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Antioxidant and Cytotoxic Activities of *Fumaria parviflora* Lam. and *Fumaria capreolata* L.

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ABSTRACT
This study was carried out to examine the in vitro antioxidant and cytotoxic effects of methanol extracts of Fumaria parviflora Lam. and Fumaria capreolata. Antioxidant assays carried out by employing 1,1-diphenyl-2-picrylhydrazyl (DPPH) radical scavenging and phosphomolybdate assays. Cell viability was quantified by employing MTT assay for 24, 48 and 72 h of exposures to 25-250 µgmL-1 concentrations of the extracts. F. parviflora methanol extract possessed the highest antioxidant activities in both free DPPH scavenging and total antioxidant capacity assays. Results from MTT assay revealed that F. capreolata methanol extract significantly increased the cell proliferation in human hepatocellular carcinoma (Hep3B) cells after 24 and 48 hours of treatment. After 72 hours of treatment, methanol extract slightly inhibited the proliferation of Hep3B cell lines, in a time-and dose-dependent manner. While F. parviflora extract had the highest cytotoxic effect on Hep3B cells after 72 hours of the treatment. F. capreolata did not cause any significant cytotoxic effect on these cell lines.

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Fumaria parviflora Lam. ve *Fumaria capreolata* L.'nin Antioksidan ve Sitotoksik Aktiviteleri

ÖZET
Bu çalışmada, *Fumaria parviflora* ve *Fumaria capreolata* metanol özütlerinin *in vitro* antioksidan aktiviteleri ile iki farklı insan hepatosellüler karsinoma (Hep3B ve HepG2) hücre hattı üzerindeki sitotoksik etkileri araştırılmıştır. Özütlerin antioksidan aktiviteleri 1,1-diphenyl-2-picrylhydrazyl (DPPH) radikalini süpürme ve fosfomolibdat testi ile belirlenmiştir. Hücre canlılığı ise 24, 48 ve 72 saatlik farklı özüt konsantrasyonları (25-250 µgmL-1) ile muamele sonrasında MTT testi ile belirlenmiştir. F. parviflora ve F. capreolata metanol özütleri serbest DPPH süpürme aktivitesi ve toplam antioksidan kapasitesi açısından karşılaştırıldığında, F. parviflora metanol özütü hem DPPH serbest radikalini süpürme hem de toplam antioksidan kapasitesi bakımından yüksek aktiviteye sahiptir. MTT assay sonuçları, *F. capreolata* metanol özütünün 24 ve 48 saatlik muameleden sonra Hep3B hücrelerinde hücre çoğalmasını önemli ölçüde artırdığını ortaya çıkarmıştır. 72 saatlik uygulama sonrası, metanol özütü Hep3B hücrelerinin çoğalmasını zamana ve konsantrasyona bağlı şekilde hafif inhibe etmiştir. *F. parviflora* özütü, Hep3B hücreleri üzerinde sadece 72 saatlik uygulamada yüksek sitotoksik etkiye sahipken, *F. capreolata* bu hücreler üzerinde önemli bir sitotoksik etki göstermemiştir.

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INTRODUCTION

Fumaria known as fumitory or earth smoke, are generally regarded as weeds in many regions; several species such as *F. indica* (Hausskn.) Pugsley, *F. officinalis* L., *F. capreolata* L., *F. parviflora* Lam., *F. densiflora* DC., and *F. vaillantii* Loisel., however, have been used for a long time as medicinal plants in folk medicine. (Zhang et al., 2020). As a result of phytochemical studies, it has been reported *Fumaria* species contain a large number of chemical components, including alkaloids, flavonoids, saponins, steroids, triterpenoids, anthraquinones, tannins, glycosides and amino acids (Humayun et al., 2012; Jameel et al., 2014). Recently, it was reported that *Fumaria* species have hepatoprotective, antiviral, anti-inflammatory, antinociceptive, antimicrobial, antioxidant and antitumor activity due to their compounds like isoquinoline alkaloids. *F. parviflora Lam.(Papaveraceae)*, a small branched annual herb found in Europe, Asia, and Africa and many parts of the world including the Middle East and South Asia, are used traditionally in Turkey for the treatment of liver and bile duct disorders and dermatological disorders like acne, eczema and scabies. It was reported that the major chemical constituents of *F. parviflora* include alkaloids like protopine, parfumine, d-bicuculline, hydrastine, N-methlhydrastine, N-methylhydrasteine, microcarpine, sanguinarine, adlumiceine, coptisine, fumaritine, sinactine, N-methylstylopine and sterols like ß-sitosterol, stigmasterol and campesterol (Fafal and Önür, 2007; Paltinean et al., 2013; Kumar et al., 2018). Also, Bribi et al (2015) reported the *in vivo* and *in vitro* anti-inflammatory and antinociceptive effects of *F. capreolata* total alkaloid extract, which is another herbaceous annual *Fumaria* plant that is native to Europe, Western Asia and Northern Africa and naturalized in Southern Australia, New Zealand, and Southern America (Atlas of Living Australia, 2021). Isoquinoline alkaloids, stylopine, protopine, fumaritine, fumaricine, fumarophycine, fumariline, fumarofine were determined by GC-MS from the aerial parts of *F. capreolata* (Maiza-Benbdesselam et al., 2007). In traditional medicine, it is used as a bile enhancer, in the treatment of eczema and fungal diseases in Turkey (Güzel et al., 2015).

While numerous advances in cancer treatment approach to cancer management, cancer remains the world's leading cause of death. Hepatocellular carcinoma (HCC) is a primary malignancy commonly seen in the liver and accounts for as many as 1 million deaths annually worldwide. In certain areas of the world, HCC has the highest recurrence among internal malignancies and hence the customary reason for cancer death (Raza and Sood, 2014). The rising cost of conventional cancer therapies has forced people to focus more on traditional medicine (Sheldon et al., 1997). Herbal medication can be used as an alternative medical solution and a healthy option in chemotherapy and can be successful in the treatment of lower systemic toxicity treatment (Gao et al., 2011).

Antioxidant properties are dependent on a compound's molecular structure. Because of this, natural antioxidants are much more favorable to human health than synthetic antioxidants. Antioxidant agents are found in a different part of plants and have been used as an important protective factor for human health (Kumar et al., 2018). However, recent concern has been raised in the use of these plants based on their natural antioxidants for various uses, such as the food industry, preventive materials and therapeutic medicines (Nemzer et al., 2019). Although studies are present on the phytochemical content and antioxidant activities of *F. parviflora* and *F. capreolata,* there is no study about the chemotherapeutic potential of these plants as an alternative drug for liver cancer disease.

In the present study, as methanol extracts of these plant species have not been previously tested against human hepatocellular carcinoma (Hep3B and HepG2) cancer cell lines, cytotoxicity and antioxidant activities of *F. parviflora* and *F. capreolata* methanol extracts were evaluated in this study.

MATERIALS and METHODS

Chemicals

Dulbecco's Modified Eagle Medium (DMEM), fetal bovine serum (FBS) penicillin-streptomycin, MTT [3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide] were purchased from Sigma, Chem, Germany and methanol, dimethyl sulfoxide (DMSO) were purchased from Merck, Germany.

Collection of Plant Material

The aerial parts of both *F. parviflora* and *F. capreolata* were used in this study. From the Mediterranean flora, two species were harvested in April-2018 from the Hatay region: *F. parviflora* from İskenderun-Karayılan-36°42'28.3"N 36°14'06.0"E; *F. capreolata* from Defne-Bahçeköy-36°07'34.0"N 36°06'37.6"E. Voucher numbers are, Y. Güzel-1101 for *F. parviflora* and Y. Güzel-1102 for *F. capreolata.* Identification of the plants was made by Dr. Yelda Güzel based on Cullen (1965). Voucher specimens were deposited in the Herbarium of the Hatay Mustafa Kemal University, Department of Biology.

Extraction

The aerial parts of these plant species were separated from their stems, air-dried for a week at the room temperature and stored until extraction. The air-dried samples (25 g) were grounded into fine powder using an electric blender (HR2118 Philips, Netherlands).

These plant powders were separately extracted with 500 mL methanol at room temperature for 24-48 h. After filtration with 0.45 µm pore size syringe filter, the extract was evaporated at 40 ℃ under reduced pressure (Laborota 4002, Heidolph). The crude extracts were kept at 4 °C until the experimental studies performed.

Antioxidant Activity of Plants

1,1-diphenyl-2-picrylhydrazyl (DPPH) radical scavenging assay

The DPPH free radical scavenging potential assay is based on the stable DPPH scavenging activity. Quantitative measurements of radical scavenging assay were carried out according to the method described by Brand-Williams et al (1995). One milliliter of 0,1 mM DPPH methanol solution was added to 3 ml of various concentrations (10, 25, 50, 75, 100 and 150 µgmL^{-1}) of extracts in methanol. The mixture was vigorously shaken, then left at room temperature to stand. Using a microplate reader (Elisa Reader, Biotek Co, USA) the absorbance of the mixture was measured at λ =517 nm after 30 min. A commercially known antioxidant, ascorbic acid, was used for comparison or as a positive control. Tests were performed in triplicate (25 and 50 µg mL^{-1}).

The percentage of the DPPH free radical was calculated using the following equation:

DPPH Scavenging capacity (%) = $[(A_0\text{-}A_1)/A_0]$x100 (1)

Where A_0 was the absorbance of the control and A_1 was the absorbance in the presence of the *F. parviflora* and *F. capreolata* methanol extracts. The actual decrease in absorption induced by the test was compared with the positive controls. The IC_{50} (concentration providing 50% inhibition) values were calculated use the dose inhibition curve in the linear range by plotting the extract concentration versus the corresponding scavenging effect.

Phosphomolybdate (total antioxidant capacity) assay

The total antioxidant capacity (TAC) assay of samples was carried out by the phosphomolybdenum method (Ghafoor and Choi, 2009). A 0.1 ml aliquot of the extract (10, 25, 50, 75, 100 and 150 µgmL^{-1}) solution was shaken with 1 mL of reagent solution (0.6 M sulfuric acid, 28 mM sodium phosphate and 4 mM ammonium molybdate). The test tubes were covered with aluminum foil and incubated in a water bath at 95 °C for 90 minutes. After the samples were cooled, the absorbances were measured at 765nm. Ascorbic acid was used as standard (25 and 50 µgmL^{-1}) and the results were expressed as µgmL^{-1} of ascorbic acid equivalents. The total antioxidant capacity (TAC) of the extracts was estimated using the following formula:

Total antioxidant capacity (%) = $[(Abs.\ of\ control - Abs.\ of\ sample)/(Abs.\ of\ control)] \times 100$ (2)

Abs: absorbance

Cell culture and *in vitro* cytotoxicity assay

The two human hepatocellular carcinoma cancer cell lines Hep3B (ATCC HB-8016) and HepG2 (ATCC HB-8065) were used for cytotoxicity tests. HepG2 and Hep3B are the most widely used hepatocellular carcinoma cell lines in drug metabolism and hepatotoxicity studies. Despite their well-known similarities, there are significant differences between these two cell lines. HepG2 and Hep3B are of different ethnic origins. In literature differences have been described between HepG2 and Hep3B in several categories, including intrinsic and drug-dependent gene expressions, drug-modified cell cycle, cell growth inhibition, and signaling pathways associated with differential drug responses (Qui et al., 2015). These cells were cultured and routinely maintained in Dulbecco's Modified Eagle Medium (DMEM) with 10% fetal bovine serum (FBS), 100 U/ml penicillin, and 100 mg/ml streptomycin and were incubated at 37°C in a humidified atmosphere containing 5% Carbondioxide (CO_2) inside a CO_2 incubator until confluent. The cells were trypsinized and cytotoxicity assays were performed in 96 well-plates.

Cell viability was examined by the ability of the cells to cleave the tetrazolium salt MTT [3-(4,5-dimethylthiazol-2-yl)-2,5- diphenyl tetrazolium bromide, by the mitochondrial enzyme succinate dehydrogenase following the procedure as described earlier (Mossman, 1983). The plant extracts were tested on cancer cell lines for their cytotoxicity using MTT assay. Briefly, cells were placed as 1x10^{3}cells/plate into the 96-well plates, and the sample extract solutions were added at concentrations ranging from 40 µgmL^{-1} to 250 µgmL^{-1} for evaluation of their cytotoxicity and incubated for 24, 48, and 72 hours. The control cells only received maintenance medium. Furthermore, the anticancer agent, Farmorubicin (40 µgmL^{-1}) was used for positive control and 0.1% dimethyl sulfoxide (DMSO), treated cells served as solvent control. The treated cells were incubated with MTT (0.5 mg/ml in phosphate buffered saline) for 4 hours at 37 °C. The medium was removed and dye crystal formazan was solubilized in DMSO. The absorbance was measured at 545 nm using a multi-plate reader (Biotek Epoch, USA) (Duthie et al., 2000).

All of the absorbance values were compared to the control samples (without any compound) which represented 100% viability.

Cell viability (%)= $[(As - Ab)/(Ac - Ab)]$ x100 (3)

As: Absorbance Sample

Ab: Absorbance Blank

Ac: Absorbance Viable cell (control)

Statistical Analysis

Differences in the means of individual dosage group and the control of each extract was analyzed using one-way ANOVA (Analysis of Variance) in the SPSS 20.0 (USA) software package program. Level of significance was set at $p \leq 0.05$ level.

RESULTS and DISCUSSION

Determination of the antioxidant activities of various plant species could contribute to show the significance their as sources for new antioxidant compounds (Chaves et al., 2020). Usually, the antioxidant properties especially that of phenolic compounds in plant extracts are efficient at low concentrations and, are associated with the prevention of some diseases like cardiovascular disease and cancer in humans. (Duthie et al., 2000; Li et al., 2014; Acay et al., 2021). In recent years, herbal therapy has emerged as an important branch of complementary and alternative medicine for cancer-related targets, to reliably reduce chemotherapy-associated toxicity. Various studies have shown that natural products derived from plants can effectively regulate cancer cell proliferation, differentiation and expression for different cancers *in vitro* and *in vivo* (Barretina et al., 2014).

Antioxidant activities

The free DPPH radical scavenging activity of methanolic extract of *F. parviflora* as comparable with known antioxidant ascorbic acid (positive control) is shown in Table 1.

The total antioxidant activity of *F. parviflora* methanol extract was concentration-dependent and there were statistically significant differences ($p < 0.05$) with increasing extract concentration. The 25 and 50 μgmL^{-1} of ascorbic acid synthetic antioxidant showed high antioxidant activity in free DPPH scavenging and phosphomolybdate assays (75.00% and 76.34%, respectively). The total antioxidant capacity of *F. parviflora* was lower than free radical scavenging activity. On the other hand, the total antioxidant capacity of *F. parviflora* methanol extract was lower than free radical scavenging activity. Total antioxidant capacity increased from75 μgmL^{-1} to over 50%, reaching the highest value at 150 μgmL^{-1} (71.25%). However, these values remained low when compared to the total antioxidant capacity displayed by ascorbic acid. The absorbance was recorded at 517 nm after 30 minutes of incubation and the IC_{50} value was calculated. IC_{50} value is the concentration of the sample required to scavenge 50% DPPH.

The antioxidant activity of *F. capreolata* methanol extract was concentration-dependent and there was a statistically significant difference ($p<0.05$) with increasing extract concentration. The IC_{50} value for free DPPH scavenging activity was also quite high (125.92 μgmL^{-1}). Total antioxidant activity values were also similar to DPPH scavenging activity (Table 1).

When *F. parviflora* and *F. capreolata* methanol extracts were compared in terms of free DPPH scavenging activity and total antioxidant capacity, *F. parviflora* methanol extract was more effective for both free DPPH scavenging activity and total antioxidant capacity. The *F. parviflora* methanol extract showed significant free radical scavenging potential with an IC_{50} of 15.06 μgmL^{-1}. The *F. capreolata* methanol extract showed insignificant free radical scavenging activity with IC_{50} of 125.92. Ascorbic acid however showed better free radical scavenging activity with IC_{50} of 25 and 50 μgmL^{-1} been <25.00.

Table 1. Antioxidant activity of methanolic extract of *F. parviflora* and *F.capreolata*
Çizelge 1. F. parviflora and *F.capreolata methanol ekstrelerinin antioksidan aktivitesi*

	Concentrations (μgmL^{-1})	DPPH scavenging activity (% ±SD)	IC_{50} DPPH scavenging (μgmL^{-1})	Total antioxidant capacity (%±SD)	IC_{50} Total antioxidant capacity(μgmL^{-1})
	25	75.00 ± 0.007*		77.45 ± 0.014*	
Ascorbic acid	50	76.34 ± 0.007*	< 25.00	78.89 ± 0.031*	< 25.00
	10	47.32 ± 0.002*		22.73 ± 0.023	
	25	50.89 ± 0.021*		36.15 ± 0.006*	
F. parviflora	50	57.59 ± 0.011*	15.06	46.48 ± 0.030*	71.32
	75	60.27 ± 0.016*		51.77 ± 0.014*	
	100	69.64 ± 0.017*		65.57 ± 0.004*	
	150	70.54 ± 0.031*		71.25 ± 0.097*	
	10	24.11 ± 0.019		10.52 ± 0.003	
	25	30.36 ± 0.035*		28.04 ± 0.006*	
F. capreolata	50	34.67 ± 0.009*		32.67 ± 0.002*	
	75	45.98 ± 0.017*	125.92	39.29 ± 0.004*	94.75
	100	46.88 ± 0.018*		61.14 ± 0.023*	
	150	50.89 ± 0.017*		66.50 ± 0.014*	

***p<0.05,** All comparisons were made with untreated control cells.

When *F. parviflora* and *F. capreolata* methanol extracts were compared in terms of free DPPH scavenging activity and total antioxidant capacity, *F. parviflora* methanol extract was more effective for both free DPPH scavenging activity and total antioxidant capacity. This difference between the antioxidant activities of the extracts may due to these two plants having different phytochemical contents. Moghaddam et al. (2018) investigated the antioxidant activity of *F. vaillantii* with various antioxidant methods (Moghaddam et al., 2018). They reported that the total phenolic content and antioxidant activity of the plant reached the highest value in the vegetative period. In another study, the antioxidant activity of *F. parviflora* root, stem, leaf and fruit ethanolic extracts were determined by the 2,2-diphenyl-1-picrylhydrazil method and it was determined that the extract with the highest antioxidant activity was leaf ethanol extract (Kumar et al., 2018). Researchers have suggested that *F. parviflora* Lam leaf ethanolic extract has more polyphenols and flavonoid compounds, which may be responsible for antioxidant activity. Noureddine et al. (2013) reported that the total alkaloid fraction from *F. capreolata* showed antioxidant activity without any toxicity *in vivo.* Maiza-Benabdesselam et al, (2018) measured the antioxidant activity, reducing power, inhibiting ability of linoleic acid peroxidation and 2,2-diphenyl-picrylhydrazyl (DPPH) of alkaloid extracts of *F. capreolata* (L.) and *F. bastardii* (L.) and determined that the extracts obtained from the aerial parts of these plants contained isoquinoline alkaloids, stylopine, protopin, fumaritine, fumaricine, fumarophycine, fumarilin, fumarofin by GC-MS method. The results of the study showed that both herbal extracts showed strong total antioxidant activity, but *F. bastardii* extract was more effective than *F. capreolata*. The results of our study are similar to the results of these researchers.

Cytotoxic activities

Cytotoxic activity of *F. parviflora* methanol extract

Cell viability assay results indicated that *F. parviflora* methanol extract caused a concentration-dependent increased in the proliferation of Hep3B cells (Table 2). After 24 hours of incubation, the methanol extract of *F. parviflora* started to increase proliferation from 25 μgmL^{-1} and showed the most increase at the highest concentration (250 μgmL^{-1}; 257.15%) compared to the control and solvent control (DMSO) group ($p<0.05$). Depending on the increase in the treatment time, the viability of Hep3B cells decreased and a high cytotoxic effect was obtained after 72 hours at 250 μgmL^{-1} concentration (33.65%.)

Table 2. The effect of *F. parviflora* methanol extract on the viability of Hep3B and HepG2 cells
Çizelge 2. F. parviflora methanol özütününHep3B ve HepG2 hücrelerinin canlılığı üzerindeki etkisi

Concentrations (μgmL^{-1})	Cell viability % ± SD					
	Hep3B cells			HepG2 cells		
	24h	48h	72h	24h	48h	72h
Control	100±0.00	100±0.00	100±0.00	100±0.00	100±0.00	100±0.00
Solvent control (DMSO)	97.48±0.035	96.19±0.014	96.00±0.174	96.21±0.036	96.02±0.060	95.80±0.106
Farmorubicin 40	0.50±0.022*	0±0.000*	0±0.000*	0±0.000*	0±0.000*	0±0.000*
25	124.39±0.107*	96.19±0.078	72.90±0.206*	108.38±0.253	90.17±0.050	81.41±0.099*
50	153.65±0.041*	91.61±0.282	68.43±0.374*	103.82±0.045	85.42±0.105*	75.92±0.248*
75	165.04±0.059*	89.90±0.035	59.49±0.095*	99.66±0.059	84.26±0.071*	69.87±0.180*
100	179.94±0.035*	88.69±0.415	50.55±0.268*	99.32±0.566	83.15±0.154*	67.65±0.064*
150	226.28±0.014*	88.52±0.353	50.09±0.457*	98.36±0.081	79.01±0.012*	64.69±0.061*
200	250.67±0.085*	86.54±0.048	39.38±0.051*	94.99±0.326	76.91±0.028*	64.01±0.219*
250	257.15±0.104*	78.04±0.012*	33.65±0.089*	91.78±0.034	76.09±0.021*	53.45±0.075*

***p<0.05,** All comparisons were made with untreated control cells.

The effect of *F. parviflora* methanol extract on viability and proliferation of HepG2 cells is shown in Table 2. 25 and 50 μgmL^{-1} concentrations of *F. parviflora* methanol extract slightly increased the viability and proliferation of HepG2 cells after 24 h treatment. The other extract concentrations after 24 h treatment had no significant effects on cell viability and proliferation compared to the control and solvent control (DMSO) group ($p<0.05$). After 48h treatment, HepG2 cell viability and proliferation decreased by increasing concentration. All the extract concentrations, except for 25 μgmL^{-1}, decreased cell viability and proliferation significantly in comparison with control and solvent control. All concentrations of *F. parviflora* methanol extract showed the cytotoxic effect on HepG2 cells after 72 h treatment ($p<0.05$). The cytotoxic effect reached 53.45% with 250 μgmL^{-1} extract treatment.

Cytotoxic activity of *F. capreolata* methanol extract

The effects of *F. capreolata* methanol extract on Hep3B cell viability and proliferation is presented in Table 3. After 24 hour treatment, *F. capreolata* methanol extracts concentrations increased the viability and proliferation of Hep3B cells at a very high level in comparison with the control group ($p<0.05$). The highest cell viability was observed after 25 μgmL^{-1} (195.13%). 48 hour treatment of *F. capreolata* methanol extracts also increased cell viability and proliferation. However, *F. capreolata* methanol extract reduced the viability and proliferation of Hep3B cells after 72 h treatment, concentration-dependently. However, this reduction was not statistically significant in comparison with control and solvent control ($p<0.05$).

Table 3. The effect of *F. capreolata* methanol extract on the viability of Hep3B and HepG2 cells
Çizelge 3. F. capreolata methanol özütünün Hep3B ve HepG2 hücrelerinin canlılığı üzerindeki etkisi

Concentrations (μgmL^{-1})	Cell viability % ± SD					
	Hep3B cells			HepG2 cells		
	24h	48h	72h	24h	48h	72h
Control	100±0.00	100±0.00	100±0.00	100±0.00	100±0.00	100±0.00
Solvent control (DMSO)	97.76±0.015	97.55±0.043	96.50±0.005	98.30±0.051	97.08±0.051	96.00±0.258
Farmorubicin 40	0±0.000*	0±0.000*	0±0.000*	0±0.000*	0±0.000*	0±0.000*
25	195.13±0.20*	147.54±0.530*	98.54±0.196	92.09±0.117	83.46±0.109*	75.95±0.071*
50	192.70±0.21*	146.87±0.125*	97.19±0.384	91.87±0.043	79.40±0.027*	73.42±0.115*
75	174.32±0.05*	127.90±0.175*	95.68±0.004	91.02±0.016	76.49±0.003*	72.91±0.083*
100	167.83±0.17*	105.35±0.287	93.02±0.426	84.02±0.012*	76.19±0.307*	71.86±0.106*
150	167.02±0.06*	104.91±0.312	88.39±0.266	81.32±0.076*	73.98±0.113*	70.24±0.268*
200	161.62±0.04*	102.90±0.021	87.61±0.111	78.21±0.419*	73.20±0.021*	66.53±0.210*
250	155.13±0.0 *	97.63±0.069	86.32±0.041	70.71±0.150*	67.49±0.378*	64.14±0.123*

***p<0.05,** All comparisons were made with untreated control cells.

F. capreolata methanol extract decreased the HepG2 cell viability in a time and concentration manner. After 24hour treatment, methanol extract reduced cell viability and proliferation significantly in comparison with the control groups. After 48 and 72 hour treatments, all extract concentrations significantly reduced the viability and proliferation of HepG2 cells compared to the control groups ($p < 0.05$).

Finally, when the cytotoxic effect of *F. parviflora* and *F. capreolata* methanol extracts on Hep3B and HepG2 cancer cell lines were compared, it was determined that the cytotoxic effect of *F. capreolata* methanol extract was more effective than that of *F. parviflora* methanol extract, concentration- and time-dependent.

The cytotoxic effect of *F. parviflora* methanol extract on Hep3B cells decreased depending on the treatment time and concentration. All extracts exhibited minimal cytotoxic effect, especially at high concentrations, after 72 hours of treatment and did not affect the viability of Hep3B cells with increasing incubation time (Table 1). However, extract of this plant showed cytotoxic activity after 48 and 72 hours of treatment on HepG2 cells (Table 1). This may be because Hep3B and HepG2 cells have different sensitivities against the compounds that are present in the extract. The cytotoxic effect of *F. parviflora* methanol extract on HepG2 cells also decreased depending on the treatment time and concentration.

Results from MTT assay revealed that *F. capreolata* methanol extract significantly increased cell proliferation in Hep3B cells after 24 and 48 hour of treatment. After 72 hour of treatment, methanol extract slightly inhibits the proliferation of Hep3B cells, in a time-and dose-dependent manner. The cytotoxic effect of *F.capreolata* methanol extract on HepG2 cells also decreased depending on the concentration and treatment time (Table 2). Many phytochemicals show a non-monotonic dose/concentration response called a biphasic dose response, depending on the dose and the time of administration. For example, while stimulating cell proliferation at low doses and short time applications, they show a cytotoxic effect in high doses and longer administration times. These phytochemicals that cause biologically opposite effects at different doses and /or durations are called hormetic compounds (Siddiqui et al., 2015, Jodynis-Liebhert and Kujawska, 2020). It can also be described as the induction of multiple cellular functions with different hormetic properties (DNA repair, antioxidant defenses, autophagy or others), whose actions are controlled by the interaction of multiple receptors and signaling pathways to produce an integrated cellular response (Calabrese and Mattson, 2017; Jalal et al., 2021). Hormesis is a synchronized reaction of any living organisms (plants and animals) to self-imposed adversity that influences certain signal-transduction

processes (hormetic) and biological plasticity to correlate endpoint response (Calabrese and Mattson, 2017). This biological plasticity serves as a foundation for adaptation to adversity (Ho and Zhang, 2018). The reason for the increase in proliferation observed in Hep3B cells as a result of the application of *F.parviflora* and *F. capreolata* methanol extracts for 24 hours may be the hormetic compounds found in the extracts and showing biphasic dose response. When the cytotoxic effect of *F. parviflora* and *F. capreolata* methanol extracts on Hep3B and HepG2 cancer cell lines were compared, it was determined that the cytotoxic effect of *F. capreolata* methanol extract was more effective than that of *F. parviflora* methanol extract, concentration-and time-dependent. This difference may have arisen due to different sensitivity of Hep3B and HepG2 cells against phytochemical compounds in methanol extract against different *Fumaria* plants and some cytotoxic compounds in these extracts.

Previous studies on different *Fumaria* species suggested that the effects of these plants on cell proliferation vary according to the type of extract and cell type used in the studies. Recently, anti-proliferative activity of *F. vaillantii* hexane, chloroform, ethyl acetate, methanol and total (water:ethanol (80:20 v/v)) on SKMEL-3 malignant melanoma, MCF-7 human breast adenocarcinoma, and K562 human myelogenous leukemia cells was reported. Researchers suggested this effect occurred by the necrotic activity of extracts (Tabrizi et al., 2016). In contrast, hepatoprotective effects of various *Fumaria* species were reported previously (Seyed et al., 2007; Rathi et al., 2008; Hussain et al., 2012; Ezzat et al., 2012). It has been shown that *F. parviflora* can counteract CCl_4-induced hepatotoxicity due to its antioxidant activities (Jamshidzadeh and Niknahad, 2006). By blocking the critical control points of apoptosis the *in vitro* protective effect of *F. parviflora* on nimesulide induced cell death in primary rat hepatocyte cultures (Triphati et al., 2010). In another study, it was reported that *F. parviflora* extract possess selective protective effect against hepatotoxicity due to MDME inhibition mediated paracetamol (Gilani et al., 1996). Furthermore, it was suggested *F. parviflora* ethanol extract prevents lead-induced testicular toxicity in male adult rats and it improves reproductive parameters in lead-treated male rats (Dorostghoal et al., 2013). Also, Bribi et al (2015) reported the *in vivo* and *in vitro* anti-inflammatory and antinociceptive effects of *F. capreolata* total alkaloid extract (Bribi et al., 2015). Furthermore, it was reported that the total alkaloid fraction of *F. capreolata* has antioxidant activity and no toxic effect on mice, when orally administered up to 2000 mg mL^{-1} of the total alkaloid extract (Bribi et al., 2013). Several studies have been conducted on the short-term effects (up to 4 days) of *F. parviflora* for the treatment of different organs. Some previous studies showed the beneficial effects of the *F. parviflora* in the treatment of hepatobiliary system diseases (Nabavizadeh et al., 2009; Adewusi and Afolayan, 2010). On the other hand, the hepatoprotective effects of this plant have been reported by Gilani et al, (1996) and Heidari et al (2004).

CONCLUSION and RECOMMENDATIONS

The results of our study showed that *F. parviflora* and *F. capreolata* methanol extracts possess antioxidant activity. Furthermore, *F. parviflora and F. capreolata* methanol extract increased the proliferation of Hep3B and HepG2 cancer cells in 24 hours. They have cytotoxic effect after 48h and 72h treatment. The cytotoxic effects of the extract may differ depending on the cell type. This difference may have arisen due to the different sensitivity of Hep3B and HepG2 cells against different phytochemical and cytotoxic compounds in methanol extracts. We believe that these preliminary results will contribute to more detailed studies to determine the mechanisms by which different *Fumaria* species elicit their anticancer activities on human cancer cells.

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Researchers' Contribution Rate Statement Summary

The authors declare the contribution of the authors is equal.

Conflict of Interest

The authors declare no conflicts of interest

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