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In Vitro Cytotoxic Effects of Some *Fumaria* L. (Papaveraceae) Species Methanolic Extracts on Cancer Cell Lines

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Abstract: The *Fumaria* L. species (Papaveraceae), is known popularly as "Şahtere". This species, which has been proven to contain significantly rich components, is used by people to treat a number of different diseases. This study investigated the cytotoxic effects of the methanolic extracts of above-ground portions of *Fumaria asepala* Boiss. and *Fumaria schleicheri* Soy.-Will. subsp. *microcarpa* Boiss. ex Hausskn. taxa against BEAS-2B, SH-SY5Y, HCT116 and A549 cell lines. The changes in cancer cell vitality were identified using the 3-(4.5-dimethylthiazol -2-il)-2.5-diphenyltetrazolium bromide (MTT). While the results showed that *F. asepala* and *F. schleicheri* subsp. *microcarpa*'s methanolic extracts presented a meaningful decrease in the vitality of colon (HCT 116) cancer cells, no cytotoxic effect was achieved on lung (A549) cancer and brain (SH-SY5Y) cancer cells. Also, the herbal extracts did not create any toxic effect on healthy lung cells (BEAS-2B).

Bazı *Fumaria* L. (Papaveraceae) Türlerinin Metanolik Ekstraktlarının Kanser Hücre Hatları Üzerindeki İn Vitro Sitotoksik Etkileri

Anahtar Kelimeler
Fumaria, Sitotoksik, BEAS-2B, SH-SY5Y, HCT116, A549

Öz: *Fumaria* L. cinsi (Papaveraceae) halk arasında "Şahtere" olarak bilinir. Son derece zengin bileşiklere sahip olduğu kanıtlanmış olan cins, halk arasında birçok rahatsızlığın tedavisinde kullanılmaktadır. Bu çalışmada, *Fumaria asepala* Boiss. ve *Fumaria schleicheri* Soy.-Will. subsp. microcarpa Boiss. ex Hausskn. taksonlarının toprak üstü kısımlarının metanolik ekstraktının BEAS-2B, SH-SY5Y, HCT116 ve A549 hücre hatlarına karşı sitotoksik özellikleri araştırıldı. Kanser hücrelerinin canlılığındaki değişiklikler 3-(4,5-dimetiltiazol-2-il)-2,5-difeniltetrazolyum bromür (MTT) yöntemi kullanılarak tespit edildi. Sonuçlar, *Fumaria asepala* ve *F. schleicheri* subsp. *microcarpa*'nın metanolik ekstraktının özellikle kolon (HCT 116) kanseri hücrelerinin hücre canlılığında istatistiksel olarak anlamlı bir azalma olduğunu gösterirken, akciğer (A549) kanseri ve beyin (SH-SY5Y) kanseri hücreleri üzerinde sitotoksik etkinlik sağlamamıştır. Ayrıca bitkisel ekstraktlar, sağlıklı akciğer hücreleri (BEAS-2B) üzerinde ise herhangi bir toksik etki yaratmamıştır.

1. INTRODUCTION

Even though herbal drugs have been used to combat diseases since ancient times, information about their effective compounds and action mechanisms have only been known since the middle of the 19th century [1]. Some of the disadvantages of chemotherapy, one of the most preferred methods in cancer treatment, decrease the success rate of the treatment. Therefore, researching the anti-cancer efficacy of herbal products has recently become one of the fields of interest [2].

Also, the search to support treatment with natural products due to synthetic drugs having many side-effects has brought traditional and complementary medicine practices to the forefront. This presents the consideration that using herbal products rich in biologically active components may be an important alternative to reducing high drug costs [3, 4].

The World Health Organization (WHO) has reported that the number of medicinal and aromatic herbs being used worldwide is approximately 20.000, that about 4.000 of these are widely used for therapeutic purposes, and about 2000 medical/aromatic herbs are traded worldwide while around 500 are traded in Western Europe [5, 6]. In Türkiye, the number of plants traded in the domestic market, together with the species and varieties collected from nature, is about 350 [7].

The *Fumaria* species (Papaveraceae), which is represented by 16 taxa in in Türkiye, consists of about 60 taxa worldwide [8] and has a cosmopolitan distribution in the entire European continent, especially in the Mediterranean region and Eastern and Western Europe [9]. Since *Fumaria* taxa are frequently used by communities in the treatment of many diseases, researchers have conducted research on the chemical contents of these taxa, which have shown that they are extremely rich sources of alkaloid content [10]. The presence of different types of flavonoids, steroid compounds and organic acids has also been identified in addition to alkaloid content [11]. As a result of studies conducted on different species of the genus, the literature shows that analgesic, antioxidant, hepatoprotective, antiproliferative, antiplasmodial, antibacterial, antifungal, and anti-inflammatory activities of *Fumaria* species have been proven [12, 13-18]. These activities are especially due to the isoquinoline alkaloids found in the plant, and protopine is the most common among them [19].

In this research, we aimed to contribute to the studies on the supply of raw materials as a new therapeutics from plants that grow naturally in in Türkiye. The cytotoxic activity of two taxa belonging to the genus *Fumaria* were investigated through using methanolic extracts and testing on the BEAS-2B (Healthy Human Bronchial Epithelial), SH-SY5Y (Human Neuroblastoma), HCT116 (Human Colorectal Carcinoma) and A549 (Human Lung Cancer) cell lines. To our knowledge, this the first study conducted to reveal the cytotoxic effect of *Fumaria asepala* Boiss. and *Fumaria schleicheri* Soy.-Will. subsp. *microcarpa* Boiss. ex Hausskn.

2. MATERIAL AND METHOD

2.1. Plant Material Collection and Identification

Plant materials were collected in May 2021 from Elazig: Baskil-Sancakli village inner road, 1410 m. in Türkiye (Lat: 38°35'13.061" Long: 38°55'23.055"). The taxonomic identification of plant materials was performed by Prof. Dr. Semsettin Civelek, who is a systematic-botanic specialist from Firat University. The collected plants were ventilated and dried in the shade for suitability for the study. Some of the specimens have been made into herbarium material and are kept under the herbarium number FUH8408 (*F. asepala*) and FUH8409 (*F. schleicheri* subsp. *microcarpa*). General appearances of plants are shown in Figure 1.

Figure 1. General appearance of *Fumaria* species: (**A.** *F. asepala*, **B.** *F. schleicheri* subsp. *microcarpa*)

2.2. Extraction Technique

All the root, leaf and flower parts of the plants were ground into powder, and 1 g of the plant sample was weighed on a precision scale and set to wait in a shaking incubator inside 100 ml 80% methanol at 35 °C for 72 hours. The mixture obtained from this was filtered using Whatman No 1 filter paper, and this process was repeated 3 times. Then, the obtained extracts were poured into sterile petri dishes and dried in a sterile cabinet until the solvent had evaporated. The dried extracts were dissolved with 99% DMSO (Dimetilsülfoksit-molecular grade), the concentrations were adjusted with DMEM (Dulbecco's Modified Eagle's Medium), and the stocks were kept at +4°C [20].

2.3. Cytotoxic Activity Assessment

The cytotoxic analysis of *F. asepala* and *F. schleicheri* subsp. *microcarpa*'s methanolic extract was done using healthy lung cells (BEAS-2B) and brain cancer (SH-SY5Y), colon cancer (HCT116) and lung cancer (A549) cell lines.

2.4. Cell Culture

Cells were grown in 25cm² flasks inside DMEM (25 mM L-Glutamine, 1% Penicillin-Streptomycin and 10% FBS (Fetal Bovine Serum)) at 37°°C in 5% CO2 atmospheric setting. When the flask base was covered with at least 95% cells, the cells were included in the experiments. A 0.25% trypsin-EDTA (Etilendiamin tetraasetik asit) solution was used for the removal of cells from the surface.

2.5. MTT Reduction Assay

The potential cytotoxicity of the tested extracts was evaluated as indicated by the MTT (3-(4.5-dimethylthiazol-2-yl)-2.5-diphenyltetrazolium bromide) Assay. In order to do this, cells were placed into the plates with 96 wells so that there were 10^4 cells in each well. Then they were left to incubate in an incubator with 5% CO_2 at 37°°C for 24 hours. After incubation, the medium in the wells was removed and added to the cells in 3 replicates at 5 different concentrations. (800, 400, 200, 100 and 50 µg/ml) from the methanolic extract of *Fumaria* species prepared in the medium. Healthy lung cell line (BEAS-2B) was used as positive control, and cell lines that no application was done on were used as negative control. The cells were left to incubate in an

incubator with 5% CO_2 at 37°C for 24 hours. After the incubation period, MTT solution (5mg/ml) was added to the wells the cells were in and incubated in a dark environment containing 5% CO_2 at 37°C for 3 hours.

After incubation, the medium was removed, and formazan crystals were dissolved with 100µl DMSO. Absorbance measurements were then made at a wavelength of 570 nm with the color change ELISA micro-plate reader (KHB ST-360) device, and cell vitality levels were calculated [21]. All MTT analyses were repeated 3 times.

2.6. Statistical Analysis

The results of the study were analyzed using SPSS statistical programming (version 22.0). The data were presented as mean ± SD. As a result of the SPSS analyses, the mean ± SD of the numerical data was provided. One-Way ANOVA test was used in the study to analyze the distributed data in the comparisons between multiple groups. A P-value of less than 0.001 was considered to be statistically significant. Tukey's tests were used for post hoc comparisons between the groups.

3. RESULTS

The cytotoxic activity of the methanolic extract of *F. asepala* and *F. schleicheri* subsp. *microcarpa* against SH-SY5Y, HCT116, A549, BEAS-2B cell lines is presented in the diagram. The cytotoxic activity of methanolic extract of *F. asepala and F. schleicheri* subsp. *microcarpa* is quite significant on colon cancer-HCT116 among the cell lines that were studied. This effect is stronger in *F. asepala* and relatively less in *F. schleicheri* subsp. *microcarpa*. At a concentration of 800 µg/ml, cell viability reduced to 57.08 ± 5.12% (p<0.001) in *F. asepala* and 63.10± 14.18% (p<0.001) in *F. schleicheri* subsp. *microcarpa* and it is the strongest concentration in terms of cell death rate. It was also observed that both *F. asepala* and *F. schleicheri subsp. microcarpa* have cytotoxix effect at 400 µg/ml, 200 µg/ml concentrations to reduce 57.42±2.28 % (p<0.001), 88.39±6.61% (p<0.001) and 80.40±8.60% (p<0.001), 85.35±6.01% (p<0.001) cell viability, respectively. While an increase was observed in the survival rate of cells due to the decrease in concentration, it was observed that the cytotoxic activity of the extracts continued down to the lowest concentration (50 µg/ml) for HCT116.

There were not significant cytotoxic activity on the other cell lines studied within both plant groups. For example, almost all concentrations of the herbal extract had a proliferative effect on both healthy lung cells and brain and lung cancer cell lines via increasing the number of living cells by almost 20%-30%. The herbal extract served as a nutrient for the cell groups that were studied, and while it was predicted to suppress cell proliferation and even kill cells, it was observed to be encouraging them to multiply. However, no toxic effects of the extracts on healthy cells were observed; on the contrary, the extracts were observed to be promoting cell growth in both healthy and cancerous cell lines. The proliferative effect of plant extracts on cell groups is expected for healthy cells, but the fact that these extracts encourage cancerous cells to divide is evidence concluding that such extracts should not be used indiscriminately. The achieved results are presented in the live cell percentage diagram in Figure 2a-2b.

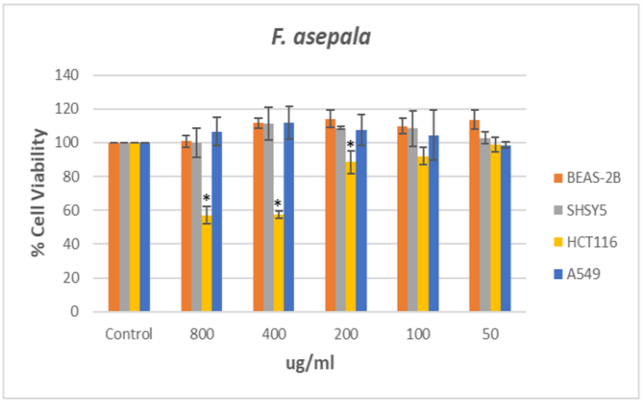

Figure 2a. Viable cell percentage diagram of *F. asepala (*p<0.001).*

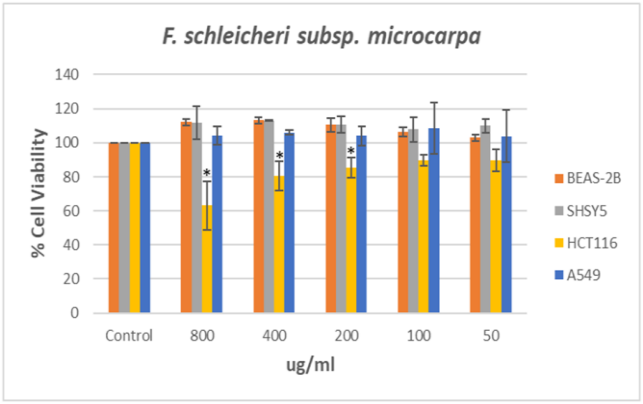

Figure 2b. Viable cell percentage diagram of *F. schleicheri* subsp. *microcarpa (*p<0.001).*

4. DISCUSSION AND CONCLUSION

Many plant species belonging to the genus *Fumaria* are reported to have anticancer components that make them medically significant. Many *Fumaria* species have been utilized for biological properties such as essential oil, cytotoxicity, antimicrobial and antioxidant capacity, and very good results have been achieved. In studies conducted in this regard, it has been shown that the effects of herbal extracts on cell proliferation vary according to the type of extract used in the study and the type of cancerous cell [22].

Although there is no cytotoxic study conducted with *F. asepala* and *F. schleicheri* subsp. *microcarpa* taxa in the literature, there are studies that have been conducted with different *Fumaria* members.

Tabrizi et al. [23] have researched the antiproliferative activity of the hexane, chloroform, ethyl acetate and methanol extracts of *F. vaillantii* species on SKMEL-3, MCF-7 and K562 cell lines and suggested that the chloroform extract of the plant showed necrotic activity

against the [24-27]. Adham et al. [28] studied the effects of chloroform and ethyl acetate fractions of *F. officinalis* on CCRF-CEM, CEM/ADR 5000, NCI-H929, OPM-2 cell lines. According to their results, they discovered that the chloroform fraction of the plant was more effective against CCRF-CEM, CEM/ADR 5000 and NCI-H929 cell lines, and the ethyl acetate fraction was more effective against the OPM-2 cell line. Celik et al. [22] conducted research on Hep3B and HepG2 cell lines using methanolic extracts of *F. parviflora* and *F. capreolata* species; as a result, while no serious cytotoxic activity was determined on the cell lines studied, when compared in terms of DPPH scavenging activity and total antioxidant capacity, *F. parviflora* methanol extract was observed to have high activity in DPPH free radical scavenging and total antioxidant capacity. Similarly, Yılmaz Sancar [33] revealed the rich chemical content of *F. asepala* and *F. schleicheri* subsp. *microcarpa* taxa and found that methanol extracts had antioxidant effect at a concentration of 1 mg/ml.

Paltinean et al. [18] determined the antioxidant, anticholinesterase, and cytotoxic potential of the F. schichleiri species by evaluating its alkaloid content. According to the results of their study on BJ and DLD-1 cell lines, they discovered that it did not have any cytotoxic activity against the studied cell line. Sattari et al. [29] conducted research on human breast cancer by combining AgNP (silver nanoparticles) and *F. parviflora* extract and showed that the resulting new compound had strong cytotoxic activity and could be used as strong potential nanodrugs against cancer. In the study done by Aljanaby [30], the antiparasitic, antimicrobial and cytotoxic potential of *F. officinalis* alcoholic extract and alkaloids extracted from the plant were studied. According to his results, alkaloids isolated from *F. officinalis* were more effective than alcoholic extract and showed strong effects as antiparasitic, anticancer and antibacterial agents.

Sadaoui et al. [31] researched the cytotoxic activity of protopine isolated from the F. agraria plant on two different breast cancer (MCF-7 and MDA-MB-231) cell lines and both of the cell lines examined displayed cytotoxicity on the examined cell lines. They also reported at the same time that MCF10A did not show any positive or negative effects on healthy breast cells. The same researcher later [32] evaluated the same extract on two different lung cancer (NCI-H23 and NCI-H460) cell lines and showed that it had strong cytotoxicity on both of the cell lines examined for protopine.

In conclusion, the cytotoxic activity of *F. asepala* and *F. schleicheri* subsp. *microcarpa* taxa was reported for the first time in this study. The genus *Fumaria* is a single-year herbaceous genus and has a cosmopolitan distribution. These plants that grow in areas such as fields, roadsides and slopes up to 700 m above sea level are not plants with special demands. Despite this, they contain a large amount of valuable compounds such as alkaloids, salt, and tannins, and are also a source of fumaric acid. All these compounds they possess and how easily they grow make them medically valuable. According to the study we conducted as well as the studies that already exist in literature, we can claim that the compounds in the *Fumaria* species are very valuable and can be used as potential therapeutics and as a source of raw materials in antiproliferative studies.

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