



SYNTHESIS AND ANTIMICROBIAL PROPERTIES OF HONEY-MEDIATED SILVER NANOPARTICLES

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Abstract: Nanoparticles are Nano scale materials with distinct physicochemical properties and different applications in biomedical, environmental, agricultural and industrial fields. Silver nanoparticles (AgNPs) are of significant interest due to their unique optical, catalytic and broad-spectrum antimicrobial properties. Nanoparticles using biological resources like honey is an eco-friendly, cost-effective and sustainable alternative to the conventional synthesis of nanoparticle. In the current study, Acacia honey was used for the synthesis of AgNPs and optimum conditions for the production of nanoparticles were studied. The optimum synthesis conditions were found to be honey to silver nitrate ratio of 2:3, AgNO₃ (2.5 mM), incubation temperature of 70°C at pH 9. UV-Vis spectrophotometric analysis revealed the successful synthesis of AgNPs with characteristic absorption peaks in the range of 400-440 nm. The synthesized AgNPs showed concentration-dependent antibacterial activity against *Staphylococcus aureus* and *Escherichia coli*. Scanning electron microscopy (SEM) revealed the presence of mostly globular and uniformly distributed nanoparticles in the size range of 25-69 nm. The formation of AgNPs was confirmed by energy-dispersive X-ray (EDX) analysis with the presence of silver and carbon. Fourier-transform infrared spectroscopy (FTIR) spectra showed the characteristic C-O vibrations at 1307 cm⁻¹, which indicates the participation of functional groups related to fructose and glucose existing in honey in nanoparticle synthesis and stabilization. The Acacia honey-mediated synthesis provides an overall simple, sustainable and eco-friendly approach for the production of AgNPs with promising antibacterial activity and potential applications in the biomedical and antimicrobial fields.

Key words: Green synthesis, Acacia Honey, Antibacterial agent, Nanoparticles, Silver nanoparticles.

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INTRODUCTION

Nanoparticle synthesis has been of great interest in the domain of nanotechnology due to its broad applications in diverse fields ranging from medicine and electronics to environmental remediation. Among the numerous methodologies used for nanoparticle synthesis, the use of environmentally friendly and biocompatible agents has become an attractive pathway for the preparation of sustainable and bio-medically relevant nanomaterials (Abuzeid *et al.* 2023). Honey is one such agent that has been explored recently. It is a natural product of great historical and therapeutic significance with a novel reducing agent and stabilizer for the synthesis of silver nanoparticles (AgNPs) (Ghramh *et al.* 2023). AgNPs are generally between 1 and 100 nanometers (nm) and have a nano-scale structure, which greatly increases their surface area to volume ratio. This special morphology offers unique physical, chemical and biological properties that are significantly different from bulk

silver. Hence, AgNPs are of great importance in many scientific and industrial fields (Fahim *et al.* 2024).

Silver nanoparticles (AgNPs) exhibit unique physicochemical properties, such as high surface area, tunable morphology, distinct optical characteristics, and surface plasmon resonance (SPR), which facilitate their use in sensing, imaging, photothermal therapy, catalysis, and various biomedical and industrial applications (Zhang *et al.*, 2016; Zhang *et al.*, 2024). The morphology of AgNPs ranges from spheres and rods to cubes and triangles, allowing for the customization of their properties for targeted applications. AgNPs demonstrate broad-spectrum antimicrobial activity against bacteria, viruses, and fungi through mechanisms that include disruption of microbial membranes, generation of reactive oxygen species (ROS), and interference with essential cellular processes (Mahmud *et al.*, 2022). AgNPs have attracted a lot of interest, for drug delivery, diagnostics, environmental cleanup and fighting microbes

(Bruna *et al.*, 2021). Traditional physical and chemical ways to make AgNPs often need a lot of energy or dangerous chemicals, which can pollute the environment make cleaning and pose health risks to people and animals (Ijaz *et al.*, 2020; Zhang *et al.* 2016).

Because of these problems people now use synthesis methods, which produce AgNPs in a safer and more eco-friendly way.

Acacia Honey has been identified as a valuable green biological resource to synthesize AgNPs, mainly because of its abundance in sugars, organic acids, antioxidants and phytochemicals. Honey can be an efficient, rapid and simpler alternative compared to microbial synthesis. Also, it eliminates steps required for the microorganism's cultivation and extraction (Balasooriya *et al.*, 2017). The reducing agents and natural capping agents in honey regulate the size of nanoparticles and control their morphology and biocompatibility (Bedlovičová, 2022; Sidhu, Verma and Kaushal, 2022). During the synthesis of AgNPs, glucose and fructose reduce Ag^+ , and organic acids and phytochemicals of honey stabilize and cap the nanoparticles (Hosny *et al.*, 2017; Bahari *et al.*, 2023). Acacia honey has a high content of fructose which contributes to its slow crystallization and favorable physicochemical characteristics (Machado *et al.*, 2025), and also includes antioxidant and antibacterial activities. Therefore, the present study aimed to optimize the synthesis of AgNPs using Acacia honey and characterize the resulting nanoparticles using UV-Vis spectroscopy, SEM, EDX, and FTIR, with particular emphasis on evaluating their antibacterial potential against bacterial pathogens.

MATERIALS AND METHODS

Acacia Honey Sample Collection and Preparation

Acacia Honey was procured from a local market. Milli-Q water was utilized in the preparation process.

Synthesis of Honey solution and silver nitrate concentrations

Initially, 5mL of acacia honey sample was mixed with 45mL of Milli-Q water and stirred briefly to form a homogeneous 10% (v/v) dilute solution. It was placed in a refrigerator at 25°C until use. Different concentrations of AgNO_3 solutions were prepared using Milli-Q water in dark bottles, and the following optimization conditions were tested.

Ratio of Acacia Honey and AgNO_3 concentration

To optimize ratio of 10% Acacia Honey to AgNO_3 , different ratios were used to synthesize AgNPs including 1:9, 1:4, 3:7 and 2:3. Representing different proportions of Acacia honey to AgNO_3 were meticulously prepared to achieve a final volume of 50mL. They were then incubated at 70°C for 1 hour at 100rpm.

Molarity of AgNO_3

Various molarities of silver nitrate (AgNO_3) were utilized in the experimental setup. Specifically, concentrations of 0.5 millimolar (mM), 1 mM, 2.5 mM, 5 mM, 7.5 mM, and 10 mM were prepared. The preparation of these varied molarities of AgNO_3 involved exact additions of 0.5 mL, 1 mL, 2.5 mL, 5 mL, 7.5 mL, and 10 mL to a corresponding volume of Milli-Q (M.Q) water, resulting in a standardized total volume of 100 ml for each concentration.

Temperature Optimization

Temperatures at which the sample batches were incubated in the shaking incubator for the synthesis of AgNPs were 40°C, 50°C, 60°C and 70°C consecutively for 1 hour at 100 rpm.

pH Optimization

The following pH was maintained for sample batches consecutively which are 7, 8, 9 and 10. These pH values were attained by the addition of 1 M NaOH solution. As soon as the pH was maintained, the reaction started taking place and the samples were then quickly shifted to shaking incubators for incubation for 1 hour at 70°C at 100 rpm.

Detection of AgNPs by Spectrophotometer

For the characterization of Honey AgNPs, the synthesized AgNPs UV-visible absorption spectra were collected across the wavelength range from 300 to 700nm. This was achieved by dispensing 200 μL of samples from the flasks into a well plate using a pipette.

Collection of the synthesized AgNPs solution

The samples were poured into Falcon tubes from flasks and subjected to centrifugation at 25°C at 10,000 revolutions per minute (rpm) for 15 minutes. Following this, the supernatant was discarded. The resultant pellet underwent three washes with Milli-Q water and two washes with ethanol. Each washing step was followed by centrifugation at 25°C and 16,000 rpm for 5 minutes. Subsequently, the samples were subjected to oven drying at 40°C.

Characterization of AgNPs

Characterization of silver nanoparticles (AgNPs) involves several techniques to understand their properties and structure. This process allows us to examine various aspects of these nanoparticles, such as their morphology, size, shape, stability, and composition.

Antibacterial Activity

Bacterial cultures were graciously given to us by Professor Mushtaq Hussain, who is associated with his Culture Collection in Dow College of Biotechnology. The antibacterial activity of the Acacia honey was carried out using the Kirby-Bauer technique (Hudzicki 2009). At first, the preparation of bacterial cultures was done carefully. These cultures were incubated overnight on LB-agar, attaining a turgidity of $1-2 \times 10^8$ colony-forming units per milliliter (CFU/ml). Next, the sterile agar plates were prepared under aseptic conditions and the holes on the agar plates were prepared using sterile tips. The bacterial colonies were revived and a suspension with a titer of 106 cells per mL containing both *Staphylococcus aureus* (as Gram-positive) and *Escherichia coli* (as Gram-negative) was introduced in each well. The honey-facilitated AgNPs were diluted and concentrations of 1000 $\mu\text{g/mL}$, 750 $\mu\text{g/mL}$, 500 $\mu\text{g/mL}$, and 250 $\mu\text{g/mL}$ were introduced in the respective wells. The AgNPs were incubated at 37°C for 24 hours after which the inhibition zones formed were measured.

SEM and EDX

AgNPs produced from honey are nicely distributed on the conductive coating of the small beads and then gold-coated using an Auto Fine Coater (JEC-3000FC) to enhance the quality of the imaging at a 30 mA current level for 30 seconds after the

sample is dried and ready for analysis. After drying, the sample is taken to the SEM chamber (JEOL JAPAN), Model No. JSM-IT 100, which is then evacuated to create vacuum conditions within the chamber. The electron beam that produces various signals due to interaction with the nanoparticles is directed on the sample surface. The interaction between the electrons and the nanoparticles results in signals which give information regarding the nanoparticles. The SEM makes it possible to visualize some details of the nanoparticle's surface structure.

The EDX technique looks at the elemental composition of AgNPs helping to determine the presence of silver and other additional elements if needed. The EDX detector is part of the SEM setup consisting of the main working element of capturing emitted X-rays to analyze them. There is an electron beam that is directed towards the nanoparticles in the SEM. The interactions of this beam with the nanoparticles lead to emission of characteristic X-rays by the atoms in the nanoparticles. Each different element will emit X-rays in specific energies providing a unique pattern of the respective element. Subsequently, EDX takes these emitted X-rays and detects them.

FTIR

FTIR is important for determining the characteristics of silver nanoparticles (AgNPs) made from acacia honey. The production of AgNPs begins by dispersing the AgNPs onto a substrate, probably in suspension. The next step involves the use of FTIR equipment in which the sample is subjected to infrared radiation for producing vibration. The interaction results in an interferogram that is transformed using Fourier transformation from time to frequency domain. Thus brings about the IR absorption spectrum, which provides an idea about the chemical composition of AgNPs. The peaks appearing in certain frequency intervals with the distinctive vibrations for the particular bonds and functional groups for AgNPs. Thus the characteristics for the chemical composition of the AgNPs and the reaction leading to their production are obtained.

RESULTS AND DISCUSSION

Optimization of Acacia honey-mediated synthesis of silver nanoparticles

This research studied the eco-friendly preparation of silver nanoparticles (AgNPs) by utilizing 10% (v/v) Acacia honey as both a reducing agent and stabilizer. The conditions for generating nanoparticles were systematically adjusted by changing the relative quantities of honey and AgNO₃, the concentration of AgNO₃, the temperature of the process, and the pH. The formation of AgNPs was initially measured by UV-vis spectroscopy based on the emergence of a characteristic SPR peak. UV-VIS spectroscopy is widely used as an effective tool in the control of AgNP synthesis since various factors, such as the size and morphology of nanoparticles, aggregation state, and solvent properties affect the position of SPR peak (Tarannum *et al.* 2019). The findings are consistent with the data obtained by Fiddaroini *et al.* (2025) that emphasize the impact of honey chemical content, especially reducing sugars, on the formation of silver nanoparticles from honey. The changes in the honey formulation resulted in changes in the UV-Vis absorbance and

properties of nanoparticles confirming the importance of biological reducing medium in controlling AgNP synthesis.

Effect of Acacia honey-to-AgNO₃ ratio

The influence of the Acacia honey-to-AgNO₃ ratio on AgNP formation was evaluated using ratios of 2:3, 1:4, 3:7, and 1:9. All reaction mixtures exhibited a pronounced absorption band in the approximately 430 nm region, confirming the formation of silver nanostructures. The maximum absorbance was observed for the 2:3 honey-to-AgNO₃ ratio, followed by 1:4, 3:7, and 1:9 (Figure 1a). Thus, the 2:3 ratio produced approximately 25% higher absorbance than the 1:9 ratio, indicating a greater relative formation of optically detectable AgNPs under these conditions. The gradual change of absorbance with the honey/AgNO₃ ratio indicates that a proper balance of reducing/capping biomolecules supplied by the honey and Ag⁺ ions is required for efficient nanoparticle formation. Honey contains reducing sugars and other biomolecules that can participate in the reduction of Ag⁺, as well as proteins and other organic constituents that can contribute to the stabilization of the resulting nanoparticles. Philip (2010) showed that honey can be used as a reducing and stabilizing medium in the synthesis of AgNPs and pH has a strong influence on the formation and size of the nanoparticles.

The present observation is consistent with recent reports that the concentration and composition of honey reducing compounds influence the AgNP formation and optical properties. In another study using different honey sources, the researchers reported that the reducing sugars influenced the characteristics of AgNPs and the difference in honey composition resulted in altered UV-Vis response and antibacterial properties (2025). Thus, the ratio 2:3 was selected as the optimized ratio of honey to AgNO₃ for the further experiments.

Effect of AgNO₃ concentration

The effect of precursor concentration was examined using AgNO₃ concentrations ranging from 0.5 to 10 mM. The absorption spectra showed a distinct SPR band between approximately 430 and 440 nm for all tested concentrations, confirming AgNP formation. Among the tested concentrations, 2.5 mM AgNO₃ produced the highest absorbance (Figure 1b). Increasing it from 0.5 to 2.5 mM resulted in an increase of absorbance of about 47%. However, increasing the precursor concentration further to between 5 and 10 mM, did not result in a similar increase. Instead, absorbance was decreased at 5, 7.5 and 10 mM, respectively. This means that with the current experimental conditions, the increase in the AgNO₃ concentration above 2.5 mM did not necessarily increase the formation of nanoparticles.

This behavior can be explained by the presence of molecules that reduce and stabilize the concentration of Ag⁺ ions. At the right concentration of the precursor, enough Ag⁺ ions can be reduced and stabilized by biomolecules from honey. However, at higher concentrations of precursors the reducing/capping capacity of the biological matrix may become relatively limiting and could affect nucleation, growth, aggregation and particle-size distribution. Similar studies have shown that the concentration of precursor can influence the SPR intensity and

wavelength of biosynthesized AgNPs (Tarannum *et al.*, 2019). Thus, the results indicate that 2.5 mM AgNO₃ is the optimum concentration of precursor under the experimental conditions used in this study.

Effect of temperature on AgNP synthesis

Temperature had a pronounced effect on AgNP formation. The reaction was evaluated at 40, 50, 60, and 70 °C for 1 h at 100 rpm. The maximum absorbance increased progressively with temperature at 440 nm at 70 °C, compared with 40, 50, and 60 °C, respectively (Figure 1c).

The absorbance at 70 °C was approximately 94% higher than that obtained at 40 °C, demonstrating a substantial enhancement of nanoparticle formation with increasing temperature within the investigated range. The temperature-dependent increase may be associated with enhanced molecular mobility and accelerated reduction of Ag⁺ ions by honey-derived reducing constituents, thereby promoting nucleation and growth of AgNPs.

Comparable temperature dependence has been reported for honey-mediated AgNP synthesis. Keskin *et al.* (2023), investigated chestnut honey-mediated AgNP synthesis at different temperatures and reported changes in nanoparticle characteristics with increasing temperature, including a reduction in particle size from approximately 55 to 27 nm under their experimental conditions.

Therefore, 70 °C was identified as the optimum temperature among the tested conditions. It should be noted, however, that temperature optimization is system-specific because excessive heating can also alter biomolecules responsible for nanoparticle reduction and stabilization.

Effect of pH on AgNP synthesis

The effect of pH was evaluated between pH 7 and 10. The UV–Vis spectra demonstrated that alkaline conditions enhanced AgNP formation, with the maximum absorbance recorded at pH 9 (Figure 1d).

The absorbance at pH 9 increased by ca. 43% compared to pH 7, which indicates increased AgNP formation at moderately alkaline pH. However, a further increase to pH 10 resulted in a reduction in absorbance. Thus, the results suggest that pH 9 provided a more favorable chemical environment for Ag⁺ reduction and nanoparticle stabilization than either neutral or more strongly alkaline conditions.

The importance of pH in honey-mediated AgNP synthesis was recently demonstrated by Philip (2010) who reported pH-controlled synthesis using honey as a reducing as well as stabilizing agent and obtained highly crystalline, almost spherical nanoparticles at pH 8.5. The present optimum pH 9 is in fair agreement with previous evidence that efficient honey-mediated AgNP formation can be promoted under mild alkaline conditions.

Analysis of Antibacterial Activity of Synthesized AgNPs

The antibacterial activity of the synthesized AgNPs was evaluated against the Gram-negative bacterium *Escherichia coli* ATCC 35218 and the Gram-positive bacterium *Staphylococcus aureus* ATCC 25923 using the agar well diffusion approach (Figure 2a). The inhibition-zone measurements demonstrated

concentration-dependent antibacterial activity, although the magnitude of inhibition differed between the two organisms.

For *E. coli*, inhibition zones of 2, 2, 2, and 3 mm were recorded at 250, 500, 750, and 1000 µg/mL, respectively. In contrast, *S. aureus* exhibited inhibition zones of 3, 4, 4, and 4 mm at the corresponding concentrations. Thus, the largest observed inhibition against *E. coli* was 3 mm at 1000 µg/mL, whereas *S. aureus* showed a maximum inhibition of 4 mm from 500 to 1000 µg/mL (Table 1). These experimental measurements are consistent with the inhibition-zone results presented for the tested AgNP concentrations.

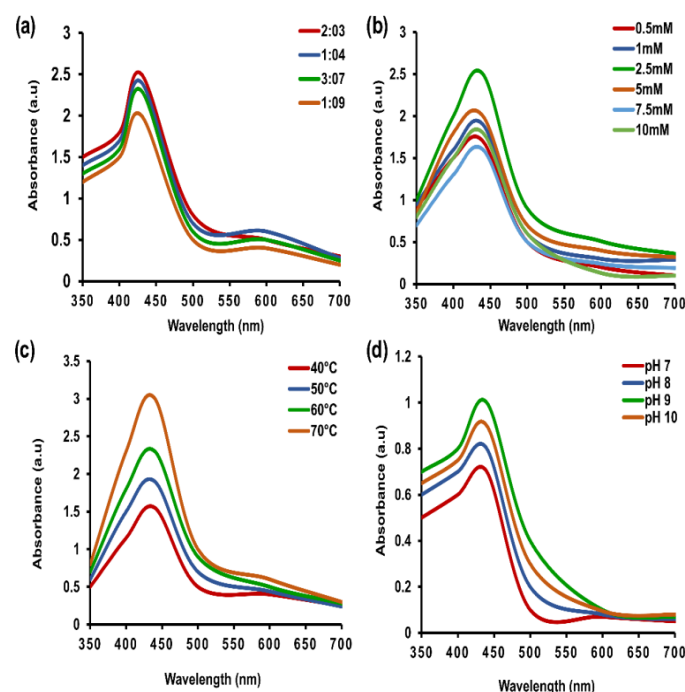


Fig. 1. Synthesis of honey-mediated AgNPs (a) Analysis of different ratios (b) Analysis of different molarities of AgNO₃ solution (c) Analysis of different temperatures (d) Analysis at different pH.

The comparatively greater inhibition observed against *S. aureus* than *E. coli* may be related to differences in bacterial envelope architecture. Gram-positive and Gram-negative bacteria possess substantially different cell-envelope structures, which can influence interaction with nanoparticles. AgNP antibacterial activity has been associated with several mechanisms, including interaction with bacterial cell surfaces, membrane disruption, generation of reactive oxygen species, release of Ag⁺ ions, and interference with cellular processes.

The present findings are consistent with previous reports that honey-mediated AgNPs can inhibit both Gram-positive and Gram-negative bacteria (Al-Brahim and Mohammed, 2020). Similarly, Fiddaroini *et al.* (2025) reported inhibition zones of approximately 14–16 mm against *S. aureus* and *Pseudomonas aeruginosa* for honey-derived AgNPs, demonstrating the antibacterial potential of honey-mediated AgNPs.

Table 1. Zone of Inhibition at different concentrations of AgNPs against Gram-Positive (*S. aureus*) (ATCC 25923) & Gram-Negative (*E. coli*) (ATCC 35218) bacteria

Concentrations ($\mu\text{g/mL}$)	<i>E. coli</i> ATCC 35218 (mm)	<i>S. aureus</i> ATCC 25923 (mm)
250	2	3
500	2	4
750	2	4
1000	3	4

Analysis of SEM & EDX of Synthesized AgNPs

Scanning electron microscopy (SEM) provided morphological evidence for the formation of nanoscale structures in the synthesized AgNP sample. The SEM micrographs show irregular-to-round particulate structures distributed across the examined field. The measured dimensions shown in the micrographs fall approximately within the 25-69 nm range, with several particles/features occurring in the lower nanometer range (Figure 2b). These observations indicate that the biological synthesis route generated particles within the nanoscale regime.

The observed particle-size range is comparable with that reported for other honey-mediated AgNP systems. A recent study using stingless bee honey reported AgNPs within a size range of approximately 25-50 nm and demonstrated significant antimicrobial activity, supporting the potential of honey-derived biomolecules as both reducing and stabilizing agents during AgNP synthesis (Bahari *et al.* 2025). It was reported that chestnut honey-mediated AgNPs with temperature-dependent particle sizes of about 55-27 nm (Keskin *et al.* (2023). Differences among studies may be due to variations in honey composition, AgNO₃ concentration, pH, temperature, reaction time, purification procedures, and instrumental measurement conditions.

The elemental composition of the synthesized material was further confirmed by energy-dispersive X-ray spectroscopy (EDX). The EDX spectrum displayed distinct Ag signals, and the quantitative analysis in Figure 2c revealed 79.89 mass% and 30.67 atomic% Ag, as well as 20.11 mass% and 69.33 atomic% carbon.

The strong Ag signal provides elemental evidence of silver in the synthesized material. The carbon signal is consistent with the presence of organic material associated with honey-derived biomolecules, although EDX alone cannot establish the specific chemical identity of these compounds. The use of SEM coupled with EDX is widely established for assessing morphology and elemental composition of biologically synthesized AgNPs.

Analyses of FTIR of synthesized AgNPs

FTIR spectroscopy was employed to investigate changes in the chemical groups associated with the AgNP preparation and to assess the possible participation of honey-derived biomolecules in nanoparticle reduction and stabilization. The FTIR spectra show clear differences between the AgNO₃ reference and the

synthesized AgNP sample, particularly in the broad O-H/N-H region and in the fingerprint region (Figure 2d).

The broad absorption region around $\sim 3300\text{ cm}^{-1}$ can generally be associated with O-H and/or N-H stretching vibrations, whereas features in the region of approximately $\sim 2900\text{ cm}^{-1}$ can be related to C-H stretching. Bands in the fingerprint region are consistent with oxygen- and carbon-containing functional groups originating from organic constituents of honey. These spectral changes suggest that biomolecules present in Acacia honey may have participated in the reduction of Ag⁺ ions and/or stabilization of the resulting nanoparticles.

The SPR bands observed at approximately 430-440 nm in the present study are within the characteristic visible absorption region reported for green-synthesized AgNPs. Gong *et al.* (2024) emphasized that the position and intensity of the SPR band are influenced by nanoparticle size, synthesis conditions and the nature of the biological reducing agent.

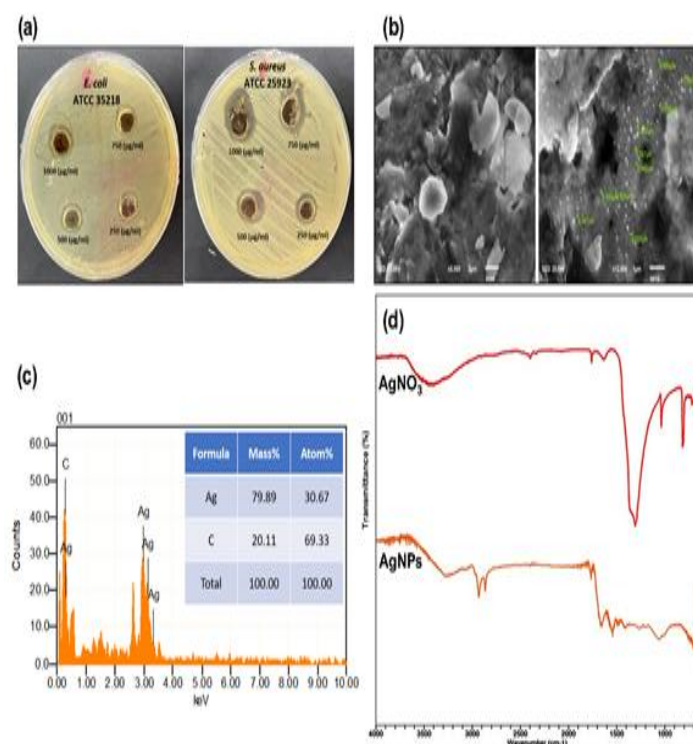


Figure 2. Characterization of honey-mediated silver nanoparticles: (a) antibacterial activity against *Escherichia coli* ATCC 35218 and *Staphylococcus aureus* ATCC 25923; (b) scanning electron microscopy (SEM) micrographs acquired at $\times 5,500$ (scale bar: 2 μm) and $\times 12,000$ (scale bar: 1 μm); (c) energy-dispersive X-ray spectroscopy (EDX) spectrum and elemental composition; and (d) Fourier-transform infrared (FTIR) spectra of AgNO₃ and the synthesized AgNPs preparation.

CONCLUSION

This research presents the eco-friendly synthesis of silver nanoparticles using Acacia honey, showcasing an eco-friendly way of synthesizing this compound with the help of natural

means rather than using traditional chemical approaches. The parameters like temperature, pH, and concentration of silver nitrate determine the size and shape of nanoparticles. The reactions are confirmed to be taking place through characterization, and the antibacterial tests demonstrate the ability of these nanoparticles to fight both Gram-negative and Gram-positive bacteria.

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