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# Chemical constituents and potential pleiotropic activities of Foeniculum vulgare (fennel) ethanolic extract; in vitro approach

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**Abstract**

Due to the growing need for more natural medicines as a result of population growth and lifestyle improvement as well as reducing unwanted side effects of fully synthetic medicines. Plants are very important sources of many valuable bioactive compounds that benefit human and animal health. So, this study aimed to investigate *Foeniculum vulgare* seeds extract to estimate its antimicrobial, antitumor, antioxidant, and antiviral activities. The extraction process was performed by grinding the plant powder in ethanol (60%). A wide range of bioactivities was evaluated as antimicrobial activity against indicator bacterial, and fungal strains followed by antioxidant activity evaluation by 2,2-diphenyl-1-picrylhydrazyl (DPPH) scavenging method and finally, MTT assay to determine antitumor activity using 5 different cell lines; the breast tumor cell line (MCF-7), cervical cancer cell line (HELA), colon cancer cell line (CACO-2), lung cancer cell line (A549), and hepatocellular carcinoma cell line (HEPG-2) in corresponding to the normal cell line (Wi-38).. As well, antiviral activity was evaluated against HSV and CoxB4. Fortunately, ethyl extract exhibited promising antimicrobial activity against the selected pathogenic microorganisms, antioxidant activity exhibit $IC_{50}$ at 28.71 µg/ml. Regarding its antitumor activity, it exhibited promising anticancer activities against all tested carcinoma cell lines and their $IC_{50}$ ranged from 248.0 to 815.1 µg/ml in corresponding to 828.1 µg/ml for the normal cells reflecting a potential anticancer potential. Regarding antiviral activities of fennel extract, it showed moderate antiviral activities 21.95% and 13.14% against the HSV and CoxB4 viruses respectively. Conclusively, fennel exhibited promising results as antitumor agents with significant activity as antimicrobial, antioxidant, and antiviral which encourages us to recommend the administration of fennel as a drink or food to protect the body and provide it with valuable compounds.

*Keywords*: GC-MS; *Foeniculum vulgare*; Antimicrobial; antioxidant; antitumor; antiviral.

## 1. Introduction

Traditionally, plants were used as a defense line for human health against various diseases in order to their inspiring pharmacological activities particularly infectious diseases, plants possess diverse antimicrobial activity [1]. *Foeniculum vulgare* is generally so-called fennel that was listed as a member of the family Apiaceae, Fennel is principally used as a carminative agent but also it has antibacterial, antifungal, antioxidant, anti-inflammatory, hepato-protective activity. Various studies revealed that *F. vulgare* has other pharmacological actions like antidiabetic, and antineoplastic [2]. *F. vulgare* (Fennel) is a widely acclaimed medicinal plant used to treat more than 40 medical conditions. *F. vulgare* contains various substances, namely, phenolic compounds, fatty acids, amino acids, flavonoids, and volatile compounds which are responsible for various medicinal properties. Literature evidence with a compilation of various data shows the antioxidant, antimicrobial, antiviral, antiplatelet, antithrombotic, and anticancer properties of fennel [3]. Fennel seeds were proven to be an effective renoprotective agent in an animal study conducted on polycystic ovary syndrome rats [4]. Fennel seeds can be used prophylactically as well as curatively in the treatment of urolithiasis [5]. Fennel is an essential component of several industrial applications that range from food to cosmetics to pharmaceutical products. It is used for flavoring, as a spice, and the oil of fennel is a key

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ingredient in liquors, toothpaste, and as a flavoring agent in different food products. It is used as folk medicine in different countries [6]. Fennel extracts exhibit anti-cancer effects on malignant tumors such as skin cancer and prostate cancer. However, the anti-tumor activity of *F. vulgare* and its underlying molecular mechanisms towards HCC is unknown. Here, we provide fundamental evidence to show that the 75% ethanol extract of *F. vulgare* seeds reduced cell viability [7].

Preliminary phytochemical screening confirmed the occurrence of flavonoids, tannins, saponins, steroids, glycosides, terpenoids besides its antimicrobial activity due to its potential essential oil constituents [8] as well as several pharmacological advantages through its bioactive constituents that are very important for human health [9].

Many previous articles had extensively studied the pleiotropic activities of fennel using different solvents, and all of them recommended methanol, ethanol, or hydro-alcoholic extracts [6-8] so, the current study aimed to assess the ability of ethyl extract (dissolved in 60% ethyl alcohol) of fennel seeds to inhibit the growth of a wide range of indicator microorganisms including Gram-positive bacteria, Gram-negative bacteria, yeasts, and filamentous fungi. Additionally, antioxidant, antitumor, and antiviral activities would be also reported. Chemical constituents would be predicted by GC-MS as a preliminary characterization tool.

## 2. Experimental

### 1. Plants collected

Seeds of fennel plant were online purchased through Al-Shark Office for Scientific Services, Alkasr Al-Ainy St. Cairo-Egypt. The voucher number was (10951), and the seeds had been finely ground by aggressive blender to a fine powder.

### 2. Extraction by ethanol 60%

Twenty grams of the plant powder was extracted by grinding in 60% ethanol, macerated plant powder was kept on the working bench at room temperature to permit solvent evaporation until complete dryness was measured by reaching to constant weight.

### 3. Antimicrobial activity of fennel extract

Agar well diffusion technique of ethyl fennel seed extract against 12 test microorganisms including Gram-positive bacteria, Gram-negative bacteria on Mueller-Hinton agar medium (code; CM0337, Thermo Scientific, Oxoid Microbiology products), and fungal strains including yeasts and filamentous fungi on malt extract agar MEA medium (code; CM0059, Thermo Scientific, Oxoid Microbiology products). Gentamycin was used as a positive control antibacterial and ketoconazole as a positive control antifungal [10].

### 4. Antioxidant activity depending upon DPPH assay

Freshly prepared (0.004% w/v) methanol solution of DPPH was prepared and stored at 10 °C in the dark. A 3 ml of freshly prepared DPPH solution was added to 40 ul of seed extract with well shaking, absorbance was recorded immediately with a UV-visible spectrophotometer (Milton Roy, Spectronic 1201). The reduction in absorbance at 515 nm was recorded at 1 and 16 min in corresponding to the control and ascorbic acid as a reference standard. All the determinations were performed in three replicates and averaged [11].

### 5. Antitumor activities assessment against 5 different cell lines

In vitro determination of antitumor activities of fennel ethanolic extract against 5 different cell lines; the breast tumor cell line (MCF-7), cervical cancer cell line (HELA), colon cancer cell line (CACO-2), lung cancer cell line (A549), and hepatocellular carcinoma cell line (HEPG-2) in corresponding to the normal cell line (Wi-38) to give a satisfactory impression of cytotoxicity the fennel ethanolic crude extract. MTT protocol was applied to evaluate cytotoxicity as follow; inoculation of 1 x $10^5$ cells/ml (100 µl/well) for each well in the 96 well tissue culture plate and incubated at 37 ˚C for 24 h, washing twice, two-fold dilutions of the tested sample were made in maintenance medium (RPMI) medium with 2% serum, added to the wells in corresponding to the negative control, incubation at 37 ˚C and examined. MTT solution was prepared (5 mg/ml in PBS) (BIO BASIC CANADA INC), 20 µl of MTT solution were added, shaking at 150 rpm for 5 min, incubate (37 ˚C, 5% $CO_2$) for 1-5 h, the media was discarded, re-suspend formazan in 200 µl DMSO, shaking at 150 rpm for 5 min, read optical density at 560 nm [12].

### 6. Assessment of antiviral activities against two viral cell lines

Assessment of antiviral activities of the ethanolic extract of fennel was achieved stepwise by detecting

the maximum non-toxic concentration (MNTC) of the extract on the Vero cell line (normal cell line) by the same MTT protocol mentioned previously, while the second step dealing with testing the effect of MNTC against both HSV, and CoxB4 viruses. Antiviral activities assessment was performed as follows; transferring 200 ul media into each well in a 96 well ELISA plate, adding $10^4$ cells in corresponding to blank and control. Incubation at 37 ˚C, 5% $CO_2$ overnight. Mixing of MNTC and viral suspension in a ratio of 1:1, and incubate this mixture for 1 h, adding 100 µl of this mixture into the wells, shaking at 150 rpm for 5 min, incubation at 37 ˚C, 5% $CO_2$ for 24 h. Adding of 20 ul MTT solution to each well, shaking at 150 rpm for 5 min, incubating at 37 ˚C, 5% $CO_2$ for up to 5 h. Throwing out the fluids by a clean towel, re-suspend formazan in 200 µl DMSO, shaking at 150 rpm for 5 min, read optical density at 560 nm.

### 7. Chemical composition forecast by GC-MS analysis

GC-MS was implemented on direct probe controller inlet part to single quadrupole mass analyzer (Thermo Scientific; GC-MS model ISQ LT - Thermo X-Caliber software). The fennel extract was applied into the injector part to go through RTX-2330 (fused silica) 30 m capillary column of 0.25 µm internal diameter and df (µm) 0.20 µm. The initial temperature of 160–250 ˚C at the rate of 5 ˚C/min and was held for 30 min. The injector and detector temperatures were 240 ˚C and 250 ˚C, respectively. Nitrogen, as a carrier gas was supplied at a flow rate of 50 ml/min with a split ratio of 20:0, and the subcomponents were identified by comparison with a linked library [1].

## 3. Results and Discussion

The dry residue of the fennel crude extract was almost 43 mg with an extraction coefficient equal to 0.17%, only 10 mg was used to be the initial concentration to carry out the MTT assay involved in both antitumor and antiviral assays. While antimicrobial and antioxidant assays involved using 15 mg of the plant extract residue.

### Antimicrobial activity determination

Bacterial and fungal resistance increased by different mechanisms so, it is very important to incorporate new members especially those belong to natural origin particularly to minimize the cytotoxic side effect [13, 14] as well as offer safe antimicrobial agents to fight the resistant invaders in different cases like human, animal and plant diseases [15, 16]. Antimicrobial activity of fennel ethanolic extract was reported in the table (1), which revealed that fennel extract showed a magnificent activity against all tested strains with a diameter of inhibition zone ranging from 11-20 mm in corresponding to 17-30 for the positive control. This result in a harmony with Rather et al. (2016) [17] who stated that the fennel essential oil exhibited antifungal activity against *Candida albicans* [18], *Aspergillus niger*, *A. flavus*, *Fusarium graminearum*, and *F. moniliforme* [19]. As well, using water base extraction methods offers a smooth and inexpensive system to get extracts with antifungal potential [20]. Regarding the strong potential antibacterial activity of fennel ethanolic extract, this result was in an accordance with Ben Abdesslem et al. (2021) [21] who reported that ethanolic extract of fennel showed strong inhibitory potential against all tested strains except *Salmonella enteritidis*.

**Table 1.** The values of inhibition zones (mm) representing the antimicrobial activity of fennel ethanolic extract against some variable test microorganisms.

| Test organisms | Fennel Extract | Positive Control |
|---|---|---|
| Filamentous fungi; Ketoconazole 100 µg (Positive control) | | |
| *Aspergillus flavus* (Local isolate) | 11 | 17 |
| *Fusarium oxysporum* (Local isolate) | 13 | 19 |
| Pathogenic yeasts; Ketoconazole 100 µg (Positive control) | | |
| *Candida albicans* ATCC 10231 | 18 | 20 |
| *Candida krusei* ATCC 14243 | 15 | 18 |
| Gram-positive bacteria; Gentamycin 4 µg (Positive control) | | |
| MRSA clinical isolate | 11 | 30 |
| *Enterococcus faecalis* ATCC 51299 | 18 | 26 |
| *Streptococcus mutans* ATCC 35668 | 13 | 20 |
| *Micrococcus luteus* ATCC 10240 | 13 | 22 |
| Gram-positive bacteria; Gentamycin 4 µg (Positive control) | | |
| *Enterobacter cloaca* ATCC 13047 | 19 | 27 |
| *Klebsiella pneumonia* ATCC 13883 | 17 | 17 |
| *Escherichia coli* ATCC 25922 | 20 | 25 |
| *Pseudomonas aeruginosa* ATCC 27853 | 14 | 17 |

**Antioxidant activity of the crude extract of *F. vulgare* seeds**

The crude ethanolic extract of fennel was evaluated as an antioxidant, and the value of $IC_{50}$ of the free radical scavenging activity was 28.71 µg/ml in corresponding to ascorbic acid which was recorded 11.2 µg/ml as $IC_{50}$. This result is in agreement with Ahmed et al. (2019) [22] who compared the potentiality of different types of fennel (Egyptian and Chinese) as an antioxidant, and he found that there are significant variations in the activity obtained from the fennel essential oils and the fennel ethanolic extract. Our findings referred to moderate antioxidant activity as evidence to demonstrate the cause of antioxidant, antimicrobial activity is belonging to the polar portion. High values of phenols and flavonoid compounds as well as an effective degree of the antioxidant capacity of the hydro-ethanolic extract of the fennel fruit have been obtained [23].

**Antitumor activity the crude extract of *F. vulgare* seeds**

Figure 1 (A-F) represents the cytotoxicity of ethanolic fennel extract as an antitumor agent against A549, HELA, CACO-2, HEPG-2, and MCF-7 whereas $IC_{50}$ values were mentioned in table (2) as follow; 347.5, 517.5, 248.0, 815.1, 474.6 µg/ml, respectively and when the same crude extract was investigated against normal cell line (Wi-38), it caused a cytotoxic effect in a value of $CC_{50}$ of 828.1 µg/ml. These findings are very promising because the value which causes killing for half number of tumor cells (in the case of A549, HELA, MCF-7) is approximately half-value which kills the half number of normal cells. While CACO-2 cell line was affected in a value of $IC_{50}$ of 248.0 µg/ml which represents approximately the quarter-value of the $CC_{50}$ of the normal cell line which leads to the feasibility of the fennel hydro-alcoholic extract as anti-colon cancer. Finally, the $IC_{50}$ value in the case of HEPG-2 is approximately the same as that belongs to the normal cell line.

This result is in harmony with the findings which reported that fennel seeds methanolic extract might have remarkable anticancer potential against MCF-7 and HEPG-2 [24]. It also found that the ethanol extract of *F. vulgare* seeds remarkably reduced lung cancer cell growth *in vitro* and *in vivo* [25]. As well, antitumor activities of fennel, asafetida, and ginseng ethanolic extracts may decrease the proliferation activity of the 4T1 cell line *in vitro* [26]. Fennel has numerous pharmacological activities and its bioactive molecules may show a significant role in human health, henceforth, it might be used for diverse drug productions [27].

**Table 2.** Effect of fennel ethanolic extract against 6 different cell lines.

| ID | Conc. ug/ml | Mean OD | Viability % | Toxicity % | $IC_{50}$ |
|---|---|---|---|---|---|
| Wi-38 | | 0.281 | 0.008 | 100.000 | ug/ml |
| Fennel | 10000 | 0.022 | 0.002 | 7.948 | 828.1 |
| | 5000 | 0.026 | 0.003 | 9.134 | |
| | 2500 | 0.051 | 0.003 | 18.268 | |
| | 1250 | 0.091 | 0.005 | 32.503 | |
| | 625 | 0.161 | 0.002 | 57.177 | |
| | 312.5 | 0.227 | 0.006 | 80.902 | |
| | 156.25 | 0.276 | 0.007 | 98.221 | |
| | 78.125 | 0.279 | 0.003 | 99.170 | |
| A549 | | 0.313 | 0.006 | 100.000 | |
| Fennel | 10000 | 0.019 | 0.003 | 5.964 | 347.5 |
| | 5000 | 0.021 | 0.001 | 6.709 | |
| | 2500 | 0.021 | 0.001 | 6.709 | |
| | 1250 | 0.025 | 0.002 | 7.987 | |
| | 625 | 0.067 | 0.009 | 21.406 | |
| | 312.5 | 0.165 | 0.005 | 52.609 | |
| | 156.25 | 0.290 | 0.008 | 92.758 | |
| | 78.125 | 0.314 | 0.007 | 100.213 | |
| HELA | | 0.235 | 0.007 | 100.000 | |
| Fennel | 10000 | 0.021 | 0.001 | 8.794 | 517.5 |
| | 5000 | 0.019 | 0.001 | 8.227 | |
| | 2500 | 0.021 | 0.001 | 8.936 | |
| | 1250 | 0.021 | 0.003 | 8.936 | |
| | 625 | 0.093 | 0.003 | 39.433 | |
| | 312.5 | 0.166 | 0.007 | 70.496 | |
| | 156.25 | 0.202 | 0.003 | 85.816 | |
| | 78.125 | 0.230 | 0.003 | 98.014 | |
| CACO-2 | | 0.290 | 0.024 | 100.000 | |
| Fennel | 10000 | 0.001 | 0.000 | 0.230 | 248.0 |
| | 5000 | 0.002 | 0.000 | 0.805 | |
| | 2500 | 0.003 | 0.000 | 1.149 | |
| | 1250 | 0.010 | 0.001 | 13.333 | |
| | 625 | 0.045 | 0.004 | 25.517 | |
| | 312.5 | 0.070 | 0.002 | 44.138 | |
| | 156.25 | 0.099 | 0.001 | 64.023 | |
| | 78.125 | 0.192 | 0.006 | 86.207 | |
| HEPG-2 | | 0.371 | 0.002 | 100.000 | |
| Fennel | 10000 | 0.030 | 0.002 | 8.086 | 815.1 |
| | 5000 | 0.037 | 0.002 | 10.063 | |
| | 2500 | 0.074 | 0.003 | 19.946 | |
| | 1250 | 0.102 | 0.007 | 27.529 | |
| | 625 | 0.215 | 0.004 | 58.167 | |
| | 312.5 | 0.296 | 0.004 | 79.730 | |
| | 156.25 | 0.302 | 0.002 | 97.543 | |
| | 78.125 | 0.374 | 0.003 | 100.719 | |
| MCF-7 | | 0.316 | 0.010 | 100.000 | |
| Fennel | 10000 | 0.010 | 0.001 | 3.270 | 474.6 |
| | 5000 | 0.014 | 0.001 | 4.430 | |
| | 2500 | 0.019 | 0.002 | 6.118 | |
| | 1250 | 0.055 | 0.003 | 17.278 | |
| | 625 | 0.142 | 0.004 | 44.895 | |
| | 312.5 | 0.184 | 0.010 | 58.186 | |
| | 156.25 | 0.258 | 0.011 | 81.751 | |
| | 78.125 | 0.312 | 0.003 | 98.629 | |
| | 39.06 | 0.320 | 0.003 | 101.160 | |
| | 19.530 | 0.318 | 0.001 | 100.738 | |

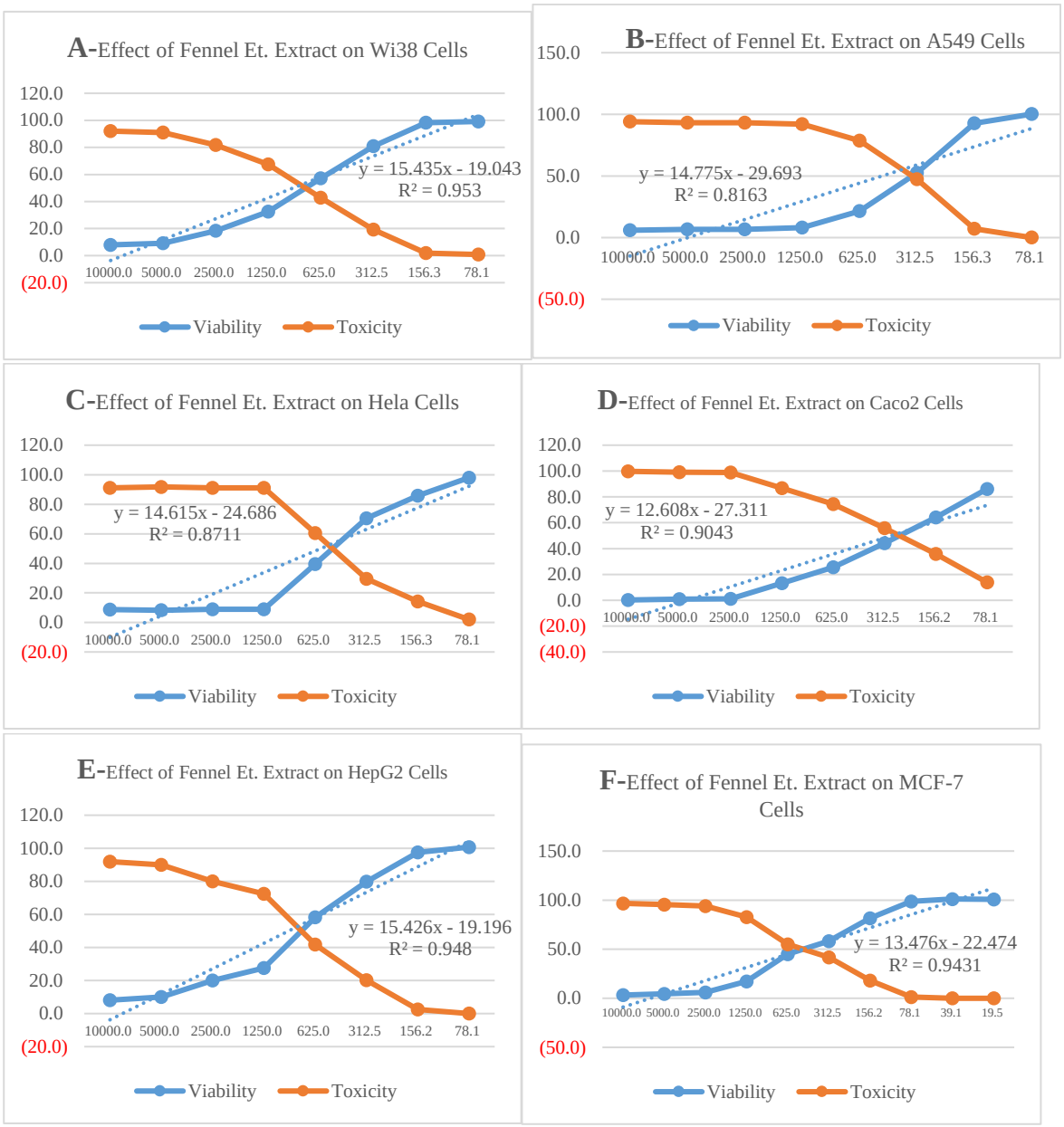


**Figure 1.** Line chart showing the effect of fennel ethanolic extract on both viability and toxicity of the tested cell lines (A-F); A-normal cell line (Wi-38), B-lung carcinoma cells (A549), C-cervical cancer cell line (HELA), D-colon cancer cells (CACO-2), E-hepatocellular carcinoma cells (HEPG-2), F- breast cancer cells (MCF-7).

**Antiviral activity assessment of the crude extract of *F. vulgare* seeds**

Figure 2 (A, B) showed the viability and toxicity responses of the two viruses were involved in this test; HSV and CoxB4. Maximum non-toxic concentrations of fennel extracted were determined as a first step. Table (3) revealed that the CC50 values were 993.8 and 1500 ug/ml respectively for HSV and CoxB4 Vero cell lines. While the MNTC was the same for both Vero cells; 156.25 ug/ml.

*F. vulgare* is officially noted in Ayurvedic Pharmacopoeia as an important part of polyherbal formulations in the treatment of different diseases and disorders. Several biological-pharmacological studies have been undertaken to evaluate the indigenous uses of *F. vulgare*. Few extracts of *F. vulgare* and isolated compounds have been evaluated for several activities [28]. It was reported that *F. vulgare* showed a high percentage of inhibition value at the maximum non-cytotoxic concentration with 83 percentage of inhibition against influenza virus [29]. It was also reported that the antiviral activity of the essential oil of fruit sample of *F. vulgare* along with 12 other Turkish medicinal plants was evaluated against the

DNA virus Herpes simplex type-1 (HSV-1) and *F. vulgare* mostly displayed strong antiviral effects against HSV-1 than the other plants included in the experiment [3, 30].

**Table 3**. Determination of MNTC of fennel ethanolic extract on two Vero cells; HSV and CoxB4.

| ID | Conc. ug/ml | Mean OD | Viability % | Toxicity % | $CC_{50}$ |
|---|---|---|---|---|---|
| HSV Vero | | 0.371 | 100.000 | 0.000 | ug/ml |
| Fennel | 10000 | 0.034 | 9.164 | 90.836 | 993.752 |
| | 5000 | 0.068 | 18.329 | 81.671 | |
| | 2500 | 0.097 | 26.146 | 73.854 | |
| | 1250 | 0.171 | 46.092 | 53.908 | |
| | 625 | 0.231 | 62.264 | 37.736 | |
| | 312.5 | 0.275 | 74.124 | 25.876 | |
| | 156.25 | 0.368 | 99.191 | 0.809 | |
| | 78.125 | 0.379 | 102.156 | 0.000 | |
| CoxB4 Vero | | 0.421 | 100.000 | 0.000 | ug/ml |
| Fennel | 10000 | 0.037 | 8.789 | 91.211 | 1500 |
| | 5000 | 0.077 | 18.289 | 81.711 | |
| | 2500 | 0.121 | 28.741 | 71.259 | |
| | 1250 | 0.238 | 56.532 | 43.468 | |
| | 625 | 0.329 | 78.147 | 21.853 | |
| | 312.5 | 0.412 | 97.862 | 2.138 | |
| | 156.25 | 0.427 | 101.425 | 0.000 | |
| | 78.125 | 0.428 | 101.662 | 0.000 | |

Table (4) revealed the influence of the MNTC of fennel extract (156.25 ug/ml) on the viral activities on their Vero cells. The results presented a promising impression in order to showing mild activities whereas HSV was reduced to 78% by mixing with fennel ethyl extract that reflected the antiviral activity of approximately 22%, while CoxB4 was reduced to 87% by adding fennel extract exhibiting approximately 13% viral activity. Figure 3 (A, B) showed the viral activities (HSV and CoxB4) on their Vero cells in the presence and absence of fennel extract corresponding to the control Vero cells.

**GC-MS analysis for the ethanolic extract of *F. vulgare***

Figure (4) displayed the GC-MS chromatography of the ethanolic extract of *F. vulgare* was analyzed by GC-MS apparatus to preliminary characterize its components. Table (5) listed the ingredients of the crude fennel ethanolic extract in which detection of 17 subcomponents their molecular weights ranged from 146 to 410 including fatty acids and their precursors, some of them were classified as ploy unsaturated fatty acids (PUFAs) like Linoleic acid. Eugenol and trans isoeugenol are classified as phenolic compounds, also squalene is alkene that belongs to isoprenoid compounds.

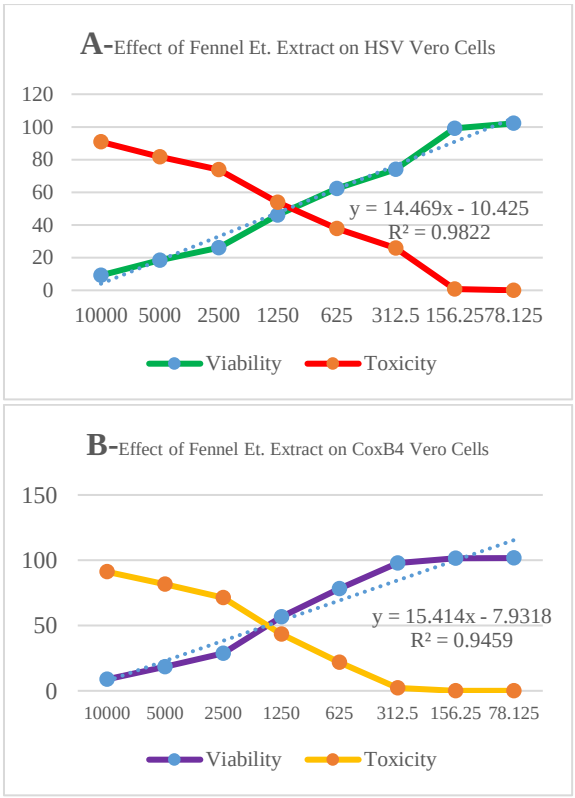


**Figure 2.** Line chart showing the effect of fennel ethanolic extract on both viability and toxicity of the tested cell lines; A-normal Vero cell line of HSV, B-normal Vero cells of CoxB4 viruses.

**Table 4.** Assessment of the antiviral activities of the MNTC of fennel ethanolic extract on two viruses; HSV and CoxB4.

| ID | MNTC ug/ml | Mean OD | viability | toxicity | Viral activity % | Antiviral effect % |
|---|---|---|---|---|---|---|
| Control Vero | | 0.371 | 100 | 0 | | |
| HSV | | 0.207 | 55.795 | 44.205 | 100 | 0 |
| Fennel Extract | 156.25 | 0.243 | 65.498 | 34.502 | 78.05 | 21.95 |
| Control Vero | | 0.421 | 100 | 0 | | |
| CoxB4 | | 0.284 | 67.458 | 32.542 | 100 | |
| Fennel Extract | 156.25 | 0.302 | 71.734 | 28.266 | 86.86 | 13.134 |

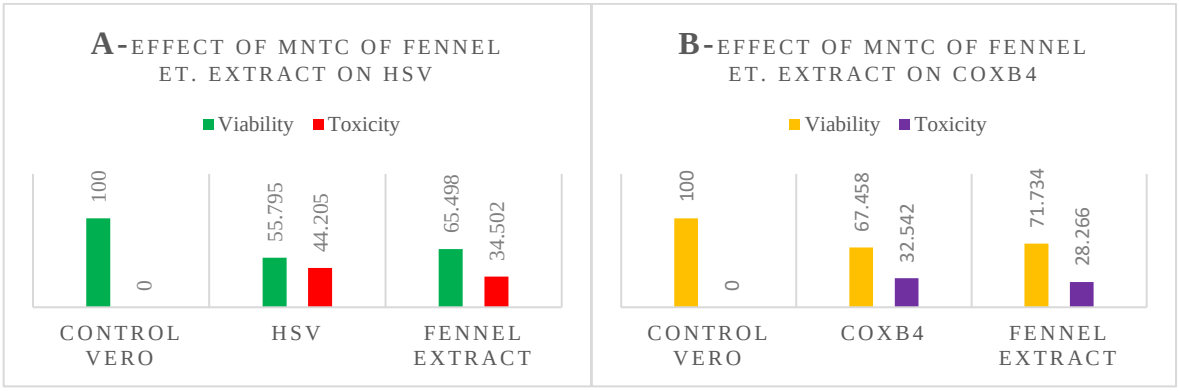


**Figure 3.** Column chart showing the viral activities in presence or absence of fennel ethanolic extract in corresponding to control Vero cells; A-response of HSV, B-response of CoxB4 virus.

It had been reported that GC-MS analysis of the essential oils of fennel and chamomile revealed the presence of 15 major monoterpenoids but their percentages in each plant were greatly different. *Trans*-anethole, estragole, fenchone, and limonene were highly abundant in all of the examined oils [31]. Fifty-seven different phytoconstituents were identified in the methanolic extract of *F. vulgare* using the GC–MS technique. The main compounds identified were trans-anethole (31.49%), 2-pentanone (25.01%), fenchone (11.68%), and benzaldehyde-4-methoxy (8.01%). Several other compounds were also identified in higher amounts and some compounds were identified in trace amounts. Many compounds have been reported for the first time in the methanolic extract of *F. vulgare*. The amount of fenchone was found to be maximum in plant extract (9.789 mg/g) in comparison with other commercial formulations by the proposed GC–MS technique. In three different commercial formulations (F, G, and H), the amount of fenchone was obtained as more than 1.0 mg/g. However, in five different commercial formulations (A, B, C, D, and E), the amount of fenchone was recorded as less than 0.1 mg/g. This method could be utilized for the analysis of fenchone contents in commercial formulations containing fenchone as an active ingredient. The results obtained in this work could be useful in the standardization of commercial formulations containing fenchone [32].

This outcome of the feasibility of the fennel hydroalcoholic extract to be recruited to serve the medicinal field with multi-advantageous properties such as antimicrobial, antitumor, antioxidant, and antiviral could lead us to deep studying other organisms rather than plants to present new natural generations of bioactive medicinal materials. Fungi, bacteria plus sea organisms could also contribute to presenting new bioactive compounds with interesting applications [33, 34].

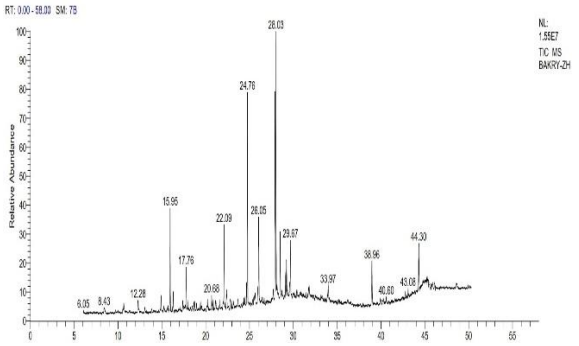


**Figure 4.** GC-MS chromatograph of the fennel ethanolic extract.

## 4. Conclusion

*Foeniculum vulgare* has a very important health value in order to the strong antimicrobial activity of its crude ethanolic extract, also, it has a significant antitumor activity especially against colon cancer cell line with low cytotoxic effect on the normal cell line, it has also a moderate antioxidant, and antiviral activity. Extraction methods, or extracting solvents had a great effect on the quality of the crude extract and its efficacy as pleiotropic activity. There is a very crucial need for antimicrobial agents to be merged to the pharmaceutical market particularly those belonging to natural origin to conquer the microbial resistance crisis, and the ongoing study presented a spectacular plant extract offering pleiotropic activity which may contribute to complementary and alternative medicine. Also, Finally, this study recommends drinking this plant may protect the

human against emerging of cancer or viral attacks, and it also could provide the body with valuable compounds that act as antioxidants and antimicrobials. Furthermore, chromatographic purification for the bioactive components and attempting to estimate its pleiotropic potentiality in vivo study as a future perspective in addition to the possibility to obtain its nano form to get maximum benefits.

**Table 5.** List of all subcomponents of the fennel ethanolic extract predicted by GC-MS.

| Compound predicted | RT | M. wt. | M. formula |
|---|---|---|---|
| Trans isoeugenol | 12.28 | 164 | $C_{10}H_{12}O_2$ |
| Eugenol | 16.31 | 164 | $C_{10}H_{12}O_2$ |
| Dihydro butyl bezodoxepin | 15.95 | 206 | $C_{13}H_{18}O_2$ |
| Hexadecanol | 17.76 | 242 | $C_{16}H_{34}O$ |
| Tridecanoic acid methyl ester | 20.68 | 256 | $C_{15}H_{30}O_2$ |
| Tetradecanoic acid methyl ester | 20.68 | 256 | $C_{16}H_{32}O_2$ |
| Retinal | 22.82 | 284 | $C_{20}H_{28}O$ |
| Hexadecenoic acid methyl ester | 24.76 | 270 | $C_{17}H_{34}O_2$ |
| Octadecenoic acid methyl ester | 28.03 | 296 | $C_{19}H_{36}O_2$ |
| 1-Eicosanol | 26.05 | 298 | $C_{20}H_{42}O$ |
| Methyl stearate | 28.50 | 298 | $C_{19}H_{38}O_2$ |
| Linoleic acid ethyl ester | 29.11 | 308 | $C_{20}H_{36}O_2$ |
| Ethyl oleate | 29.22 | 310 | $C_{20}H_{38}O_2$ |
| Docosene | 29.66 | 308 | $C_{22}H_{44}$ |
| Erucic acid | 29.67 | 338 | $C_{22}H_{42}O_2$ |
| Benzene dicarboxylic acid | 38.95 | 390 | $C_{24}H_{38}O_4$ |
| Squalene | 44.30 | 410 | $C_{30}H_{50}$ |
| **Total** | | | **17** |

## 5. Conflicts of Interest

The authors declare that they have no conflict of interest regarding this article.

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