plates, and the cells were then treated with varying concentrations of *D. pentaphyllum* extract (0-500 µg/mL) and cisplatin (0-10 µg/mL) for 72-h. At the end of the incubation, the crystals formed by adding 10 µL of MTT dye (0.25 mg/mL) to each well were then dissolved with DMSO. Finally, absorbance values in the wells were measured with a microplate reader (Molecular Devices Versamax, California, USA) at 570 nm. Absorbance values were compared with negative control cells and %cell viability values were calculated. Using these values, the IC_{50} values of the extract and cisplatin in each cell series were determined. IC_{50} values determined in both cell lines of extract and cisplatin were used to calculate the selectivity index with the following formula (Demir et al., 2019b):

Selectivity Index = Fibroblast cells IC_{50}/HeLa cells IC_{50}

Flow Cytometry Analysis

HeLa cells were treated with 37.5-150 µg/mL concentrations of *D. pentaphyllum* extract for 72 h, before being harvested, and washed twice with buffer solution. All procedures were performed according to the manufacturer's recommendations (BD Biosciences, Cat No: 340242, San Diego, CA). 30000 cell counts were performed on a flow cytometer (BD Accuri C6, MI, USA) for each group. The results were finally compared with those of the negative control cells.

Determination of Mitochondrial Membrane Potential (MMP)

Cells were seeded into a flat-bottomed 96-well black-walled plate and then treated with 37.5-150 µg/mL concentrations of *D. pentaphyllum* extract for 72-h. At the end of the time, the cells were washed with phosphate buffered saline solution and stained with 10 nM 3,3′-dihexyloxacarbocyanine iodide [DiOC6(3)] for 30 min in the dark. At the end of the incubation, fluorescence measurement was performed on a plate reading fluorometer (Molecular Devices SpectraMax Paradigm Multi-Mode, Sunnyvale, CA, USA) at an excitation wavelength of 484 nm and an emission wavelength of 525 nm. Fluorescence values were compared with negative control cells and results were expressed as relative MMP (Demir et al., 2017a; Turan et al., 2018).

Statistical Analysis

All experiments were carried out in triplicate and the results were expressed as a mean±standard deviation. Compatibility with normal distribution was determined using the Kolmogorov-Smirnov test. One-way ANOVA analysis was used to compare differences among the groups. $p<0.05$ was regarded as significant.

RESULTS and DISCUSSION

Oxidative stress is caused by the overproduction of reactive oxygen species (ROS), which is due to the insufficient antioxidant capacity of the cell. The effect of ROS on cells depends on the level at which they are present. Low levels of ROS contribute to cell proliferation and survival, while high levels of ROS may damage macromolecules and even cause cell death (Aliyazicioglu et al., 2011; Di Domenico et al., 2012). It is therefore asserted that oxidative stress is involved in the etiology of many pathological conditions, such as cancer, diabetes, and cardiovascular diseases (Mentese et al., 2014; Yalcin et al., 2016). Antioxidant activity is therefore important for human health and it is claimed that many biological activities have been caused by the antioxidant effect. It is also believed that phenolics in natural products can provide protection against chronic diseases associated with oxidative stress due to their antioxidant activities. Therefore, the determination of the antioxidant activity of a natural product whose biological properties are investigated is considered as a starting point for more comprehensive studies (Aliyazicioglu et al., 2017; Demir et al., 2017a; Ozkan et al., 2017). Various *in vitro* methods are used to determine the antioxidant activity of natural product extracts and it is recommended to use at least two different methods in researches (Demir et al., 2019b). We therefore determined the antioxidant properties of extract using TPC and TFC methods in this study, and the results are shown in Table 1.

Table 1. Antioxidant properties of *D. pentaphyllum* extract (n=3)

Antioxidant Parameters	
Total Polyphenolic Content (mg GAE/g sample)	68.9±2.3
Total Flavonoid Content (mg QE/g sample)	18.8±0.2

Consistent with our results, Stefanović *et al.* (2015) reported that the TPC and TFC value of ethanolic extract of *D. herbaceum* was 75.77 mg GAE, and 110.07 mg rutin equivalent per g sample, respectively. In another study, the TPC and TFC value for the

methanolic extract of *Pterocarpus erinaceus*, a member of *Fabaceae* family, was reported as 814.7 mg tannic acid equivalent, and 10.3 mg QE per g sample, respectively (Noufou et al., 2016). It is known that there is a strong relationship between the amount of phenolic compounds and antioxidant activity (Turan et al., 2017b). Therefore, after determining the total levels of phenolic compounds by TPC, we used RP-HPLC analysis to identify the phenolic compounds responsible for the antioxidant properties of the extract. A chromatogram of phenolic standards and the extract is shown in Figure 1.

The phenolics found in *D. pentaphyllum* are summarized in Table 2, and the values are expressed as mg/g sample. Quercetin was the most abundant compounds in *D. pentaphyllum* (Figure 2).

It is reported that *Fabaceae* family rich in phenolics, such as gallic acid, fumaric acid, chlorogenic acid, 4-hydroxybenzoic acid, caffeic acid, cinnamic acid, *p*-coumaric acid, catechin derivatives, quercetin, hesperidin, taxifolin, naringenin, myricetin, resveratrol, apigenin, and galangin (Sobeh et al., 2016; Bencherchar et al., 2017). Our phenolic composition results are not exactly compatible with those from previous studies. This may have arisen from the plant type, type of extraction method and solvent employed, environmental factors, soil, geographic region, harvest season, and post-harvesting conditions. In addition, the compounds determined may be altered by the method used in phytochemical analysis. We think that further studies with other standard compounds may reveal the phenolic composition of *D. pentaphyllum*.

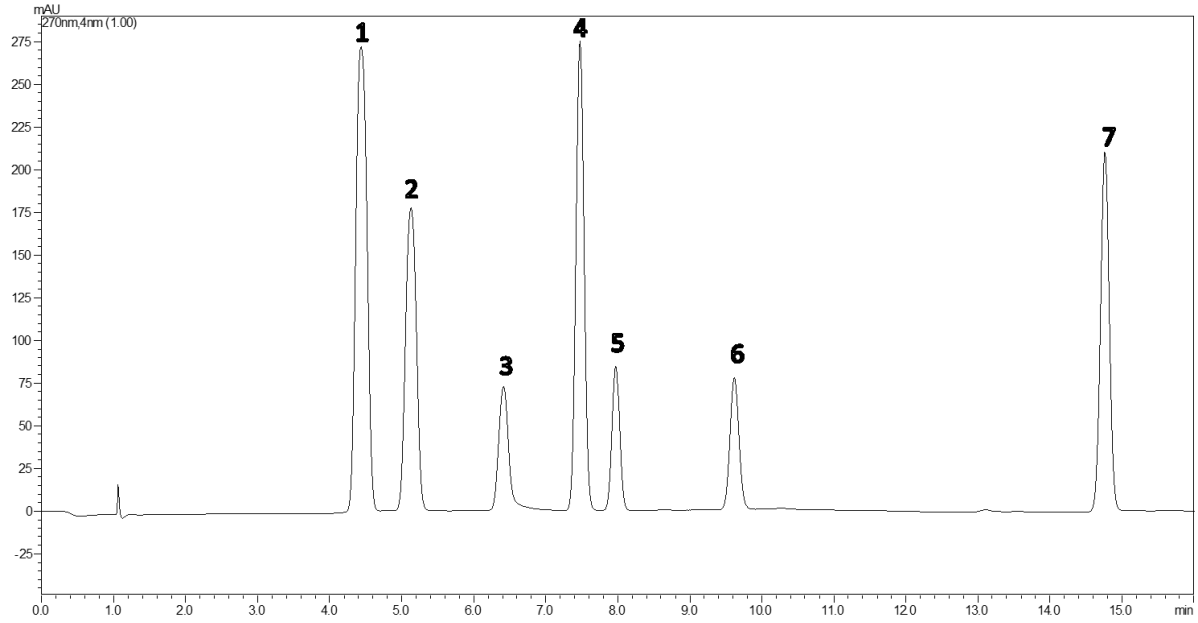

Figure 1. RP-HPLC chromatogram of phenolic standards investigated in *D. pentaphyllum* samples. Peak identification: (1) *p*-OH benzoic acid, (2) vanillic acid, (3) syringaldehyde, (4) *p*-coumaric acid, (5) sinapic acid, (6) benzoic acid, and (7) quercetin.

Table 2. Phenolic composition of *D. pentaphyllum* extract analyzed using RP-HPLC

Phenolic compound assignment	Retention time (min)	Standard curves	Correlation coefficient (r)	Amount (mg/g)
p-OH-benzoic acid	4.43	y = 56.637x+70148	0.9999	ND
Vanillic Acid	5.12	y = 39.126x+6315.9	0.9996	ND
Syringaldehyde	6.41	y = 4.0135x-1263.2	0.9999	ND
Coumaric Acid	7.47	y = 14.202x-4598.7	0.9999	ND
Sinapic Acid	7.97	y = 18.75x-4448.1	0.9999	ND
Benzoic Acid	9.62	y = 14.671x-1614	0.9998	3.52
Quercetin	14.76	y = 57.65x-33068	0.9997	3.16

ND: Not determined.

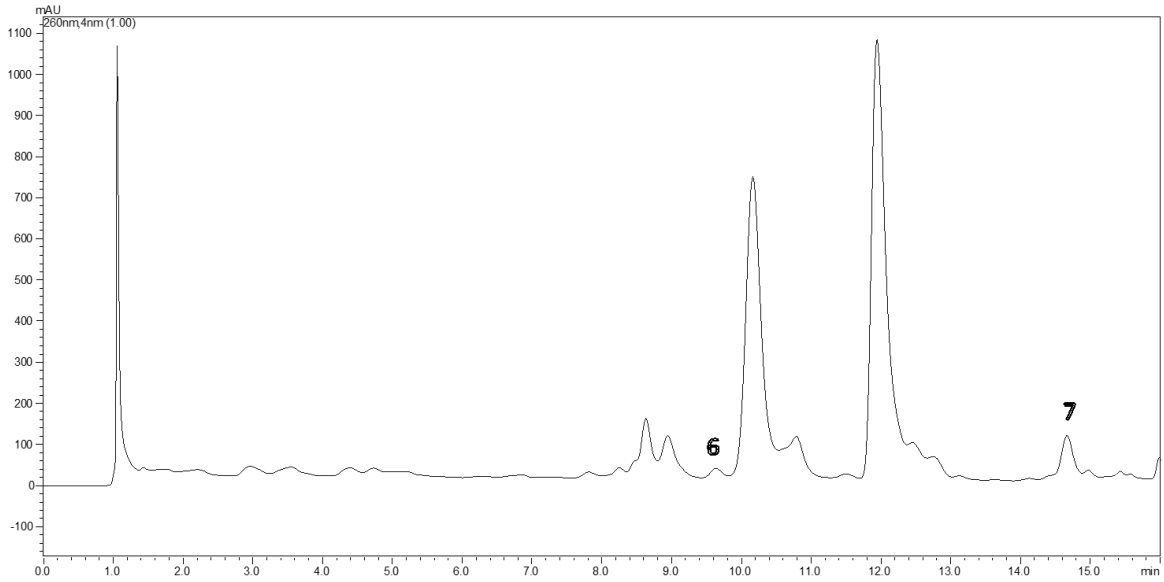

Figure 2. Chromatogram of *D. pentaphyllum* sample

Cancer is one of the leading cause of disease related deaths and is a major public health problem worldwide (Demir et al., 2018a). Gynecological (uterus, cervix, and ovary) and breast cancers constitute 19% of the cancers in the world (Zingue et al., 2016). Although chemotherapy is one of the most widely used methods of cervical cancer, it has many serious side effects such as drug resistance, toxicity, low specificity, bone marrow suppression, nausea and vomiting, hair loss and loss of appetite (Naghibi et al., 2014; Demir et al., 2018a). Recent studies have therefore focused on the discovery of more effective chemotherapeutics with lower side effects (Naghibi et al., 2014; Demir et al., 2018b). Natural products are a great source of potential for anticancer research due to their selective cytotoxic effects in cancer cells compared to normal cells (Demir et al., 2016b). *Dorycnium* species are medicinal plants with a long history of use in traditional medicine (Bremner et al., 2009; Lacerda et al., 2014; Kumar et al., 2017). There are several reports of biological activities including anti-inflammatory, antimicrobial, cytotoxic, and antioxidant properties and these beneficial activities being attributed to their phenolic compounds (Bremner et al., 2009; Stefanović et al., 2015; Demir et al., 2019a). Numerous studies have reported *in vitro* cytotoxic effects of different *Fabaceae* species in recent years (Soni et al., 2013; Zingue et al., 2016), but there have been only limited studies on the cytotoxic effects of *D. pentaphyllum* (Demir et al., 2019a). In addition, no previous research has determined the cytotoxic effect and mechanism of action of *D. pentaphyllum* on HeLa cells. To investigate the potential selective cytotoxic effect of *D. pentaphyllum*, HeLa and normal fibroblast cells were treated with varying concentrations of extract and the results were determined by the MTT method. *D. pentaphyllum* extract significantly inhibited HeLa cell growth in a dose-dependent manner (Figure 3).

The IC_{50} values and selectivity index of extract and cisplatin are given in Table 3.

Cancer is a cellular disease and is mainly caused by an imbalance between normal cellular proliferation and death. Stopping the cell cycle and increasing apoptosis are the two main strategies in cancer therapy (Demir et al., 2016a; Turan et al., 2018). In order to determine whether the antiproliferative effect of extract was associated with changes in cell cycle regulation and apoptosis, we treated HeLa cells with 37.5-150 µg/mL concentrations of *D. pentaphyllum* extract for 72-h and analyzed propidium iodide-stained cells in a flow cytometer. The cell cycle analysis results are summarized in Figure 4 and 5.

All the concentrations of *D. pentaphyllum* extract significantly increased the cell numbers at the sub-G_1 phase ($p=0.0001$). Additionally, all the extract concentrations (except with 37.5 µg/mL) significantly increased the cell numbers at the S phase and decreased the cell numbers at the G_0/G_1 phase compared to the untreated cells ($p=0001$).

The DNA content analysis also showed that the hypodiploid sub-G_1 cell population was increased with extract treatment, this proves the apoptotic effect of this extract. Since decreased MMP is an indicator of activation of the intrinsic apoptosis pathway (Demir et al., 2016a), we investigated the effects of the extract on MMP. All the concentrations of *D. pentaphyllum* extract significantly reduced MMP in HeLa cells ($p=0.0001$). The percentage reductions in MMP caused by *D. pentaphyllum* extract were 42.5%, 50.5%, 66.7%, and 82.1% for the concentrations of 37.5, 75, 112.5, and 150 µg/mL, respectively (Figure 6).

Totally, our results demonstrate that extract induces

S phase arrest followed by mitochondrial dependent cell death in HeLa cell lines. Previous studies with *Fabaceae* species have shown that the *Gymnocladus dioicus* extract exhibits a cytotoxic effect on HeLa cells in a concentration-dependent manner (Jantova et al., 2001).

Bremner *et al.* (2009) reported that *Ononis ramosissima* extract exhibits a cytotoxic effect on HeLa cells, while Rathi *et al.* (2009) demonstrated that *Glycyrrhiza glabra* extract exhibits a cytotoxic effect on human breast cancer (MCF-7) cells.

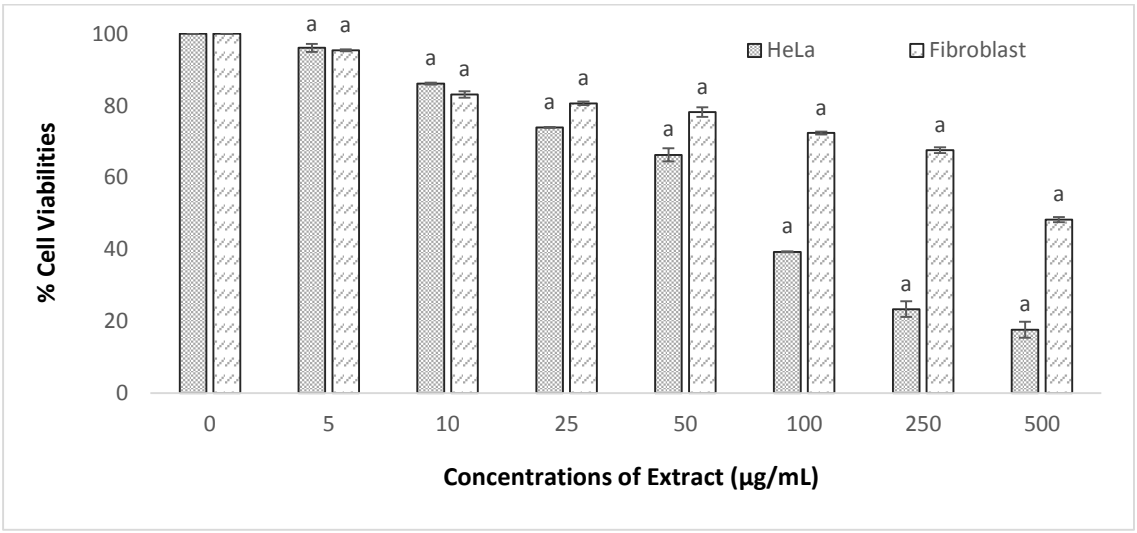

Figure 3. Anti-growth effect of the *D. pentaphyllum* extract. Viability of HeLa and fibroblast cells after the treatment with varying concentrations of extract for 72-h was measured by MTT assay. The results are mean of three independent experiments and the error bars indicate SD. *Denotes statistically significant differences in comparison with untreated cells ($p<0.05$).

Table 3. IC_{50} values calculated for extract and cisplatin on HeLa and fibroblast cells

Test Compound	HeLa Cells	Fibroblast Cells	Selectivity Index
Extract	74.2±0.4	483.7±9.5	6.5
Cisplatin	0.76±0.03	5.27±0.3	6.9

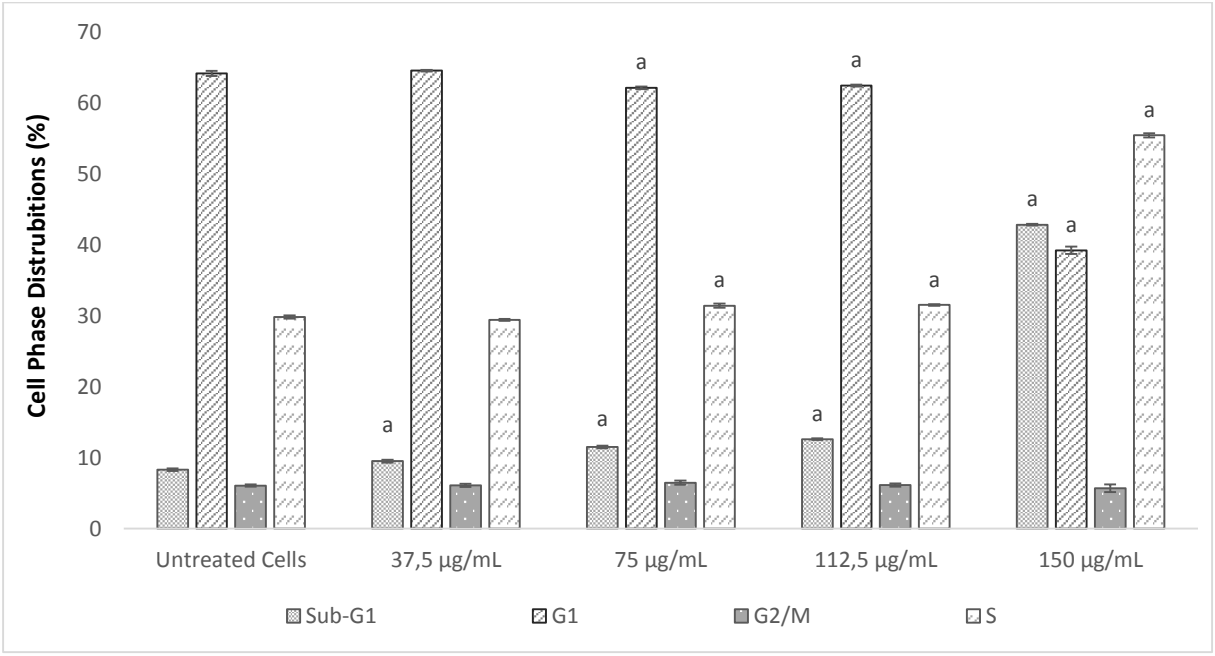

Figure 4. Cell cycle analysis of HeLa cells treated with increasing concentrations of *D. pentaphyllum* extract for 72-h. [a]Represents significant result ($p<0.05$) compared with untreated cells.

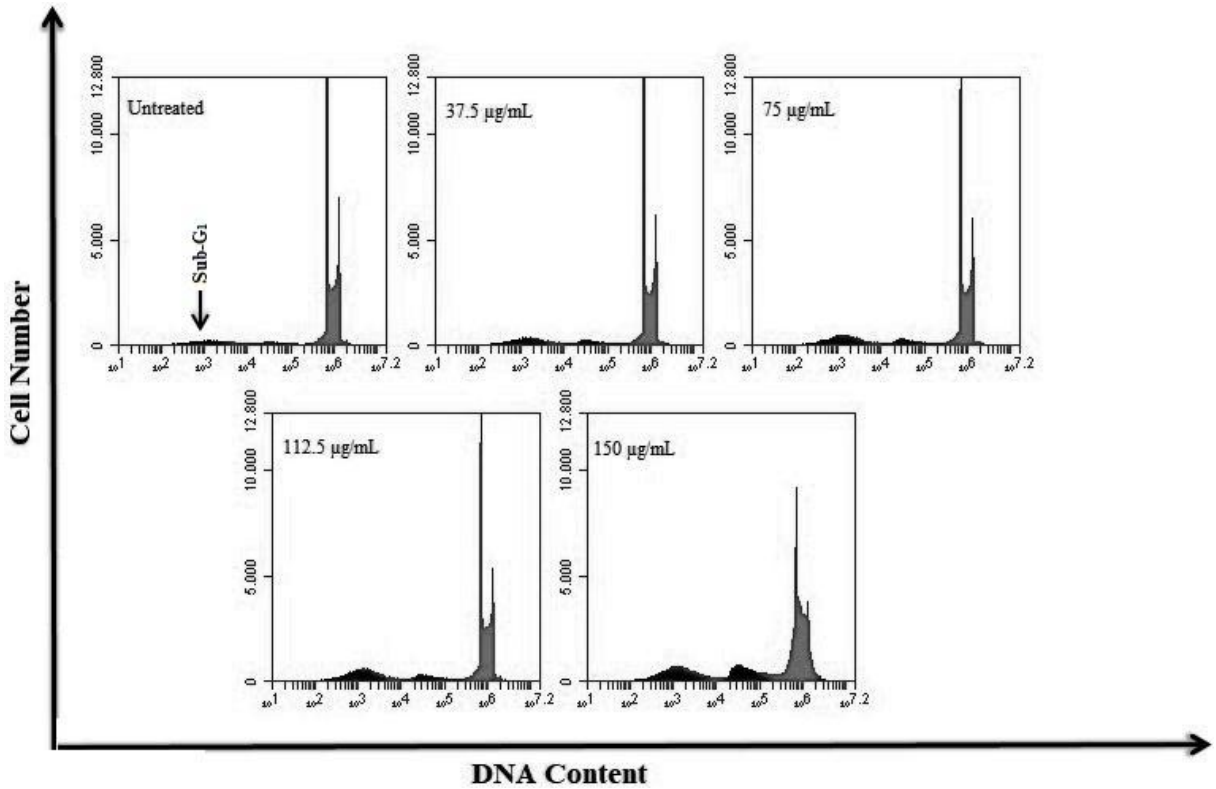

Figure 5. Effect of *D. pentaphyllum* extract on cell cycle phase distribution. Cells were treated with 37.5-150 µg/mL extract for 72-h and analyzed for DNA content using PI staining assay.

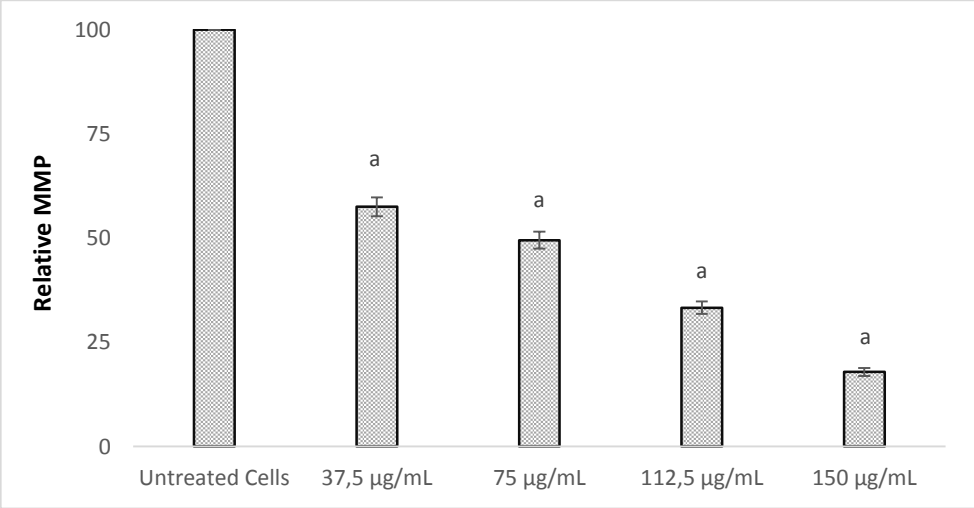

Figure 6. The effect of *D. pentaphyllum* extract on mitochondrial membrane potential in HeLa cells. [a]Represents significant result ($p<0.05$) compared with the untreated cells.

Zhang *et al.* (2013) reported that ethyl acetate and butanol fractions of aqueous extract of *Peltophorum pterocarpum* exhibits cytotoxic effect on human leukemia (HL-60), and stomach cancer (AZ521) cells, while Hussein *et al.* (2016) demonstrated that *Caesalpinia ferrea* Martius extract exhibits a cytotoxic effect on human liver (HepG2), breast (MCF-7), colon (HCT-116), larynx (Hep2) and prostate (PC-3) cancer cells.

Recently, Bencherchar *et al.* (2017) reported that chloroform, ethyl acetate, and methanolic extract of *Genista ferox* exhibits a cytotoxic effect on HeLa cells.

Polyphenols are an important class of secondary plant metabolites and are known to have strong antioxidant character (Turan et al., 2015; Demir et al., 2017b). These strong antioxidant properties of phenolic compounds are reported to provide cytotoxic, anticarcinogenic, antidiabetic, antibacterial and anti-

inflammatory effects (Demir et al., 2019b). Anticancer properties of phenolics may derive from their ability to increase apoptosis and the cell cycle arrest, inhibit DNA synthesis, and modulate signal transduction pathways (Demir et al., 2017b; Demir et al., 2018b; Oyenihi and Smith, 2019). In both our characterization studies and other previous studies, *Fabaceae* species have been shown to be rich in phenolics, such as gallic acid, fumaric acid, chlorogenic acid, 4-hydroxybenzoic acid, caffeic acid, cinnamic acid, *p*-coumaric acid, catechin derivatives, quercetin, hesperidin, taxifolin, naringenin, myricetin, resveratrol, apigenin, and galangin (Sobeh et al., 2016; Bencherchar et al., 2017). There are many studies on the anticancer effects of these phenolic compounds on various cancer cells (Di Domenico et al., 2012; Oyenihi and Smith, 2019). Therefore, we think that the selective cytotoxic activity of the extract in HeLa cells may be due to its phenolic content.

CONCLUSION

This study is the first to evaluate the antiproliferative properties of *D. pentaphyllum* extract on human cervical cancer cells. Additional *in vivo* and clinical investigations are now necessary to obtain a more detailed understanding of the exact interaction of the signaling pathways.

Conflicts of Interest Statement

None of the authors had any financial or personal relationships with other individuals or organizations that might inappropriately influence their work during the submission process.

REFERENCES

Aliyazicioglu Y, Demir S, Turan I, Cakiroglu TN, Akalin I, Deger O, Bedir A, 2011. Preventive and protective effects of Turkish propolis on H_2O_2-induced DNA damage in foreskin fibroblast cell lines. *Acta Biol Hung*, 62(4): 388-396.

Aliyazicioglu R, Demir S, Badem M, Sener SO, Korkmaz N, Demir EA, Ozgen U, Karaoglu SA, Aliyazicioglu Y, 2017. Antioxidant, antigenotoxic, antimicrobial activities and phytochemical analysis of *Dianthus carmelitarum*. *Rec Nat Prod*, 11(3): 270-284.

Bencherchar I, Demirtas I, Altun M, Gul F, Sarri D, Benayache F, Benayache S, Mekkiou R, 2017. HPLC analysis, anti-oxidant activity of *Genista ferox* and its antiproliferative effect in HeLa cell line. *Bangladesh J Pharmacol*, 12: 260-267.

Bremner P, Rivera D, Calzado MA, Obón C, Inocencio C, Beckwith C, Fiebich BL, Muñoz E, Heinrich M, 2009. Assessing medicinal plants from South-Eastern Spain for potential anti-inflammatory effects targeting nuclear factor-Kappa B and other pro-inflammatory mediators. *J Ethnopharmacol*, 124(2): 295-305.

Chaddha V, Jain PK, Dixit ND, Rathor H, Zama R, 2018. An importance of herbal medicines in treatment of cancer disease. *Int J Pharm Sci Rev Res*, 51(2): 80-82.

Demir S, Aliyazicioglu Y, Turan I, Misir S, Mentese A, Yaman SO, Akbulut K, Kilinc K, Deger O, 2016a. Antiproliferative and proapoptotic activity of Turkish propolis on human lung cancer cell line. *Nutr Cancer*, 68(1): 165-172.

Demir S, Turan I, Aliyazicioglu Y, 2016b. Selective cytotoxic effect of *Rhododendron luteum* extract on human colon and liver cancer cells. *J Buon*, 21(4): 883-888.

Demir S, Turan I, Aliyazicioglu Y, Kilinc K, Yaman SO, Demir EA, Arslan A, Mentese A, Deger O, 2017a. *Morus rubra* extract induces cell cycle arrest and apoptosis in human colon cancer cells through endoplasmic reticulum stress and telomerase. *Nutr Cancer*, 69(1): 74-83.

Demir S, Turan I, Demir F, Demir EA, Aliyazicioglu Y, 2017b. Cytotoxic effect of *Laurocerasus officinalis* extract on human cancer cell lines. *Marmara Pharm J*, 21(1): 121-126.

Demir S, Turan I, Aliyazicioglu R, Yaman SO, Aliyazicioglu Y, 2018a. *Primula vulgaris* extract induces cell cycle arrest and apoptosis in human cervix cancer cells. *J Pharm Anal*, 8(5): 307-311.

Demir S, Turan I, Aliyazicioglu Y, 2018b. Cytotoxic effect of *Rhododendron luteum* leaf extract on human cancer cell lines. *KSU J Agric Nat*, 21(6): 950-956.

Demir S, Turan I, Misir S, Aliyazicioglu Y, 2019a. Selective cytotoxic effect of *Dorycnium pentaphyllum* extract on human breast, liver, and lung cancer cells. *KSU J Agric Nat*, 22(3): 473-479.

Demir S, Turan I, Aliyazicioglu Y, 2019b. Antioxidant properties of *Primula vulgaris* flower extract and its cytotoxic effect on human cancer cell lines. *KSU J Agric Nat*, 22(1): 78-84.

Di Domenico F, Foppoli C, Coccia R, Perluigi M, 2012. Antioxidants in cervical cancer: chemopreventive and chemotherapeutic effects of polyphenols. *Biochim Biophys Acta*, 1822(5): 737-747.

Hussein SAM, El-Mesallamy AMD, Souleman AMA, Mousa MA, 2016. Cytotoxic activity of bioactive compound from *Caesalpinia ferrea* Martius, *Fabaceae*. *IJPPR*, 8(12): 2080-2084.

Jantova S, Nagy M, Ruzeková L, Grancai D, 2001. Cytotoxic effects of plant extracts from the families Fabaceae, Oleaceae, Philadelphaceae, Rosaceae and Staphyleaceae. *Phytother Res*, 15(1): 22-25.

Kim C, Kim B, 2018. Anti-cancer natural products and their bioactive compounds inducing ER stress-mediated apoptosis: A review. *Nutrients*, 10(8): 1021.

Kumar RS, Murugananthan G, Rajkapoor B, 2017. Anticarcinogenic potential of ethanol extract of

Indigofera cordifolia Roth. (Fabales: Fabaceae) on diethylnitrosamine induced hepatocarcinogenesis in rats. *Braz J Biol Sci*, 4(7): 53-66.

Lacerda LP, Malaquias G, Peron AP, 2014. Antiproliferative action of aqueous extracts of *Hymenaea stigonocarpa* Mart. (*Fabaceae*) on the cell cycle of *Allium cepa* L. *An Acad Brasil Cienc*, 86(3): 1147-1150.

Mentese U, Dogan OV, Turan I, Usta S, Dogan E, Mentese SO, Demir S, Ozer T, Aykan AC, Alver A, 2014. Oxidant-antioxidant balance during on-pump coronary artery bypass grafting. *Scientific World Journal*, Article ID: 263058, doi: 10.1155/2014/263058.

Moreno MI, Isla MI, Sampietro AR, Vattuone MA, 2000. Comparison of the free radical- scavenging activity of propolis from several regions of Argentina. *J Ethnopharmacol*, 71(1-2): 109-114.

Mosmann T, 1983. Rapid colorimetric assay for cellular growth and survival: Application to proliferation and cytotoxicity assays. *J Immunol Methods*, 65: 55-63.

Naghibi F, Irani M, Hassanpour A, Pirani A, Hamzeloo-Moghadam M, 2014. Cytotoxic effects of selective species of Caryophyllaceae in Iran. *RJP*, 1(2): 29-32.

Noufou O, Sawadogo WR, Tibiri A, Lompo M, Dijoux MG, Diederich M, Guissou IP, 2016. Phenolic contents and in vitro pharmacological activities of methanolic extract of *Pterocarpus erinaceus* Poir. stem bark (Fabaceae). *BJPR*, 10(2): 1-7.

Oyenihi AB, Smith C, 2019. Are polyphenol antioxidants at the root of medicinal plant anti-cancer success? *J Ethnopharmacol*, 229: 54-72.

Ozkan MT, Aliyazicioglu R, Demir S, Misir S, Turan I, Yildirmis S, Aliyazicioglu Y, 2017. Phenolic characterisation and antioxidant activity of *Primula vulgaris* and its antigenotoxic effect on fibroblast cells. *Jundishapur J Nat Pharm Prod*, 12(1): e40073.

Rathi SG, Suthar M, Patel P, Bhaskar VH, Rajgor NB, 2009. In-vitro cytotoxic screening of *Glycyrrhiza glabra* L. (Fabaceae): a natural anticancer drug. *J Young Pharm*, 1(3): 239-243.

Saha S, Shilpi JA, Mondal H, Hossain F, Anisuzzman M, Hasan MM, Cordell GA, 2013. Ethnomedicinal, phytochemical, and pharmacological profile of the genus *Dalbergia* L. (*Fabaceae*). *Phytopharmacology*, 4(2): 291-346.

Slinkard K, Singleton VL, 1977. Total phenol analyses: automation and comparison with manual methods. *Am J Enol Viticult*, 28: 49-55.

Sobeh M, El-Hawary E, Peixoto H, Labib RM, Handoussa H, Swilam N, El-Khatib AH, Sharapov F, Mohamed T, Krstin S, Linscheid MW, Singab AN, Wink M, Ayoub N, 2016. Identification of phenolic secondary metabolites from *Schotia brachypetala* Sond. (Fabaceae) and demonstration of their antioxidant activities in *Caenorhabditis elegans*. *Peer J*, 4: e2404.

Soni S, Vaidya S, Jain AK, Sharma A, 2013. A comparative study on anticancer potential of different extracts of *Mucuna pruriens* Linn. seeds against sertoli (GC) and ZR-75 cell lines. *IJPCBS*, 3(2): 305-314.

Stefanović OD, Tešić JD, Čomić LR, 2015. *Melilotus albus* and *Dorycnium herbaceum* extracts as source of phenolic compounds and their antimicrobial, antibiofilm, and antioxidant potentials. *J Food Drug Anal*, 23(3): 417-424.

Turan I, Deger O, Aliyazicioglu Y, Demir S, Kilinc K, Sumer A, 2015. Effects of Turkish propolis on expression of hOGG-1 and NEIL-1. *Turk J Med Sci*, 45(4): 804-811.

Turan I, Demir S, Kilinc K, Burnaz NA, Yaman SO, Akbulut K, Mentese A, Aliyazicioglu Y, Deger O, 2017a. Antiproliferative and apoptotic effect of *Morus nigra* extract on human prostate cancer cells. *Saudi Pharm J*, 25(2): 241-248.

Turan I, Demir S, Kilinc K, Aliyazicioglu Y, Alver A, Misir S, Yaman SO, Akbulut K, Mentese A, Deger O, 2017b. *Morus rubra* extract induces G_1 cell cycle arrest and apoptosis in human lung and prostate cancer cells. *IJPER*, 51(1): 51-58.

Turan I, Demir S, Kilinc K, Yaman SO, Misir S, Kara H, Genc B, Mentese A, Aliyazicioglu Y, Deger O, 2018. Cytotoxic effect of *Rosa canina* extract on human colon cancer cells through repression of telomerase expression. *J Pharm Anal*, 8(6): 394-399.

Yalcin CO, Aliyazicioglu Y, Demir S, Turan I, Bahat Z, Misir S, Deger O, 2016. Evaluation of radioprotective effect of Turkish propolis on foreskin fibroblast cells. *J Cancer Res Ther*, 12(2): 990-994.

Zhang J, Nishimoto Y, Tokuda H, Suzuki N, Yasukawa K, Kitdamrongtham W, Akazawa H, Manosroi A, Manosroi J, Akihisa T, 2013. Cancer chemopreventive effect of bergenin from *Peltophorum pterocarpum* wood. *Chem Biodivers*, 10(10): 1866-1875.

Zingue S, Tchoumtchoua J, Ntsa DM, Sandjo LP, Cisilotto J, Nde CBM, Winter E, Awounfack CF, Ndinteh DT, Clyne C, Njamen D, Halabalaki M, Creczynski-Pasa TB, 2016. Estrogenic and cytotoxic potentials of compounds isolated from *Millettia macrophylla* Benth (Fabaceae): towards a better understanding of its underlying mechanisms. *BMC Complement Altern Med*, 16(1): 421.