**In Vitro** Novel Study the Effect of Scopoletin Compound and Silver Nanoparticles of Fenugreek Extract on Focal Adhesion Kinase Enzyme as Anti-Metastatic in Serum of Iraqi Patients of Stage ΙΙ and Stage ΙΙΙ with Pancreatic Cancer

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**ABSTRACT**

**Background:** Experts consider pancreatic cancer one of the most deadly types. For unknown reasons, pancreatic cancer incidence and mortality are rising. Scopoletin and Ag-NPs compounds have therapeutic properties and are utilized experimentally in vitro as chemotherapies for numerous disorders.

**Objective:** This study manufactures silver nanoparticles from fenugreek extract and analyzes their anti-metastatic effects with scopoletin at different doses to find the best combination to lower FAK enzyme concentration.

**Patient and Methods:** Sixty pancreatic cancer serum samples were divided: 30-Stage ΙΙ patient samples (male and female, 45-65 years old), 30 stage IV patients of both sexes and ages (52-79), Thirty serum samples from healthy 23-45-years-olds served as a control group. Silver nanoparticles and scopoletin solutions were tested to find the appropriate concentration to reduce the focal adhesion kinase (FAK) enzyme.

**Results:** Researchers showed that patients with stage ΙΙ and stage ΙΙΙ with pancreatic cancer and the control group had enzyme concentrations of 1.620.11 ng/ml, 1.790.06 ng/ml, and 0.940.41 ng/ml, respectively, when treated with a 2 ppm solution of nanoparticles. Stage ΙΙ and stage ΙΙΙ with pancreatic cancer patients had lower enzyme concentrations than controls (1.570.15 ng/ml and 1.410.07 ng/ml), when treated with a 8 ppm solution of Scopoletin solution.

**Conclusion:** Based on their potential to decrease focal adhesion kinase enzyme concentrations in the blood serum of patients with stageΙΙ and stageΙΙΙ Iraqi pancreatic cancer, scopoletin and silver nanoparticles of fenugreek can be recommended as anticancer materials.

**Keywords:** Pancreatic cancer, Scopoletin, Fenugreek, Silver nanoparticles, Focal adhesion kinase.

**INTRODUCTION**

Cancer is an idiom for a disease in which unusual cells divide in the absence of control and can pervade nearby tissues (1). Cancer is the unrestricted growth of cells that have damaged deoxyribonucleic acid manifestation. The cancerous cells repeatedly divide, displacing normal tissue (2, 3).

The broad spectrum of pancreatic cancer is typically divided into benign, pre-malignant, and malignant neoplasms based on biological characteristics and histological differentiation into epithelial or non-epithelial neoplasms. Ductal and acinar neoplasms are additional categories for epithelial tumours that exist (4).

The disease is often discovered at an advanced stage because pancreatic cancer has a high mortality rate and relatively poor detection rates. This is a result of the disease's quick progression and inadequate diagnosis (5). Pancreatic cancer can be described as follows (6).

Stage I: Pancreatic tumour that has not spread to other organs. Tumour infiltrates the bile duct and other nearby tissues in stage II, but lymph nodes are negative. Any lymph nodes with a positive stage three reach neighbouring organs such as the stomach, liver, diaphragm, and adrenals at stage IVA. Stage IVB: The tumour has spread to distant organs (7).

The most significant risk factors are smoking (which accounts for 20% of pancreatic cancer cases), age over 55, diabetes, obesity, chronic pancreatitis, liver cirrhosis, helicobacter pylori infection, work exposure to chemicals in the dry cleaning and metalworking industries, males more than females, African Americans more than whites, and family history. 10% of instances are genetic in nature, either due to genetic mutations or to illnesses such as Lynch syndrome, Peutz-Jeghers syndrome, VonHippal Lindau syndrome, or MEN1 (multiple endocrine neoplasia type1). Heavy alcohol consumption, coffee consumption, physical inactivity, a high intake of red meat, and two or more soft drinks per day are all risk factors (7).

Focal adhesion kinase (FAK) is an Enzyme with a commission number (EC 2.7.10)(8). FAK, also known as PTK2 (protein tyrosine kinase 2), is a cytoplasmic protein kinase that is essential for embryonic development and cell adhesion, migration, survival, and proliferation (9). The 125kD FAK protein is composed of a central catalytic kinase domain, a central FERM (four-point-one, ezrin, radixin, moesin) domain, and a C-terminal domain. Integrins regulate focal adhesions, which are points of interaction between the cell surface and the extracellular matrix. Cell adhesion, migration, proliferation, differentiation, and survival are influenced by the coordinated creation and dissolution of focal adhesions as...
well as by intracellular signaling from these sites. An important stage in the control of cell migration, the modification of these adhesion sites in tumors, is mediated by FAK. (10). According to biological research, FAK overexpression and enhanced FAK activity are crucial to the initiation and development of human malignancies. Angiogenesis, cell migration and invasion, cancer stem cell production, cancer cell survival and proliferation, and angiogenesis are just a few of the ways that FAK promotes tumor growth (11).

Since the beginning of time, people have employed plants for medical purposes, and they still do today. Most cancer chemotherapy drugs are made of compounds that can be found and extracted from plants or their synthetic equivalents (12). The coumarin phenolic compound scopoletin (7-hydroxy-6-methoxy coumarin) is extensively present in plants and comprises coumarin derivatives that are superior in many different types of plants and have a wide range of medicinal characteristics (13, 14). It is also found in the fenugreek plant and extracted from it (15). Scopoletin is a light-yellow amorphous powder with a melting point of 202-204° C, the chemical formula C10H8O4 and a molecular weight of 192.17 g/mol. Scopoletin has a low solubility in water and aqueous buffers (13).

Scopoletin has many biological activities such as anticancer activity (16). Scopoletin has been found to prevent cancer cell proliferation (17). It has anti-metastatic effects, the investigation found that it significantly inhibits the invasion of cancer cell (18).

Fenugreek is a medicinal plant that has long attracted interest in traditional medicine in many countries of the world for its remarkable pharmaceutical properties (19).

Phytomining is the process of converting metal ions into nanoparticles using plant extracts. Plant extract investigation for nanoparticle synthesis has become a popular approach in recent years. Plant extracts operate as reducers, capping agents, and stabilizers. A multidisciplinary scientific discipline called nanotechnology seeks to develop atomic, molecular, and supramolecular materials with improved properties (20, 21, 22). Nanomaterials are employed in the detection of glucose, DNA and RNA, heavy detection, disease diagnosis, disease control, and microelectronics (23).

This study aimed to prepare silver nanoparticles from fenugreek extract and compare its effect as an anti-metastasis with scopoletin, which is one of the components of fenugreek extract with different concentrations, to find the best concentration of both compounds that gives the best decrease in the concentration of FAK enzyme, because reducing the activity of the enzyme leads to stopping the growth of cancer cells, their reproduction and division, and these compounds Scopoletin and AgNPs can reduce one type of tyrosin kinas or more than one type.

**MATERIALS AND METHOD**

During the period from January 2022 to June 2022 blood samples were collected from patients complaining of pancreatic cancer in the Oncology Teaching Hospital and Gastroenterology and Hepatology Teaching Hospital. The blood samples were classified into three groups as the follows:

- G1- 30 samples from patients of stageII and of both sexes with an age range of (45-65) years.
- G2- 30 samples from patients of stage III and of both sexes with an age range of (52-79) years
- G3- 30 samples from serum of healthy subjects as a control group with an age range of (23-45) years.

**Specimen collection and preparation**

Eight millilitres of venous blood were drawn using ten millilitres (ml) disposable plastic syringes. Blood samples were taken from each individual and the control group and put into regular plastic tubes without any anticoagulant. Blood was then allowed to coagulate for 20 to 30 minutes at 37°C. After 10 minutes of centrifugation at 3000 rpm, serum was collected, put in tiny Eppendorf tubes, and kept at -20°C until analysis.

**Materials**

- AgNO3 (Silver Nitrate,99.0%) from India supplied the product, Thomas baker.
- Scopoletin from England supplied the product, Sigma-Aldrich.
- Focal adhesion kinase kit from china, fine Test.
- Fenugreek plant from Baghdad Iraq Country.

**Preparation of Fenugreek extract**

After being picked, the fenugreek was cleansed with distilled water to remove impurities. It was then minced into little pieces and dried in the sun for 10 days. The dried samples were crushed into a fine powder using an electric mixer. The following step involved mixing 4 g of fenugreek powder with 200 ml of distilled water. The mixture was heated at 80 °C for two hours with magnetic stirring, and the finished solution was filtered through Nomination paper (24).

**Synthesis of AgNPs from fenugreek extract**

A common approach involved dissolving calibrated volumes of fenugreek extract and silver nitrate (4 gram each) in 50 mL of water to create two aqueous solutions. As fenugreek extract was gradually added to the AgNO3 aqueous solution, the color of the solution abruptly changed. The resultant mixture was then heated to 60 °C and agitated for 1 hour. It was then transferred to an autoclave lined with teflon and the hydrothermal reaction was carried out there for 18 hours at 100 °C (25). The
samples were then dried at room temperature in a vacuum. 25 ml of the solution were steamed at 200 °C for two hours to create nano-powder (26).

Preparation of solutions
In this work, a stock standard solution was created by dissolving (0.005 mg) of each scopoletin and silver nanoparticle in (100 ml) of water. This solution was then used to determine the concentrations of each substance. Working solutions with varying concentrations were made by diluting stock solutions in water (2 ppm, 4 ppm, 8 ppm and 10 ppm). Just prior to usage, stock standard solutions and diluted solutions were made.

Determination of the concentration of FAK in serum
This assessment relies on the sandwich enzyme-linked immune-sorbent assay. The capture antibody was previously coated on 96-well plates. The biotin-conjugated antibody was also used as a detection tool. The wells were cleaned using a wash buffer after the addition of the standards, test samples, and detection antibody were conjugated to biotin. After adding HRP-Streptavidin, unbound conjugates were removed using a wash buffer.

To observe the HRP enzymatic process, TMB substrates were utilized. A blue product was made when TMB was catalyzed by HRP. Later, it turned yellow when an acidic stop solution was introduced. The density of yellow reflects the desired sample volume that was gathered on the plate. To calculate the target concentration, use a microplate reader to calculate the O.D. absorbance at 450 nm (27).

Ethical consent: The research project received approval from the University of Baghdad's Academic and Ethical Review Board. All participants signed a written consent forms indicating that they understood the nature of the study and agreed to take part in it. All operations involving human participants in this study must adhere to the guidelines outlined in the World Medical Association’s Declaration of Helsinki on the conduct of scientific research involving humans.

Statistical analysis
The means ± standard deviation can be used to represent the results’ data from the current investigation. Additionally, the t-test was employed when a P < 0.05 indicated that the difference was statistically significant to compare two groups with different mean values and to emphasize the significance of that difference. All of the values of the results in all of the groups included in the current study were performed using the office program (Excel 2010).

RESULTS
Characterization of Ag NPs
UV visible spectra of synthesized fenugreek as nanoparticle
The silver ions were reduced and the solution’s color changed when silver nitrate solution was added to the fenugreek extract solution. Surface Plasmon Resonance phenomenon is the name for the change in color of the solution brought on by the reduction of silver ions (SPR). Due to the simultaneous oscillation of the electrons of the metal nanoparticles in resonance with a light wave, the silver nanoparticles have free electrons, which create the SPR absorption band. AgNPs’ crisp bands were seen at 448 nanometers, much like they were for fenugreek, which is seen in Figure (1). A peak absorption of about 448 nm was discovered. The ability of the fenugreek plant to decrease Ag+ to Ag was thus tested by comparing the peak of absorbance that was achieved (28).

FT-IR spectrum of synthesized fenugreek as nanoparticle
The biomolecules for capping and efficient stability of the metal nanoparticles produced were identified using FT-IR analysis. The findings of the FT-IR analysis of the plant extract (Figure 2a) and the sample AgNPs (Figure 2b) are displayed below. The broad band between 3200 and 3500 cm⁻¹ in the FT-IR spectra of AgNPs is associated with hydroxyl group stretching, hydrogen-bonded solvent residue, and phenols. NH2 is responsible for the faint peak at 3413 cm⁻¹ and 3471 cm⁻¹. The C=O bond was stretched in the peak at 1635 cm⁻¹. The peak at 1080 cm⁻¹ is indicative of the presence of ether or glycoside (C-O-C) groups in the extract. The bioreduction of silver ions to atoms and nanoparticles may be caused by functional groups present in the sample, such as the hydroxyl, carbonyl, -NH2, and -C-O-C- groups. While Ag-NPs were found to stretch at 428 cm⁻¹ (29).

XRD analysis of synthesized fenugreek as a nanoparticle
XRD analysis was used to confirm that the produced AgNPs are crystalline. Figure (3) used a plant extract from Trigonella to display the XR-Diffraction pattern of the generated silver nanoparticles. The prominent diffraction peaks at 38.2, 44.3, 64.5, and 77.4 correspond to the diffraction from the lattice planes (111), 200, 220, and 311 correspondingly. These describe the properties of a metallic silver face-centric cubic structure (FCC). It was noted that the peak corresponding to the (111) plane is the highest; as a result, this became the cubic structure's preferred orientation. According to figure (3) XRD, the average crystal size was 40 nm. The outcomes can be contrasted with those of Ajitha et al. (30), who likewise observed a face-centric cubic structure with orientation
(111) as the predominate direction.

**FESEM analysis of synthesized fenugreek as a nanoparticle**

Figure (4) showed FE-SEM pictures with scales of 500 and 10 nm provide a clear picture of the morphology and structure of silver of nanoparticles. Images clearly showed that AgNPs have diverse nano-sizes and are unevenly distributed in solution, with the shape of spherical assemblies and nano-semispherical structures. The presence of aggregation of nanoparticles was observed due to the different nanoparticle sizes and solvent evaporation during sample preparation. The outcomes we attained are remarkably similar to those indicated by Ajitha *et al.* (30) who talked about the particle size and a homogeneous combination of spherical nanoparticles.

![Figure 1](https://ejhm.journals.ekb.eg/)

**Figure (1):** UV-visible spectra of silver nanoparticles prepared using Trigonella extract

![Figure 2 a](https://ejhm.journals.ekb.eg/)

**Figure (2 a):** FTIR spectrum of fenugreek extract.
Figure (2 b): FTIR spectrum of AgNPs synthesised by fenugreek extract.

Figure (3): XR-Diffraction pattern of prepared AgNPs by Fenugreek extract.
Figure (4): FESEM image of the AgNP sample synthesized by fenugreek extract.

Anti-metastatic effects of scopoletin and silver nanoparticles of fenugreek extract on FAK in serum of patients with stage II and stage III pancreatic cancer.

From table (1) and figure(5) a significant increase (P≤ 0.05) in the value of FAK concentration was observed in the patient's groups G1 and G2 compared to the control group G3, without any addition and after adding solutions of silver nanoparticles of fenugreek extract with concentration of 2 ppm and scopoletin with 8 ppm. It was also observed that the values of FAK concentration after adding the solution of scopoletin with concentration of 8 ppm reduced more than compared to the values of FAK concentration after adding ANPs solution with concentration of 2 ppm and without any addition in all study groups.

Table (1): Concentration FAK in sera of three studies groups

<table>
<thead>
<tr>
<th>Groups</th>
<th>No.</th>
<th>FAK (ng/ml)</th>
<th>P</th>
<th>FAK (ng/ml)</th>
<th>P</th>
<th>FAK(ng/ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>without added Mean±SD</td>
<td></td>
<td>With Scopoletin Mean±SD</td>
<td></td>
<td>With NAPs of fenugreek Mean±SD</td>
</tr>
<tr>
<td>G1</td>
<td>30</td>
<td>2.19±0.42</td>
<td>P&gt; 0.05</td>
<td>1.41±0.07</td>
<td>P≤ 0.05</td>
<td>1.62±0.11</td>
</tr>
<tr>
<td>G2</td>
<td>30</td>
<td>3.38±1.05</td>
<td>P≤ 0.05</td>
<td>1.57±0.15</td>
<td>P≤ 0.05</td>
<td>1.57±0.15</td>
</tr>
<tr>
<td>G3</td>
<td>30</td>
<td>1.99±0.46</td>
<td>P≤ 0.05</td>
<td>0.17±0.08</td>
<td>P≤ 0.05</td>
<td>0.94±0.41</td>
</tr>
</tbody>
</table>

*Data for (mean ± SD) of the concentration FAK in serum of patients with stage II and stage III pancreatic cancer compared to control groups, without any added and with added a solution of scopoletin in concentration of 8 and the solution AgNPs of fenugreek extract in concentrated (2ppm).*
DISCUSSION

From table (1) and figure(5) a significant increase (P≤ 0.05) in the value of FAK concentration was observed in the patient's groups G1 and G2 compared to the control group G3. without any addition and after adding solutions of silver nanoparticles of fenugreek extract with concentration of 2 ppm and scopoletin with 8 ppm. It was also observed that the values of FAK concentration after adding the solution of scopoletin with concentration of 8 ppm reduced more than compared to the values of FAK concentration after adding ANPs solution with concentration of 2 ppm and without any addition in all study groups. These findings are consistent with those of earlier research, which indicated that pancreatic cancer is associated with a higher level of FAK in numerous studies. Other studies showed that the reduction of FAK by drug compounds applied in vivo or synthetic compounds applied in vitro leads to inhibiting the development of pancreatic cancer (31).

Given that its chemical structure contains functional groups like hydroxyl group, methoxy group and carbonyl ketone group aromatic rings. It has anti-cancer properties such as triple-negative breast cancer (TNBC) and its efficacy is by inhibiting FAK. Chalcone-syringaldehyde hybrid (CSH1) of chemical importance was designed and synthesized from reaction of syringaldehyde and 1-2,4-dimethoxy. CSH1 treatment hindered DNA replication, cell cycle arrest, and cell death by impairing signal transducer and activator of transcription 3 (STAT3) phosphorylation. A full genome RNA-seq analysis found that 4% of the affected genes were related to DNA damage and repair and that 18% of the altered genes were functionally related to cell adhesion and migration. In tests, CSH1 administration was observed to significantly alter FAK distribution and phosphorylation, which stopped TNBC cells from migrating and decreased cell-matrix adhesion (32). The compound under study scopoletin has function groups (hydroxyl group, methoxy group and carbonyl group aromatic ring) similar to CSH1, so we expect it to act in the same mechanism as CSH1, for that we agree with this study. While, the effectiveness of the silver nanoparticles of fenugreek extract containing scopoletin is slightly reduced, which blocks some of the active groups from affecting the enzyme. Through the results of this study, we can suggest a compound of scopoletin and silver nanoparticles of fenugreek extract as anticancer materials that inhibit and kill cancer cells through their ability to reduce FAK concentration.

CONCLUSION

In conclusion, Scopoletin is an important coumarin and AgNPs fenugreek extract with anticancer properties. Scopoletin and AgNPs fenugreek extract inhibit the growth of pc-cells by preventing cells from metastasizing. Hence, it may be beneficial in treating pancreatic cancer stage II and stage III and therefore warrants in vivo evaluation.

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Author contribution: Authors contributed equally in the study.

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