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# Anticancer Activities of *Trigonella foenum-graecum* against Pancreatic Ductal Adenocarcinoma Cell Line

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**Abstract:** One of the most prevalent malignancies in the world is pancreatic ductal adenocarcinoma (PDAC), and the majority of available treatments are only marginally effective. According to epidemiological research, the survival rates of diabetic cancer patients receiving various antidiabetic therapies vary. Although its exact mechanisms of action are yet unknown, *Trigonella foenum-graecum* is a traditional medicinal plant having anticancer activity. In this study the anticancer potentials of extract of *Trigonella foenum-graecum* seeds was investigated against PANC-1 and MiaPaCa2 cells. Cytotoxicity of extracts was determined by MTT assay. The results showed that the ethanolic extract *T. foenum-graecum* possessed a moderate amount of anticancer activity and the $IC_{50}$ value was recorded. The most potent anticancer activity was observed with the ethanolic extract of *T. foenum-graecum* with $IC_{50}$ values of 54.50 µg/ml and 43.75 µg/ml on PANC-1 and MiaPaCa2 cells, respectively. According to phytochemical tests, the strong plant extracts contained significant amounts of flavonoids and phenols, which may be crucial to their anticancer properties. These findings imply that *T. foenum-graecum* is a viable natural product source that presents chances for the creation of innovative anticancer medications.

**Keywords:** Pancreatic ductal adenocarcinoma, *Trigonella .foenum-graecum*, PANC-1, MiaPaCa2, MTT assay

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## Introduction

Diabetes mellitus (DM) refers to a group of metabolic disorders characterized by prolonged hyperglycemia. The World Health Organization (WHO) reported that, as of 2015, approximately 422 million individuals worldwide were affected by diabetes, and by 2017, 1.6 million deaths were directly attributed to the disease. These numbers have shown a steady increase over recent decades. DM is classified primarily into type 1 diabetes mellitus (T1DM), caused by complete insulin deficiency due to autoimmune-mediated destruction of beta cells, and type 2 diabetes

mellitus (T2DM), which arises predominantly from insulin resistance, impairing the ability of target cells to utilize glucose efficiently.

It is widely acknowledged that diabetes significantly elevates the risk of developing severe and potentially life-threatening complications. These complications, which may be microvascular (e.g., nephropathy, retinopathy, neuropathy) or macrovascular (e.g., atherosclerosis, hypertension, stroke), result from the dysfunction and damage of multiple organs, including the kidneys, heart, skin, blood vessels, and nerves. Most diabetes-related deaths are due to these vascular complications. Beyond these, hyperglycemia has been shown to facilitate cancer cell growth and metastasis. Research has established a strong association between diabetes mellitus and cancer, with T2DM displaying the most substantial link. Although the association is less prominent in T1DM, cancer risk remains elevated in both types of diabetes.

Globally, cancer caused approximately 9.6 million deaths in 2018, making it the second leading cause of death. Nearly one in six deaths worldwide is attributed to cancer. The significant global burden of both diabetes and cancer has led researchers to explore the shared environmental and pathophysiological factors that may link these two conditions.

Fenugreek (*Trigonella foenum-graecum*), a member of the Leguminosae family, is an annual plant with seeds recognized for their medicinal properties. Fenugreek seeds possess hepato-protective, antidiabetic, antibacterial, appetite-regulating, gastric stimulant, and anticancer activities. They are rich in bioactive compounds, including diosgenin, saponins, coumarins, alkaloids (such as trigonelline, gentianine, and carpaine), polyphenols (like rhaponticin and isovitexin), and flavonoids (Wani *et al*., 2018).

These bioactive molecules, with their functional groups, facilitate the synthesis of nanoparticles. The phenolic compounds in fenugreek seed extract may act as capping and stabilizing agents, enhancing nanoparticle stability and preventing aggregation. Additionally, the galactomannan polysaccharides in fenugreek extract, which possess reducing functional groups, play a significant role in nanoparticle synthesis (Ramamurthy *et al*., 2013). Traditionally, fenugreek has been widely used in Ayurvedic and other Indian medicinal systems to treat a variety of ailments.

This study aimed to evaluate the antiproliferative effects of the ethanolic extract of *T. foenum-graecum* on pancreatic ductal adenocarcinoma cell lines.

## Materials and Methods

### *Plant Material and Extraction*:

Trigonella foenum-graecum seeds were procured from a local market, dried, and ground into coarse powder. The powdered material was subjected to extraction with 95% v/v ethanol using a Soxhlet apparatus. The obtained extracts were evaporated under controlled temperatures (35–40°C) until dry. These dried extracts were stored in airtight containers under refrigerated conditions and dissolved in respective solvents for subsequent analyses.

### *Phytochemical Screening*:

The Soxhlet-obtained extracts, prepared using sequential extraction methods, were used for preliminary phytochemical screening. After vacuum evaporation, the dried extracts were analyzed for the presence of various phytoconstituents, such as alkaloids, carbohydrates, phenolics, flavonoids, proteins, amino acids, saponins, mucilage, and resins. Chemical tests were conducted following standard protocols described by Trease and Evans (1983) and Harborne (1973).

### *Tumor Cell Lines*:

Human pancreatic cancer cell lines, PANC-1 and MiaPaCa-2, were used for the study. The cells were cultured in Minimum Essential Media (MEM) supplemented with sodium bicarbonate, EDTA, and fetal calf serum (FCS). Incubation was carried

out at 37°C in a humidified environment with 5% $CO_2$. The culture medium was refreshed every two days. For experiments, cells were grown in T-75 tissue culture flasks with 10 ml of media under 5% $CO_2$ and 95% air at 37°C. Cells were passaged weekly, and the culture medium was replaced biweekly to ensure optimal growth.

*MTT Assay* (*Mossman, 1983*):

*Cell Viability and Antiproliferative Assay*

The antiproliferative effects of the extracts were quantified using the MTT assay, which measures cell viability through the reduction of [3-(4,5-dimethylthiazol-2-yl)-2, 5-diphenyltetrazolium bromide]. After treatment, 20 µl of 5 mg/ml MTT solution was added to the cells, and absorbance was measured at 545 nm using a microplate reader. Untreated cells were used as controls.

To distinguish between antiproliferative and cytotoxic effects, the assay conditions were varied. For antiproliferative effects, 5000 cells/well were seeded, and the extracts were incubated for 72 hours. For cytotoxic effects, 25,000 cells/well were seeded, and the extracts were incubated for 24 h.

Stock solutions of the extracts were prepared in dimethyl sulfoxide (DMSO), ensuring that the final DMSO concentration in the medium did not exceed 0.3%, as this concentration did not significantly affect cell growth. Extract concentrations ranging from 10 to 1000 µg/ml were tested to determine their effects.

Extracts that demonstrated more than 50% growth inhibition at any concentration were selected for further in vitro studies, including cytotoxicity and dose-response analyses.

*Drug Interaction Study*:

The interaction between doxorubicin and acridones was evaluated using the checkerboard method. Serial twofold dilutions of acridones were tested in combination with twofold dilutions of doxorubicin. Cell growth rates were determined via MTT assay. Drug interactions were analyzed using the fractional inhibitory index (FIX) to quantify the combined effects.

| | | | |
|---|---|---|---|
| FIX < 0.5 | Synergism | 1 < FIX < 2 | Indifferent effect |
| FIX = 0.51-1 | Additive effect | FIX > 2 | Antagonism |

## Results and Discussion

*Trigonella foenum-graecum* seed ethanolic extracts were subjected to phytochemical screening, which showed the presence of alkaloids, flavonoids, phytosterols, tannins, and phenols (Table 1). Based on its potential medical benefits, the *T. foenum-graecum* seed extract employed in this study was selected. Phytochemical analysis was used in a previous work on *T. foenum-graecum* naturally occurring ethanolic extracts. The crude extract's phytochemical screening indicated the presence of alkaloids, cardiac glycosides, terpenoids, saponins, tannins, flavonoids, and steroids; however, reducing sugars, carbonyl (aldehyde), and phlobatannins were found to be absent (Makinde *et al*., 2007).

The range of secondary metabolites found in these naturally growing plants includes phenols, flavonoids, quinones, coumarins, tannins and their glycosides, alkaloids, essential oils, and more. It has been underlined how crucial these compounds are as microbial agents against the infection (Sofowora, 1993). It was evident from this investigation that the greatest quantity of the various metabolites found in *T. foenum-graecum* was extracted by ethanol. Boominathan and Ramamurthy (2009) reported that the phytochemical analysis of the *H. indicum* and *C. procumbens* extracts showed the presence of tannins, alkaloids, flavonoids and phenolic compounds. Tannins have been found to form irreversible complexes with proline-rich proteins.

*T. foenum-graecum* anticancer properties were investigated using various mammalian cell lines. The MTT cytotoxicity test was used to assess the anticancer activity of both the standard and the ethanolic extract of *T. foenum-graecum*. The ethanolic extract showed a good producing

Table 1: Qualitative Phytochemical screening on seed extracts of *Trigonella foenum-graecum*

| S. No. | Name of Test | Test applied / Reagent used | Ethanol |
|---|---|---|---|
| 1 | Alkaloids | A] Mayer's<br>B] Wagner's<br>C] Hagner's<br>D] Dragndorff's test | +++<br>+++<br>+++<br>++ |
| 2 | Flavonoids | HCl and magnesium turnings | +++ |
| 3 | Carbohydrate | Molisch's test | ++ |
| 4 | Tannins & Phenols | A] 10% Lead acetate<br>B] $Fecl_3$ | +++<br>+++ |
| 5 | Test for Steroids | A] Salkowski's Test<br>B] Libermann-Burchard's Test | ++<br>++ |
| 6 | Gums & Mucilages | Alcoholic Precipitation | - |
| 7 | Fixed oil & Fats | Spot test | + |
| 8 | Saponins | Foam test | ++ |
| 9 | Phytosterols | LB test | + |
| 10 | Volatile oils | Hydro distillation method | + |
| 11 | Protein & free amino acids. | A] Biuret test<br>B] Ninhydrin test<br>C] Xanthoprotein test | +++<br>+++<br>+++ |

Table 2: Anticancer activity of pancreatic cancer cells treated with ethanolic seed extracts of *T. foenum-graecum*

| Concentrations (µg/ ml) | PANC-1 | | MiaPaCa2 | |
|---|---|---|---|---|
| | % of Cell viability | % of Cytotoxicity | % of Cell viability | % of Cytotoxicity |
| | 100 | 0 | **100** | **0** |
| 7.8 | 68 | 32 | **79** | **21** |
| 15.6 | 53.1 | 46.9 | **72.1** | **27.9** |
| 31.2 | 49.5 | 50.5 | **63.8** | **36.2** |
| 62.5 | 40.8 | 59.2 | **57.9** | **42.1** |
| 125 | 33.9 | 66.1 | **45.2** | **54.8** |
| 250 | 28.2 | 71.8 | **35.5** | **64.5** |
| 500 | 20.4 | 79.6 | **26.4** | **73.6** |
| 1000 | 9.5 | 90.5 | **5.5** | **94.5** |
| Vehicle control (DMSO) | 1.8 | 98.2 | **3.8** | **96.2** |

capability of phytocompound activity in the preliminary investigation. PANC-1 and MiaPaCa2 cell lines were used in the current study of the ethanolic extract of *T. foenum-graecum*. The results are depicted in Table 2.

The concentration of *T. foenum-graecum* at 1000 µg/ml was found to have the highest cell inhibition (90.5%) and the lowest cell viability (9.5%). The anticancer activity of the ethanolic extract of *T. foenum-graecum* against PANC-1 and MiaPaCa2 cell lines was determined by calculating the $IC_{50}$ values, which were 54.50µg/ml and 43.75µg/ml. For this investigation, tamoxifen served as a benchmark. Higher concentrations were found to have the lowest cell viability and the highest cell inhibition in the standard.

Numerous natural chemicals extracted from various plant extracts have been demonstrated to possess anticancer effects. Research is being done all around the world to identify a lead molecule

that can prevent human cancer from developing. This objective has always benefited greatly from the contributions of nature. Natural substances derived from plants, such as terpenoids, flavonoids, and steroids, have drawn a lot of interest because of their many pharmacological characteristics, which include cytotoxic and chemopreventive actions (Abdullaev, 2001). They were the first agents to advance into clinical use for the treatment of cancer (Cragg and Newman, 2005).

*Withania somnifera* as a potential source of new molecules that can curtail cancer growth were studied (Abdullaev, 2001). Similar to tamoxifen, *T. foenum-graecum* has also been demonstrated to suppress the growth of human cancer cell lines. The seed extract inhibited the growth of PANC-1 and MiaPaCa2 tumor cell lines. Jayaprakasam *et al.* (2003) reported that the inhibitory concentrations obtained was 25.1±0.91 against colon cell line HCT-116, but in this study *T. foenum-graecum* extracts shown more than 90% inhibition against human cell lines. Additionally, this investigation has documented the growth-inhibiting potential of *T. foenum-graecum* against cancer cell lines, including PANC-1 and MiaPaCa2 tumor cell lines. Thus, this investigation has demonstrated that the *T. foenum-graecum* extract possesses exceptional anticancer properties.

## Conclusion

The extract's capacity to bind to phytocompounds generated during ethanol metabolism in PANC-1 and MiaPaCa2 cells may be the cause of its protective action. Nature has always played a significant role in achieving this objective. Additionally, this investigation demonstrated that *T. foenum-graecum* ethanolic seed extract possesses cytotoxic effects that can be tested in clinical trials on cancer patients. According to the current investigation, *T. foenum-graecum* has anticancer activity against the PANC-1 and MiaPaCa2 cell lines. The results of this investigation demonstrated the strong anti-cancer effects of *T. foenum-graecum* extract on cell lines. Phytocompounds are most likely the cause of the anti-cancer action. The study's results also support the use of the plant in traditional medicine to treat diabetes mellitus.

## Conflict of Interest

The authors declare no conflicts of interest.

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