Ultrasound-Assisted Extraction and Cytotoxic Activity of Fennel Leaves: Process Optimization and Thin Layer Chromatography

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Abstract

*Foeniculum vulgare* commonly known as fennel belonging to the family Umbelliferae, is cultivated throughout the world as a spice as well as a medicinal plant possessing antimicrobial, antidiabetic, antioxidant, anti-inflammatory and others. A Single Factor Experiment analysis has been employed in this study to optimize caffeic acid extraction from fennel leaves by ultrasound assisted extraction method in which three variables single factor examined which were ethanol concentration, extraction time and ultrasound frequency. The study's objective was to assess the effects of variables chosen on caffeic acid's extraction performance, preliminary evaluated by thin layer chromatography (TLC) and evaluate the cytotoxic effect of optimized ethanolic extract of this plant on human breast cancer (MDA-MB-468) cell line. The experimental results revealed that the most influential extraction variables were: 80% ethanol as extracting solvent, 15 min was the time to achieve the good extraction efficacy and 40 KHz ultrasound frequency and The IC50 value of the cytotoxic effect (P < 0.05) of optimized ethanolic extract of *Foeniculum vulgare* leaves on MDA-MB-468 cell line after 72 h of incubation were significantly high (46.89 µg/ml). The results of the study will be used to create a natural medicine for the symptoms of various diseases and to standardize the processes for extracting caffeic acid from natural items, particularly fennel.

Keywords: Caffeic acid, Cytotoxic activity, Fennel, Single factor experiment, Ultrasound assisted extraction.

Introduction

Plants have been shown to have significant concentrations of a number of secondary metabolites, such as phenolic compounds, flavonoids, carotenoids, terpenoids and alkaloids which serve as nutrients, antimicrobials and antioxidants that can be utilized to cure and prevent a wide range of human ailments. They are also crucial to the defensive mechanism of the plant; Particularly, polyphenolic compounds which are the most prevalent bioactive components found in edible plants. These compounds are important sources of metabolites that promote human health because they act as non-enzymatic antioxidants in cells to stop damage from oxidative stress and reduce the risk of developing various chronic diseases including cancer. For these naturally occurring bioactive compounds to be successfully obtained, extraction methods must be carefully examined, there have been several suggested techniques for isolating natural bioactive substances.
Traditional techniques including maceration, percolation, Soxhlet extraction, reflux extraction, and infusion are frequently employed, but they take a long time and require a lot of efforts. Furthermore, extracting solvents are not eco-friendly, so in order to address these issues, eco-friendly alternatives have recently been developed. (4) Examples of these eco-friendly procedures include supercritical fluid extraction, microwave-assisted extraction, and ultrasonic-assisted extraction (4.5).

Due to its usefulness and affordability, ultrasonic-assisted extraction (UAE), among other things, is very promising for the food and herbal industries, also enables for low-temperature extraction, uses less time and energy and preserves the extract's quality. (6) UAE extracts bioactive compounds from its natural sources using high-intensity sound waves in which plant interstitial tissues are damaged by the sonic cavitation produced by UAE, increasing the solvent-plant material contact area leading to a higher extraction yield of bioactive compounds compared with traditional extraction methods, saving time, cost and labor (7).

This research focuses on evaluating fennel's anticancer properties and using the UAE approach to extract phenolic components, particularly caffeic acid; due to its flavor, Foeniculum vulgare, a medicinal plant in the Umbelliferae (Apiaceae) family, has been utilized and known to humans since ancient times and was grown in practically every nation (8). It has more than 100 names in addition to the common name "fennel" with a lengthy history of usage as medicine and is a well-known and traditional herb. (9) Studies have shown that F. vulgare is capable of efficiently treating a wide range of infectious diseases with bacterial, fungal, viral, mycobacterial and protozoal origins (9-11).

It has hepatoprotective (12), cytoprotective (13), antitumor (14), anticancer (14) and hypoglycemic effects (15). According to Koppula S. and Kumar H., F. vulgare can lower stress and has a unique memory-enhancing function (16). Limited clinical trials and animal studies indicate that long-term usage of F. vulgare is not hazardous. (13) Fennel can be eaten on a regular basis in a variety of ways, including fresh in salads and snacks, cooked in stews, boiled, grilled or baked foods or even used to make herbal teas or alcoholic beverages. Due to its valuable nutritional constituents rich in polyphenolic chemicals, a diet containing the reasonable amounts of fennel may have positive effects on health (17).

Through the use of single-factor experiment analysis, preliminary chemical tests, thin layer chromatography (TLC), and cytotoxic activities that were specifically examined to validate the ideal UAE conditions for fennel leaves, this research makes a significant contribution by being the first to evaluate the application of the UAE technique to extract biologically active compounds like caffeic acid from fennel leaves crude extract, one of the plants that are most frequently used in traditional medicine and foods.

**Materials and Methods**

**Plant Collections**

Foeniculum vulgare leaves were procured in October 2021 from local markets in Baghdad/Iraq and then verified in the Department of Pharmacognosy and Medicinal Plants/College of Pharmacy/ University of Baghdad. The leaves were then subjected for series of workup, water-washed, shade-dried for two weeks at room temperature then grounded with electric blender, weighted and processed to the extraction techniques.

**Experimental work**

The experimental work was divided into sequential steps:

**Extraction of plant material**

Sixty grams of the powdered plant material was extracted by maceration in n-hexane for 2 days with frequent shaking. At room temperature, the extract was filtered off, this procedure was repeated three times for defatting purpose (10), then the plant material was left to dry and subjected to extraction by Ultrasound assisted extraction (UAE) method that was done by using probe ultrasonicator with ethanol as a solvent. The UAE was carried out under the following experimental conditions:

Plant material is directly in touch with ultrasonic waves in a probe ultrasonicator at a temperature of 25 °C, for 10 minutes, with a solvent to solids ratio of 10:1 ml/gm, 60% ethanol as extracting solvent and a sonication frequency of 40 Hz. (10). Later on, the crude ethanol extract was filtered and evaporated to dryness using a rotary evaporator, as shown in Figure 1.
The impact of each factor on fennel extraction was examined using a single-factor experiment. The other special factors remained the same, but one of them altered. Four factors (solvent/solid ratio, ethanol concentration, time of extracting, and ultrasound frequency) were reported to be different for similar phytoconstituents from different plant varieties; for all of the above a single factor experiment was chosen each time; the level gradients of each factor were: solvent/solid ratio was preset as 10:1 ratio since the study was done in small scale; ethanol concentrations were 60%, 70%, and 80%; extracting times were 5, 10, and 15 min; ultrasound frequencies were 20, 40, and 60 KHz.

Preliminary phytochemical examination of crude extract

The dried extract from crude ethanolic extract had been subjected to preliminary phytochemical assay for initial screening of possible phytochemicals applying chemical tests for detecting polyphenols and flavonoids in the extracts.

Test for phenolic

One milliliter of crude ethanolic extract was dissolved in 1 ml of 5% ferric chloride, the color was observed, a deep green to black coloration indicates the presence of phenolic acids.

Test for flavonoids

One milliliter of crude ethanolic extract was dissolved in 2 ml 1% potassium hydroxide in a test tube, and the color was observed. A yellow color indicates the presence of flavonoids.

Qualitative identification by thin layer chromatography

The optimized ethanolic extract of Iraqi Foeniculum vulgare leaves were subjected to thin layer chromatography for the qualitative estimation of phenolic acid (caffeic acid). This was done using ready-made aluminum silica gel plates GF 254, detection by UV light detector at 254 nm and 366 nm wave lengths, standard caffeic acid (sigma-aldrich, USA), and five different solvent systems for identification. The dried extract from crude ethanolic extract had been subjected to preliminary phytochemical assay for initial screening of possible phytochemicals applying chemical tests for detecting polyphenols and flavonoids in the extracts. (20, 21).

$$S_1: \text{Toluene: ethyl acetate: formic acid (9:3:1.25)}$$
$$S_2: \text{Ethyl acetate: toluene: formic acid: methanol (3:3:0.8:0.2)}$$
$$S_3: \text{Toluene: dioxan: acetic acid (9:2:4:0.6)}$$
$$S_4: \text{Toluene: chloroform: acetone: formic acid (8:4:3:3)}$$
$$S_5: \text{Chloroform: acetone: formic acid (75:16.5:8.5)}$$

Cytotoxic assay method

On the viability of the human MDA-MB-468 cancer cell line, an optimal ethanolic extract of Iraqi Foeniculum vulgare leaves was tested using the MTT colorimetric assay. The human MDA-MB-468 breast cancer cell lines were donated by ATCC. They were kept in the cell bank of the tissue culture research center at College of Pharmacy/ University of Al-Mustansiriyah. The cells were placed in liquid nitrogen storage, frozen at (80°C, 24 h), thawed at 37°C, and then given 10 ml of fresh media. The cells were obtained using centrifugation. After that, the cells were moved into a flask of 75 cm² and cultivated in 25 ml of fresh medium. A549 cells were maintained in 500 cc of RPMI-1640 Medium. Penicillin-streptomycin-ampicillin B100X as an antiseptic and 50 ml of 10% FBS from complete medium were administered as supplements. Cells were cultivated in flasks with a 75 cm² surface area. Incubated at 5% CO₂ and 95% humidity was (37 °C).

Flasks containing A549 cells were passaged under sterile conditions as soon as the cells reached 90% confluence. PBS 5ml solution was used to wash the cells. After that, cells were incubated in a trypsin solution at 37°C for two minutes to allow them to separate from the flask’s bottom. A conical tube was then filled with the cell suspension and an equivalent volume of complete growth medium (50ml). The cells were also centrifuged (3 min, 1200 rpm). The cell pellet was then resuspended in enriched growth media.
after the supernatant was discarded (fresh). In order to calculate the number of cells needed, a haemocytometer was employed under a microscope (25).

**Cell Viability by MTT Colorimetric Assay**

The vitality of MDA-MB-468 cancer cells was examined using the MTT test in order to determine the effects of the optimized ethanolic extract of Iraqi *Foeniculum vulgare* leaves. In 96-well flat-bottom tissue culture plates, cell suspensions (100 μl) were added at concentrations of 5 x 10³ cells per well and incubated for 24 hours under standard conditions, 4 x 10³ cells for 48 hours, and 3 x 10³ cells for 72 hours. After 24 hours, the cells were treated with various concentrations of the optimal (80% ethanol as extracting solvent, solvent to solids ratio of 10:1 ml/gm, temperature of 25 °C with 15 min as the time to achieve the good extraction efficacy and 40 KHz ultrasound frequency) ethanolic extract of Iraqi *Foeniculum vulgare* leaves (0.15, 0.35, 0.75, 1.52, 3.125, 6.25, 12.5, 25, 50 and 100 μM).

The cell culture medium was removed after a recovery period of 24 hours, 48 hours, and 72 hours, and cultures were then incubated for 4 hours at 37 C° with a medium containing 30 μL of MTT solution (3 mg/ml of MTT powder in PBS). This medium was removed after 4 hours by gently inverting it and taping on paper. To the control wells, just 100 μL of growth medium were introduced. Then, 100 μL of DMSO were added to each well, which were then left in the dark at room temperature for about (15-20 min) By utilizing a multi-scan reader with a wavelength of 540 nm to detect each well's absorbance and 650 nm to account for background absorption, the absorbance of each well was calculated (24,26).

The percentage of cytotoxicity, or the rate at which cell growth is inhibited, was estimated as follows:

\[
\text{Percentage Inhibition Rate} = \left( \frac{A-B}{A} \right) \times 100
\]

Where \([A, \text{ and } B]\) denoted, respectively, the optical densities of the control and tested substances.

**Determination of the Half-Maximal Inhibitory Concentration (IC₅₀)**

By creating a dose-response curve and analyzing the impact of various antagonist concentrations on reversing agonist activity, it is possible to establish the IC₅₀ of the optimized ethanolic extract of Iraqi *Foeniculum vulgare* leaves. By figuring out the concentration required to inhibit 50% of the maximum biological response of the agonist, the IC₅₀ values for a particular antagonist can be derived. The environment in which IC₅₀ values are measured has a significant impact on those results. In general, agonist activity will be decreased more as inhibitor concentration increases. As agonist concentration rises, IC₅₀ value rises. Furthermore, several factors may affect the IC₅₀ value depending on the kind of inhibition. The IC₅₀ shows the concentration of the optimum ethanolic necessary for a 50% suppression of cell viability, according to the in vitro MTT test.

Based on the results of the in vitro MTT experiment, the optimum ethanolic IC₅₀ values were determined 72 hours after the cells were exposed to the compounds. 1.52–100 μM concentrations were used (27).

**Data Analysis**

The statistical analysis of the optimized ethanolic extract of Iraqi *Foeniculum vulgare* leaves on MDA-MB-468 cancer cell was performed using the nonlinear regression curve fitting software Graph Pad Prism 8.1. One-way ANOVA with Post hoc Tukey was used to compare all groups within a single MTT plate Statistics were judged significant at \(p < 0.05\).

**Results and Discussions**

**Extraction outcome**

During last decades, aromatic plants widely used in food as herbs and spices, are extensively studied for their phenolics and volatile compounds. Among aromatic herbs and spices, fennel (*Foeniculum vulgare*) has a long history of use, both for its sensory and its functional (carminative, stomachic, antispasmodic and antimicrobial) properties (28). In spite of the importance of *Foeniculum vulgare* as an aromatic herbs and spices, the phytochemical studies concentrated on the volatile oils secondary metabolites especially fennel trans-anethole, estragole, fenchone, and limonene (29). Therefore, the chosen of phenolic compounds especially caffeic acid in this study is the main target because they have different pharmacological activities.

In this study, the first step involved as much fat removal as possible by maceration with n-hexane then the extraction was done by Ultrasound assisted extraction (UAE) probe type which is a quick, safe, and environmentally responsible method of extracting natural compounds that yields high-quality extracts (2,5). The findings revealed that probe UAE produced the higher percentage yield in the Iraqi *Foeniculum vulgare* leaves after each single optimizing factor tested with different variables so as to get to the best yielding condition for it, then they were summited with each other to verify the optimized condition (80% ethanol as extracting solvent, solvent to solids ratio of 10:1 ml/gm, temperature of 25 °C with 15 min as the time to achieve the good extraction efficacy and 40 KHz ultrasound frequency) to get the higher extraction yield which equals to 2.78 gm.

**Single factor experiment analysis**

Due to the complexity of the herbs' chemical composition and the components' affinities towards various changeable parameters, (30) numerous experiments had been done to obtain an appropriate optimal condition. The UAE parameters were optimized using single factor experimental analysis.
Effects of ethanol concentration

The superiority of hydro-alcoholic combinations over other common solvents can be explained by the fact that they contain water, which may expand the plant material and make it easier for the solvent to penetrate the solid matrix and improve extractability \(^{(31)}\). Ethanol is the most efficient solvent for the recovery of phenolic acid \(^{(32)}\) especially caffeic acid; target of this study, therefore ethanol/water was chosen as the extracting solvent. Different concentrations of ethanolic solutions (60% to 80%) were examined using a 10:1 solvent/solid ratio, 10 min of extraction time, and 40 KHz ultrasonic frequency in order to examine the influence of ethanol/water concentrations on extraction yield. Figure 2 shows the effect of the solvent on the fennel extraction, when the ethanol concentration increased, the extraction efficiency (percentage yield) gradually increased. Thus, 80% ethanolic solvent was applied in the later experiments.

![Figure 2. The effect of ethanol concentration on the extraction efficiency (percentage yield) of the fennel leaves extracts.](image)

Effect of extracting time:

Time of extraction is an important parameter effecting on the extraction efficiency, in order to evaluate the influence of extraction time on Iraqi Foeniculum vulgare leaves, extractions were performed in the range of 5-15 min with 80% ethanol, 10:1 solvent/solid ratio and 40 KHz ultrasound frequency as shown in Figure 3, when the extracting time increased from 5 min to 15 min, the extraction efficiency (percentage yield) increased. The results showed that ultrasound could accelerate the release of bioactive constituents from the material when the ultrasound time is prolonged \(^{(33)}\). Therefore, 15 min was chosen in the later experiments.

![Figure 3. The effect of extraction time on the extraction efficiency (percentage yield) of the fennel leaves extracts.](image)

Effects of Ultrasound frequency

Another crucial factor that affects extraction effectiveness is ultrasound frequency, the higher ultrasonic frequency applied, the more bubbles formed. \(^{(32)}\) Every point of the extraction solution can be reached by ultrasonic waves of greater amplitude (ultrasonic frequency) which can improve the effectiveness of the extraction, while a very high frequency can disturb the plant in the extracts. \(^{(34)}\) The effects of ultrasound frequency on fennel extraction in the range of 20 KHz to 60 KHz were examined with 80% ethanol, 10:1 solvent/solid ratio and 10 min extraction time, the results are presented in Figure 4. when the ultrasound frequency rose from 20 to 40 KHz the extraction efficiency increased. As the ultrasound frequency rose from 40 to 60 KHz, the extraction efficiency slightly decreased, as high frequency low power density generates cavitations with producing large number of reactive radicals like hydroxyl (OH) and hydrogen peroxide (H\(_2\)O\(_2\)) radicals causing the degradation of compounds. \(^{(35)}\) Thus, 40 KHz was chosen as the preferred ultrasound frequency for the UAE process.

![Figure 4. The effect of ultrasound frequency on the extraction efficiency (percentage yield) of the fennel leaves extracts.](image)

Preliminary phytochemical examination of fennel extract:

After obtaining the aqueous ethanolic extract under optimized conditions, phytochemical screening assays were performed with the appropriate tests as they are simple, quick and inexpensive procedures.
Test for phenolic: a positive result was found as deep green to black coloration indicate the presence of phenolic acids.

-Test for flavonoids: a positive result was present a yellow color indicates the presence of flavonoids.

**Qualitative evaluation by thin layer chromatography (TLC)**

Thin layer chromatography (TLC) can be used on an analytical scale to track the course of a reaction and quickly determine how many components are in a combination, or on a preparative scale to purify small amounts of a product. The objective of TLC is to produce well-defined and well-separated spots that will support the identity of the chemical in the mixture when a compound's R<sub>f</sub> is compared to the R<sub>f</sub> of reference compound that is known to exist.

**Table 1. The R<sub>f</sub> values of the best three developing solvent systems.**

<table>
<thead>
<tr>
<th>Solvent systems</th>
<th>Caffiec acid standard 60%</th>
<th>Ethanal concentration 70%</th>
<th>Ethanal concentration 80%</th>
</tr>
</thead>
<tbody>
<tr>
<td>S1</td>
<td>0.49</td>
<td>0.488</td>
<td>0.488</td>
</tr>
<tr>
<td>S2</td>
<td>0.827</td>
<td>0.814</td>
<td>0.82</td>
</tr>
<tr>
<td>S3</td>
<td>0.642</td>
<td>0.636</td>
<td>0.63</td>
</tr>
</tbody>
</table>

**Figure 7:** TLC chromatogram of aqueous ethanolic extract of fennel leaves at three different concentrations (60%, 70%, 80%) using silica gel GF<sub>254nm</sub> as adsorbent and the three mobile phase S1, S2, S3 respectively, UV detection at 254 and 366 nanometers. S1: toluene: ethyl acetate: formic acid (9: 3: 1.25), S2: ethyl acetate: toluene: formic acid: methanol (3: 3: 0.8: 0.2), S3: toluene: dioxan: acetic acid (9.2: 4 :0.6)

B. The presence of numbers of phytoconstituents in aqueous ethanolic extracts obtained from *Foeniculum vulgare* leaves at different extraction times (5, 10, 15 min). One of these phytoconstituents, appeared as a single compact spot having the same color and R<sub>f</sub> value as that caffeic acid standard on TLC plates which was identified by using three different developing solvent systems (S1, S2 and S3) and detected by using UV-light at 254nm and 366nm. as shown in Figure 7.

The R<sub>f</sub> values of this spot and its corresponding standard (caffeic acid) in three developing solvent systems were calculated and listed in Table 2.

**Table 2. The R<sub>f</sub> values of the best three developing solvent systems.**

<table>
<thead>
<tr>
<th>Solvent systems</th>
<th>Caffiec acid standard 5 min</th>
<th>Etraction time 10 min</th>
<th>Etraction time 15 min</th>
</tr>
</thead>
<tbody>
<tr>
<td>S1</td>
<td>0.462</td>
<td>0.46</td>
<td>0.458</td>
</tr>
<tr>
<td>S2</td>
<td>0.731</td>
<td>0.725</td>
<td>0.737</td>
</tr>
<tr>
<td>S3</td>
<td>0.532</td>
<td>0.528</td>
<td>0.53</td>
</tr>
</tbody>
</table>
C. The presence of numbers of phytoconstituents in aqueous ethanolic extracts of *Foeniculum vulgare* leaves at different ultrasound frequency (20, 40, 60 KHz). One of these phytoconstituents, appeared as a single compact spot having the same color and R_f value as that caffeic acid standard on TLC plates which was identified by using three different developing solvent systems (S1, S2 and S3) and detected by using UV-light at 254nm and 366nm. as shown in Figure 9.

The R_f values of this spot and its corresponding standard (caffeic acid) in three developing solvent systems were calculated and listed in Table 3.

![Figure 8: TLC chromatogram of aqueous ethanolic extract of fennel leaves at three different extraction times (5,10,15 min) using silica gel GF 2.54nm as adsorbent and the three mobile phase S1, S2, S3* respectively, UV detection at 254 and 366 nanometers. S1: toluene: ethyl acetate: formic acid (9: 3: 1.25), S2: ethyl acetate: toluene: formic acid: methanol (3: 3: 0.8: 0.2), S3: toluene: dioxane: acetic acid (9.2: 4: 0.6)](image)

![Figure 9.TLC chromatogram of aqueous ethanolic extract of fennel leaves at three different ultrasound frequency (20, 40, 60 KHz) using silica gel GF 2.54nm as adsorbent and the three mobile phase S1, S2, S3 respectively, UV detection at 254 and 366 nanometers. S1: toluene: ethyl acetate: formic acid (9: 3: 1.25), S2: ethyl acetate: toluene: formic acid: methanol (3: 3: 0.8: 0.2), S3: toluene: dioxane: acetic acid (9.2: 4: 0.6)](image)

<table>
<thead>
<tr>
<th>Solvent systems</th>
<th>Caffiec acid standard</th>
<th>Ultrasound frequency 20 KHz</th>
<th>Ultrasound frequency 40 KHz</th>
<th>Ultrasound frequency 60 KHz</th>
</tr>
</thead>
<tbody>
<tr>
<td>S1</td>
<td>0.468</td>
<td>0.456</td>
<td>0.46</td>
<td>0.46</td>
</tr>
<tr>
<td>S2</td>
<td>0.667</td>
<td>0.66</td>
<td>0.654</td>
<td>0.655</td>
</tr>
<tr>
<td>S3</td>
<td>0.735</td>
<td>0.736</td>
<td>0.736</td>
<td>0.729</td>
</tr>
</tbody>
</table>

Table 3. The R_fvalues of the best three developing solvent systems.

From the above, caffeic acids TLC qualitative evaluation results can run in the same path with the Single factor experiment analysis of UAE results proposing that the optimized UAE is a simple, trouble-free and effective option for recovering and assessing the quality of phenolic acids, particularly caffeic acid recovered from fennel species leaves.

**Cytotoxic assay**

The optimized ethanolic extract of Iraqi *Foeniculum vulgare* leaves, obtained by probe UAE method as it gave the highest concentration of the bioactive constituents (caffeic acid) were monitored by TLC, was subjected to the cytotoxic assay for the first time on human tumor cell line; pleural effusion breast metastatic adenocarcinoma MDA-MB-468 cells by the MTT test.

With a maximum concentration cytotoxic activity at 100 µg/ml and a minimum concentration cytotoxic activity at 0.15 µg/ml. Figure 9 illustrated the cytotoxic effect of the optimized ethanolic extract on the MDA-MB-468 cell line and demonstrated that the cytotoxic effects of the optimized ethanolic extract had a concentration and time-dependent pattern. Its cytotoxic effect on MDA-MB-468 breast cancer cell lines was 28.37% at a dosage of 12.5 µg/ml after 48 hours of incubation. With a rise in the optimal ethanolic extract concentration and incubation period, the cytotoxic effects were more pronounced. As a result, the cytotoxic effect on the MDAMB- 468 breast cancer cell lines after 72 hours of incubation was 72.64% on a concentration of 100 µg/ml.
Ultrasound-assisted extraction and cytotoxic activity of fennel leaves

The cytotoxic effects of conventional medicinal compounds are categorized into four groups based on the IC₅₀ value. The classifications of very active, relatively active, weakly active, and inert cytotoxic chemicals are based on the IC50 values of 0-20, 20-100,100-1000, and >1000 µg/ml, respectively (24). On the MDA-MB-468 cell line, the optimized ethanolic extract of Iraqi Foeniculum vulgare leaves had a cytotoxic impact with an IC₅₀ value of 46.89 µg/ml. The optimized ethanolic extract of Iraqi Foeniculum vulgare leaves is categorized as a "relatively active" compound by the anticancer compound classification criteria. Plots of the concentrations of log-transformed Foeniculum vulgare extract were made to show the dosage response. Through the use of nonlinear regression analysis, IC₅₀ values were calculated (Prism Pad 8.1). Results represent the standard error of the mean (SEM) for triplicate data p<0.05, as shown in Figure 10.

Conclusion and Perspectives

The findings demonstrate that the optimum UAE conditions for fennel extraction were 80% ethanol, 15 min. extraction time and 40 KHz ultrasound frequency; in addition to, the high cytotoxic potential of Foeniculum vulgare for in vitro manner, which is attributed mainly to the occurrence of caffeic acid and other related polyphenolic compounds that were detected using the MTT cytotoxic assay. Further investigations are needed to complete the phytochemical profile, pharmacology mechanisms and pharmacokinetics studies of the optimized ethanolic Foeniculum vulgare leaves extract.

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Conflicts of Interest

The authors declare no conflict of interest regarding the publication of the manuscript.

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Ethics Statements

The manuscript did not include human and/or animal studies, so ethical approval is not needed for this research.

Author Contribution

The author (Thukaa Zuhair Abdul-Jalil) planned the experiments, accomplished the planned procedures, including sample preparation, extraction process, phytochemical analysis by TLC, evaluation of cytotoxic activity, statistical analysis, and wrote the manuscript.

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