# Biosynthesis of silver nanoparticles using *Basil Lamiaceae* leaf extract and screening its antimicrobial activity

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## Abstract:

*The area of nanotechnology is the most widely used today, despite the existence of chemical and phytochemical methods because of the recent discoveries of many advanced devices for nano-scale measurements. Nanostructures are high-speed, easy-to-install and highly environmentally friendly for their low use of environmentally friendly solutions and chemicals.The synthesis of silver nanoparticles, AgNPs, was studied to demonstrate their antibacterial effect using basil leaf extract ofBasil Lamiaceae, after a qualitative evaluation of the active phytochemical components that act as anti-microbial, anti-oxidant and inhibiting blood albumin.The synthesized AgNPshave been characterized by UV-Vis spectroscopy, Scanning electron microscopy (SEM), transmission electron microscopy (TEM), andFourier-transform infrared spectroscopy (FTIR).The guide of silver nanoparticle synthesis is the color change from yellowish green to reddish brown and the surface Plasmon absorption band was measured by the UV-Visible spectroscopy.The size of the uniform spherical molecule ranged between 25 - 30 nm, the prepared nano- particle have a highest activity against pathogenic bacteria such asStaphylococcus sp., Proteuinsts sp. spseudomonas sp. Escherichiacoli. We found that nano particle appeared highest activity againstEscherichia coli followed byStaphylococcus sp, pseudomonas sp., Proteussp,respectively.*

**Keywords:** Basil Lamiceae (Ryhan) planextractt- Silver nano-particles (AgNPS)-Anti-microbial activity.

## Introduction

Recent studies and scientific research have focused on nanoparticlesWith important properties such as large surface area compared to nanoscale, optical stimulation, nanoscale and high electrical conductivity of nanoparticles (Vasilescu, et al. 2012; Solgi and Taghizadeh, 2012) and their use in various fields: medical, pharmaceutical, food, agricultural, engineering, electronics as well as friendly Of the environment and free of harmful chemical wastes of the environment(Wonsawat, 2014).Many noble elements noble metals, that is, silver, gold, platinum and palladium (Roy and Barik, 2010). Silver nanoparticles (AgNPs) has acquired great importance in many scientific applications in the field of therapeutic medicine, biology and ecology (Zuas, 2014).Plant extracts contain many biopharmaceuticals that can reduce silver ions to stable and more stable nanoparticles than harmful chemicals to the environment (Shankar, et al. 2004; 8-10). It was found that silver had a inhibitory effect on pathogenic microorganisms (Simon, 1984; Lok, 2007). Therefore, the tendency to use plants in the preparation of nanoparticles was considered to be inexpensive and did not require the development of microbes such as bacteria or fungi (Simon,1984).Basil plant belongs to the oral family, Lamiaceace and is used in folk medicine and is an effective antifungal (Simon, 1984).Also known since ancient times, the basil plant (Ocimumbasilicum), which dates back to theLamiaceae family with its flavor and aromatic taste and dates back to India and the countries of the Middle East with its therapeutic benefits.The Basil plant is grown and used extensively in Iraq as vegetables and served on the table daily for sweet taste and tender taste known as Rayhan.One of the benefits of the synthesis of nanoparticles using plant extracts is the synthesis of particles of the silver element using the aqueous extract of the Rayhan plant to study surface properties and optical stimulation, to determine the size, shape. In addition,the purpose of present study is to synthesis a silver nanoparticles from the aqueous extract of Basil leaf, then conducted to see its effects the inhibition of different bacterial species and their effects the antioxidant and albumin inhibition.

## Materials and methods

### Materials

It was used the analytical grade of all reagents and deionized water throughout this study.Also, all the chemical analysis were settled in the Biological and Chemical labs in Al-Mustansyria University/Biology Dep./Baghdad/Iraq.

#### Basil leaf and its extract preparation

The leaves of the Basil plant*(Ocimumbasilicumis*) were obtained from the local markets of the grocery in the city of Baghdad andit was thoroughly washed with tab water to remove any foreign matter, especially soil and dust.It also excluded any other impurities such as grass or any other varieties of plants, and then leaves were dried in the shade under a ceiling fan for two full days and grinded very finely with the electric household mill, then saved in a sealed glass container and saved in the refrigerator until it wasused. The extraction process was carried out by the method of (Abdel-Aziz et al. 2014). Twenty grams of dried basil leaf powder was weighed and placed in 50 ml of distilled water in a 500 ml Erlenmeyer bottle,

boiled for 30 minutes, then the leaf residue was filtered through a sterile quiet cloth and re-filtered through What' man filter paper (No. 1). Then the extract was vaporized at 50 ° C until we got 25 ml, the precipitates were collected and stored at 4 ° C until the chemical analyzes were performed.

**Phytochemical Scanning**

The aqueous extract of the Basil leaves were screened for the presence of active phytochemicals such as flavonoids, tannins, saponins, resins, glycosides, alkaloids and the phenols using standard color tests (Jeffery, 2007).

**Synthesis of silver nanoparticles**

It was added0.25 ml of the prepared Basil extract to 50 mM of the silver nitrate solution in a conical flask with a rubber stopper and left for 12 hours at room temperature as shown in Fig. 1. After that, the color of the extract was changed from yellowish green to brownish red, and this indicates the synthesis of silver nanoparticles (AgNPs). Sample was carried out after 30 minutes to monitor the formation of AgNPs as by (Saifuddin, et al. 2009; Deepa, et al. 2017 ).

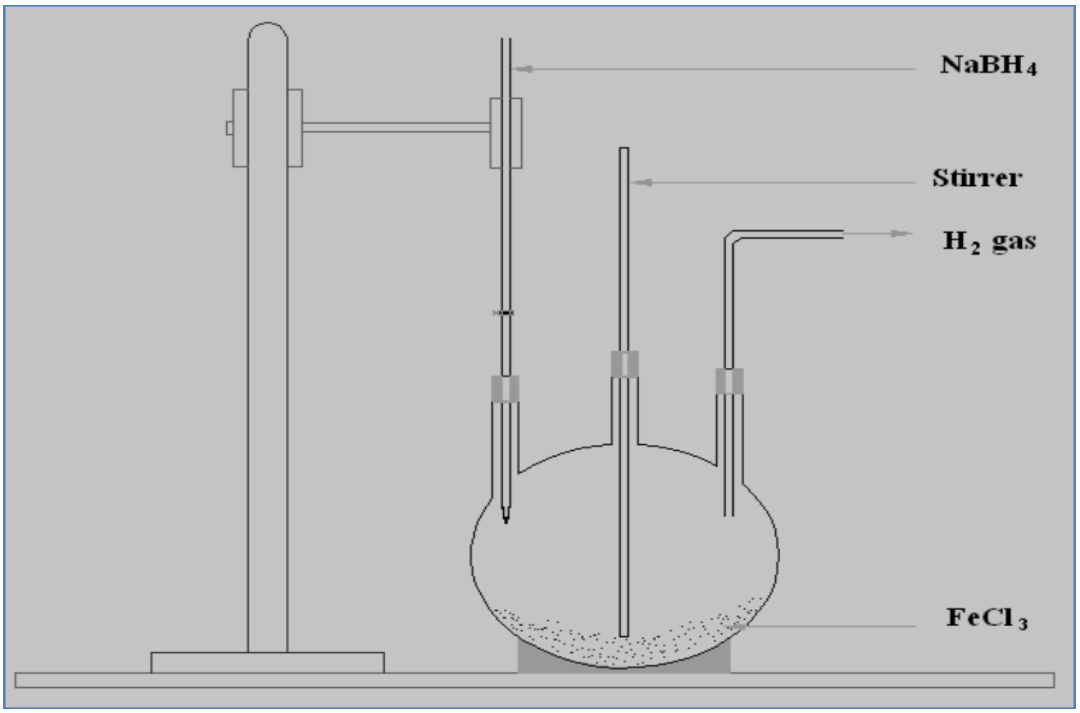



**Figure (1): shows the AgNPssynthesis .**

**Analysis of bio reduced silver nanoparticles**

**Use UV-Vis spectroscopy:**

Use UV-Vis spectroscopy to diagnose and analyze the optical properties of biodegradable silver nanoparticles with basil plant extract (AgNPs) with (Thermo Scientific Spectrascan UV 2700) at room temperature operating at 1 nm with the range between 200 and 800 nm.

**Scanning electron microscopic (SEM):**

Scanning electron microscopic of the electronic survey was done using the Zeiss EV-18 model by preparing a thin film of the sample on a carbon-coated copper network by placing a small amount of the nanoparticle sample on the copper grid, until the film on the SEM network and dried by using the device's mercury lamp for 5 minutes.

**Use Transmission Electron Microscopy (TEM)**

To determine the size and shape of the bio-structure of the formed silver nanoparticles by exposing a drop of this solution on carbon-coated copper grids to evaporate under infrared light for 30 minutes and on voltage at 200 kV.

**Fourier transform infrared spectroscopy**

FTIR analysis was used to characterize the nature of strains that are instrumental in stabilizing the silver nanoparticles formed through the biosynthesis process.The mixture of synthesized silver nanoparticles was centrifuged at 15,000 rpm for 15 min after complete reduction reaction of AgNO3 by the Basil leaf extract to separate the silver nanoparticles from biomagnetic compounds or other compounds that may interfere with the analysis of protein that interacted with AgNPs synthesis. Then the silver nanoparticles pellets were dispersed with distilled water and centrifuged at the same previous conditions in terms of rotational speed and room temperature. Washing or dispersion was repeated three times to obtain the pure silver particles, and the obtained samples were dried and ground using KBr pellets and analyzed on Nicolet IR 200 (Thermal Electron Model) by Fourier's Infrared Spectroscopy (FTIR).

**Microbial efficacy test**

Common human pathogenic bacterial strain of*Klebsiella sp., Staphylococcus sp., Proteus sp. spseudomonas sp. Escherichia coli, Staphylococcus sp., Bacilljous sp.,* were used for assessment of antibacterial activity of synthesized AgNPs. They were clinical isolates and are used as international reference standards for disc susceptibility assessment of many antibiotics. Nutrient agar was used for growth and maintenance of bacterial strains. Nutrients Agar has been used for the growth and maintenance of bacterial strains used in this study. The feed broth was also used to prepare a suspension cultures. Muller Hinton-agar (MHA) was then used to assess the potential for microbial inhibition. It was used a various concentrations (200, 300 and 100 lg/mL) of aqueous dispersions of silver nanoparticles. Use a minimal inhibitory concentration measurement (MIC) for the biologically manufactured nanoparticles using all the previous bacterial strains according to(Mayr-Hartinget al. 1972). The MIC was determined as the lowestconcentration indicated that inhibited the visible growth of the used bacterium.

## Result and Discussion

### Phytochemical Screening

Standard phytochemical screening was conducted to find the presence of metabolites like alkaloids, flavonoids …etc. in the aqueous extract of fresh Basil plant in this study. It was founded thatthe aqueous extract was rich Alkaloids, Flavonoids, Tannin, Phenols, Tannins, Phenols, Glycosides and Saponins as shown clearly in Table 1. The effectiveness of these metabolic components has been proven to bind silver nitrate and reduce its ratio in silver nanoparticles and makes it pure, thus increasing its effectiveness in microbial inhibition and oxidation (Jayapriya1 and Lalitha, 2013).

**Table 1: Compounds & active groups in Basil leaf powder**

| Active compounds | Used Detector (Indicator) | Index of detector | Basil leaf powder |
|---|---|---|---|
| Alkaloids | Dragenroph index | Orange deposit | + |
| Flavonoids | Ethyle alcoholic+ NaoH | Yellow color | + |
| Tannin | + | | + |
| Phenols | 1% Fe $(CL)_3$ | Bluish green color | + |
| Glycosides | Fehlinic index | Red deposit | + |
| Saponins | Shaking the aqueous extract | Dense foam and lengthens for a long time | + |
| Resins | 4% HCL | Turbidity | + |

### Synthesis of aqueous basil Silver Nanoparticles

Bio-biological materials were used with noble elements such as silver, gold, platinum and other noble elements in the synthesis of nanoparticles for silver on a large scale to synthesize such silver colloids. The present study emphasizes the use of fresh Basil leaf plant for the Synthesis of Silver nanoparticles, which act as reducing and coating agents in silver nanoparticles synthesis as in fig. 2. Previous studies have indicated that phytochemicals like

phenols, and flavonoids, tannin and saponin not only play a role in reducing the silver ions to the nano size, but also play as coating agent of the nanoparticles (Vedpriya, 2010; Collera, et al. 2005). The decrease in silver ions during the synthesis of nanoparticles for silverwith basil leaf extract was attributed to the combination of silver ions with the biological components of the plant extract such as proteins, enzymes, vitamins, amino acids, sugars and organic acids(Collera, et al. 2005; Jagadeesh, et al. 2004) which are eco-friendly.

**Fig. 2: Coloration of the Silver Nanoparticles by using the aqueous extract of basil leaf plant**

**UV –Visible spectra analysis**

UV- Visible Spectroscopy technique was used for preliminarily properties of synthesized silver nano-particles. After the mixing of Basil leaf extract with silver nitrate solution, the color of the solution was changed from Yellow to reddish brown for as in Fig.3.The reason of coloration is attributed to the surface vibrations of the plant plasma, which indicates the formation of silver nanoparticles(Daizy, and Unni, 2011).

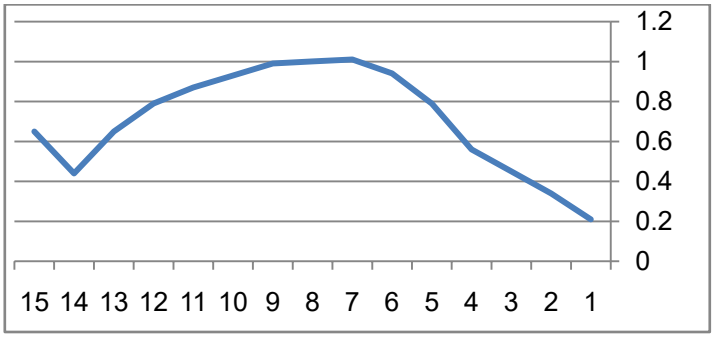



**Fig. 3: UV spectrum absorption of AgNps formed from Basil leaf extract**

**Scanning Electron Microscopy SEM:**

Scanning Electron Microscopy was used to provide information about the morphology, arrangement, and size of the created silver nanoparticles.It was found a high density of Silver nanoparticles synthesized by the basil leaf extract that studied with SEM image as in fig. 4.The image shows the formation of the porous and spherical surface layers of the silver particles with the extract of the basil leaves of nan scale between 20-30nm.

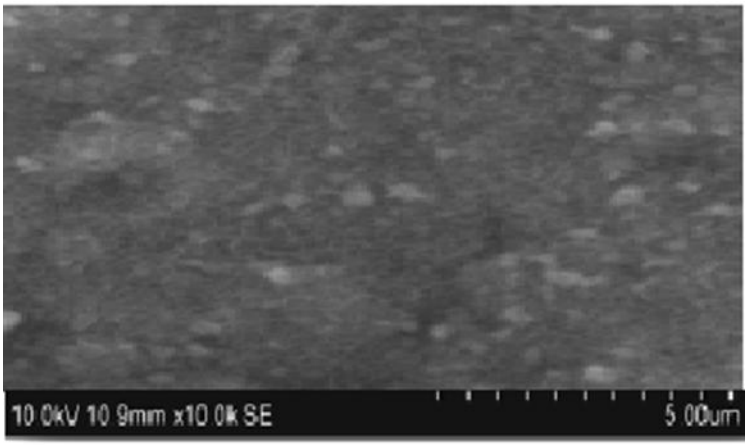



**Fig .4:SEM image of silver nanoparticles synthesis with Basil leaf extract.**

**Transmission Electron Microscopy (TEM).**

The size and shape of the synthesized silver nano-particles were confirmed by TEM technique. Figure 5 shows TEM images of AgNPs at different magnifications. The image shows that the AgNPs were often spherical, not interconnected, and sometimes merged into dense rows, possibly due to the presence of other organic materials.

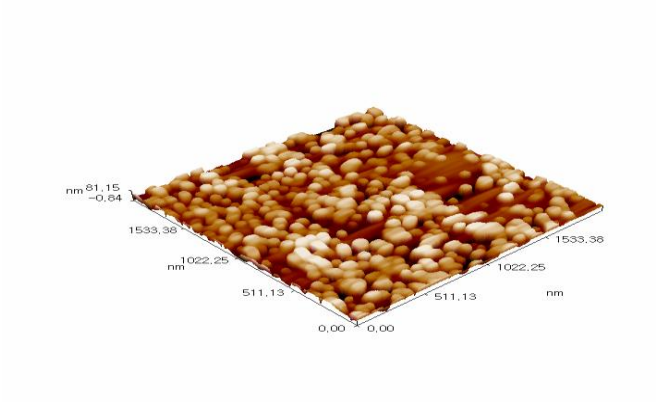



**Fig. 5: Scanning result of AgNPs synthesized by Basil leaf extract by TEM**

**Fourier-transform infrared spectroscopy:**

The technique FTIR, was used for determination the binding properties of AgNPs synthesized by Basil leaf extract. Also, it was found the properties of nanoparticle particles with the presence of plant extracts by using FTIR technique shows that active function aggregates is responsible for filling and covering the surfaces of nanoparticles of processed particles and also has a role in the stabilization and stability of such nanoparticles.Previous researchers have explained that the peak FTIR corresponds to the presence of a C-H vibration due to the aromatic ring, while the stretch vibration of C-O indicates the carbonyl and flavonoids group as clearly shown in the figure 6. (Deepa, et al. 2017; Charusheela, et al. 2013).

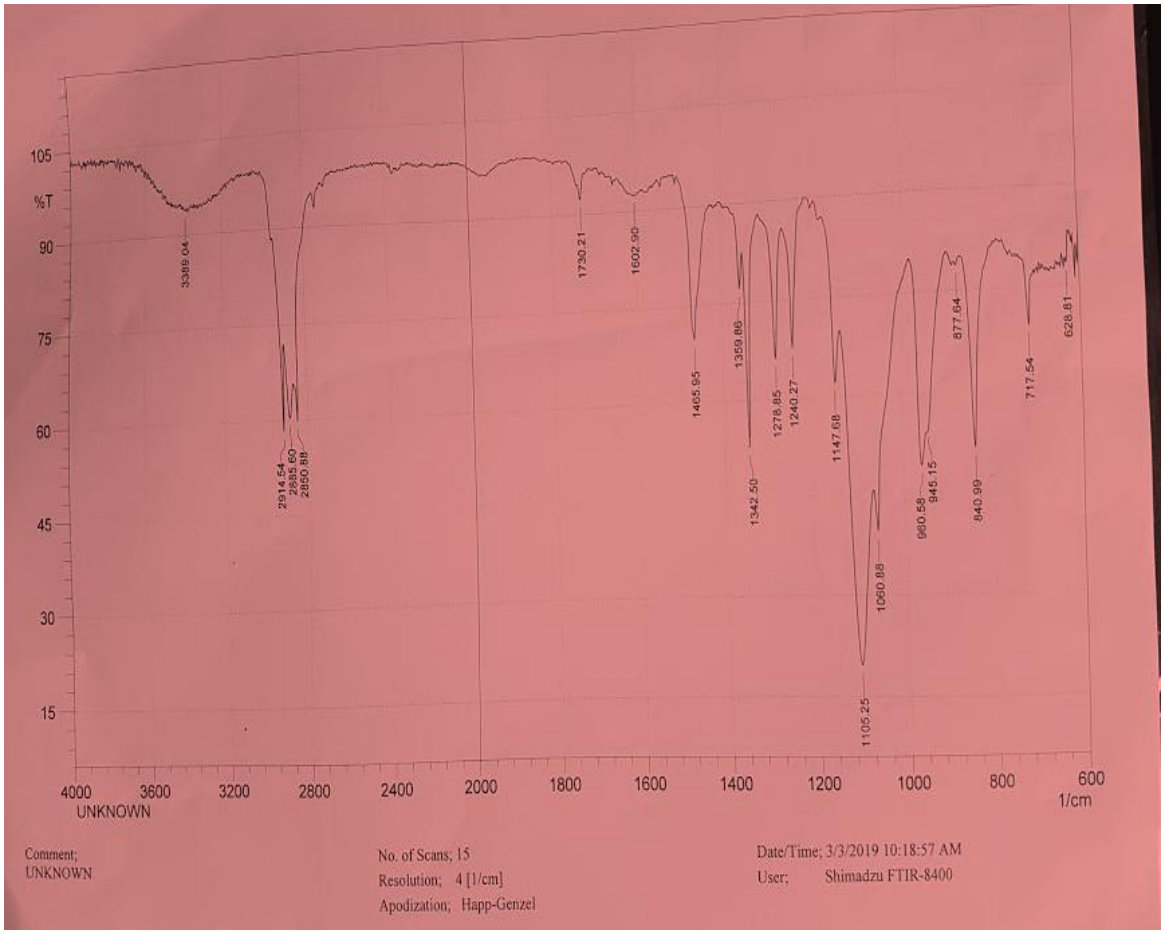



**Fig. 6: Scanning result AgNPs synthesized by Basil leaf extract in FTIR**

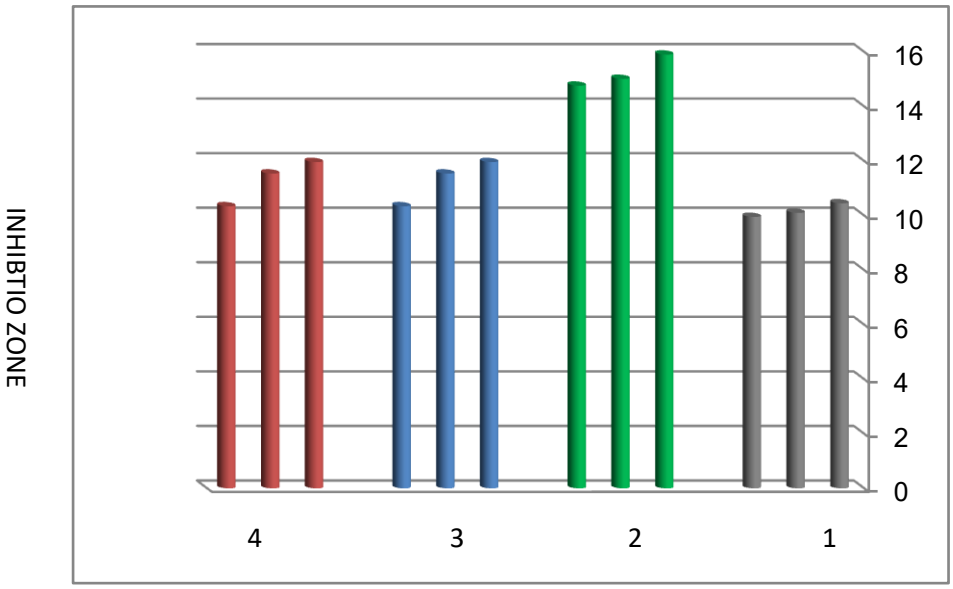
## Assessment of Antibacterial Activity:

Decades ago, the silver element's ability to inhibit the growth of many microbes, molds and fungi has been known (28).Since ancient times, Al-Rayhan plant has been known for its effective and anti-bacterial and fungal growth because it contains phenolic compounds and a lot of volatile essential oils and has been used on the traditional and commercial levels (Charusheela, et al. 2013; .Prakash and Gupta, 2005). The results of this study showed the effect of the use of basil extract on silver nano-particles synthesis and its inhibitory effect on the four types of bacteria studied by measuring values of diameter of zone inhibition compared to the control treatment as shown in Table 2. The nanoparticles prepared were found to have high inhibitory activity for microorganisms of all bacteria under positive and negative to gram stai *Staphylococcus sp., Proteus sp. spseudomonas sp. Escherichia coli* (Maliszewska, et al. 2009). The prepared nanoparticles in this study showed the highest inhibitory effect on *Escherichia coli* 15.9 at a concentration of 400 microliters per tablet followed by *Staphylococcus sp.* The *pseudomonas sp and Proteus sp*respectively were 12.96, 11.96 and 10.45 mm, respectively. Peter et al., (2013) and Charusheela, et al. (2013) found that nanoparticles made from *Achyranthesaspera L* showed a inhibitory effect against pathogenic bacteria. We conclude from this that the association of the intrinsic properties of

basil extract with nano-technolgy synthesis with silver proved its inhibitory effectiveness for microbial growth to minimum levels**.**

**Table 3: Values of zones of inhibition obtained by disc diffusion method**

| | Concentraion ( µl/disc) | | |
|---|---|---|---|
| **Bacterial St.** | **200** | **300** | **400** |
| ***1-pseudomonas sp*** | **0.13±10.34** | **0.20±11.54** | **0.12±11.96** |
| ***2-Staphylococcus sp*** | **11.14±0.14** | **0.14±12.54** | **0.23±12.96** |
| ***3-Escherichia coli*** | **14.76±0.21** | **0.19±15.01** | **0.14±15.9** |
| ***4-Proteus sp*** | **9.95±0.14** | **10.1±0.16** | **10.45±0.11** |




***1-Staphylococcus sp., 2-Proteus sp. 3-spseudomonas sp. 4-Escherichia col***

## Conclusion

The current study revealed that basil leaf is an excellent source for synthesis of silver nanoparticles and is easy to apply and configure, as well as it is environmentally friendly and its components are safe for the environment. The formation of silver nanoparticles was confirmed by changing color to reddish brown within 30 minutes due to excitation of surface plasmon vibration.The morphological appearance, arrangement of nanoparticles, nanoparticle size and silver nanoparticle density were examined using UV-Vis, SEM, TEM and FTIR

techniques.The Ag NPs formed were quite stable in the solution. Phytochemical analysis of the aqueous extract of Basil leaf presented the presence of Alkaloids, Flavonoids, Tannin, Phenols, Tannins, Phenols, Glycosides and Saponins. These phytochemicals were indicated their role in capping as well as stabilization of silver nitrate to silver nanoparticles molecules.This unique method was for its ease, low cost, rapid synthesis of nanoparticles from natural resources, less waste causing and safe for the environment.Therefore, more experiments should be conducted using medicinal plants in the nanoparticles of many mineral elements and their clinical application.

**Acknowledgements**

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# التخليق الحيوي للجسيمات النانوية الفضية باستخدام مستخلص نبات الريحانLamiaceaeوفحص نشاطه المضاد للميكروبات

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## الملخص

مجال تكنولوجيا النانو هو الأكثر استخدامًا على نطاق واسع اليوم ، على الرغم من وجود طرق كيميائية ونباتية كيميائية بسبب الاكتشافات الحديثة للعديد من الأجهزة المتقدمة لقياس النانو. الهياكل النانوية عالية السرعة وسهلة التركيب وصديقة للبيئة للغاية لاستخدامها المنخفض للحلول والمواد الكيميائية الصديقة للبيئة تم استخدام هذه الدراسة في تخليق الجسيمات النانوية للفضة المضادة للبكتيريا (AgNPs) باستخدام مستخلص نبات من الأعشاب الطبية، مثل أوراق الريحانBasil Lamiaceae. كما تم إجراء تقييم نوعي للحد من إمكانات مستخلص الأوراق ، مما يشير إلى وجود كمية كبيرة من المواد الكيميائية النباتية النشطة بتميز AgNPs المركّبة بواسطة التحليل الطيفي للأشعة فوق البنفسجية ، الفحص المجهري للإلكترون المسح ( SEM) ، المجهر الإلكتروني للإرسال ( TEM) ، التحليل الطيفي بالأشعة تحت الحمراء لتحويل فوريه ( FTIR).دليل تركيب الجسيمات النانوية الفضية هو تغيير اللون من الأخضر المصفر إلى البني المحمر و تم قياس نطاق امتصاص Plasmon السطح بواسطة التحليل الطيفي للأشعة فوق البنفسجية يتراوح حجم الجزيئات الكروية الموحدة بين 25 - 30 نانومتر ، وكانت للجسيمات النانوية المحضرة أعلى نشاط ضد البكتيريا المسببة للأمراض مثل المكورات العنقودية الذهبية Proteuinsts sp. ,Spseudomonassp الإشريكية القولونية. وجدنا أن جسيمات النانو ظهرت أعلى نشاط ضد الإشريكية القولونية يليها المكورات العنقودية الذهبية ، الزائفة الزائفة ، جنس البروتيوس لى التوالي.

**الكلمات المفتاحية:** مستخلص اوراق نبات الريحان- ذرات الفضة النانوية- نشاط مضادات الميكروبات