Effect of *Trigonellafoenum* extract and TiO2 nanoparticles against *Acinetobacter baumannii*.

AnaamAbdulqader Hasan¹, RanaMujahid Abdullah¹*, HibaQasim Hameed¹

1. Department of Biology, College of Education for Pure Science Ibn-Al Haitham, University of Baghdad, Iraq

*Corresponding author:-Prof. Dr. RanaMujahid Abdullah
E-mail:- dr.rana_alshwaikh@yahoo.com

**Abstract**

The results of the inhibitory efficacy of nanotitanium oxide (TiO2) on the growth of three isolates of *Acinetobacterbaumannii* bacteria indicated that TiO2 nanoparticles have an anti-bacterial effect against isolates 1 and 3, while there was little effect against isolation no. 2. The effect of the hot alcoholic extract of the fenugreek seed (*Trigonellafoenum-graecum*) against three isolates of the bacteria was evaluated. The results showing an inhibitory efficacy of the extract against all of the three isolated of *Acinetobacter baumanii*, with a varying diameter of inhibition zones. Moreover, the effect of both the alcoholic extract mixed with titanium oxide nanoparticles (TiO2) was also studied, and a synergistic inhibitory effect was found against the isolates of *Acinetobacterbaumannii*. Additionally, the active substances were detected in the hot alcoholic extract of fenugreek seed and the results revealed that the extract contained alkaloids, flavonoids, tannins, and phenols. X-Ray Diffraction analysis (XRD) of titanium nano oxide was performed and results showed that the average size of TiO2 particles is 26 nanometers.

**Keywords:** *Trigonellafoenum*, TiO2 Nanoparticles, *Acinetobacter baumannii*

**How to cite this article:**Hasan AA, Abdullah RM, Hameed HQ (2020): Effect of *Trigonellafoenum* extract and TiO2 nanoparticles against *Acinetobacter baumannii*, Ann Trop Med & Public Health; 23(S18):


**Introduction**

Plants are a very important source of safe substances that involving in the medical and pharmacological industries that have been used in the treatment of many pathological cases. Many studies in several countries of the world have been indicated the inhibition effectiveness of medicinal plants and therefore plants are still classified as the cornerstone of modern medicine for the treatment of infectious diseases [1]. Interestingly, *Trigonellafoenum graecum* seed (fenugreek) is one of these important plants which endemic in North Africa [2]. Several scientific studies have been showed that fenugreek seed extracts have antibacterial efficacy, including *Staphylococcus spp* [3], whereas [4] found that the methanol and chloroform extract of fenugreek seed has an inhibiting effect on the growth of E. coli bacteria, as the diameters of inhibition zones were ranged between 25.2-35.6 mm indicating the anti-bacterial effect of these extracts which is maybe due to the presence of chemical compounds including alkaloids [5].

Nanoparticles have been used in biomedicine, pharmaceuticals and many biotechnology fields [6], and several studies have been indicated the effect of TiO2 on microorganisms such as algae, viruses, fungi and bacteria [7]. It also observed that the recent studies are interested in the field of nanotechnology and nanoparticles which are particles with a diameter of 10-9 mm; due to their small size of these particles, it possesses special characteristics such as chemical, mechanical, electrical and magnetic properties which facilitate their entry into the cell. It also interferes with the normal processes of cells, including nano oxides such as titanium nano oxides (TiO2). Interestingly, nano oxides are the most common nanoparticles used in various industries such as cooking utensils in addition to many applications in medical and technological fields [8]. In a study of [9], TiO2 was used to...
eliminate the pathogenic bacteria affecting poultry, including *E. coli*, *Klebsiella spp.*, *Staphylococcus spp.* and *Salmonella spp.*

Recently, there is an interesting toward nanoparticles due to their inhibitory properties against pathogenic bacteria for their ability to achieve treatment accurately and selectively, especially in the applications of pharmacy and life sciences, and TiO$_2$ is one of the effective nanoparticles used in this field to their special properties including shape, composition, size, stability of the surface and the transition between different phases of nanoparticles under the influence of pressure and heat; TiO$_2$ knowns as the magic of medicine according to its anti-bacterial effects that causing infectious diseases such as *Pseudomonas aerogenosa*. Therefore, this study aimed to assess the inhibition efficiency of the hot alcoholic extract of fenugreek seed in combination with TiO$_2$ nanoparticles on the studied bacteria growth.

**Materials and Methods**

Sources of isolates: *Acinetobacterbaumannii* was isolated from the Laboratory of Microbiology and Molecular Biology at the Faculty of Education for Pure Science, Ibn al-Haitham- Graduate Studies section. *Trigonellafoenum* seeds were collected from local markets and then grind with an electric grinder and the powder was kept in the refrigerator with sealed glass and sterile until use. For preparation of hot alcoholic extract: The method was used according to $^{[11]}$ protocols by weighting (20) g of fenugreek seed powder was dissolved in 150 ml of ethanol in Thumble in the extractor (Soxhlet apparatus), and the extraction process continued for three hours, and after that, the extract dried at (60-50) ° C in sterile tubes and the extract preserved at 4°C until use.

The following methods have been used to detect the active compounds, Alkaloids $^{[12]}$, Flavonoids $^{[13]}$, tannins $^{[14]}$, Glycosides $^{[14]}$, Polynosides Phenols $^{[15]}$, Fatty acid $^{[15]}$ and Terpenst steroid $^{[16]}$ method. For the preparation of concentrations of fenugreek seed hot alcoholic extract: Stock solution of fenugreek seed hot alcoholic extract was prepared as (1) g of extract was dissolved in 100 ml of distilled water and then sterilized using filter papers with a diameter of 0.22 μm$^{[17]}$.

Preparation of nanoparticles solutions, The nanoparticles were suspended in distilled water using an ultrasound device for 15 minutes and attended at a concentration of (1) g/100 ml $^{[18]}$. For the Preparation of the mixture of nanoparticles and *Trigonellafoenum* extract solution, 1 ml of the hot alcoholic extract of the fenugreek seeds was mixed with 1 ml of the nanoparticles solution (1g/100 ml) in ultrasound device for 15 minutes $^{[19]}$. The agar wells Diffusion method was used to study the effect of both nanoparticles and hot alcoholic extract of fenugreek seeds alone and in combination together. X-Ray Diffraction (XRD) Analysis:- TiO$_2$ powder used to measure X-ray diffraction (XRD) (Shimadzu XRD-6000); the analysis was conducted in the Service Laboratory at the Faculty of Education for Pure Science, Ibn Al-Haitham, University of Baghdad.

**Results and discussion**

The inhibitory efficiency of TiO$_2$ nanoparticles was estimated against three isolates of *Acinetobacterbaumannii* bacteria via measurement of inhibition zone; the results showed that these nanoparticles have inhibition effectiveness against isolates 1 and 3 while there was no effect was seen on isolate 2(Table 1).

Table (1): The diameters of the inhibition zones of bacteria growth that treated with TiO$_2$ nanoparticles and *Trigonellafoenum* extract alone and in combination.

<table>
<thead>
<tr>
<th>Bacterial isolates</th>
<th><strong>Control</strong></th>
<th><em>Trigonellafoenum</em> extract</th>
<th>TiO$_2$ nanoparticles</th>
<th><em>Combination</em></th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Acinetobacterbaumannii</em> 1</td>
<td>-</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td><em>Acinetobacterbaumannii</em> 2</td>
<td>-</td>
<td>+</td>
<td>-</td>
<td>+</td>
</tr>
<tr>
<td><em>Acinetobacterbaumannii</em> 3</td>
<td>-</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
</tbody>
</table>

* Combination (mixture of TiO$_2$ nanoparticles and *Trigonellafoenum*)

** Distilled water
Many recent and previous studies have been indicated the ability of nanoparticles, particularly TiO2, to inhibit certain types of bacteria \cite{21}. The study by \cite{22} proved the inhibitory efficacy of TiO2 against the pathological bacteria including *Staphylococcus aureus* and *Klebsiella aerogenes*. Furthermore, TiO2 was shown as an anti-bacterial agent against gram-negative and -positive bacteria, and its effect on gram-negative bacteria was higher than its effect on the gram-positive bacteria.

The inhibitory efficacy of hot alcoholic extract of fenugreek seeds was studied and the results revealed that it had a high inhibition effect against the three isolates of *Acinetobacter baumannii*; these results are in agreement with \cite{23} study which found that there is a high inhibitory efficacy of fenugreek seed extracts against *Staphylococcus aureus* and *E. Coli* where the diameters of the inhibition zones ranged between 15±0.57 and 17 ±0.33 mm. Additionally, \cite{24} observed that the methanol extract of fenugreek seed exerted a high inhibitory effect against *Pseudomonas aeruginosa*, with a diameter of 30 mm.

On the other hand, the alcoholic extract of fenugreek seed was TiO2 and the results indicated a synergistic action against the growth of three isolates of studies bacteria; this is consistent with a study \cite{25} that reported the inhibitory effect of silver nanoparticles (ELE-Ag) after mixing with the extract and the inhibition zone diameter was (19-21) mm in comparison to the effect in the case of using the extract alone with diameter (8-10) mm. The detection of active substances was carried out, and the results showed the presence of alkaloids, flavonoids, tannins and phenols in the hot alcoholic extract of fenugreek seeds as shown in Table (2).

**Table (2):** Detection the active substances in the crude hot alcoholic extract of *Trigonella foenum* (fenugreek) seeds.

<table>
<thead>
<tr>
<th>Active substances</th>
<th>The crude hot alcoholic extract of <em>Trigonella foenum</em></th>
</tr>
</thead>
<tbody>
<tr>
<td>Alkaloids</td>
<td>+ve</td>
</tr>
<tr>
<td>Flavonoids</td>
<td>+ve</td>
</tr>
<tr>
<td>Tannins</td>
<td>+ve</td>
</tr>
<tr>
<td>Glycosides</td>
<td>-ve</td>
</tr>
<tr>
<td>Poly Phenols</td>
<td>+ve</td>
</tr>
<tr>
<td>Fatty acid</td>
<td>-ve</td>
</tr>
<tr>
<td>Terpenst steroid</td>
<td>-ve</td>
</tr>
</tbody>
</table>

Moreover, the researchers confirmed that the fenugreek seeds contained some active compounds as it was found by \cite{26} that this plant contains flavonoids, alkaloids, steroids, terpenes, tannins, and saponins. The study \cite{27} also reported that the fenugreek seeds contain flavonoid and phenolic compounds that increase their antioxidant effectiveness and their role in eliminating bacteria.

X-ray (XRD) diffraction was performed for TiO2 as shown in Figure (1), and the values of the diffraction indicated that the size of TiO2 crystals is very small and the average size of its nanoparticles can be calculated using the Scherrer's equation. Interestingly, the results showed that the average size of TiO2 is 26 nanometers (nm) and these results are consistent with \cite{29} study as the size of TiO2 was found to be 22.41 nm according to Scherrer's equation, while the size of these nanoparticles was 13 nm in another study using Scherrer's equation is also \cite{30}.

**Conclusion**

The combination of TiO2 nanoparticles and hot alcoholic extract of Trigonellafoenum seeds showed that they have high inhibition effectiveness against *Acinetobacter baumannii* bacteria.

**References**


