

## Comparative Antimicrobial Activity of Silver Nanoparticles Synthesized by *Corynebacterium glutamicum* and Plant Extracts

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### Abstract:

Biosynthesis of nanoparticles has received considerable attention due to the growing need to develop environmentally benign nanoparticle synthesis processes that do not use toxic chemicals. Therefore, biosynthetic methods employing both biological agents such as bacteria and fungus or plant extracts have emerged as a simple and a viable alternative to chemical synthetic and physical method. It is well known that many microbes produce an organic material either intracellular or extracellular which is playing important role in the remediation of toxic metals through reduction of metal ions and acting as interesting Nano factories. As a result, in the present study Ag NPs were synthesized by two methods biosynthetic technique using supernatant of *Corynebacterium glutamicum* that isolated from soil and green synthesis method by using plant extracts of fresh green plants. Ag NPs which synthesized by two methods were investigated visually by monitoring the color shift of reaction mixture from pale yellow to brown color, UV-Visible spectrophotometer was used to measure maximum absorbance of synthesized Ag NPs. The nanoparticles synthesized from *Corynebacterium glutamicum* exhibited maximum antimicrobial activity against selected pathogenic and environmental strains more than Ag NPs synthesized by green synthesis method from *Spinacia oleracea*, *Malva parviflora* and *Eruca sativa*. plant extracts

**Key words:** Antimicrobial activity, *Corynebacterium glutamicum*, Plant extracts, Silver nanoparticles, UV-Visible spectrophotometer.

### Introduction:

Nanotechnology has revolutionized the modern research with method to design, manufacture and dominate on arrangement of the particles ranging (1-100) nm. Also it holds enormous promise for designing and development many kinds of new products with its possible medical applications that could uses for early detection ,treatment and prevention of disease .Silver is chosen for its ability to kill microbes , furthermore it is known recently for being antimicrobial agent that is acting on abroad range of target sites both intracellular and extracellular (1,2) .Thus, metal nanoparticles have attracted intensive research interest because of their advantageous application like biomedical, drug delivery , food industries ,agriculture ,textile industries, chemical sensing, water treatment ,as an antimicrobial and antifungal agent and catalysis for dye degradation (3,4,5,6,7). Metal nanoparticles especially Ag NPs are important material which have been studies extensively because of possess

distinctive properties including optical, electrical, chemical, magnetic and biological properties in addition to its specific surface area and its high fraction of surface atom. (8, 9, 10). These nanoparticles can be synthesized by several chemical and physical methods. But these process are dangerous to the environment because of relay on hazardous and toxic material and require high energy, temperature and pressure also these methods cannot avoid the generation of toxic byproduct in the synthesis protocol.(11,12,13). Therefore, a great deal efforts have been made for search about novel methods for fabrication nanoparticles without using toxic chemical. As a result, biosynthetic methods employing either microorganism or plant extract are emerged as a simple and alternative way to chemical and physical methods. Recently, the utilization of biological system has emerged as a novel method for fabrication of metal nanoparticles, several biological systems including bacteria ,fungi, yeast, and algae are extremely good candidates for synthesis inorganic material like silver, gold, iron, zinc, cadmium, copper, platinum, and titanium nanoparticles either intra or extracellular

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(14,15,16). In the recent years, using plants as reducing and capping agent for fabrication of Ag NPs got attention of researchers as a rapid, low cost, eco-friendly, and a single step method and safe for human therapeutic use. There are studies that point out the rate reduction of metal ions by plant extract was faster and stable for formation of metal nanoparticles than other biological methods. Different parts of plant materials such as plant extracts, fruit, bark, fruit peels, root, and callus have been studied so far for synthesis of silver, gold, platinum, and titanium nanoparticles in different sizes and shapes (17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28). In the current study, Ag NPs were synthesized using a type of bacteria which has been isolated from *Corynebacterium glutamicum* and three environmental plant extracts of *Eruca sativa*, *Spinacia oleracea* and *Malva parviflora*. Their antibacterial activity was examined against some environmental and pathogenic bacteria.

### Materials and Methods:

#### Preparation of Silver Nitrate Solution with 1mM:

1mM of silver nitrate solution was prepared by adding 0.0849 g of  $\text{AgNO}_3$  to 500 ml of sterile distilled water and mixing the solution thoroughly by the stirrer to dissolve the material. The final solution was kept in a fuscous coloured bottle to prevent the auto-oxidation of silver nitrate solution (29).

#### Source of Microorganisms:

The bacterial strain *Corynebacterium glutamicum* that was used in the current study was isolated from soil and characterized depending on morphological and biochemical tests. The pure culture was inoculated on nutrient agar slant and incubated at  $37^\circ\text{C}$  for 24 hours. Finally, the bacterial strain is preserved in a refrigerator at  $4^\circ\text{C}$  on nutrient agar slants for further use.

#### Bacterial Culture:

A loop full of *Corynebacterium glutamicum* culture was inoculated in 250 ml conical flask containing 100 ml sterile nutrient broth. The inoculated medium was incubated at  $37^\circ\text{C}$  for 24 hours in orbital shaker at 150 rpm. Later, the culture was centrifuged at 5000 rpm for 15 minutes (30,31). Culture supernatant was used for synthesis of silver nanoparticles.

#### Synthesis of Silver Nanoparticles from Bacterial Filtrate:

Silver nanoparticles solution was synthesized by adding 10ml of the cultural supernatant of *Corynebacterium glutamicum* to a sterile reaction vessel containing 90 ml of silver nitrate solution  $\text{AgNO}_3$  at two concentrations 1mM and 3mM. The reaction mixture was incubated in

coloured brown bottle for 3 days at  $37^\circ\text{C}$  in orbital shaker at 150 rpm. The metal processed bacterial filtrate was centrifuged at 10000 rpm for 15 minutes and kept the pellet for checking the antibacterial effect (30,31).

#### Preparation of Plant Extracts:

Plant extracts of *Eruca sativa*, *Spinacia oleracea* and *Malva parviflora* which were collected from local market were prepared by adding 25g of fresh green leaves of each plant separately into clean Erlenmeyer conical flask containing 100 ml of sterile distilled water. The plant's leaves were washed several times with tap water to remove the dusts and other pollutants and cut it into small pieces. Later, the extract was incubated in water bath at  $80^\circ\text{C}$  for 30 minutes to facilitate the formation of aqueous plant extracts. Finally, plant extract was centrifuged at 8000 rpm for 20 minutes and stored at  $4^\circ\text{C}$  in refrigerator for further experiment (32,33,34).

#### Silver Nanoparticles Preparation from Plant Extract:

Preparation of Ag NPs from plant extracts was achieved by adding 10ml of plant extract to Erlenmeyer conical flask containing 90 ml of 1mM  $\text{AgNO}_3$  solution. The mixture was kept in water bath at  $80^\circ\text{C}$  for 30 minutes, the colour change of reaction mixture is an indication proving the formation of silver nanoparticles. Finally, the reaction mixture was centrifuged at 5000 rpm for 10 minutes for getting the pellet which is used for checking antimicrobial activity. (1,32,33,34).

#### UV-Visible Spectrophotometric Analysis

Formation of Ag NPs by both methods which was said previously was characterized by using UV-Visible spectrophotometer which has proved to be a very important technique for analysis of nanoparticles beside the colour change indication (10). The solution of silver nitrate was kept a sample for controlling and comparing, the absorption spectra was measured between 200-800 nm.

#### Antibacterial Activity of Silver Nanoparticles

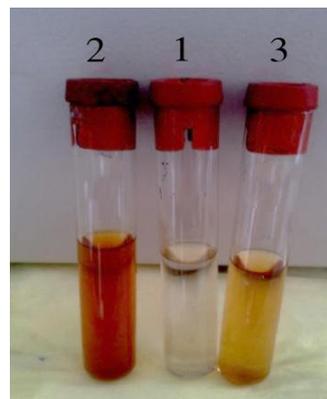
The antibacterial activity of synthesized Ag NPs was done by agar well diffusion method against some pathogenic and environmental bacteria strains like *Escherichia coli*, *Klebsiella spp.*, *Salmonella spp.*, *Staphylococcus aureus*, and *Enterobacter fecalis*. The bacterial strains were kindly provided by (biology department, college of science, university of Mosul) and grown in nutrient broth at  $37^\circ\text{C}$  for 24 hours. Bacterial density was fixed at  $1.5 \times 10^8$  by comparison with McFarland No.(0.5). 20 ml of Muller Hinton Agar was poured in sterilized petri dishes and allowed to solidify. Then, wells in the agar plates were made by using sterile cork borer with diameter of 6.0 mm, the bacterial

strain used in the current research was swabbed uniformly onto the surface of plates separately by sterile cotton swabs. The inoculated plates were left for moments to dry. Subsequently, we added volume  $10\mu\text{L}$  for each of the  $\text{AgNO}_3$  (1mM) used as (standard) and pellet of Ag NPs which have been synthesized by *Corynebacterium glutamicum* reacted with  $\text{AgNO}_3$  at 1 mM, 3mM and three plant extracts that treated with 1mM of  $\text{AgNO}_3$  separately into wells of inoculated Mueller-Hinton Agar plates by using micropipette. The treated plates were left for one hour for allowing the diffusion to occur. Then, these treated plates were incubated at  $37^\circ\text{C}$  for 24 hour. Finally, the clear area around the wells was measured. The diameter of inhibition zone was calculated using a metre ruler, and the values for each type of bacteria were recorded (29,31).

### Result and Discussion:

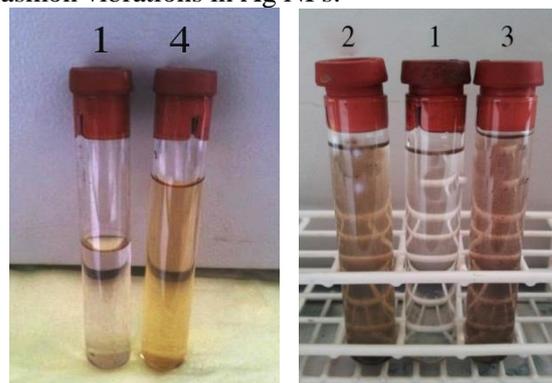
#### Characterization of Synthesized Silver Nanoparticles

The synthesized Ag NPs was characterized by colour shift of reaction mixture from almost pale yellow to yellowish brown. Whereas no change was noted in the media with  $\text{AgNO}_3$  alone. It is well-known that Ag NPs are appearing yellowish brown colour in reaction mixture (35). Also, the appearance of brown colour is due to the excitation of surface plasmon vibrations and provides a convenient spectroscopic signature of their formation (36,37). The colour change is shown in the Fig.(1). Brown colour was more intense in test tube containing 3mM  $\text{AgNO}_3$  solution reacted with culture supernatant of *Corynebacterium glutamicum* than the test tube containing 1mM  $\text{AgNO}_3$  solution reacted at the same condition. Increasing intensity may be due to the formation of more nanoparticles also it depended upon size of Ag NPs (28,38). This observation agrees with previous study (30), when cell free of *Corynebacterium spp.* was put in  $\text{AgNO}_3$  solution and incubated for 72 hour. Also study of researcher (39) which were synthesized successfully Ag NPs from dried cells of *Corynebacterium glutamicum* which it is isolated from soil and treated with diamine silver solution.



**Figure 1.** Indicates formation of nanoparticles by using *Corynebacterium glutamicum* (1)  $\text{AgNO}_3$  solution, (2) 3mM of  $\text{AgNO}_3$  solution+ bacteria (3) 1mM of  $\text{AgNO}_3$  solution +bacteria

Ag NPs exhibit yellowish brown colour in aqueous solution of fresh plant extract such as *Eruca sativa*, *Spinacia oleracea* and *Malva parviflora* which is treated with 1 mM  $\text{AgNO}_3$  by using green synthesis technique, the colour change is shown in the Fig.(2). The results are in agreement with studies (2,32,34) which were confirmed the appearance of a brown colour in the reaction mixture for plant extract of *Spinacia oleracea* treated with  $\text{AgNO}_3$  due to the formation of Ag NPs, the reaction mixture was changed from yellowish green to brown. Also our results on plant extract of *Malva parviflora* are conformable to a study which was accomplished by researcher (40), which exhibited the visual change of colour from yellow to reddish brown due to excitation of surface plasmon vibrations in Ag NPs.

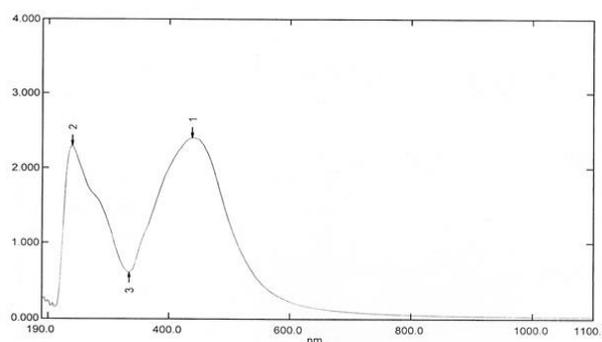


**Figure 2.** Indicates formation of nanoparticles using plant extracts:(1)  $\text{AgNO}_3$  solution, (2)  $\text{AgNO}_3$  solution + *Spinacia oleracea*(3)  $\text{AgNO}_3$  solution + *Eruca sativa* (4)  $\text{AgNO}_3$  solution + *Malva parviflora*

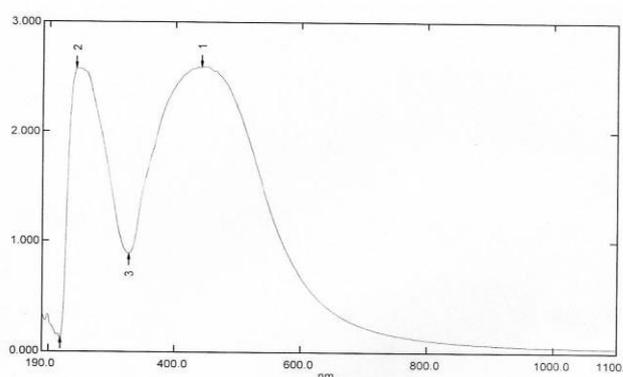
#### UV-Visible Spectrophotometric Analysis:

UV-Visible spectroscopy is one of techniques used to characterize Ag NPs in reaction mixture. UV-Vis spectroscopy can be used to examine the size and shape of nanoparticles in aqueous suspensions (41). Ag NPs synthesized by supernatant of *Corynebacterium glutamicum*

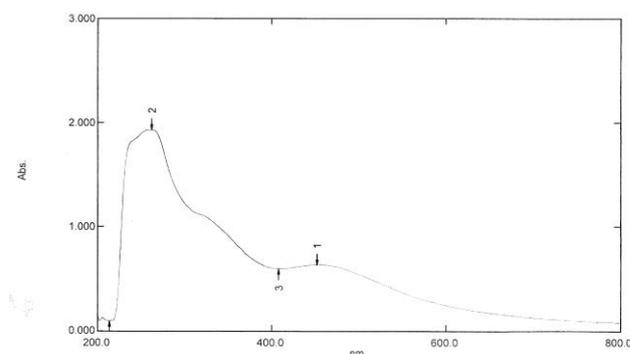
showed a absorbance peak at 442 nm when the supernatant was reacted with (3mM)  $\text{AgNO}_3$  solution while Ag NPs showed absorbance peak at 438nm when the supernatant reacted with (1mM) $\text{AgNO}_3$  solution Fig. 3 and 4, this band is related to absorbance by Ag NPs which take place between (380-450)nm and due to the occurrence of the exterior plasmon sensations, while  $\text{AgNO}_3$  solution did not display this characteristic peak .Variation in absorption peaks (442, 438 nm) indicates formation of numerous nanoparticles in reaction mixture as well as surface plasmon peak for Ag NPs become distinct with increasing concentration of  $\text{AgNO}_3$  in reaction mixture and led to broad plasmon bands which indicated enhancement in size of the particle ,this is clear through increasing in absorption value with increasing concentration of  $\text{AgNO}_3$  from 1mM to 3mM (28,38) .The current study is in agreement with the study (30) which recorded a strong absorption peak at 420 nm indicating the presence of Ag NPs in the filtrate of *Corynebacterium spp.* treated with 1mM of  $\text{AgNO}_3$  solution which represents typical absorption spectrum of spherical Ag NPs due to their surface plasmon resonance. The absorption of this study agrees with the study of researchers (31, 39) which observed maximum absorption spectrum located between(410- 440) nm for reaction solution containing dried cells of *Corynebacterium glutamicum* added to diamine silver nitrate complex. Figure 5, 6 and 7 showing the reduction of silver ions into Ag NPs by plant extracts of *Eruca sativa*, *Spinacia oleracea* ,and *Malva parviflora* which exhibited maximum absorption spectrum at 404, 432 and 452 nm respectively, due to excitation of surface plasmon vibration of Ag NPs. The current study is in agreement with a previous studies(1,32,34) which observed maximum absorption spectrum between 422 and 440 nm of the entire reaction mixture for Ag NPs synthesized by *Spinacia oleracea* .Also, the absorption of this study was approximately close to the study of researcher (40) which showed an absorption band at 445nm for *Malva parviflora* extract treated with 1mM  $\text{AgNO}_3$  solution. The appearance of numerous absorption peaks due to natural component in the plant extracts which showed peaks of absorbance in measure wavelengths but not in the range of characteristic surface plasmon absorption band because it didn't participate in the formation of Ag NPs.



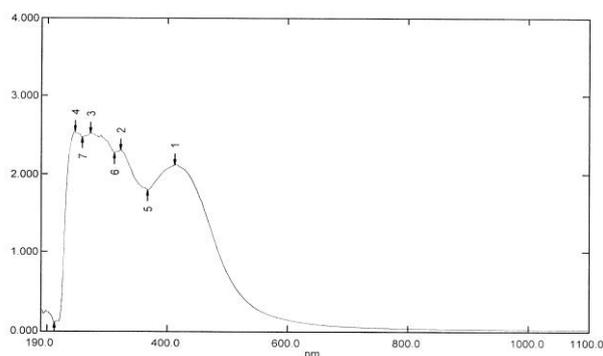
**Figure 3. UV-Visible spectra recorded maximum absorbance at 438 nm of silver nanoparticles which is synthesized by culture supernatant of *Corynebacterium glutamicum* at (1mM) of  $\text{AgNO}_3$  solution.**



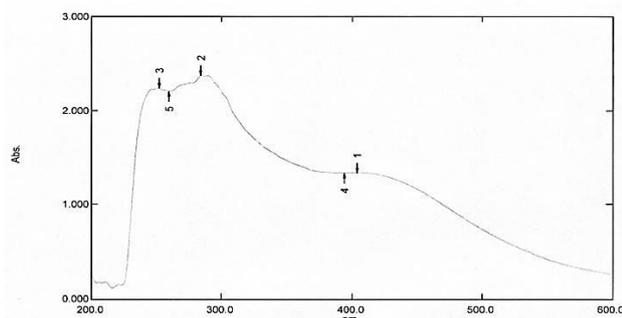
**Figure 4. UV-Visible spectra recorded maximum absorbance at 442 nm of silver nanoparticles which is synthesized by the culture supernatant *Corynebacterium glutamicum* at(3Mm) of  $\text{AgNO}_3$  solution**



**Figure 5. UV-Visible spectra recorded maximum absorbance at 452 nm of silver nanoparticles synthesized by plant extract of *Malva parviflora* treated with (1mM)  $\text{AgNO}_3$  solution.**



**Figure 6. UV-Visible spectra recorded maximum absorbance at 432 nm of silver nanoparticles synthesized by plant extract of *Spinacia oleracea* treated with (1mM) AgNO<sub>3</sub> solution.**



**Figure 7. UV-Visible spectra recorded maximum absorbance at 404 nm of silver nanoparticles synthesized by plant extract of *Eruca sativa* treated with (1mM) AgNO<sub>3</sub> solution**

**Antibacterial Activity of Silver Nanoparticles**

Antibacterial activity of synthesized Ag NPs was investigated against some bacteria using well diffusion method Fig. (8). Ag NPs were biosynthesized using 3mM of AgNO<sub>3</sub> solution reacted with bacterial filtrate of *C. glutamicum* which exhibited maximum zone of inhibition against *Staphylococcus aureus*, *Staph epidermids*, *Environmental E. coli*, and *Salmonella spp.* with inhibition zone diameter of 23, 22, 20 and 20 mm respectively. The antibacterial activity results are shown in Table (1). This results previously published in the studies (30, 31, 39) which observed maximum antibacterial activity of Ag NPs against *Staphylococcus aureus* and *E. coli* compared to other bacterial pathogens. Numerous researches

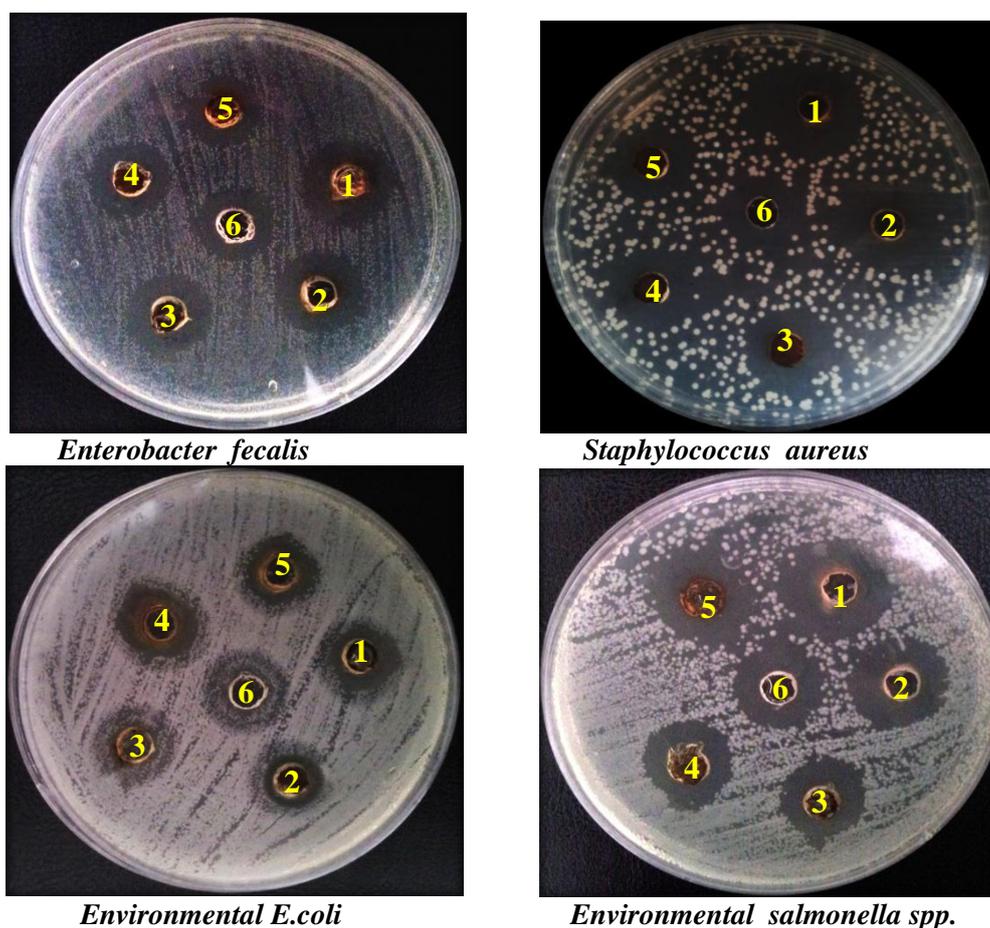
were demonstrated the capability of microorganisms of synthesis metal nanoparticles by either enzymatic or non- enzymatic mechanism, the process involves the reduction of silver ions into Ag NPs in the presence of NADH and NADPH - dependent nitrate reductase (42). The researcher(30) detected nitrate reductase enzyme in the culture filtrate of *Corynebacterium spp.* using nitrate reductase assay. This enzyme is induced by nitrate ions and thus silver ions are reduced to metallic silver(10).The green synthesis of Ag NPs by plant extracts of *Spinacia oleracea*, *Malva parviflora* and *Eruca sativa* is exhibited highest antimicrobial activity on environmental *E. coli*, *salmonella spp.*, and *staphylococcus aureus*. On the other hand, lower antibacterial activity was observed against two strain *Enterobacter fecalis* and *Staphylococcus epidermis*, the results are shown in Table (2). Our results are conformable to studies accomplished by researchers (1,32,34).Which indicated that Ag NPs synthesized from plant extracts of both *Eruca sativa* and *Spinacia oleracea* was exhibited more efficient antibacterial property for gram-negative bacteria than gram-positive due to differences in the structure of bacterial cell well .Therefore, gram-positive bacteria may allow less silver ion (Ag<sup>+</sup>) to reach the cytoplasmic membrane than gram negative bacteria(32,42).

**Table 1. Antibacterial activities of silver nanoparticles against some bacteria which produced from supernatant of *Corynebacterium glutamicum* treated with AgNO<sub>3</sub>**

Species of bacteria	Ag NPs synthesized from 3mM AgNO <sub>3</sub>	Ag NPs synthesized from 1mM AgNO <sub>3</sub>	Standard AgNO <sub>3</sub> (control)
<i>Environmental E. coli</i>	20	19	14
<i>Environmental Salmonella spp.</i>	20	19	16
<i>Enterococcus fecalis</i>	18	17	15
<i>Staph aureus</i>	23	20	17
<i>Staph epidermids</i>	22	20	14

**Table 2. Antibacterial activities of silver nanoparticles against some bacteria which produced from plant extract treated with 1mM AgNO<sub>3</sub>**

Species of bacteria	<i>Eruca sativa</i>	<i>Spinacia oleracea</i>	<i>Malva parviflora</i>	StandardAgNO <sub>3</sub> (Control)
<i>Environmental E. coli</i>	19	17	18	14
<i>Environmental Salmonella spp.</i>	16	17	19	16
<i>Enterococcus fecalis</i>	17	16	16	15
<i>Staph aureus</i>	16	17	18	17
<i>Staph epidermids</i>	12	14	14	14



**Figure 8. Antimicrobial activity of silver nanoparticles against some bacteria**

(1)-*Corynebacterium* + 3mM AgNO<sub>3</sub>, (2)-*Corynebacterium* +1mM AgNO<sub>3</sub>, (3)-*Eruca sativa* +1mM AgNO<sub>3</sub>, (4) *Spinacia oleracea* +1mM AgNO<sub>3</sub>, (5)-*Malva parviflora* + 1mM AgNO<sub>3</sub>, (6)- Standard (1m M AgNO<sub>3</sub>)

### Conclusion:

This study, investigated a simple, convenient, eco-friendly method for fabrication Ag NPs by using biological technique. Ag NPs have been synthesized successfully by both *Corynebacterium glutamicum* and plant extracts of *Eruca sativa*, *Spinacia oleracea* and *Malva parviflora*. The biological method for fabrication Ag NPs represented using supernatant of *Corynebacterium glutamicum* showing antibacterial activity against pathogenic and environmental bacterial strain.

**Conflicts of Interest: None.**

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## مقارنة الفعالية ضد ميكروبية لجسيمات الفضة النانوية المصنعة من قبل *Corynebacterium glutamicum* والمستخلصات النباتية

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### الخلاصة:

نال البناء الحيوي للجسيمات النانوية اهتمام كبير يعود ذلك للضرورة المتزايدة لتطوير عمليات غير خطيرة بيئياً لتصنيع جزيئات نانوية دون استخدام مواد كيميائية سامة، لذا برزت طرق البناء الحيوي بمساعدة العوامل البيولوجية كالبكتريا والفطريات أو المستخلصات النباتية كطرق بسيطة ومغايرة لطرق البناء الكيميائية والفيزيائية. من المعروف ان العديد من الميكروبات تنتج مواد عضوية اما خارج او داخل خلوي التي تلعب دور مهم في ازالة الايونات المعدنية السامة وتعمل كمصانع نانوية. نتيجة لذلك، في الدراسة الحالية تم بناء جزيئات الفضة النانوية باستخدام الراشح البكتيري لبكتريا *Corynebacterium glutamicum* التي عزلت من التربة والمستخلص النباتي للنباتات الخضرية كالجرجير، السبانخ والخباز المعاملة بالمحلول المائي لنترات الفضة بتركيز 1ملي مولر. تم التحري عن البناء الحيوي لجزيئات الفضة النانوية من خلال التغير اللوني لمزيج التفاعل من اللون الاصفر الشاحب الى اللون البني واثبت ذلك بواسطة جهاز المطياف الضوئي ذي الاشعة فوق البنفسجية. جزيئات الفضة النانوية المبنية من قبل *C. glutamicum* ابدت اعلى تأثير على السلالات المرضية والبيئية قيد الدراسة من جزيئات الفضة النانوية التي تم بنائها بطريقة البناء الخضري من المستخلص النباتي لنبات الخباز، السبانخ والجرير.

**الكلمات المفتاحية:** فعالية ضد الميكروبية، *Corynebacterium glutamicum*، مستخلصات نباتية، جسيمات الفضة النانوية، مطياف الاشعة فوق البنفسجية.