Green synthesis of silver nanoparticles using aqueous leaf extract of Lemon balm (*Melissa officinalis*) and its antibacterial activity

Hamady Nazhan M. and Mohammed Abdulalah T.*
Department of Chemistry, College of Science, University of Anbar, IRAQ
*atm952@yahoo.com

Abstract

Green synthesis of silver nanoparticles has gained wide attention due to the advantages of these reactions as eco- friendly and their cheap cost. In this present study, easy and rapid synthesis of silver nanoparticles (Ag NPs) was successfully synthesized as stable particles for more than three months by using leaf extract of Lemon balm (Melissa officinalis) as reducing agent. The formed yellow solution was identified as evidence on formation of silver nanoparticles. UV–Vis spectrophotometer showed peak at 438-440 nm. The size of Ag NPs was found at range of 1- 80 nm using Atomic Force Microscope (AFM) and also nanoparticles distribution was measured. Scanning electron microscopy (SEM) showed the size of Ag NPs in diameter ranging 12- 38 nm.

The stability of silver nanoparticles was analyzed by zeta potential measurements. A negative zeta potential value of -15.11 mV proved the stability of these nanoparticles. X-ray diffraction (XRD) was employed to confirm the crystalline nature of silver nanoparticles which implied the presence of (100), (111) and (220) lattice planes of the face centered cubic (fcc) structure of metallic silver. Effectiveness was studied as antibacterial agents for both silver nanoparticles and Melissa extract alone against the growth of Escherichia coli (Gram negative), Staph urease (Gram positive) and Proteus (Gram positive). Silver nanoparticles showed highly effective in inhibiting these types of bacteria, but the extract did not show any significant activity.

Keywords: Silver nanoparticles, herbal extract, antibacterial activity.

Introduction

Nanotechnology has contributed to the development of practical science through new nano materials manufactured in medicine and industry. The size of these nanoparticles is between 1-100 nm which means a higher surface area thus increasing the efficiency of these materials[9]. Silver nanoparticles are used in nanotechnology because of their unique properties such as chemical stability, good conductivity, antimicrobial, antiviral, antifungal and anticancer[6,8,14]. There are many methods used in the preparation of silver nanoparticles (Ag NPs), including physical methods such as micro-waves, X-rays and chemical methods which depend on the reduction of silver salts by reducing agents such as sodium borohydride.[5-8]

In recent years, it has been observed that green synthesis is widely used compared to chemical and natural methods because it is easy to prepare, cheap, simple and one step, in addition to being economical and environment friendly. In this method, the plant extract is used as a reducing agent for silver ions and thus to obtain its nanoparticles.[10-12,16-18,21,23]

The goal of this study is to synthesize stable Silver nanoparticles (Ag NPs) by using simple method and cheap cost. A locally herbal extract Lemon balm (*Melissa officinalis*) is used as reducing agent.

Material and Methods

UV-Vis absorption spectra were recorded on UV-VIS Spectroscopy (England), while size and shape of the particles were measured on Scanning Electron Microscopy SEM (Zeiss Scanning electron microscope, England) and Atomic Force Microscopy AFM (Phywe, Germany). Stability of the particles was recorded on Zeta Analyzer (Malven zeta seizer 2000, Malvern, UK). The crystallographic structure of the prepared sample was determined using a high-resolution X-ray diffractometric system JDX-3532 using monochromatic Cu-Kα radiation of wave length 1.5418 ˚A.

Silver nitrate crystals were purchased from Merck in Germany; Melissa leaves and Arabic gum were supplied from local market.

Melissa leaves were washed with distilled water (fig. 1), dried at 40^{o}C for 2h and then crushed by mortar. In a Pyrex (200 mL) conical flask, 60 mL of distilled water was added and then heated to boiling point. To this boiling water 0.5 grams of the crushed *Melissa* leaves were added and placed far from the heater and covered with watch glass for 10 minutes. The mixture is then filtered by a suction pump to obtain the extract solution of the *Melissa*. 0.15 g from the Arabic gum was added to 50 mL of distilled water which was heated to 70^{o}C in a 100 mL Pyrex beaker. This solution was added to the extract of *Melissa* with stirring.

Synthesis of silver nanoparticles: The reaction was carried out in a place away from direct light where the mixture of *Melissa* extract and the Arabic gum was placed in a 250 mL conical flask and heated to 60-70 °C. The pH of the mix was

adjusted to 7.5. Then, 50 mL of 1mM silver nitrate solution was added gradually with stirring. The color of the solution was converted directly to yellow indicating the formation of silver nanoparticles. The reaction lasted for twenty minutes, then the heater was raised and the solution remained stirring until it reached room temperature. The color of the yellow solution did not change even after three months (fig. 2).

Preparation of Mueller Hinton Agar (MHA) medium: 8 grams of medium (Mueller Hinton Agar MHA) were suspended in 500 mL of distilled water and mixed thoroughly and then heated with frequent agitation and boiled for 1 minute to completely dissolve the components and then autoclaved at 121°C for 15 minutes. The agar was cooled to 45°C and 25 ml of agar was poured in each of the six sterile Petri dishes (100-mm) to a 4 mm level in the horizontal depth to give a uniform depth. The medium was allowed to solidify at room temperature for 24 h.

Six Petri dish were cleaned and washed with distilled water and then 20 mL of MHA solution was placed in each dish. These Petri dishes were placed in autoclave to sterilization at temp. =120^{o}C and pressure =1 bar for 15 minutes. MHA was dried after 24 h at room temperature. The Ag NPs and Melissa extract were injected in three well and kept at 37^{o}C for 24 h. Distinct inhibition of bacteria by form zone contains Ag NPs which did not form about well contains in case of Melissa extract.

Another technique was used to test the activity of Ag NPs against the same three types of bacteria. This technique is based on measurements of difference in optical density between two solutions. One contains only the bacteria and the other the same solution but with the addition of Ag NP_S. The activity of Ag NPs in inhibition of bacteria can be confirmed by decreasing the low optical density values compared to the original solution that does not contain Ag NPs[13].

Results and Discussion

UV- VIS spectroscopy is an important technique used to demonstrate the formation of silver nanoparticles. The appearance of the yellow color in the solution was attributed to Surface Plasmon Resonance (SPR) which proves the composition of these particles. The spectra were carried out in the range of 350-600 nm and the max. peaks were observed at 436-440 nm confirming the formation of Ag NPs as shown in fig. 3.[1]

The XRD measurement of the prepared silver nanoparticles was given broad peaks due to their small size. The spectrum also confirmed the crystalline structure of nanoparticles as shown in fig. 4.

Five peaks were observed at degree of 2θ equal to 27, 38, 44, 54, 65, which are corresponding to five diffraction components of silver nanoparticles (table 1) corresponding to the (111), (200), (210) and (311) lattice planes. The unit cell of the crystal structure of Ag NPs was Face Cubic Center (FCC) face centered cubic[5,19].

The mean size of Ag NPs was 10 nm as shown in table 2 which was calculated using the Debye-Scherer's equation.

Atomic Force Microscopy (AFM) is used to obtain three-dimensional images of the surface as well as the distribution of nanoparticles. The 2D and 3D images of the Ag NPs show that the size of the particles ranges from 1-90 nm with a spherical shape as shown in fig. 5. The resulted average size of Ag NPs is 12 nm.

Ag NPs were visualized by SEM which indicated that the particles are spherical and have size ranged between 12-38 nm as shown in fig. 6.

The measurement of zeta potential is based on the micro electrophoresis technique, which is the best known method for the determination of nanoparticles in the solution. The value of Zeta potentiol for the synthesized silver nanoparticles equals to -15.2 which is an evidence of the stability of these particles (fig. 7). If zeta potential values are near to zero, that is an indication to agglomerate the Ag NPs in the solution, while if the values have high negative value, this confirms that Ag NPs were stable[3].

Anti-bacterial activity for synthesized Ag NPs and Melissa extract against *E. coli*, *S. aureus* and *proteus* bacteria were studied by two techniques; optical density assay and disc diffusion method. Both gave positive activity results with Ag NPs while negative results with Melissa extract. There is decrease in value of the optical density for the silver nanoparticles (fig. 8). The optical density values are inversely proportional with the inhibited bacteria[7].

The zone of inhibition (ZOI) values for the above three bacteria was recorded as shown in fig. 9. The activity of Ag NPs was measured as anti-bacterial and the ZOI appeared around the disc with a diameter of about 12-18 mm. The Melissa extract did not show any inhibition ability[20].

Fig. 1: Lemon balm (*Melissa officinalis*) plant

Table 1
The values of 2θ against the intensity

2θ°	Plane	Element	Shape
27	100	Ag	FCC
38	111	Ag	FCC
44	200	Ag	FCC
54	220	Ag	FCC
65	311	Ag	Hexagonal

Table 2
The calculated sizes of the Ag NPs

2θ°	FWHM	β= (π / 180) × FWHM	Cos θ	D = K λ / β.Cos θ nm
27	0.055	0.0017	0.97	13.86
38	0.06	0.001	0.94	15.40
44	0.052	0.0022	0.93	7.40
54	0.11	0.0019	0.89	8.15
65	0.14	0.0024	0.84	7.00

(a) **(b)**

Fig. 2: Synthesized Ag NPs solution; (a) after 20 minutes, (b) after three months

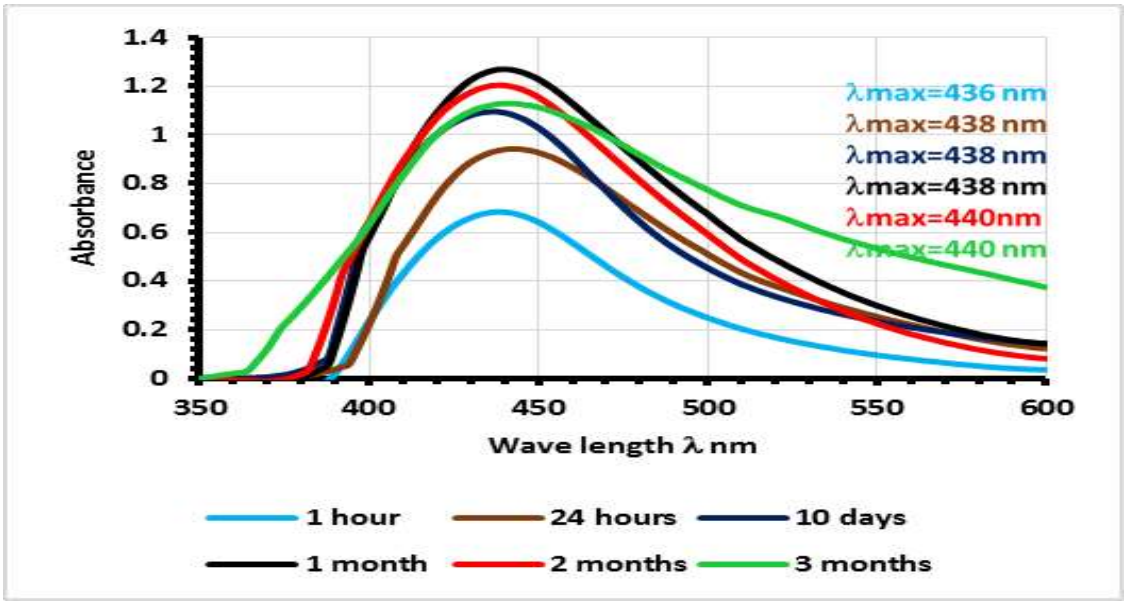

Fig. 3: UV- VIS spectra of synthesized Ag NPs at different times

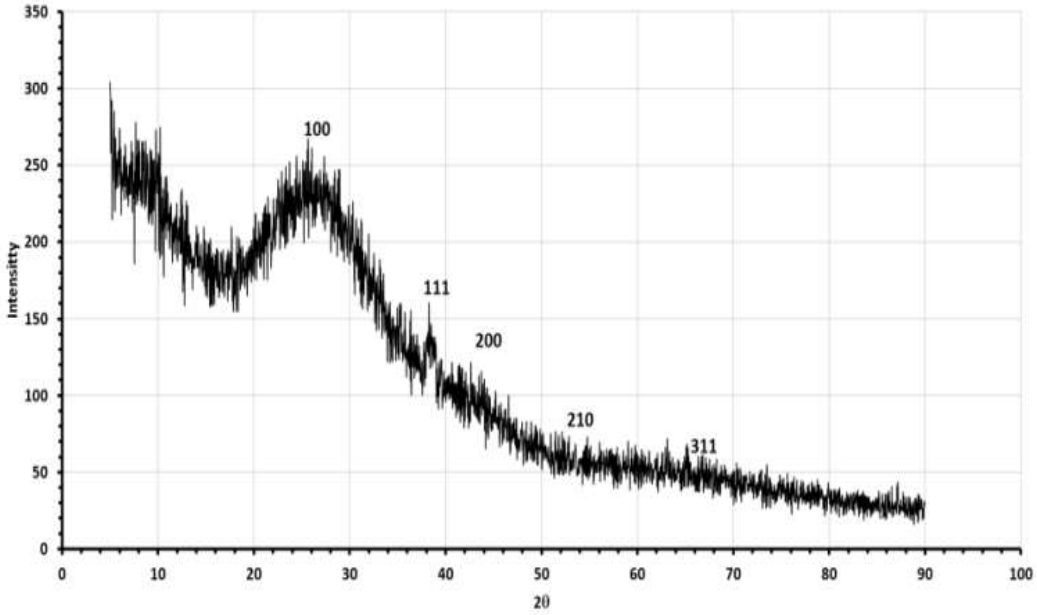

Fig. 4: XRD spectra for synthesized Ag NPs

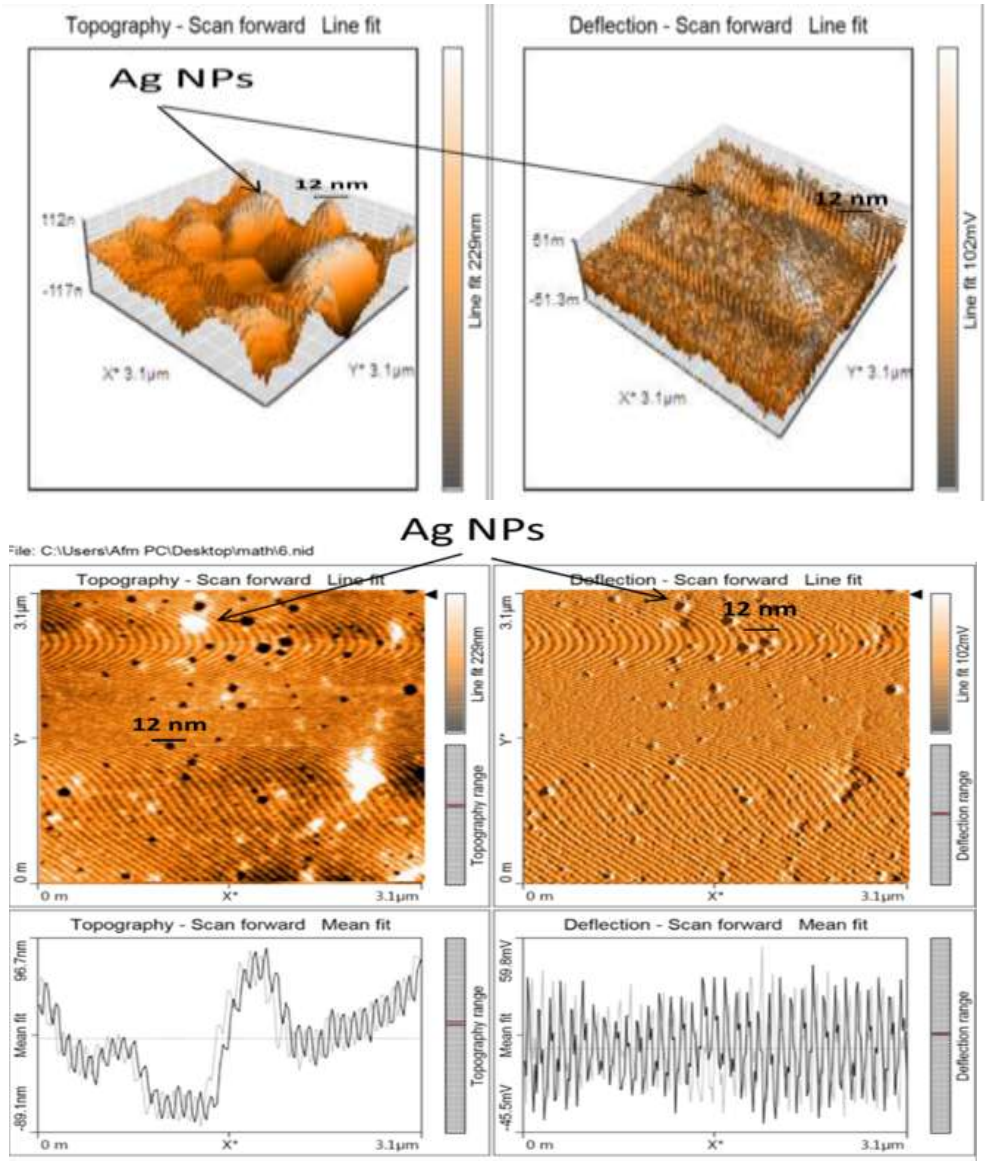

Fig. 5: AFM image (topography) of Ag NPs

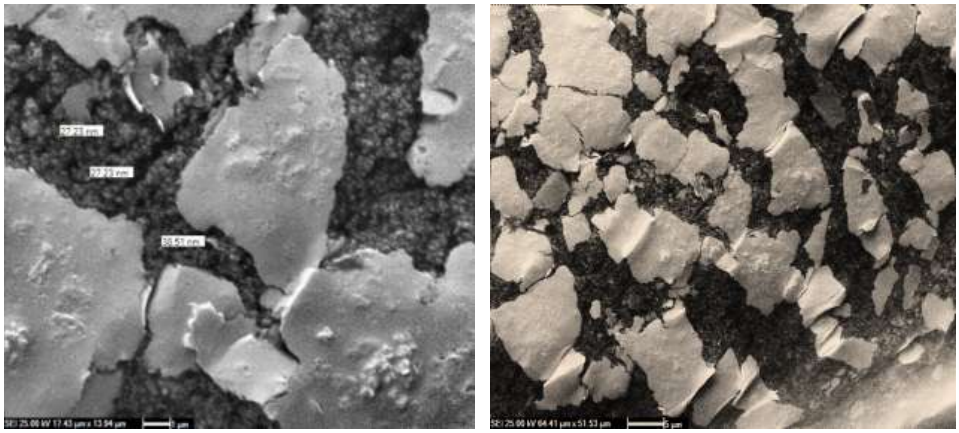

Fig. 6: SEM images for Ag NPs

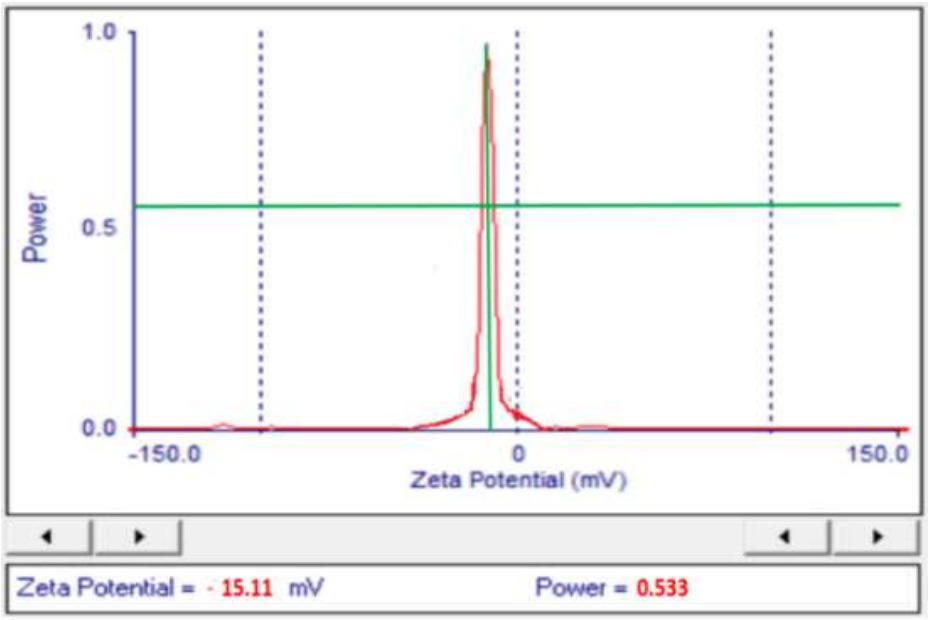

Fig. 7: Zeta potential of synthesized Ag NPs by *Melissa* extract

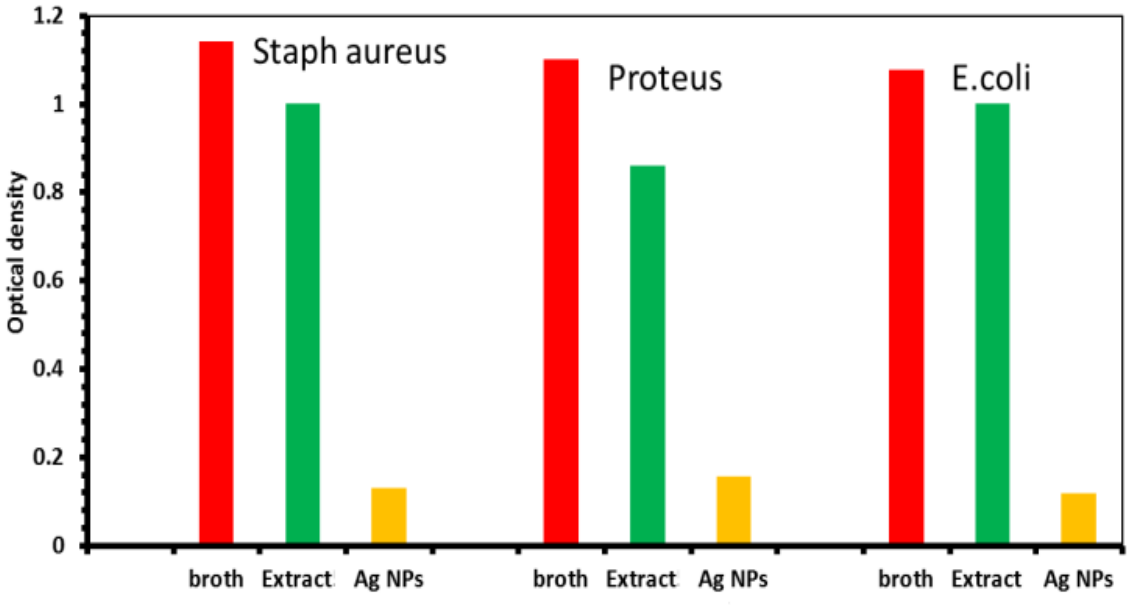

Fig. 8: Optical density for the synthesized Ag NPs

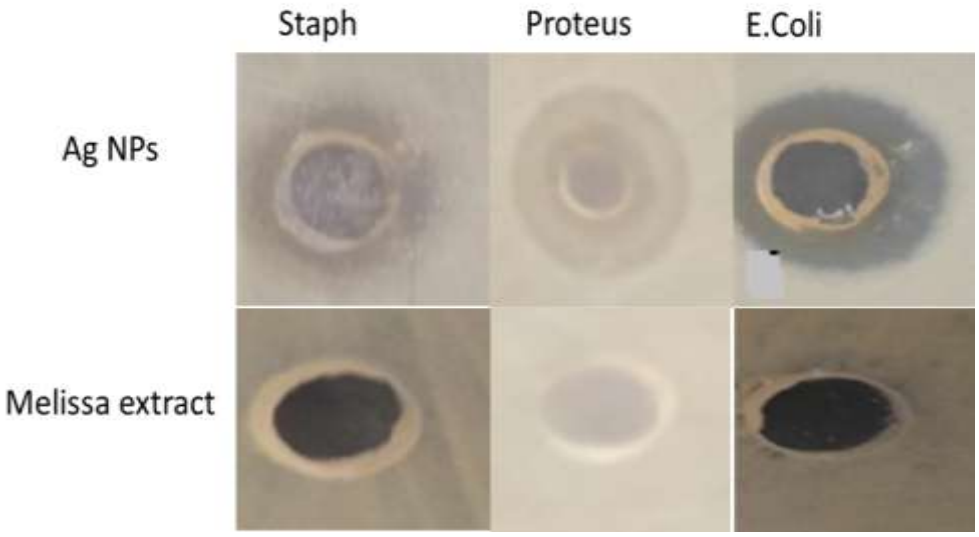

Fig. 9: Biological activity for Ag NPs and *Melissa* extract

Conclusion

This study showed an easy way to produce Ag NPs with desirable properties from *Melissa* extract using the Arabic gum as stabilizing agent. UV-Visible spectroscopy results confirmed the existence of Ag NPs through the observation of the particular peak at 438 nm. The SEM analysis confirmed spherical and uniform Ag-NPs with diameter ranging 12- 38 nm. The biosynthesized Ag NPs showed considerable antibacterial activity and could be used as an effective treatment in wound and burn infections.

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