# GREEN SYNTHESIS OF SILVER NANOPARTICLES AND THEIR ANTIBACTERIAL ACTIVITY

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**Abstract**— The silver nanoparticles (AgNPs) were synthesized by using honey as reducing and stabilizing agent. The synthesized AgNPs were characterized by UV-visible spectroscopy; Fourier transforms infrared spectroscopy (FTIR analysis) and transmission electron microscopy (TEM). The UV-visible spectral analysis of colloidal exhibited the maximum absorption peak between 400 — 450 nm. The FTIR spectra showed absorption bands in regions ranging from 3269.15 to 418.70 $cm^{-1}$ it indicates that the nanoparticles were bound to protein. The colloidal is found to consist of silver nano-particles of an average size of 20nm and nearly spherical which was evident through TEM images. The synthesized silver nanoparticles were evaluated for their antibacterial activity against five human pathogenic bacteria. AgNPs solution (50 µl) inhibited Gram positive as well as Gram negative bacteria by different extents. Maximum zone of inhibition was observed against S. aureus (MRSA) (23.7 mm), followed by S. pyogenes (16.7 mm), E. coli (13.0 mm), B. subtilis (12.7 mm) and P. aeruginosa (12.0 mm). MIC value was found less than 12.5 µl/ml for S. aureus, while, it was <25.0 µl/ml for S. pyogenes, B. subtilis and E. coli. However, the MIC value for P. aeruginosa was 70µl/ml. The present study reveals that AgNPs had broad spectrum antibacterial property thus it can be explored further for the the eco-friendly production of silver nanoparticles for medical and therapeutic uses..

**Keywords-** Silver nanoparticles, green synthesis, UV-visible spectrophotometer, TEM, FTIR, antimicrobial assay.

## I. INTRODUCTION

The emergence of bacterial infectious diseases and the development of antibiotic resistance in the pathogenic bacteria is a problem of great concern. Therefore, there is a continuous need for the development of new antimicrobial agent to combat pathogenic bacteria. Silver has been used as an excellent antimicrobial agent since ages. The silver nanoparticles (AgNPs) have unique physical and chemical properties which increases the efficiency of silver. Now days, synthesis of nanoparticles is attracting the attention of the researches due its myriad uses [1]. Currently the biological or green syntheses of nanoparticles have been explored extensively as the chemical synthesis has several environmental hazards. Biological synthesis of silver nanoparticles is eco-friendly, cost effective and maintains the homogeneity and stability of the synthesized nanoparticles [2]-[3].

## II. MATERIALS AND METHODS

### A. Synthesis of silver nanoparticles using honey

Fresh honey was procured from the local market of Riyadh, Saudi Arabia. For the synthesis of AgNPs, the procedure described by Philip [4] was followed. Briefly, 20g honey was diluted with in 80ml deionized water. An aliquot of 20ml aqueous solution of $AgNO_3$ (10 mM) was mixed to 15ml of honey solution and pH was maintained to 7.5. This reaction mixture was incubated at room temperature for up to 5 h. The changes in color ranging from light greenish to dark brownish were recorded after each hour of incubation; reduction was indicated by golden yellow color of the solution [4]. To screen the reducing power of the Ag+ synthesized in the reaction mixture, the UV-Vis spectrum of the colloidal was measured by using UV-Vis Spectrophotomer, (Shimadzu 1800, Japan) at the absorbance of 300 nm to 600 nm. FTIR analysis was done to identify the capping and stabilizing compounds responsible for the formation of silver nanoparticles using Bruker-Vertex-80 Machine. TEM analysis was performed for further characterization of the AgNPs. Samples for TEM studies were prepared by placing a drop of the AgNPs colloidal suspension on carbon-coated grids. TEM images were obtained by using a transmission electron microscope, JEOL JEM-1011.

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### B. Antibacterial activity of AgNPs

Five bacterial strains i.e. Staphylococcus aureus (MRSA) ATCC 29213, Bacillus subtilis ATCC 6633, Pseudomona aeruginosa ATCC 27853, Streptococcus pyogenes ATCC 12384, Escherichia coli ATCC 25922 were used in the present study; all the tested strains were obtained from King Khalid University Hospital, K.S.U. Saudi Arabia. These Bacterial cultures were grown in Muller Hinton broth at 37ºC and maintained on Muller Hinton slants at 4ºC.

Antimicrobial activity of the AgNPs was evaluated by employing agar well diffusion assay. In this method, 10 ml aliquots of nutrients broth (Sigma-Aldrich, Germany) was inoculated with the bacterial pathogens and incubated at 37°C for 24 h. Following day cultured broth was adjusted to a turbidity of 0.5 Mac Farland standards ($10^8$ CFU/ml). 100 µl bacterial suspensions were evenly spread over the entire surface of the nutrient agar plate to obtain uniform inoculums. Four wells per plate were made and were each well was filled with 50 µl AgNPs extract. Augmentin was

used as positive control. Each extract was analysed in triplicate. All the plates were incubated for 24 h at 37°C. The antibacterial activity was interpreted from the size of the diameter of zone of inhibition measured to the nearest (mm) as observed from the clear zone surrounding the well.

Minimum inhibitory concentration (MIC) was determined by the streak method. To determine the MIC, AgNPs solution was dissolved in MHB (950 µl) and serially diluted in eppendorf tubes (starting with 50 µl) under a laminar flow cabinet. Bacterial cell suspension was prepared in Mueller Hinton broth corresponding to $1x10^6$ CFU/ml, the uniform volume (100 µl) of an actively growing culture of the tested pathogen was added to the different eppendorf tubes and cultures were grown overnight in an incubator at 37°C. The following morning, streaking was done from all samples on respective agar plates. MIC was rated by the lowest concentration of the test solution that inhibited the growth.

## III. RESULTS AND DISCUSSION

AgNPs exhibit yellowish brown colour in aqueous solution due to excitation of surface plasmon resonance. On mixing the extract with aqueous solution of the Ag ion complex, a change in the colour of reaction mixture from colourless to reddish brown was observed. It happens due to the splitting of $AgNO_3$ into Ag+ and $NO_3$-. The metabolites in the extracts act as e- donors and reduced Ag+ ions to Ag. As a result, the formation of AgNPs was indicated by the brown colour of the reaction mixture [5]. UV-visible spectral analysis of the reaction mixture exhibited the maximum absorption peak between 400 — 450 nm. Silver nanoparticles are known to exhibit a UV–Visible absorption maximum in the range of 400–500 nm, which was apparent in the UV-Vis spectral analysis of the colloidal of AgNPs synthesised by using honey (Fig. 1). Our results are in agreements with the findings of Philip [4].

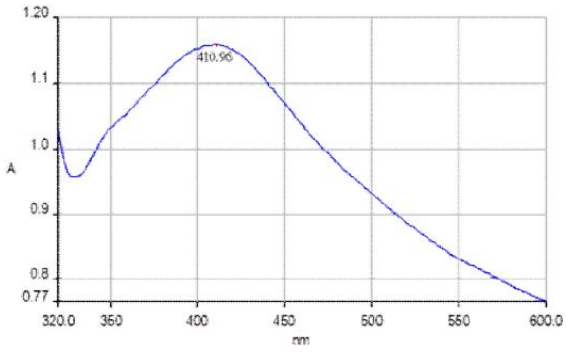



**Figure 1: UV-visible spectral analysis of honey extract with 10mM of silver nitrate solution after 24 h.**

FTIR measurement was carried out to identify the possible biomolecules in honey responsible for capping leading to efficient stabilization of the silver nanoparticles (Fig. 2). Carbonyl groups from the amino acid residues and peptides of proteins show strong affinity for binding with metals. The FTIR spectra of honey extracts showed absorption bands in regions ranging from 3269.15 to 418.70 $cm^{-1}$ (Fig. 2). The overall observation confirms the presence of protein in samples of silver nanoparticles. Therefore, it indicates stabilization of silver nanoparticles by proteins [6].

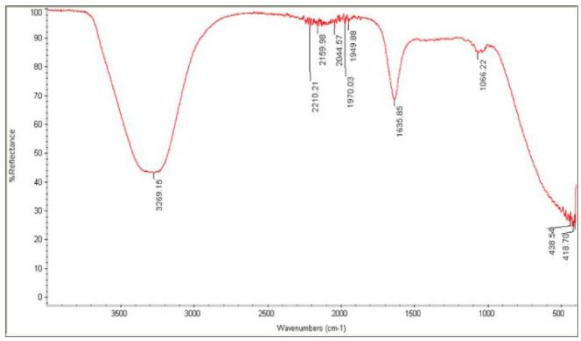



**Figure 2: FTIR spectral analysis of honey derived AgNPs.**

The TEM images obtained for honey are shown in (Fig. 3). It clearly showed that honey derived nearly spherical silver nanoparticles.

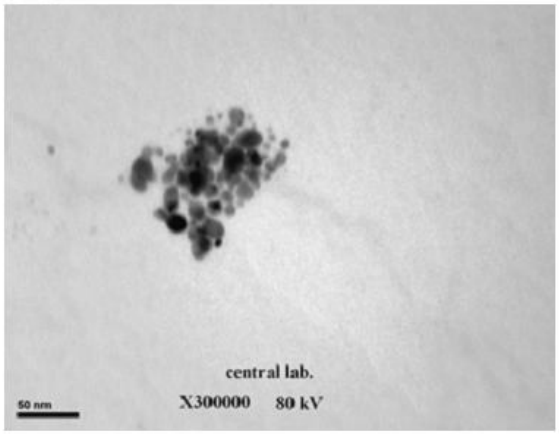



**Figure 3a: TEM micrograph of the silver nanoparticles, scale bar correspond to 50 nm.**

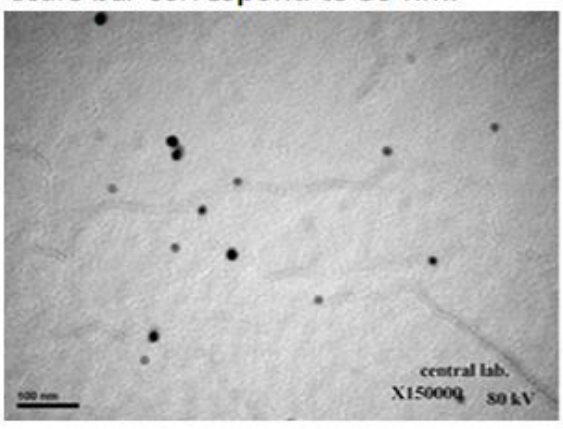



**Figure 3b: TEM micrograph of the silver nanoparticles, scale bar correspond to 100 nm.**

Antibacterial activity of AgNPs synthesized using honey showed varied zone of inhibition against the tested bacteria. AgNPs solution (50 µl) has inhibited both Gram positive and Gram negative bacteria by

different extents. Maximum zone of inhibition was observed against S. aureus (MRSA) (23.7mm), followed by S. pyogenes (16.7mm), E. coli (13.0 mm), B. subtilis (12.7mm) and P. aeruginosa (12.0 mm). MIC value of AgNPs was found to be less than 12.5 µl/ml for S. aureus, while, MIC value was <25.0 µl/ml for S. pyogenes, B. subtilis and E. coli whereas, the MIC value for P. aeruginosa was 70 µl/ml.

**Table 1: Antibacterial activity and MIC of synthesized silver nanoparticles against different human pathogenic bacteria**

| Bacteria | AgNPs | Augmentin (10 µg) | MIC (µl/ml) |
|---|---|---|---|
| | Zone of inhibition (mm)* | | |
| *S. aureus* | 23. 7±0.58 | 26.7±0.58 | <12.5±0.58 |
| *B. subtilis* | 12.7±1.15 | 20.7±0.58 | <25.0±0 |
| *E. coli* | 13.0±0 | 31.3±1.15 | <25.0±0.58 |
| *S. pyogenes* | 16.7±1.53 | 25.0±1.00 | <25.0±1.00 |
| *P. aeruginosa* | 12.0±1.00 | 32.3±0.58 | <70.0±0 |

* Zone: mean ±SD for N = 3

Silver ions as well as AgNps were known to have strong antimicrobial activities [7]. Several mode of action have been suggested by researchers, AgNPs are come in contact with the cell surface of bacteria which may lead to damaging the cell membrane consequently rendering bacterial cell more permeable [1]. It has been reported that the size and shape of the nanoparticle influences its antibacterial activity. The spherical and smaller size of the nanoparticle indicates that a large surface area will come in contact with the bacterial cells therefore it will have more impact since it has higher rate of interaction with the cell surface. The nanoparticles smaller than 10 nm interact with bacteria and produce electronic effects, which enhance the reactivity of nanoparticles [8]-[9]-[10]. The present study reveals that AgNPs synthesised from honey had broad spectrum antibacterial property thus it can be explored further for the the eco-friendly production of silver nanoparticles for medical and therapeutic uses.

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