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Selective Cytotoxic Effect of *Dorycnium pentaphyllum* Extract on Human Breast, Liver, and Lung Cancer Cells

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ABSTRACT

Although some studies have evaluated the cytotoxic activity of different *Fabaceae* species, there has been only limited research into the cytotoxic effect of *Dorycnium pentaphyllum*. The purpose of this study was to determine the antioxidant properties and cytotoxic effect of *D. pentaphyllum* extract on various human cancer cells. The total phenolic content (TPC) of the extract was determined using colorimetric method. The cytotoxic effect of the extract on human lung (A549), liver (HepG2), and breast (MCF-7) cancer cells and a normal human fibroblast cells was assessed using the MTT assay. TPC value of extract was found as 140.3±1.1 mg gallic acid equivalent per g sample. Extract showed selective cytotoxicity on all studied cancer cells compared to normal fibroblast cells, and the IC_{50} values of the extract in the cancer cells range from 100.4 to 298.5 µg/mL. This is the first study to reveal the cytotoxic effect of *D. pentaphyllum* extract on cancer cell lines. Phytomedical applications of *D. pentaphyllum* may represent promising approaches in the treatment of cancer.

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Dorycnium pentaphyllum Ekstraktının İnsan Meme, Karaciğer ve Akciğer Kanseri Hücrelerindeki Seçici Sitotoksik Etkisi

ÖZET

Fabaceae ailesine mensup bitkilerin sitotoksik etkilerini konu alan bazı çalışmalar bulunmakla birlikte, *Dorycnium pentaphyllum*'un sitotoksik etkisi ile ilgili sınırlı araştırma bulunmaktadır. Bu çalışmanın amacı, *D. pentaphyllum* ekstraktının antioksidan özelliklerinin ve çeşitli insan kanser hücreleri üzerindeki sitotoksik etkisini belirlenmesidir. Ekstraktın toplam fenolik içeriği kolorimetrik yöntem kullanılarak belirlendi. Ekstraktın insan akciğer (A549), karaciğer (HepG2) ve meme (MCF-7) kanser hücreleri ile normal insan fibroblast hücreleri üzerindeki sitotoksik etkisi MTT analizi kullanılarak değerlendirildi. Ekstraktın toplam fenolik içerik değeri g örnek başına 140.3±1.1 mg gallik asit eşdeğeri olarak bulundu. Ekstraktın çalışılan tüm kanser hücre serileri üzerinde normal fibroblast hücrelerine göre seçici sitotoksik etki gösterdiği belirlendi ve IC_{50} değerleri 100.4-298.5 µg/mL arasında hesaplandı. Bu çalışma *D. pentaphyllum* ekstraktının kanser hücre serileri üzerindeki sitotoksik etkisini ortaya koyan ilk çalışmadır. *D. pentaphyllum*'un fitokimyasal uygulamaları kanser tedavisinde umut verici yaklaşımları temsil edebilir.

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Anahtar Kelimeler
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INTRODUCTION

Cancer remains one of the main causes of worldwide deaths, and unfortunately, only reasonable progress has been made in reducing the morbidity and mortality of this disease (Bhanot et al., 2011). According to the International Agency for Research on Cancer, 14.1 million people have been diagnosed with cancer in 2012, and this number will be expected approximately 21.7 million in 2030 (Kim and Kim, 2018). The main form of cancer treatment, especially in cases of metastatic cancer, is chemotherapy involving systemic delivery of drugs to kill cancer cells. However, most of these drugs can be used at minimal levels because they cause serious side effects in patients. (Martin-Cordero et al., 2012). In particular, the low efficacy of chemotherapeutics used in the treatment of metastatic cancer requires the development of new generation agents (Martin-Cordero et al., 2012; Demir et al., 2017a). Despite the close interest of pharmaceutical companies in molecular modeling and combinatorial chemistry techniques, natural products continue to be an important source of new generation drug development. Natural products are not only used because of their therapeutic effects, they are also the main sources for the development of new active substances (Martin-Cordero et al., 2012). The natural products have been used in cancer treatment for many years and more than 60% of the currently used anti-cancer agents, such as vinca alkaloids, taxanes, camptothecin and its derivatives, are derived from natural sources (Bhanot et al., 2011; Demir et al., 2018a).

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). It is reported that *Fabaceae* family has rich content of phenolic acids and flavonoids (Sobeh et al., 2016; Bencherchar et al., 2017). The genus *Dorycnium* belongs to the family *Fabaceae* (*Leguminosae*) and is widely distributed in Europe and Asia (Stefanović et al., 2015; Kocabas and Ilcim, 2016). The genus *Dorycnium* is represented by thirteen species on the earth. The seven species of them are naturally grown in Turkey (Kocabas and Ilcim, 2016). Plants of *Dorycnium* genus produce many biologically active compounds, some of which have been shown to carry as anti-inflammatory, antimicrobial, cytotoxic and antioxidant properties (Bremner et al., 2009; Usta et al., 2014; Stefanović et al., 2015).

Many studies have demonstrated the cytotoxic actions of various species *of Fabaceae* family. Jantova *et al.* (2001) demonstrated that the ethanolic extract of *Gymnocladus dioicus* has a cytotoxic effect on human cervix cancer (HeLa) cells in a concentration-dependent manner, while Rathi *et al.* (2009) reported that chloroform extract of *Glycyrrhiza glabra* exhibits a cytotoxic effect on human breast cancer (MCF-7) cells. Recently, it is reported that aqueous, methanolic, and ethanolic extracts of *Dorycnium pentaphyllum* have cytotocic effect against *Agrobacterium tumefaciens* with the potato disc method (Usta et al., 2014). However, to the best of our knowledge, there are no previous studies about the cytotoxic effect of *D. pentaphyllum* in human cancer cell lines. We therefore evaluated the antioxidant properties of *D. pentaphyllum* extract and its cytotoxic effect on human breast (MCF-7), lung (A549), and liver (HepG2) cancer cells and human normal foreskin fibroblast cells.

MATERIALS and METHOD

Chemicals

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

Plant Extraction

Dorycnium pentaphyllum plant samples were collected from Sinop, Turkey. The flowers of the plant were dried at room temperature, converted to fine powder using a milling procedures. 1 g of the powdered sample was then mixed with 20 mL of dimethyl sulfoxide (DMSO). The mixture was incubated with continuous shaking at 150 rpm at 45°C for 24-h. The resulting supernatant was then filtered through filter paper and then passed through 0.2 µm filters (Aliyazicioglu et al., 2017). The resulting *D. pentaphyllum* extract was aliquoted for use in experiments and stored at -20°C.

Determination of Total Phenolic Content (TPC)

The TPC of the extract was determined spectrophotometrically using the Folin-Ciocalteu method (Slinkard and Singleton, 1977). Gallic acid was used as a standard, 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 standard, and the TFC value was calculated as mg quercetin equivalent (QE)/g sample.

Cell Culture

Human liver carcinoma (HepG2), breast carcinoma (MCF-7), lung carcinoma (A549), and normal foreskin fibroblast cells were purchased from the American

Type Culture Collection (Manassas, VA, USA). All cells were grown in Eagle's Minimum Essential Medium supplemented with 10% fetal bovine serum and 1% antibiotic solution with a 5% CO_2 supply at 37°C.

Cytotoxicity Experiments

MTT assay was used to determine the cytotoxic effects of *D. pentaphyllum* extract, and cisplatin (as a refence chemotherapetutic drug) on three cancer and one normal cell lines (Mosmann, 1983; Demir et al., 2016b). Briefly, cells were seeded into a flat-bottomed 96-well cell culture plates, and then treated with varying concentrations of *D. pentaphyllum* extract (0-500 µg/mL), and cisplatin (0-10 µg/mL) for 72-h. Next, 10 µL of MTT dye was added to each well. The crystals formed were dissolved with DMSO. Finally, absorbances were measured at 570 nm with a microplate spectrophotometer (Molecular Devices Versamax, California, USA), and the absorbance values obtained were used to determine cell viability. Dose-response curves were drawn using log-concentration versus cell viability values. IC_{50} values were determined using these curves (Demir et al., 2017b). Selectivity index in all cancer cell lines were determined by the following formula using IC_{50} values obtained for extract and cisplatin. (Demir et al., 2018b):

Selectivity Index = Normal cells IC_{50}/Cancer cells IC_{50}

Statistical Analysis

All experiments were repeated three times and the results were expressed as mean±standard deviation. The conformity of the data to the normal distribution was evaluated with the Kolmogorov-Smirnov test. ANOVA and post-hoc Tukey tests were used for statistical analysis of the data sets that fit the normal distribution. $p<0.05$ was considered as statistically significant.

RESULTS and DISCUSSION

Oxidative stress is a pathological condition resulting from the deterioration of balance between reactive oxygen species (ROS) formation and antioxidant defense system. Oxidative stress has been implicated in the etiology of many pathological conditions, such as cancer, diabetes, and cardiovascular diseases (Aliyazicioglu et al., 2011; Mentese et al., 2014; Yalcin et al., 2016). Antioxidant activity is therefore important for human health, and in recent years it has been argued that numerous biological activities are caused by antioxidant effects. It is also believed that, due to their antioxidant activities, phenolics in natural products may protect against chronic diseases associated with oxidative stress. The investigation of the antioxidant property of a tested natural product extract is therefore considered a starting point for extensive studies. (Demir et al., 2016b; Aliyazicioglu et al., 2017; Ozkan et al., 2017). There are several *in vitro* analyzes to determine the antioxidant activity of natural product extracts and it is recommended to use at least two different methods. (Turan et al., 2017a). TPC and TFC methods are often used to determine *in vitro* antioxidant properties of natural product extracts because they are effective, fast, and inexpensive analyzes. (Demir et al., 2016b; Aliyazicioglu et al., 2017). We therefore determined the antioxidant properties of extract ussing TPC and TFC methods, 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)	140.3±1.1
Total Flavonoid Content (mg QE/g sample)	18.9±0.1

Consistent with our results, Stefanović *et al.* (2015) reported that the TPC and TFC value of ethanolic extract of *Dorycnium herbaceum* are 75.77 mg GAE, and 110.07 mg rutin equivalent per g sample, respectively. In another study, the TPC and TFC value for 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). Although our results are similar to some of previous studies, the differences may have been caused by plant species, extraction method, plant collection time and environmental factors.

Cancer is one of the leading causes of deaths worldwide and is characterized by certain distinctive features, including cell proliferation, differentiation, and absence of control of death (Noufou et al., 2016). The most common types of cancer in the world are lung, stomach, colon, liver and breast cancers (Chaddha et al., 2018). Although side effects and treatment costs represent two important challenges for affected patients, chemotherapy and surgery are the main treatments for cancer. These two factors lead approximately 80% of the population to use medicinal plants for basic health problems. Nowadays, there is an increasing interest in natural products, such as medicinal plants. Anticancer properties of medicinal plants are therefore further investigated (Zingue et al., 2016). *Dorycnium* species are the medicinal plants that have been used in traditional treatment for a long time. (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 polyphenolic compounds (Bremner et al., 2009; Usta et al., 2014; Stefanović et al., 2015). Although there are some studies reporting the cytotoxic effects of different

Dorycnium species in recent years (Jantova et al., 2001; Rathi et al., 2009), there are limited studies on the cytotoxic effect of D. *pentaphyllum* (Usta et al., 2014). Especially, it has been revealed that some natural product extracts can increase apoptosis in cancer cells without harming normal cells in recent years. It is therefore important to screen for the cytotoxic effects of natural product extracts or compounds isolated from natural products in cancer cells. (Demir et al., 2018b). Selectivity (no toxic effects on normal cells) and effectiveness (high efficacy against multiple cancers) are the desired two main proterties from an effective anticancer agent (Turan et al., 2017b; Demir et al., 2016b). The cytotoxic effect of the *D. pentaphyllum* extract was therefore examined on three cancer and a normal cell line using the MTT assay. The concentration-dependent effect of the extract on cell viability is shown in Figure 1.

The IC_{50} values obtained from the growth curves are presented in Table 2. The IC_{50} values of the extract in the cancer cells range from 100.4 to 298.5 µg/mL.

The selectivity index of the extract and cisplatin are shown in Table 3. The most selective cytotoxic effect of the extract was seen on HepG2 cells. The term selectivity index indicates how selectively the extract or drug molecule can eliminate cancer cells compared to normal cells (Demir et al., 2016a). The IC_{50} values of the extract in cancer cells were higher than cisplatin, but the selectivity index of the extract on HepG2 cells was higher than cisplatin.

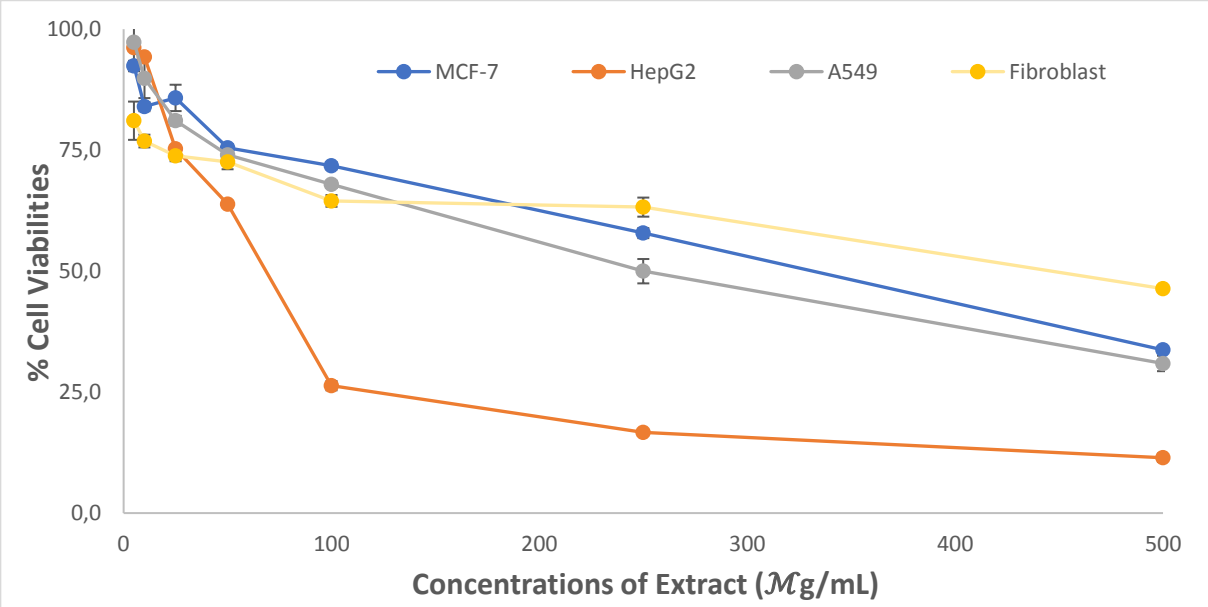

Figure 1. The anti-growth effect after the treatment with the extract for 72-h against human cancer and normal fibroblast cells using the MTT assay (n=3).

Table 2. Cytotoxic activity (IC_{50}, µg/mL) of *D. pentaphyllum* extract and cisplatin (n=3)

	Test Compounds	
Cell Lines	***D. pentaphyllum* extract**	**Cisplatin**
A549	272.2±4.7	0.72±0.04
MCF-7	298.5±5.6	1.65±0.08
HepG2	100.4±1.9	2.47±0.11
Fibroblast	441.6±2.5	5.20±0.23

Table 3. Selectivity index of *D. pentaphyllum* extract and cisplatin

	Test Compounds	
Cell Lines	***D. pentaphyllum* extract**	**Cisplatin**
A549	1.6	7.2
MCF-7	1.5	3.2
HepG2	4.4	2.1

Previous studies with *Fabaceae* species have shown that the ethanolic extract of *Ononis ramosissima* exhibits a cytotoxic action on HeLa cells (Bremner et al., 2009).

Lopes *et al.* (2011) demonstrated that alkaloidal fraction of methanolic extract of *Indigofera suffruticosa* shows a dose-dependent cytotoxic effect on murine lung (LP07) and breast (LM2) cancer cells, while Teyeb *et al.* (2012) investigated the cytotoxic effects of different extracts of *Astragalus gombiformis* Pomelon on A549 cells and they found that the most effective cytotoxic effect was in dichloromethane extract. Phani Kumar *et al.* (2014) demonstrated that aqueous extract of *Delonix elata* has a concentration-dependent cytotoxic effect in MCF-7 and HepG2 cells, while Hussein *et al.* (2016) reported that ethanolic extract of *Caesalpinia ferrea* Martius exhibits a cytotoxic effect on HepG2, MCF-7, colon (HCT-116),

larynx (Hep2), and prostate (PC-3) cancer cells. In recent years, it has been reported that not only effect of plant extracts from the *Fabaceae* family but also the cytotoxic effects of various other compounds isolated from different plants were found. Zhang *et al*. (2013) demonstrated that bergenin isolated from *Peltophorum pterocarpum* exhibits strong cytotoxic effect on melanoma cells, while it has no cytotoxic effect on HL-60, AZ521, A549, and SK-BR-3 cells, while Gbaweng *et al*. (2018) reported that excelsanone isolated from *Erythrina excelsa* has a dose-dependent cytotoxic effect on human prostate cancer (DU145) cells.

Phenolics are an important class of secondary herbal metabolites and are known to exhibit strong antioxidant properties (Turan et al., 2015). Phenolics have been reported to exhibit antioxidant, anticancer, antimutagenic, anti-atherosclerotic, antimicrobial, and anti-inflammatory effects (Demir et al., 2019). It is suggested that the anticancer effect of phenolics is due to their ability to modulate carcinogen metabolism and gene expression, and to arrest the cell cycle and induce apoptosis. (Demir et al., 2017a). In previous studies, *Fabaceae* family 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 have been numerous reports of the selective cytotoxic effects of these phenolics on different cancer cells (Ravishankar et al., 2013; Anantharaju et al., 2016). We therefore think that the selective cytotoxic effect of the extract on cancer cells may derive from its phenolic content.

CONCLUSION

This is the first study about the cytotoxic effect of *D. pentaphyllum* extract on human cancer cells. More extensive studies are needed to elucidate the cellular mechanisms underlying this cytotoxic effect of the extract.

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.

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