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ANTIOXIDANT, ANTICANCER ACTIVITY AND CHEMICAL COMPOSITION OF *FOENICULUM VULGARE* MILL. METHANOLIC EXTRACT*

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This current study investigated the antioxidant, anticancer, and chemical profiles of methanolic extract of *Foeniculum vulgare* Mill. aerial parts. Quantitative analysis demonstrated that *F. vulgare* extract contained 107.84 mg GAE/g and 78.18 CE/g total phenolics and flavonoids respectively. The extract of *F. vulgare* was derivatized with trimethylsilyl (-TMS) for gas chromatography-mass spectrometry (GC-MS) analysis and chemical composition was evaluated. Forty-three compounds were identified in the methanol extract (72.4%). The major compounds of the extract were D-Mannitol, 6TMS derivative (7.7%), and Sucrose, 8TMS derivative (7.1%). Carbohydrate derivatives (43.8%) were the dominant group of the extract. The methanol extract demonstrated potent antioxidants; DPPH, FRAP, and ABTS with a 28.7, 69.63 and 81.83 mg TE/g, respectively. The methanol extract demonstrated dose-dependent cytotoxicity against C6 glioma cells, showing significant viability reduction at 400 µg/mL with an IC_{50} of 128.42 µg/mL, while exhibiting minimal toxicity toward L929 normal fibroblasts. This selective anticancer activity aligns with bioactive flavonoids and carbohydrate derivatives, potentially enhancing drug solubility and targeting efficacy. The results position *F. vulgare* as a promising source of selective anticancer agents, particularly for gliomas, with its unique carbohydrate profile warranting further mechanistic and preclinical exploration.

Keywords: *Foeniculum vulgare,* GC-MS, Antioxidant Activity, Phenolics, Cancer cell line, Cytotoxicity.

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Introduction

Cancer is a broad term encompassing a large group of diseases capable of affecting any part of the body, with over 277 identified types [1]. Globally, cancer ranks as the second leading cause of death, following cardiovascular diseases. According to the World Health Organization (WHO), approximately 20 million new cancer cases were reported in 2022, resulting in 9.7 million deaths [2]. It is anticipated that nearly 1 in 5 individuals will develop cancer during their lifetime. Lung cancer is the most common form of cancer worldwide, followed by female breast cancer, and colorectal cancer ranks third [3]. The high mortality rates associated with cancer have made it a primary focus of biomedical research. Given the significant potential of herbal medicine in cancer research.

F. vulgare, commonly known as fennel, is a perennial herb belonging to the Apiaceae family. Due to its culinary and medicinal significance, fennel is extensively cultivated in various parts of the world, including the Mediterranean region and Western Asia [4]. Fennel has been utilized for its pharmacological properties, particularly

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in treating various diseases, and its diverse bioactive components contribute to its therapeutic potential, which includes antibacterial, antifungal, antioxidant, anti-inflammatory, hepatoprotective, and antidiabetic activities [5]. Among these, the anticancer potential of fennel has gained significant attention in recent years.

Although antioxidants play a critical role in combating oxidative stress and reducing cancer progression, the use of synthetic antioxidants has raised significant concerns due to their potential toxicity and long-term side effects. Many synthetic compounds have been associated with harmful biological responses, limiting their clinical use in oncology. In this context, plants have emerged as a promising and safer alternative source of natural antioxidants. Plant-derived antioxidants, rich in polyphenols, flavonoids, and other bioactive molecules, offer potent free radical scavenging activities with low toxicity profiles. In addition, due to their ability to regulate key cellular pathways involved in cancer development, plant-based compounds have become attractive candidates for holistic cancer prevention and treatment strategies. *F. vulgare* has been valued for its antioxidant, anti-inflammatory, antimicrobial, and anticancer activities. This plant is particularly rich in bioactive constituents, including flavonoids, phenolic acids, and volatile oils (most notably anethole) which contribute to its potent free radical scavenging and cytoprotective effects [6, 7]. Recent research has further emphasized the potential of *F. vulgare* extracts in modulating oxidative stress and inhibiting cancer cell proliferation, underscoring its promise as a natural therapeutic agent in oncology [8, 9]. Given its favorable safety profile and broad spectrum of biological activities, *F. vulgare* remains a significant focus of scientific investigation as a potential source of novel compounds for integrative health and therapeutic applications.

Anethole, the primary active compound in fennel seeds, has been extensively studied for its anticancer properties [10]. Anethole effectively suppresses tumor necrosis factor (TNF)-dependent responses, which are essential in malignancy and inflammation. Studies have demonstrated that fennel extracts, prepared with ethanol, significantly induce apoptosis in cancer cells, including leukemia and breast cancer cell lines. This apoptotic effect is mainly due to lipid peroxidation regulation, the enhancement of the antioxidant defence system enhancement, and the elimination of free radicals. In addition to anethole, fennel contains many phenolic compounds, including *trans*-anethole, estragole, fenchone, and quercetin, which contribute to its potent antioxidant activity [5, 11]. These compounds play a crucial role in retarding lipid peroxidation, thereby protecting cells from oxidative stress and potential damage. Various studies support the idea that the high content of phenolic compounds in fennel is associated with its antioxidant capacity [4, 12]. *In vivo* and *in vitro* studies of 75% ethanol extract of fennel have shown inhibitory effects on hepatocellular carcinoma cells by reducing cell viability, inducing apoptosis, and effectively inhibiting cell migration, all while exhibiting no apoptotic toxicity in healthy liver cells, which indicates its safety and selectivity for cancerous cells [13]. Fennel's methanol extract has also been shown to significantly reduce the growth of breast cancer cells by enhancing the antioxidant defense system and neutralizing free radicals. The quality and effectiveness of fennel extracts highly depend on the extraction methods and solvents used, as these can affect the concentration of bioactive compounds and their health benefits [4, 14].

This study aims to further investigate the anticancer potential of *F. vulgare* by evaluating its cytotoxic effects on various cancer cell lines and characterizing its chemical composition through GC-MS analysis.

Materials and methods

Plant material and extraction. Aerial parts of *F. vulgare* were collected from Ikitelli-Başakşehir, İstanbul, Turkey, during June 2017. The plant specimen authenticated and deposited in the Marmara University Herbarium (MARE20233). The plant was dried in shadow under appropriate conditions in laboratory. The dry plant materials were crushed and powdered. The plant was extracted with methanol (99.8% purity, Merck) by the maceration method (three times for three days). The extract was concentrated at 45 °C under reduced pressure using a rotary evaporator (Heidolph) to give crude methanol extracts. Previous studies have recommended methanol, ethanol, or hydro-alcoholic extracts for the activities of *F. vulgare* [10, 13, 15]. For this reason, the methanol used as solvent for the extraction.

Gas chromatography-mass spectrometry (GC-MS). The methanol extract of *F. vulgare* was derivatized with trimethylsilyl (-TMS) for gas chromatography-mass spectrometry (GC-MS) analysis. Briefly, 50 µL of chloroform and 50 µL of MSTFA were added to the extract. After vortexing for 20 s, it was incubated for 60 min at 65 °C. The methanol extract was analyzed by GC-MS using a non-polar column: HP-5MS (5% phenyl, 95% methyl polysiloxane; 30 m × 0.25 mm, 0.25 m film thickness). The oven temperature was programmed as follows: isothermal at 60 °C for 1 min, then increased to 246 °C at a rate of 3 °C $min^{-1,}$ and subsequently held isothermal for 30 min.

Carrier gas was helium, with a flow rate of 0.9 mL min^{-1}. The methanol extract was injected (1 µL) in splitless mode. The compounds were identified by comparing the relative retention indices of *the n*-alkane series to the literature and by comparing the mass spectra (NIST17 Mass Spectra library) [16].

Determination of total phenolic content (TPC). Quantified using the Folin-Ciocalteu (FC) assay, a redox-based method that measures electron transfer from phenolic antioxidants to phosphomolybdic/phosphotungstic acid complexes under alkaline conditions. Briefly, 2.5 µL of the extract was added to 195 µL of distilled water, followed by sequential addition of 2.5 µL of FC reagent and 50 µL of 7% sodium carbonate (Na_2CO_3) to adjust the pH to ~10. The reaction mixture was incubated in darkness for 30 minutes, and the resulting blue chromophore was measured spectrophotometrically at 765 nm. Results were standardized as gallic acid equivalents per gram of extract (mg GAE/g) using a calibration curve [17, 18].

Determination of total flavonoids content (TFC). TFC was determined via aluminum chloride colorimetry as described by Dewanto et al. [19] with modifications. Briefly, 250 µL of extract was reacted with 75 µL of 5% $NaNO_3$ (w/v) for 5 min, followed by addition of 150 µL of 10% $AlCl_3$ (w/v). The mixture was vortexed (30 sec), then alkalinized with 500 µL of 1 M NaOH. After 30 min dark incubation (25±1 °C), absorbance was recorded at 510 nm. TFC was expressed as catechin equivalents (mg CE/g extract) based on a standard curve prepared with serial dilutions of (+)-catechin.

In vitro antioxidant assays. The hydrogen atom donation capacity was evaluated using the stable DPPH (2,2-diphenyl-1-picrylhydrazyl) radical (1 mM in methanol). Test samples (100 µL) were reacted with 2.9 mL DPPH solution (final conc. 0.1 mM) under light-protected conditions (30 min, 25 °C). Absorbance decay at 515 nm was quantified against a Trolox standard curve, with results expressed as Trolox equivalents per gram extract (mg TE/g) [20]. The ABTS (2,2′-azino-bis (3-ethylbenzothiazoline-6-sulfonic acid)) radical cation was generated by oxidizing 7 mM ABTS with 2.45 mM $K_2S_2O_8$. The solution was reacted with samples (1 : 20 v/v) for 6 min. Electron/hydrogen transfer capacity was measured spectrophotometrically at 734 nm and calibrated against Trolox. Reducing power was assessed using the Fe^{3+}-TPTZ complex (300 mM acetate buffer pH 3.6, 10 mM TPTZ, 20 mM $FeCl_3 \cdot 6H_2O$). Samples were incubated with FRAP reagent (3 mL, 37 °C, 10 min), and Fe^{2+} reduction was monitored at 593 nm. Data were normalized to a Trolox standard following Benzie & Strain [21].

Generation of cell lines. The mouse fibroblast cells (L929), human breast adenocarcinoma cells (MCF7), human lung adenocarcinoma cells (A549), and rat glioma (C6) cell lines used in the experiments were cultured in a medium formed by adding 10% Fetal Bovine Serum (FBS) to RPMI-1640 (Roswell Park Memorial Institute-1640) medium. The cells were propagated in the medium using 100 U/ml penicillin and 100 mg/ml streptomycin at 37 °C, 5% CO_2.

MTT Cytotoxicity Assay. The cell viability is assessed with MTT, a tetrazolium salt. This salt is specific to the succinate dehydrogenase enzyme found in the mitochondria of living cells and is based on the principle that this enzyme breaks down the tetrazolium ring of the MTT dye and forms water-insoluble formazan salts. This formation is only seen in living cells with active mitochondria. The value obtained by dissolving water-insoluble formazan crystals with DMSO and measuring them in a spectrophotometer indicates the number of living cells. Since the MTT reagent is light-sensitive, the experiments were carried out without direct exposure to light. After the cells were removed from the flask's surface, the cell suspension formed was centrifuged, and the cell pellet was collected. The pellet was resuspended in the medium, seeded in a 96-well plate at $1 \cdot 10^5$ cells/well, and incubated overnight at 37 °C.

On the second day, the cells in the 96-well plates were exposed to the test substances in 4 different concentrations (50–400 mg/mL) in 3 repetitions and incubated for 24 hours. After the 24-hour incubation period, 5 mg/ml of powdered MTT prepared in PBS was added to each well of the 96-well plates. After incubation for 4 hours, 100 µl of DMSO was added to dissolve the formazan crystals and measured at 570 nm with a spectrophotometer. Three independent experiments were conducted. The data repeated 3 times for each substance were compared, the concentration-dependent graph was drawn, and the relative % cell viability was determined. At the end of the measurement, the percentage proliferation value was calculated using the following equation:

$$\text{Viability (\%)} = 100 \text{ X (Absorbance of treated cells - Blank) / (Absorbance of control cells - Blank)} \quad (1)$$

Statistical analysis. Data were expressed as mean or fold changes ± standard deviations (SDs). Student t-test determined statistical significance, and GraphPad Prism 9.0 statistical software was used for IOS. p values indicate the probability of the means compared, being equal with * $p<0.05$, ** $p<0.01$, *** $p<0.001$, **** $p<0.0001$.

Results and Discussion

The methanol extract of *F. vulgare* was derivatized with trimethylsilyl (-TMS) for gas chromatography-mass spectrometry (GC-MS). Forty-three compounds were identified in the methanol extract (72.4%). The major compounds of the extract were D-Mannitol, 6TMS derivative (7.7%), and Sucrose, 8TMS derivative (7.1%). Carbohydrate derivatives (43.8%) were the dominant group of the extract (Table 1).

In this study, the detection of carbohydrate derivatives differs from some studies in the literature. The main reason for this difference is most likely the analytical methods used. In our study, the analysis of TMS derivatives has been optimized to identify polar compounds such as sugars. In contrast, in studies where volatile oils are analyzed directly or different methods are used, such non-volatile components are generally not detected. Therefore, the chemical profile presented here, which also includes carbohydrates, is a unique result of the derivatization method we used. Furthermore, natural variations observed in the composition depending on the geographical region where the plant grows and the cultivation conditions may also contribute to quantitative differences in common components. According to a study from Saudi Arabia, the methanol extract of fennel contains *trans*-anethole (31.49%), 2-pentanone (25.01%), fenchone (11.68%), and benzaldehyde-4-methoxy (8.01%) as major components [22]. These compounds were not detected in the current study's methanol extract. In another study, fatty acid derivatives, *n*-alkane derivatives, phenolic, and terpene groups were determined in fennel seed ethanol extract from Egypt [14]. Fatty acid derivatives were common in previous and present studies, but quantitative differences exist. The other group of the previous research was not found in the current study.

Fennel seeds' extract and essential oil composition may vary depending on geographical regions. *F. vulgare* seeds may have genetic differences in different countries. These genetic differences reveal that the plant has distinct varieties, species, and subspecies. Additionally, the variations of the extract ingredients and composition may be correlated with environmental factors such as temperature, humidity, and photoperiod. The quantitative composition of the extracts can be related to plant age and harvesting time.

Mannitol ($C_6H_{14}O_6$) is a sugar alcohol. The primary usage of mannitol is as an osmotic diuretic with strong dehydrating effects. Also, it is a hydroxyl scavenger that can decrease cisplatin-induced cytotoxicity by inhibiting oxidative stress. It can cause kidney damage [23]. Sucrose is found in all plant tissues. It is a common carbohydrate. The solubility of caffeic acid phenethyl ester (CAPE) is low in water. Sucrose fatty acid ester (SFAE) was used to nanoencapsulate CAPE in aqueous propylene glycol (PG) and improved the CAPE's cytotoxicity against breast cancer colon cells [24]. Various carbohydrate derivatives can be found in natural sources. These carbohydrate derivatives contain polyhydroxy groups, which increase water solubility and interaction between receptors and guests for molecular recognition. This property plays an essential role in drug design. Carbohydrate derivatives can be starting materials for the new compound synthesis with biological activity [25].

The methanolic extract of *F. vulgare* exhibited significant levels of phenolic and flavonoid compounds, as well as strong antioxidant activity. The TPC was found to be 107.84±1.61 mg GAE/g, while the TFC was 78.18±1.22 mg CE/g. These results indicate that *F. vulgare* is a rich source of bioactive compounds, which may contribute to its therapeutic potential. The DPPH radical scavenging activity was 28.73±1.36 mg TE/g, while the ABTS assay showed a higher value of 69.63±1.84 mg TE/g. The FRAP assay, which measures reducing power, yielded 81.83±0.98 mg TE/g, suggesting that the extract has a strong ability to donate electrons and neutralize free radicals (Table 2).

The obtained results align with previous studies on *F. vulgare*, though variations exist due to differences in extraction methods, plant origin, and growing conditions. For instance, a study by Singh et al. [26] reported a TPC of 44.11 mg GAE/g in a methanolic extract of *F. vulgare* seeds, which is dramatically lower than our findings. Another study by Beyazen et al. [27] found a TFC of 11.78 mg CE/g, which is also less than our observed value. These differences could be attributed to variations in solvent polarity, extraction time, or plant maturity. Regarding antioxidant activity, our DPPH result was higher than that reported by Mousavi et al. (2019) (11.7 mg TE/g) [28], also our ABTS value was higher than findings from Kamiloglu et al. [29], who reported ABTS scavenging in *F. vulgare* seeds. The high FRAP value further supports the potent reducing capacity of the extract, which is in agreement with Pencheva et al. [30], who highlighted antioxidant power in fennel aqueous extracts.

L929, MCF7, A549, and C6 cell lines were likely chosen in this study to evaluate the anticancer potential of *F. vulgare* against different cancer types. However, including normal cells for comparison would provide better insight into the selectivity and safety of the extracts. The data are expressed as percentage cell viability compared to the control. The control group shows 100% cell viability. The concentration of *F. vulgare* is 100 µg/mL. *F. vulgare* extract appears to exert selective cytotoxic effects, particularly against cancer cell lines, while having limited toxicity on normal fibroblast cells (L929). Among the tested cell lines, C6 glioma cells are the most sensitive to the treatment. These findings suggest the potential of *F. vulgare* as a candidate for anticancer therapy, especially for glioma (Fig. 1).

Table 1. Chemical composition of methanol extract of *F. vulgare* aerial part

RT[1]	RRI[2]	RRI[3]	Compounds	(%)	IM[4]
10.002	1045	1095	L-Alanine, 2TMS derivative	0.4	RI, MS
11.837	1093		L-Proline, trimethylsilyl ester	0.2	RI, MS
13.096	1220	1128	L-Valine, 2TMS derivative	0.8	RI, MS
14.835	1283		Glycerol, 3TMS derivative	3.2	RI, MS
15.280	1299		L-Proline, 2TMS derivative	3.9	RI, MS
15.680	1314	1323	Butanedioic acid, 2TMS derivative	0.6	RI, MS
16.300	1338	1344	Glyceric acid, 3TMS derivative	0.2	RI, MS
17.064	1367	1342	Serine, 3TMS derivative	0.2	RI, MS
17.762	1393	1367	L-Threonine, 3TMS derivative	0.4	RI, MS
20.358	1497		Malic acid, 3TMS derivative	2.7	RI, MS
20.540	1504		Hexanedioic acid, 2TMS derivative	0.2	RI, MS
20.754	1513	1512	Erythritol, 4TMS derivative	0.4	RI, MS
20.940	1521	1528	meso-Erythritol, 4TMS derivative	0.4	RI, MS
21.034	1525	1520	L-5-Oxoproline, 2TMS derivative	2.0	RI, MS
23.397	1627	1636	L-Phenylalanine, 2TMS derivative	0.3	RI, MS
23.727	1641	1677	D-(+)-Ribono-1,4-lactone (R,S,R)-, 3TMS derivative	0.2	RI, MS
24.389	1670	1682	Ribonic acid, 2-desoxy-tetrakis-O-(trimethylsilyl)-	0.2	RI, MS
25.328	1712	1698	Adonitol, pentakis(trimethylsilyl) ether	0.8	RI, MS
25.884	1738	1748	Xylitol, 5TMS derivative	2.1	RI, MS
26.969	1789	1769	D-Fucitol, 5TMS derivative	0.3	RI, MS
27.695	1824	1813	D-(-)-Tagatofuranose, pentakis(trimethylsilyl) ether (isomer 2)	3.0	RI, MS
27.871	1832	1841	D-(+)-Talofuranose, pentakis(trimethylsilyl) ether (isomer 2)	4.6	RI, MS
28.522	1864	1852	D-Allofuranose, pentakis(trimethylsilyl) ether	0.9	RI, MS
28.837	1879	1863	Quininic acid (5TMS)	3.0	RI, MS
29.446	1910	1924	α-D-Glucopyranose, 5TMS derivative	1.9	RI, MS
30.071	1942	1965	L-Tyrosine, 3TMS derivative	0.3	RI, MS
30.254	1951	1969	D-Mannitol, 6TMS derivative	7.7	RI, MS
30.398	1959	1979	D-Glucitol, 6TMS derivative	3.2	RI, MS
30.725	1975	1960	D-(+)-Glucosamine, 6TMS derivative	0.9	RI, MS
31.266	2003	1970	β-D-Glucopyranose, 5TMS derivative	3.6	RI, MS
31.867	2035		Palmitic Acid, TMS derivative	3.8	RI, MS
33.667	2133		Caffeic acid, 3TMS derivative	0.8	RI, MS
33.776	2139	2145	1-Octadecanol, TMS derivative	0.2	RI, MS
34.154	2160	2162	Phytol, TMS derivative	0.2	RI, MS
34.798	2196	2212	Linoleic acid trimethylsilyl ester	2.5	RI, MS
34.920	2203	2218	α-Linolenic acid, TMS derivative	1.3	RI, MS
35.300	2226	2248	Stearic acid, TMS derivative	0.6	RI, MS
41.197	2556		β-D-Lactose, (isomer 1), 8TMS derivative	2.5	RI, MS
41.626	2576	2598	2-α-Mannobiose, octakis(trimethylsilyl) ether (isomer 1)	0.6	RI, MS
42.737	2621	2616	D-(+)-Trehalose, octakis(trimethylsilyl) ether	1.4	RI, MS
44.277	2677		Sucrose, 8TMS derivative	7.1	RI, MS
44.514	2685	2693	D-(+)-Turanose, octakis(trimethylsilyl) ether	2.1	RI, MS
44.733	2693	2707	Maltose, octakis(trimethylsilyl) ether, methyloxime (isomer 1)	0.7	RI, MS
Fatty acid derivatives				11.7	
Carbohydrate derivatives				43.8	
Amino acid derivatives				8.5	
Others				8.4	
Total identified compounds				72.4	

[1]RT: Retention time; [2]RRI: Relative retention time; [3]IM: Identification method

Table 2. Total polyphenolic content (TPC and TFC) and free radical scavenging and reducing power potential of *F. vulgare* methanolic extract

Parameter	Result (mean ± SD)
TPC (mg GAE/g)	107.84±1.61
TFC (mg CE/g)	78.18±1.22
DPPH (mg TE/g)	28.73±1.36
ABTS (mg TE/g)	69.63±1.84
FRAP (mg TE/g)	81.83±0.98

Figure 2 shows both assessing cell viability following treatment with *F. vulgare* in C6 cell line. As the *F. vulgare* concentration increases from 400 µg/mL to 50 µg/mL, the cell viability decreases significantly, particularly at 400 µg/mL, where viability is much lower than the control group. The *F. vulgare* extract demonstrated significant cytotoxicity, especially against the C6 glioma cell line, as indicated by its substantial reduction in cell viability. The results align with those found by Shah et al. [31], who reported that *F. vulgare* exhibits significant antiproliferative effects on various cancer cell lines, including breast cancer and glioma, due to its bioactive compounds, such as flavonoids and essential oils.

Our findings are consistent with the body of research that highlights the potential of plant-derived compounds as alternative or adjunctive treatments for cancer. In particular, plants like *F. vulgare* contain various bioactive compounds that have been shown to exert anti-cancer effects through mechanisms such as apoptosis induction, cell cycle arrest, and inhibition of angiogenesis [31]. The dose-dependent effects of these extracts observed in our study further support their potential as chemotherapeutic agents. The selectivity of certain extracts for specific cancer cell lines, such as *F. vulgare* for glioma cells (C6), suggests that these extracts may have targeted effects, which is an advantageous characteristic for minimizing damage to healthy cells. This selective toxicity is an important consideration for the development of plant-based therapies that aim to reduce side effects associated with conventional chemotherapy [32]. Further studies on the molecular mechanisms underlying the anticancer effects of these extracts, as well as their safety profiles, are essential for their potential clinical applications.

The results of the MTT assay demonstrated a concentration-dependent cytotoxic effect of *F. vulgare* on the C6 glioma cell line, with higher concentrations leading to greater reductions in cell viability. Specifically, at 200, 100 and 50 µg/mL, cell viability began to recover but remained lower than the control, indicating that *F. vulgare* exhibits significant cytotoxic activity at higher concentrations (Fig. 2). The calculated IC_{50} value for the C6 cell line was 128.42 µg/mL (±0.02) after 24 hours, suggesting that the plant extract is highly effective against this cell type. These findings are consistent with previous studies that have demonstrated the anticancer properties of various plant extracts. For instance, a study by Khan et al. [33] found that extracts from medicinal plants, such as *F. vulgare*, exert dose-dependent cytotoxicity against cancer cell lines, likely due to the presence of bioactive compounds like flavonoids, phenolic acids, and terpenoids, which are known to induce oxidative stress and cell apoptosis in cancer cells.

In contrast, *F. vulgare* did not show significant cytotoxicity against the L929 cell line, as cell viability remained close to that of the control group across all tested concentrations (400, 200, 100 and 50 µg/mL). This suggests that *F. vulgare* may have a selective cytotoxic effect, targeting specific cancer cell lines like C6 while being relatively non-toxic to normal or less sensitive cell types, such as L929 fibroblasts. Similar observations have been reported for other plant extracts, which often exhibit selective toxicity toward cancer cells while sparing normal cells.

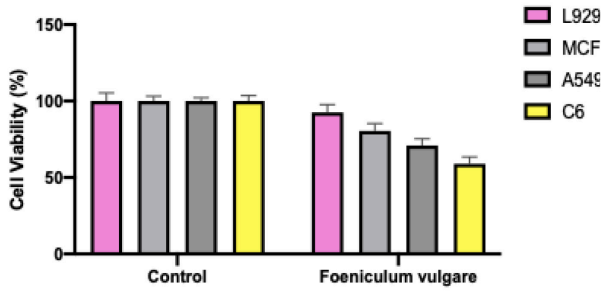

Fig. 1. Cell viability analysis was performed on cell lines using the MTT assay, following treatment with 100 µg/mL concentration of *F. vulgare*. Viability is expressed as a percentage of control

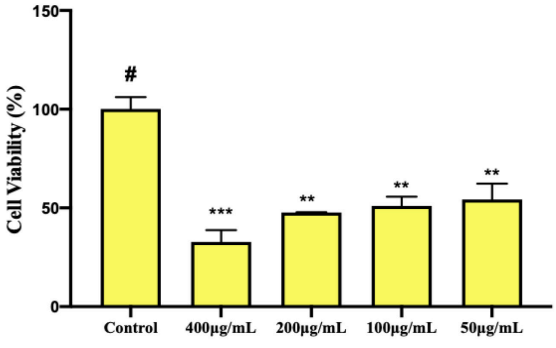

Fig. 2. Cell viability analysis was performed on a C6 cell line using the MTT assay, following treatment with various concentrations of *F. vulgare*. Viability is expressed as a percentage of control. The results show a significant decrease in cell viability at concentrations of 200, 100 and 50 µg/ml, with the most significant reduction observed at 400 µg/mL (**$p < 0.01$, ***$p < 0.001$)

Previous research has shown that *F. vulgare* contains several bioactive compounds, including flavonoids and essential oils, which have been reported to possess anticancer activity. Flavonoids, in particular have been shown to inhibit cancer cell proliferation by modulating signaling pathways involved in cell cycle regulation, apoptosis, and angiogenesis [31]. Another study demonstrated the anti-proliferative effects of the chloroform fraction of fennel on two breast cancer cell lines. The findings indicated that extract triggers apoptosis through a reactive oxygen species-mediated, mitochondrial, caspase-dependent pathway [34]. Interestingly, the lack of significant cytotoxicity in the L929 cell line suggests that *F. vulgare* might have selective anticancer effects, which is a desirable feature for developing therapeutic agents that target cancer cells without causing substantial harm to normal cells. This selective toxicity is crucial in reducing the side effects typically associated with conventional chemotherapy drugs, which can damage normal tissues and lead to undesirable side effects.

Conclusion

This study is essential in demonstrating the anti-cancer potential of *F. vulgare*. It contributes to the research on plant-based anti-cancer agents. The cytotoxic activity of extracts may be related to different extraction methods, extract concentration, extract constituents, extract storage methods, cell types and geographical regions. The chemical components of the extracts might be influenced by using different polarity solvents. Furthermore, bioactive compounds of the extract should be isolated by chromatographic techniques and checked for cytotoxic activity potential. A nano form of extract or bioactive compound should also be obtained to get maximum benefits. Based on these cytotoxicity results, although there are no similar studies in the literature on the C6 cell line, *F. vulgare* was first found to be cytotoxic at a dose of 128 µg/mL in 24h treatment. The differences between the two cell lines (C6 and L929 cell line) suggest that *F. vulgare* might have selective cytotoxicity based on the cell type. Further studies on apoptosis, autophagy, or other cellular death mechanisms should be investigated and could explore the underlying mechanisms of this selective toxicity.

The selective toxicity observed in C6 cells, along with minimal effects on L929 cells, suggests that *F. vulgare* may possess cell-type specific cytotoxicity, highlighting its potential as a candidate for targeted anticancer therapy. The differences in cytotoxicity between various cell lines may be influenced by factors such as extraction methods, solvent polarity, and the geographical origin of the plant. Future research should aim to isolate and identify the bioactive compounds responsible for its cytotoxicity using chromatographic techniques and explore their mechanisms of action, including apoptosis, autophagy, and other cellular death pathways. Furthermore, the development of nanoformulations of the extract or its bioactive compounds may enhance therapeutic efficacy. Although no similar studies on the C6 cell line exist in the current literature, these findings pave the way for further *in vivo* and clinical studies to evaluate the full therapeutic potential and safety of *F. vulgare* in cancer treatment.

Supplementary Information
The electronic supplement to the article (DOI: http://www.doi.org/10.14258/jcprm.20260117363s) provides additionalexperimental material that reveals the main points set out in the article.

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Conflict of Interest
The authors of this work declare that they have no conflicts of interest.

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