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## ORIGINAL ARTICLE

# Biosynthesized Silver Nanoparticles Induced Potential Antibacterial Activity

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## ABSTRACT

***Background:*** *Nanoparticles especially those synthesized biologically are promising in its applications in medical field. The nanoparticles synergic effect with common used antibiotics are the most targeted studies nowadays, in order to overcome the microbial antibiotic resistance problem.* ***Objectives:*** *To synthesize silver-nanoparticles (AgNPs) using desert plant Alhagi maurorum (am), and to examine their effects in improvement the antibiotics efficiency.* ***Methodology:*** *Extracellular silver-nanoparticles biosynthesis using Alhagi maurorum (desert plant). The synthesized silver-nanoparticles were characterized using UV-visible spectroscopy, scanning electron microscopy-energy dispersive spectroscopy (SEM-EDS), X–ray diffraction (XRD) and zeta potential in order to investigate their structural, and textural properties. Synergistic effect of AgNPs plus antibiotics was also measured.* ***Results:*** *The UV-visible absorbance showed a strong intense peaks at 375 nm The XRD showed diffraction peaks at $2\theta=32.12°$, $44.76°$, $46.22°$ corresponding to the (122), (200), (231) which agreed with the quality value (JCPDS card No. 04-0783). SEM–EDS and XRD analysis confirmed the crystalline nature of silver-nanoparticles with spherical, oval and hexagonal in shape ranged from 37-79 nm in size. DLS analysis showed the size distribution of silver-nanoparticles with maximum intensity at 70 nm. The silver-nanoparticles showed considerable synergistic effect with different antibiotics.* ***Conclusion:*** *Silver can be reduced to nanoparticles by Alhagi maurorum extract. Considerable synergic effects were recorded by applying nanoparticles with diversified antibiotics on different pathogenic bacteria.*

## INTRODUCTION

Metal based nanoparticles are now grooming for various fields especially in medicine and biotechnology. Silver nanoparticles is from the few nano-products raised from metal and currently in use for medical purposes, and consider the most promising product in past and present in this area. Silver nanoparticles were recognized as an effective antimicrobial agent and have many prospective applications in medicine[1-3].

Silver nanoparticles defined as those particles of which are size ranged from 1-100 nm, and has unique properties that used in many medical and other technical applications. The main methods used for synthesis of these nanoparticle are the physical and chemical methods, but the problem correlated with using these methods is the cost, since they represent expensive ways and maybe toxic as a result of the toxic substances absorbed onto them during preparation as well [4].

The medical properties of silver solution were known since over 2,000 years ago, that silver-based compounds have been used since the nineteenth century in many antimicrobial applications. For its effective antimicrobial properties, silver nanoparticles are now being used as antimicrobial agents in many public places in China such as railway stations and elevators [4]. Nowadays, nanoparticles have achieved remarkable attention as novel antimicrobial products as they possess unique physical and chemical properties helping it acting as antimicrobial agents. [5–8].

Shijing Liao *et al.*,[9] assumed that silver nanoparticles showed significant antibacterial effect on antibiotic-resistant *P. aeruginosa* strains.

The silver-nanoparticles are not only used as antimicrobial agent, but also have diversified application as bactericidal and therapeutic agent. For example, it used in preparation of dental composites; coating the medical devices; coating of water filters for its bactericidal effects; in air sanitizer sprays as an antimicrobial agent. Silver-nanoparticles can be also used in wet wipes, sock, pillows, detergents, soaps and shampoos, toothpastes, and in washing machines detergents. Wound dressings and bone cement are from the most important products include silver-nanoparticles for its antimicrobial activities. The environmental toxic effect proposed to silver nanoparticles need more studies to be conducted to conclude that there is a real problem with it [4, 10].

On the contrary of using chemical and physical methods, the major advantage of nanoparticles green synthesis (using plant extracts) is that they are easily available, safe, unexpansive, and nontoxic. Plant parts have metabolites variety that can participate in reduction of silver ions, which are faster than microbes in this case.

The main mechanism considered for this process is plant-assisted reduction due to phytochemicals, which include terpenoids, flavones, ketones, aldehydes, amides, and carboxylic acids. Flavones, organic acids, and quinones are water-soluble phytochemicals that are responsible for the immediate reduction of the ions [11].

The aim of our work is to examine the ability of *Alhagi maurorum* leave extract to synthesize nanoparticles from silver-nitrate solution. Characterization and examination of the synergistic effect of these nanoparticles were targeted as well.

## METHODOLOGY

### Plant materials and preparation of the aqueous extracts.

Leaves of *Alhagi maurorum* (Fabaceae) were collected freshly, washed thoroughly with copious amount of deionized water, shade dried for 2–3 weeks and powdered. Plant samples (5 g) were boiled in 50 mL of deionized water at 60 °C for 15 min. After cooling, the mixtures were filtered through Whatman No.1 filter paper and the filtrates were used for further experiments.

### Synthesis of silver nanoparticles (AgNPs).

Leaf extract was prepared according to Velmurugan *et al.*, [14]. The color change (from light yellow to reddish brown) confirmed the initial AgNPs formation. After the end of synthesis process, centrifugation of the mixture at 8000×g for 10 min was done, and the pellet of silver-nanoparticles were cleaned to remove impurities by dissolved them in sterile distilled water tree times and re-suspended by centrifugation.

### Characterization of AgNPs.

***UV–Vis spectrophotometer*.**

*UV-1800-Shimadzu,* Kyoto, Japan, was operated at 1-nm resolution within a wavelength ranged from 300 to 700 nm.

***X-ray powder diffraction (XRD).***

Samples was performed using a Rigaku X-ray diffractometer (Rigaku, Japan), with scanning done in the region of $2\theta$ from $2\theta = 20$ to 80° at 0.04°/min with a time constant of 2s.

***Scanning electron microscopy-Energy dispersive spectroscopy (SEM-EDS).***

The surface morphology of the nanoparticles was conducted through the spectra of SEM, JEOL-JSM 6390, Japan. The elemental composition was also confirmed by focusing the electron beam onto the particles surface.

***Dynamic light scattering (DLS).***

The particle sizes of AgNPs was evaluated using dynamic light scattering (DLS) measurements and zeta potential analysis was conducted with a Zetasizer Nanoseries compact scattering spectrometer (Malvern Instruments Ltd., UK).

### Antibiotics susceptibility test and synergic effect of silver nanoparticles.

The synergistic effect of silver-nanoparticles was evaluated with four antibiotics by using the well diffusion method (Fig. 1). Penicillin G, Tetracycline, Streptomycin, Vancomycin, and Methicillin were tested for their synergized effect on three bacterial reference strains; *E. coil*, *Pseudomonas aeruginosa*, and *staphylococcus aureus* strains as described in 2015.[12]

**Fig. 1:** Representative picture for well diffusion method

## RESULTS

***Characterization of AgNPs.***

***UV–Vis spectrophotometer*.**

During reduction reaction, the pure $Ag^+$ ions are converted to $Ag^0$ which can monitored based on a visual color change from light yellow to brown according to the particles concentration. The UV-visible absorbance of the reaction mixture showed intense peaks at 375 nm. during 3 h of incubation period.

***X-ray powder diffraction (XRD).***

The pattern of XRD of the Am reaction extract is shown in fig. 2. The diffraction peaks at $2\theta=32.12°$, 44.76°, 46.22° corresponding to the (122), (200), (231) planes for *Alhagi maurorum*, sets of grid planes and points to the cube centered on the face (fcc) structure of silver-nanoparticles. All diffraction peaks are agreed with JCPDS quality value (card No. 04-0783).

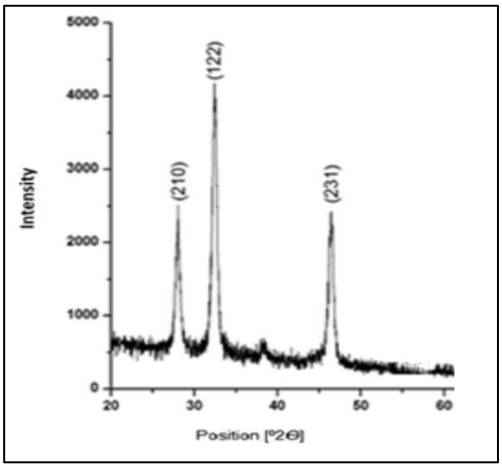

**Fig. 2:** XRD pattern of biosynthesized silver Nano-particles

### *Scanning electron microscopy-Energy dispersive spectroscopy (SEM-EDS).*

SEM images and EDS spectrum of *Am* (*Alhagi maurorum)* AgNPs are shown in Fig. 3a, and 3b, respectively. The size of nano-particle reveals that all types of NPs were various shapes like circular, rectangle and oval with smooth edges (Fig. 3a) and the particles size ranged from 37-79 nm.

The EDX signals analysis of AgNPs showed that the strong silver metal signals were in the range of 2–4 keV, while weak peaks of silicon, chlorine and oxygen were appeared, which confirm obtaining AgNPs are in pure elemental form (Fig. 3b).

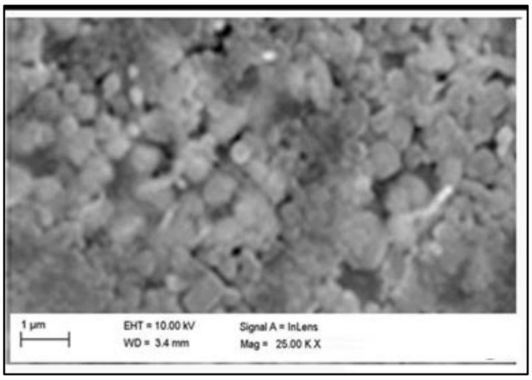

**Fig. 3a**: SEM & SEM-EDS images of synthesized AgNPs using *Alhagi maurorum*, extracts confirming the spherical and cuboids shaped nanocrystals.

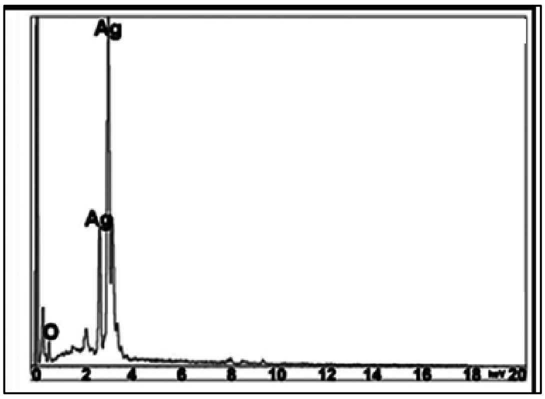

**Fig. 3b:** Energy dispersive X-ray (EDX) spectroscopy of AgNPs exhibited the strong signal of silver

### *Dynamic light scattering (DLS).*

AgNPs dispersed in Millipore water was highly stable with a zeta (ζ) potential value of −14.9 mV for Am. DLS analysis showed the size of AgNPs with maximum intensity was at 70 (table 1).

**Table 1: Zeta potential and particle size determination of biosynthesized AgNPs from *Am* extracts**

| | ***Am*AgNPs** |
|---|---|
| Zeta potential value (mV) | –14.9 |
| Particle size determination (nm) | 70 |

### *Synergistic Effect of Silver-Nanoparticles with Antibiotics.*

The synergistic effects of Ag-NPs which were investigated with different four antibiotics against three selected bacterial strains are shown in figure 4 (a, b, c). Majority of combinations studied showed positive effectiveness against *E. coil*, *Pseudomonas aeruginosa*, and *staphylococcus aureus* strains. No synergistic effect was recorded with 3 cases; Tetracycline with each of *E. coli* (fig. 4a) and *Staphylococcus aureus* (fig. 4c) and Vancomycin in case of *Pseudomonas aeruginosa* (fig. 4b) The highest synergistic ratio was recorded in case of *Staphylococcus aureus* [60% synergic effect with Penicillin G (fig. 4c)]. The synergic effect of silver-nanoparticles with Tetracycline and Streptomycin showed high ratio as well (50%) when applied on *Pseudomonas aeruginosa* strain (fig. 4b). The other antibiotics showed a synergistic effect with average ranged from 2.7% to 42% on the used bacterial strains (figs 4 a, b, c).

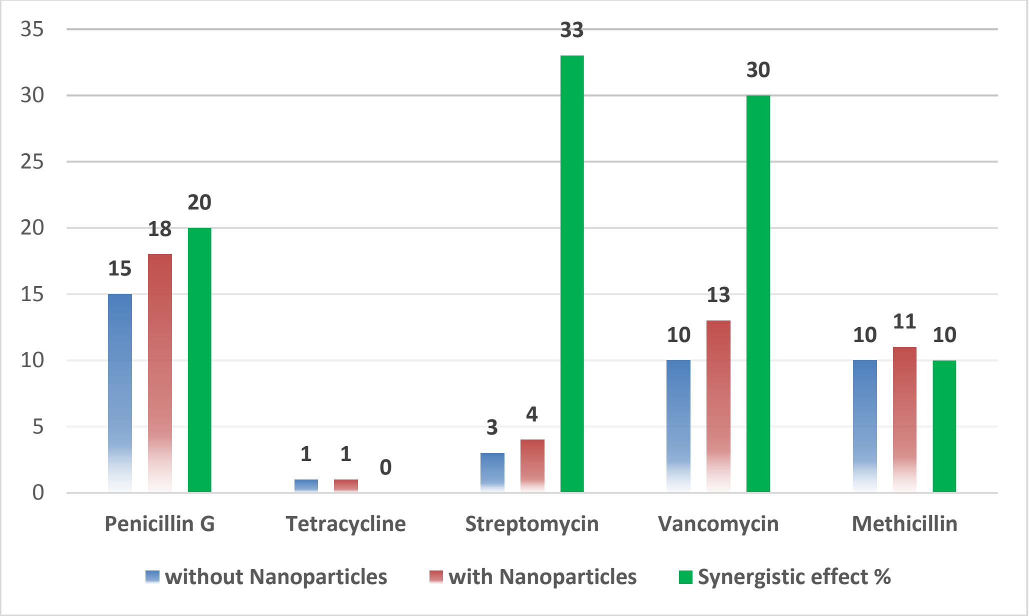

**Fig. 4a:** Average Inhibition Zone (mm) with different antibiotics, and antibiotics plus Silver Nanoparticles using *E. coli*.

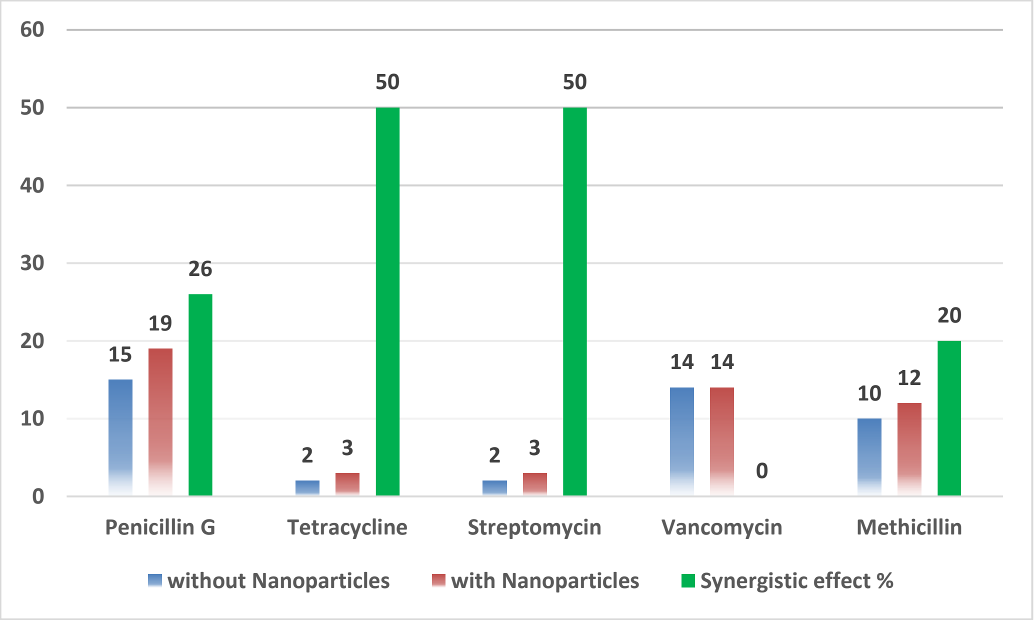

**Fig. 4b:** Average Inhibition Zone (mm) with different antibiotics, and antibiotics plus Silver Nanoparticles using *Pseudomonas aeruginosa*.

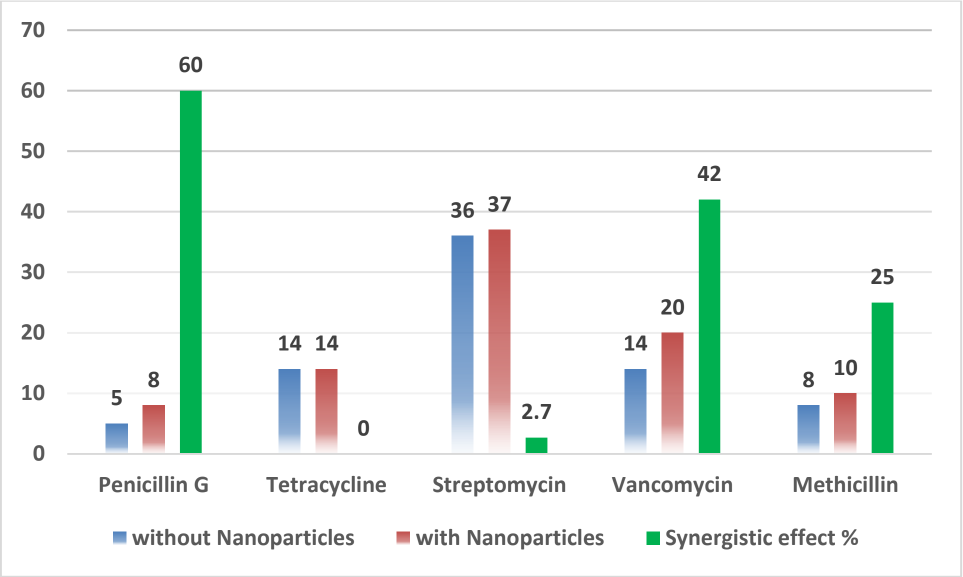

**Fig. 4c:** Average Inhibition Zone (mm) with different antibiotics, and antibiotics plus Silver Nanoparticles using *Staphylococcus aureus*.

## DISCUSSION

Using plant extracts for silver nanoparticle synthesis have major advantages, that they are easily available, safe, and nontoxic in most cases, have a broad variety of metabolites and phytochemicals that involved directly in ions reduction and silver nanoparticles formation, so they are quicker than microbes in the nanoparticle synthesis [13].

Many reports have documented the role of plant parts extract in silver-nanoparticles synthesis. Rajan *et al.*[14] reviewed the reports on silver nanoparticles synthesized with plant extract with particular concentration to their antimicrobial, antioxidant, and anticancer activities. In 2021 it was also reviewed that plant-mediated synthesis of AgNPs is faster, more convenient, and could be effective in utilization of wastes generated by fruit and vegetable producers and handlers. Plant-mediated AgNPs are effective against both Gram-positive and Gram-negative foodborne pathogens and spoilage bacteria[15]. Sur *et al.*[16] have described the green synthesis of silver nanoparticles using the plant extract of Shikakai and Reetha. They reported that the biosurfactant molecules present inside the plant extract play a significant role both as stabilizing and reducing agents of silver metal ions.

Mukherjee *et al.*[17] and Velmurugan *et al.*[18] reported that the reduction process of $Ag^+$ to $Ag^0$ nanoparticles occurred possibly in the presence of enzyme NADPH-dependent dehydrogenase and phytochemicals like phenolic group compound present in plant material could be responsible for $Ag^+$ reduction.

Our results are well coordinated with earlier reports on SEM and EDS spectrum of AgNPs obtain by leaf extracts of *Artemisia nilagirica*[١٩] ; *Ficus benghalensis*[16]; *Rauvolfia tetraphylla* [20]; *Caesalpinia coriaria* [21] and *Naringi crenulata* [22].

The XRD diffraction is consistent with Jeeva *et al.* [23] and Jayaseelan and Rahuman [24] reports for AgNPs synthesis by using aqueous leaf extracts of *Caesalpinia coriaria* and *Ocimum canum*.

Earlier results of Kumar *et al.*[25] and Sukirtha *et al.* [26] on zeta potential study using AgNPs synthesized from *Gracilaria corticata* and *Melia azedarach* leaf extract are in accordance with our results.

Despite the mechanism for the synergistic activity is not well known, the combination between silver-nanoparticles and an antibiotic can synergistically suppress bacterial growth, especially against the drug-resistant bacteria.

The combination between AgNPs and antibiotic Levofloxacin, has lower MIC value, which confirm that AgNPs-Levo combination have greater antibacterial activity. The enhancement of AgNPs antibacterial activity in Levo combination due to the hydrophobic nature of AgNPs [27].

Another studies have reported the combination effect of nanoparticles with different antibiotics, which were investigated against *S. aureus* and *E. coli*. The highest fold increases in inhibition zone according to disk diffusion method were observed for vancomycin, amoxicillin, and penicillin G against *S. aureus*. The effects of Ag-NPs on the antibacterial activity of the aforementioned antibiotics for *E. coli* were lower than *S.*

*aureus*. In contrast, the most synergistic activity was observed with erythromycin against *S. aureus* [1].

Hwang *et al.*, [28] have reported that, all combinations studied were effective against the bacteria tested. Synergistic interactions of nano-Ags and ampicillin were observed against *Enterococcus faecium*, *Streptococcus* mutans and *E. coli*, while synergistic interactions of nano-Ags and chloramphenicol were found only against *Enterococcus faecium* and *P. aeruginosa*, but synergistic interactions of nano-Ags and kanamycin were seen against *Staphylococcus aureus*, *Streptococcu*s mutans, *E. coli* and *P. aeruginosa*. They observed that other combinational activities of nanoAgs and antibiotics had partially synergistic interactions.

Deng *et al.,*[29] observed that enoxacin, kanamycin, neomycin, and tetracycline show suppressive synergistic action against the *Salmonella* bacteria when combined with AgNPs, while ampicillin and penicillin do not.

In our study, the synergistic effects of Ag-NPs were reported in its majority against *E. coil*, *Pseudomonas aeruginosa*, and *staphylococcus aureus* strains, while no synergistic effect was recorded with Tetracycline on each of *E. coli* and *Staphylococcus aureus* and Vancomycin on *Pseudomonas aeruginosa.* The highest synergistic ratio was recorded in case of *Staphylococcus aureus* with Penicillin G. The most synergic activity was observer with Tetracycline and Streptomycin (50%) against *Pseudomonas aeruginosa*. The synergistic effect of nano-Ags in presence of conventional antibiotics suggested that, it might be possible to reduce the viability of bacterial strains at lower antibiotic concentrations [30].

## CONCLUSION

This protocol showed that the environmentally renewable source of leaf extracts can used as an effective bio-reducing and capping agent for silver-nanoparticles synthesis. These biosynthesized nanoparticles can be applied to enhance the efficiency of commercial antibiotics especially on MDR bacteria and reducing the antibiotics dose used after studying the effects of nanoparticles in vivo.

This manuscript has not been previously published and is not under consideration in the same or substantially similar form in any other reviewed media. I have contributed sufficiently to the project to be included as author. To the best of my knowledge, no conflict of interest, financial or others exist. All authors have participated in the concept and design, analysis, and interpretation of data, drafting and revising of the manuscript, and that they have approved the manuscript as submitted.

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