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Green synthesis of silver nanoparticles using Sidr and Spring honey and evaluation of their antibacterial activity against clinical *Staphylococcus aureus* isolates

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Abstract

Objective

Diabetic foot infections (DFIs) are one of the most severe complications of the diabetes mellitus, and the presence of multiple drug-resistant *Staphylococcus aureus* in DFIs restricts conventional antibiotic use. The main aim of the present study was to synthesize silver nanoparticles using Sidr and Spring honey and to characterize the synthesized nanoparticles and then to assess their antibacterial activity against clinical isolates of S. aureus as well as their synergistic effect with amoxicillin/clavulanic acid against clinical S. *aureus* isolates

Materials and methods

Silver nanoparticles were prepared using natural honey as reducing and stabilizing agent with the help of silver nitrate. Characterization of the synthesized nanoparticles was done by UV–Vis spectroscopy, scanning electron microscopy (SEM), X-ray diffraction (XRD), Fourier transform infrared spectroscopy (FTIR), energy-dispersive X-ray spectroscopy (EDX), and zeta potential analysis. They were tested for their antibacterial activity by the agar well diffusion method and synergistic activity by combining with amoxicillin/clavulanic acid against clinical S. aureus isolates.

Results

The characteristic surface plasmon resonance peak at 422 nm was observed using UV–Vis spectroscopy, indicating the formation of nanoparticles. SEM analysis of the synthesized nanoparticles showed the presence of mostly spherical shaped nanoparticles in the range of 15.45 to 30.34 nm. The XRD confirmed the structure of the AgNPs as crystalline, and the zeta potential values of the AgNPs were around -23 mV, which showed good colloidal stability. The synthesized AgNPs showed concentration-dependent antibacterial activity, with highest inhibition zone of 21.8 mm by the synthesized AgNPs derived from the spring honey as compared to Sidr honey-derived AgNPs. Significant different antibacterial activity was observed between AgNPs and amoxicillin/clavulanic acid as compared to AgNPs with amoxicillin/clavulanic acid ($P < 0.05$).

Conclusion

Honey mediated silver nanoparticles demonstrates an excellent physicochemical property, possessing good antibacterial activity and exhibit a substantial synergistic effect in combination with amoxicillin/clavulanic acid in diabetic foot infection associated clinical Staphylococcus aureus strains. Natural honey is used as a biological reducing and stabilizing agent for the synthesis of nanoparticles, offering a sustainable methodology for the synthesis of nanoparticles and the use of these nanoparticles as additional antimicrobial agents that enhance the therapeutic efficacy and may decrease the doses of antibiotics used in therapy and the development of bacterial resistance.

Keywords: antibacterial activity, green synthesis, natural honey, silver nanoparticles, *Staphylococcus aureus*

Paper Type: Research Paper.

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Introduction

Nanotechnology is a cutting-edge field that focuses on manipulating matter at the nanoscale (1–100 nanometers), where materials exhibit unique physical and chemical properties that make them ideal for medical applications (Ma et al., 2024). Among the nanomaterials, silver nanoparticles have garnered significant attention due to their antimicrobial activity and potential as therapeutic agents (Naganthran et al., 2022). Recent studies have increasingly focused on natural sources for the green synthesis of nanoparticles (Ahmed et al., 2022). The nanoparticles are smaller in size, which increases their capacity to release silver ions, hence enhancing their antibacterial properties (Abbas et al., 2024). Honey is a natural product rich in phenolic compounds, enzymes and minerals. It has an impressive antibacterial and antioxidant effect (Hossain et al., 2021; Khalifa et al., 2024). and has recently entered the world of different therapeutic uses, including in the treatment of wounds and skin ulcers (Bazaid et al., 2022), which is why it is a promising medium in the biosynthesis of nanoparticles. On the other hand, diabetes is one of the chronic diseases that has been on the increase all over the world. According to the International Diabetes Federation (IDF), approximately 589 million adults were living with diabetes worldwide in 2024, and this number is expected to rise to 853 million by 2050 (Duncan et al., 2026). Among its most dangerous complications, there are diabetic foot infections that are caused by impaired blood circulation and the malfunction of immune processes (Yang et al., 2022). Diabetic foot ulcers remain one of the leading causes of hospitalization, infection, and lower-limb amputation among patients with diabetes worldwide (Deng et al., 2023). One of the most prevalent pathogens due to its ability to form biofilms and resistance to antibiotics, *Staphylococcus aureus* treatment is more complex and the likelihood of amputation is increased (Li et al., 2025; Spoială et al., 2022). While the use of honey mediated silver nanoparticles was explored earlier, comparative study was done between different types of natural honeys such as Sidr and Spring honeys and clinical isolates of *Staphylococcus aureus* from DFIs is limited. Therefore, the present study evaluated the green synthesis, characterization, and antibacterial activity of silver nanoparticles synthesized using these two honey types. This study provides an environmentally friendly approach for the synthesis of silver nanoparticles and may contribute to improving antibacterial strategies for the management of diabetic foot infections (Meher et al., 2024; Sultana et al., 2023). Thus, the current study was conducted to prepare silver nanoparticles using green synthesis from two varieties of natural honey (Sidr honey and Spring honey), to evaluate their antibacterial activity against clinically isolated *Staphylococcus aureus* from diabetic foot infections, and investigate the synergistic effects of the synthesized nanoparticles with antibiotics.

Materials and method

Sample collection: Honey samples were collected from a local apiary in al-Sharsh region in the city of Qurna in the Basra Province, Iraq. Two types of natural honey, Sidr honey and Spring honey, were used in this study. The study was approved by the Research Committee of the Basrah Health Directorate, Ministry of Health, Iraq (Research Committee Decision No. 818, dated February 24, 2025). Official permission was granted to collect clinical samples from the participating healthcare institutions.

Green synthesis of silver nanoparticles: A honey solution was prepared by dissolving 26.3 g of honey in 500 mL of deionized water (Czernel et al., 2021). Separately, 0.2105 g of silver nitrate ($AgNO_3$) (B.D.H., England) was dissolved in 500 mL of deionized water (Promega, USA) to prepare an approximately 2.48 mM $AgNO_3$ solution. All solutions were gently stirred and blended individually for a minute. The pH of the mixture was adjusted to 9 using sodium hydroxide (NaOH), and the mixture was stored under dark conditions. Subsequently, it was heated to 80°C until a color change from yellow to dark brown was observed after 25 min, indicating the reduction of silver ions and the formation of nanoparticles. After nanoparticle formation, the suspension was centrifuged (Kokusan, Japan) at 36,000 rpm for 20 min to recover the nanoparticles. The resulting pellet was washed three times with deionized water to remove residual impurities and then dried in a hot-air oven (Memmert GmbH + Co. KG, Germany) at 40°C. The re-dispersed silver nanoparticles after centrifugation and drying were used to make a stock suspension of 1000mg/l and lower concentrations (750, 500, 250mg/l) were made up individually by direct dilution of the stock suspension using sterile deionized water. These concentrations were subsequently used in the antibacterial activity assay. The same preparation procedure was followed for both Sidr honey and Spring honey.

Characterization of silver nanoparticles

Color variation of the reaction mixture: The color variation of the reaction mixture served as a key indicator that the reducing agents present in both Sidr and Spring honey reduce silver ions to silver nanoparticles. The change in the solution color from yellow to deep brown was considered the successful formation of silver nanoparticles (Abreu et al., 2026).

UV-Vis spectrophotometer: Ultraviolet–visible (UV–Vis) spectroscopy (Thermo Fisher, Finland) was used to characterize the synthesized silver nanoparticles. A characteristic surface plasmon resonance (SPR) absorption peak was observed at 422 nm within the wavelength range of 200–1000 nm (Kumar et al., 2020).

Scanning electron microscope (SEM): The morphology and surface characteristics of the synthesized silver nanoparticles were examined using a scanning electron microscope (FEI Company, Netherlands). The samples were scanned with a focused electron beam to obtain high-resolution images of the synthesized silver nanoparticles (Kaabour et al., 2025).

FTIR spectral analysis: The FTIR analysis (JASCO, Japan) was used to identify the functional groups present on the surface of the synthesized silver nanoparticles prepared using Sidr honey and Spring honey. When the particles were centrifuged and washed using deionized water and dried, the samples were crushed and mixed with KBr before being pressed into discs. The spectra were recorded over the range of 400-4000 cm^{-1} to identify the functional groups involved in the biosynthesis of silver nanoparticles (Eid, 2022; Pasieczna-Patkowska et al., 2025).

X-ray diffraction (XRD) analysis: The crystalline nature of the silver nanoparticles was characterized using X-ray diffraction (XRD) (Haoyuan, China). The sample was dried and transformed into a fine powder, then examined over a range of angles (2θ) from 20° to 80° using Cu-Kα radiation with a wavelength of 1.5406 Å. The obtained diffraction patterns were used to identify the crystalline phases and compared with the standard JCPDS cards for silver (Ag) (Naganthran et al., 2022).

Analysis of zeta potential: The Zeta potential of silver nanoparticles was measured using Dynamic Light Scattering (DLS) (Malvern Panalytical, UK) to evaluate the colloidal stability of the synthesized silver nanoparticles. The sample was diluted with deionized water before analysis, and the surface charge was measured at room temperature (Taherpour et al., 2025).

Elemental analysis (EDX): To determine the elemental composition of the silver nanoparticles, the scanning electron microscope with an energy-dispersive X-ray detector (EDX) (Bruker, Germany) was used in the elemental analysis. The dried sample was mounted on a metallic holder, after which it was exposed to an electron beam. The generated spectra were used to identify the elemental composition and determine the relative elemental percentages. A characteristic silver (Ag) peak was observed at approximately 3 keV (Keskin et al., 2023).

Biological activity testing-Preparation of bacterial isolates: Clinical isolates of *Staphylococcus aureus* obtained from diabetic foot infections were used in this study. Fresh and pure bacterial colonies were prepared by subculturing the isolates on Mueller–Hinton agar plates and incubating them at 37 °C for 24 h (Ahmad et al., 2025).

Identification and standardization of bacterial isolates: Bacterial isolates were initially cultured on selective media, including Mannitol Salt Agar. Preliminary identification was performed based on colony morphology, Gram staining, and standard biochemical characteristics. The final identification of the isolates was confirmed using the VITEK 2 Compact system (bioMérieux, France) (Faustova et al., 2025). Fresh bacterial colonies were suspended in sterile saline, and the turbidity was adjusted to 0.5 McFarland standard (Menchinelli et al., 2024). The prepared suspensions were then loaded into the appropriate identification cards according to the manufacturer's instructions. The cards were incubated for 8-12 hours and automatically analyzed by the system. The final identification results were recorded for subsequent analysis (El-Amier et al., 2024).

Preparation of concentrations: A series of concentrations of the synthesized silver nanoparticles was prepared using sterile deionized water as the diluent. The concentrations used were 250, 500, 750, and 1000 mg/L (Taherpour et al., 2025).

Biological efficacy test (agar diffusion method): The bacterial suspension was adjusted to a density of approximately 1.5×10^8 CFU/mL (0.5 McFarland standard). A sterile cotton swab was used to evenly spread the bacterial suspension over the surface of Mueller–Hinton agar plates. Five wells, each 6 mm in diameter, were made in the agar using a sterile cork borer. Each well was filled with 100 µL of the prepared silver nanoparticle concentrations. Pure honey was used as a negative control, while an amoxicillin/clavulanic acid disc was used as a positive control (Yousef et al., 2025).

Incubation and measurement of inhibition zones: The plates were incubated at 37°C for 24 hours. After the incubation period, the diameters of the inhibition zones formed around the wells were measured using a Vernier caliper, and the results were recorded in millimeters to assess the biological efficacy of the tested substance (Yousef et al., 2025).

Synergistic effect test

preparation of bacterial suspension: A bacterial suspension of *Staphylococcus aureus* was prepared, and its turbidity was adjusted to 1.5×10^8 CFU/mL (0.5 McFarland standard) using a turbidimeter (CLSI, 2024). The bacterial suspension was evenly spread over the surface of Mueller–Hinton agar plates using a sterile cotton swab (Elmehalawy et al., 2025).

Synergistic testing by agar well diffusion method: Three wells, each approximately 6 mm in diameter, were made in Mueller–Hinton agar plates previously inoculated with *Staphylococcus aureus*. Pure honey was added to the first well as a negative control. The second and third wells were filled with 100 µL of silver nanoparticle suspensions at concentrations of 1000 mg/L and 250 mg/L, respectively. In addition, three amoxicillin/clavulanic acid discs were placed on the inoculated agar surface. One disc served as the antibiotic control, whereas 0.01 mL (10 µL) of silver nanoparticle suspension at concentrations of 1000 mg/L and 250 mg/L was applied onto the second and third antibiotic discs, respectively, to evaluate the synergistic antibacterial effect between the nanoparticles and the antibiotic (Khleifat et al., 2022).

Incubation and synergistic effect assessment: The plates were incubated at 37°C for 24 h. After incubation, the diameters of the inhibition zones around the wells and antibiotic discs were measured in millimeters using a Vernier caliper. The inhibition zones produced by the combined treatment were compared with those produced by the antibiotic alone and the silver nanoparticles alone to evaluate the synergistic antibacterial effect. (Yousef et al., 2025). The percentage enhancement in antibacterial activity was calculated using the following equation:

$$\text{Enhancement (\%)} = \frac{(IZD_{combination} - IZD_{antibiotic})}{IZD_{antibiotic}} \times 100$$

Statistical analysis: Statistical analysis was performed using IBM SPSS Statistics version 25 (IBM Corp., Armonk, NY, USA). The mean values were calculated for all experimental groups. Differences among the treatment groups were analyzed using analysis of variance (ANOVA). A p-value of less than 0.05 ($P < 0.05$) was considered statistically significant.

Results and discussion

Extraction and description of silver nanoparticles

Color variation: Upon heating the reaction mixture containing Sidr honey solution and silver nitrate ($AgNO_3$) solution at 80°C for 25 min, a distinct color change from yellow to dark brown was observed compared with the negative control (Figure 1). This color change is considered the first visual indication of silver nanoparticle (AgNP) formation and results from the reduction of Ag^+ ions into metallic silver (Ag^0) by the bioactive compounds present in honey, including phenolic compounds, flavonoids, proteins, and reducing sugars. The reddish-brown colour is due to the phenomenon known as surface plasmon resonance (SPR), which is an optical property of silver nanoparticles. A similar observation was made by Fan et al. (2024) and Ahmed et al. (2022) who also noticed the development of brown colour during green synthesis of the AgNPs and attributed it to SPR which confirmed the successful formation of the silver nanoparticles.

UV-visible spectrophotometer: The UV-Visible spectrum of the prepared silver nanoparticles showed a prominent peak at 422 nm (Figure 2). The silver nanoparticles were successfully formed and confirmed by this absorption peak. This is also believed to be due to the phenomenon of surface plasmon resonance (SPR) which is a characteristic optical property of AgNPs. When light is shone on the nanoparticle, the electrons in the conduction band oscillate together to form the SPR band. The bioactive components in honey such as the phenolic compounds, flavonoids, proteins, and reducing sugars acted as reducing agents and also contributed to the stabilization of the synthesized nanoparticles by reducing the Ag^+ ions to metallic silver (Ag^0). These conclusions were echoed by de Souza et al. (2024). They noted a typical SPR absorption peak in the range 400-450 nm, indicating successful green synthesis of silver nanoparticles.

Scanning electron microscope (SEM) examination: The scanning electron microscope (SEM) images revealed that the synthesized silver nanoparticles were predominantly spherical in shape, with particle sizes ranging from 15.45 to 30.34 nm and an average particle size of 26.84 nm (Figures 3 and 4). The relatively narrow size distribution and uniform morphology suggest the effective green synthesis of AgNPs. Small particle size gives high surface area to volume ratio and thereby better interaction of the nanoparticles with the bacterial cells and as a consequence better antibacterial activity. Furthermore, the bioactive compounds present in honey act as reducing and stabilizing agents, preventing nanoparticle aggregation and contributing to the formation of well-dispersed particles.

Figure 1. Represents the change in colour in the reaction mixture. In the case of (A) -no silver nitrate salts ($AgNO_3$) is added to the system. The presence of brown color (B) represents that the enzymes and reducing agents in the solution of honey ar e reducing silver nitrate salts.

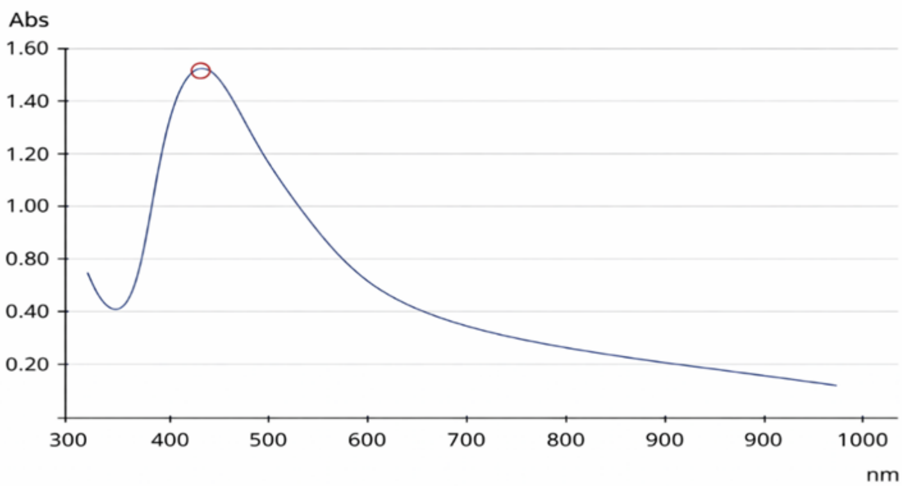


Figure 2. UV–Vis spectrum of silver nanoparticles synthesized using honey

These findings are consistent with those reported by Elmehalawy et al. (2025) and Ghramh et al. (2025). They also observed predominantly spherical silver nanoparticles with relatively uniform size distribution and attributed their enhanced biological activity to their small particle size and stable morphology.

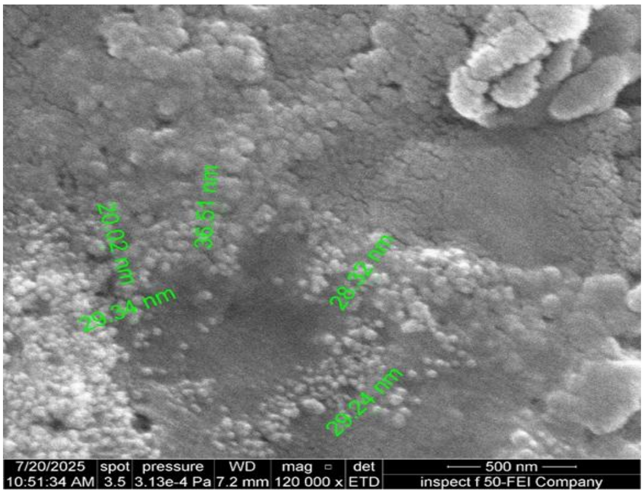


Figure 3. SEM image of silver nanoparticles prepared using Sidr honey, showcasing nearly spherical shapes and nanoscale sizes

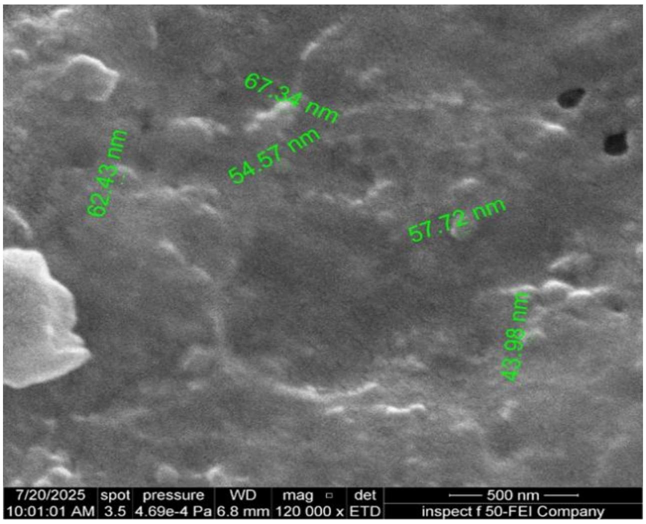


Figure 4. SEM image of silver nanoparticles prepared using Spring honey, displaying nearly spherical shapes and nanoscale sizes

Infrared Spectroscopy Analysis (FTIR): FTIR spectra of silver nanoparticles obtained from Sidr honey and Spring honey showed the presence of wide bands of hydroxyl (O–H) groups stretching vibration at 3247–3297 cm^{-1}. They are positive for phenolics and alcohols. Furthermore, presence of absorption peaks in the range of 1060–1068 cm^{-1} was attributed to the C–O stretching vibrations indicating the involvement of sugars and other organic compounds in the reduction of Ag^+ ions and stabilization of the synthesized nanoparticles. Also, the presence of absorption bands from 1631-1697 cm^{-1} was assigned to carbonyl/amide groups (C=O/Amide I), suggesting that proteins or N-containing compounds were involved in the capping and stabilization of the surface of the nanoparticle. These findings are align with those reported by de Souza et al. (2024). They identified similar FTIR absorption bands at 3200–3400 cm^{-1} (O–H), 1630–1640 cm^{-1} (C=O/Amide I), and 1060–1100 cm^{-1} (C–O), verifying the involvement of bioactive compounds in the reduction and stabilization of silver nanoparticles. Likewise, Muhammed Ameen et al. (2025) reported comparable FTIR peaks associated with hydroxyl, carbonyl, and C–O functional groups, indicating that honey-derived phytochemicals acted as natural reducing and capping agents during the green synthesis of AgNPs. The same was found by Keskin et al. (2023) who showed that the bioreduction of silver ions and stabilization of the synthesized nanoparticles in honey were due to its phenolic compounds, proteins, and carbohydrates. The close similarity between the results of the present work and other works demonstrates the success of the synthesis of silver nanoparticles by the green method and the importance of the bioactive components of honey in the process of synthesis and stabilization of nanoparticles.

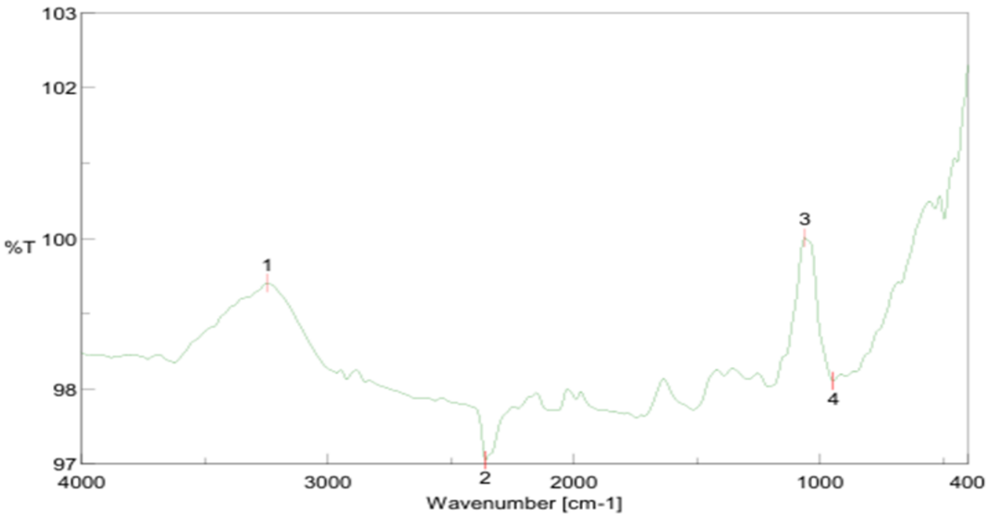


Figure 5. The FTIR spectrum of silver nanoparticles synthesized using Sidr honey

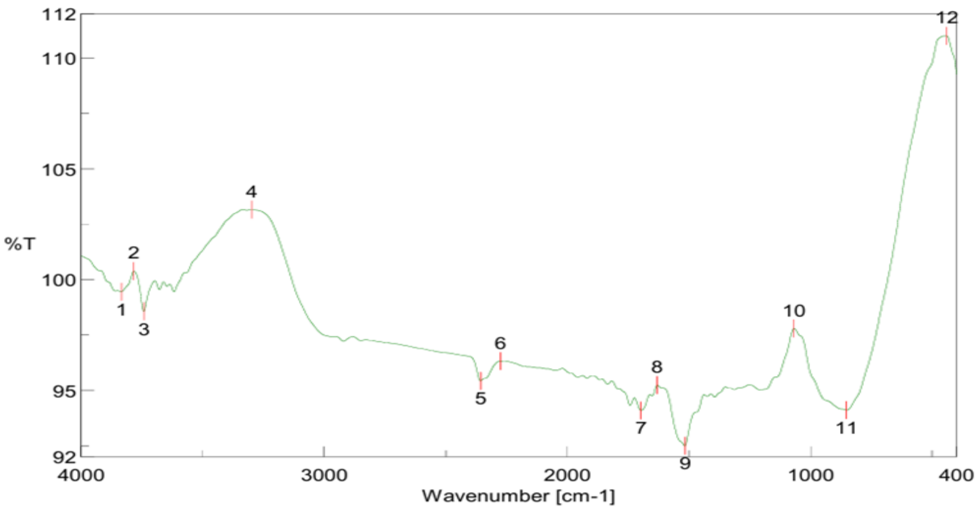


Figure 6. The FTIR spectrum of silver nanoparticles synthesized using Spring honey

X-ray diffraction analysis (XRD): The XRD patterns of silver nanoparticles synthesized using Sidr honey and Spring honey exhibited distinct diffraction peaks at 2θ values corresponding to the crystallographic planes 111, 200, 220, 222, and 311, validating the crystalline nature and successful biosynthesis of AgNPs (Figures 7 and 8). The diffraction peak corresponding to the 111 plane showed the highest intensity, indicating the preferred crystal orientation of the synthesized nanoparticles. Moreover, the peak intensity of AgNPs prepared from Spring honey was found to be higher than the peak intensity of AgNPs prepared from Sidr honey indicating higher crystallinity. The diffraction peaks observed during the XRD analysis are representative of metallic silver with face centered cubic (FCC) crystal structure, indicating structural stability of the prepared nanoparticles. Al-Mahmud et al. (2024), Moosavy et al. (2023) and Ahmed Sezae and Razouqi (2026) have also reported similar diffraction patterns. They identified the characteristic diffraction peaks of FCC crystal planes of biosynthesized silver nanoparticles, 111, 200, 220, and 311. The results of the present study are in accordance with the previous studies, suggesting that natural reducing agents such as honey and plant derived biomolecules have the potential to synthesize highly crystalline, stable and homogeneously distributed AgNPs with good physicochemical and biological properties.

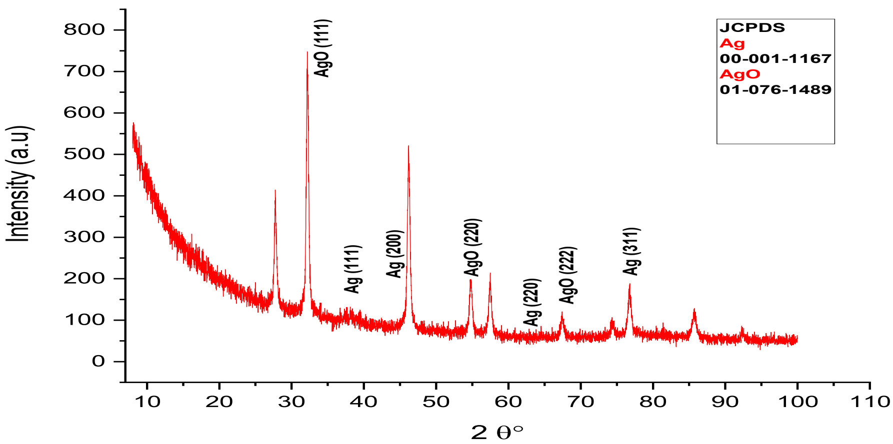


Figure 7. X-ray diffraction pattern (XRD) of silver nanoparticles synthesized using Sidr honey

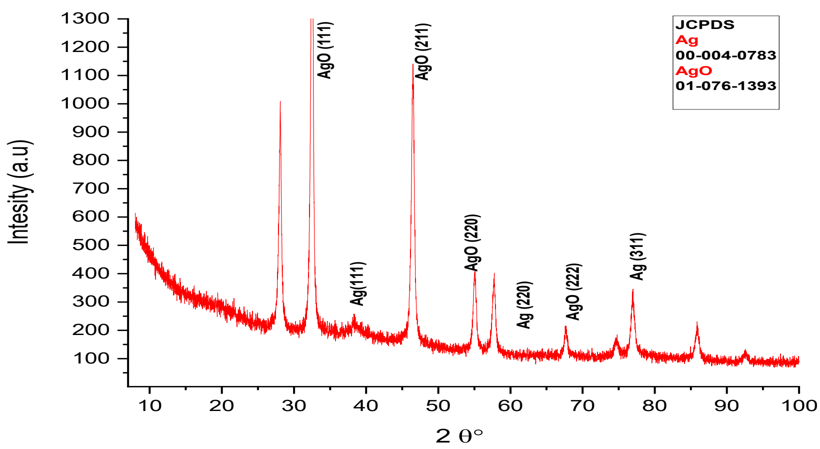


Figure 8. X-ray diffraction pattern (XRD) of silver nanoparticles synthesized using Spring honey

Zeta potential analysis: The zeta potential of the silver nanoparticles prepared with the help of Spring honey and Sidr honey were found to be -23.2 ± 4.53 mV (mean ± SD) and -23.5 ± 4.10 mV (mean ± SD), respectively (Table 9, Figure 9 and Table 10, Figure 10). The negative value of the zeta potential of the synthesized nanoparticles indicates that they have a moderate surface charge density, which would promote the stability of the colloidal solution due to electrostatic repulsion between the particles, preventing them from aggregating. Sidr honey mediated AgNPs have slightly negative zeta potential values, indicating slightly more stability in the colloidal state than the Spring honey mediated AgNPs. The results presented here are in agreement with Kaabour et al. (2025), who also found that the mediated silver nanoparticles in the presence of honey had a negative zeta potential, which means that the nanoparticles were well dispersed and did not aggregate. Likewise, Abreu et al. (2026) showed that silver nanoparticles prepared using stingless bee honey possessed negative surface charge, confirming that this natural liquid contains natural reducing and capping agents which increases the stability of silver nanoparticles. In contrast, Alharbi and Alsubhi (2022) found that the silver nanoparticles prepared with extract from the fruit of Azadirachta indica had a more negative zeta potential value (-35.6 mV), which resulted from the greater electrostatic repulsion and thus high colloidal stability. The difference in zeta potential value between the results of the above studies can be explained due to the difference in the biological origin, phytochemical composition, and the method used to synthesize the nanoparticles, which is in turn affected by the surface charge and the colloidal stability of the synthesized nanoparticles.

Elemental analysis (EDX): The EDX spectra for all prepared silver nanoparticles with Sidr honey and Spring honey showed the presence of a prominent peak of Ag (silver) in both samples, which is evidence of the successful synthesis of AgNPs. Besides Ag, the Sidr honey mediated nanoparticles also contained O, Al, Si, Cl and Cu. In contrast, the Spring honey-based nanoparticles were found to contain O, Cl, Br, Cu and Ag, which suggests that the element composition of the synthesized nanoparticles (NP) was different (Figures 11 and 12). These differences could be caused by the chemical composition, bioactive compounds, and percentage of each type of honey. The strong characteristic Ag peak was observed, which indicated the reduction of Ag^{+} to Ag^{0}. In contrast, oxygen signal can be related to the presence of oxygen functional groups present in the surface of the nanoparticles, which are resulted from biomolecules present in the honey. The same results were observed by Dua et al. (2023). They identified a characteristic peak of Ag as well as other elemental peaks, indicating successful biosynthesis of Ag nanoparticles. Similarly, Keskin et al. (2023) observed a significant Ag peak with oxygen and carbon related signals suggesting the attachment of biomolecules from the honey to the surface of the nanoparticles and confirming the successful preparation of honey-mediated silver nanoparticles. In general, the results indicate that the elemental composition of biosynthesized AgNPs depends on the type of biological reducing and capping agents in the honey that help in the formation and stability of the nanoparticles.

Identification of clinical isolates and antibiotic susceptibility patterns: The antimicrobial susceptibility test carried out on the Staphylococcus aureus isolates showed different resistance and susceptibility patterns to the antibiotic drugs tested. Linezolid, levofloxacin, tigecycline, fosfomycin and nitrofurantoin were completely susceptible (100%). High resistance rates were observed for benzylpenicillin and oxacillin, however.

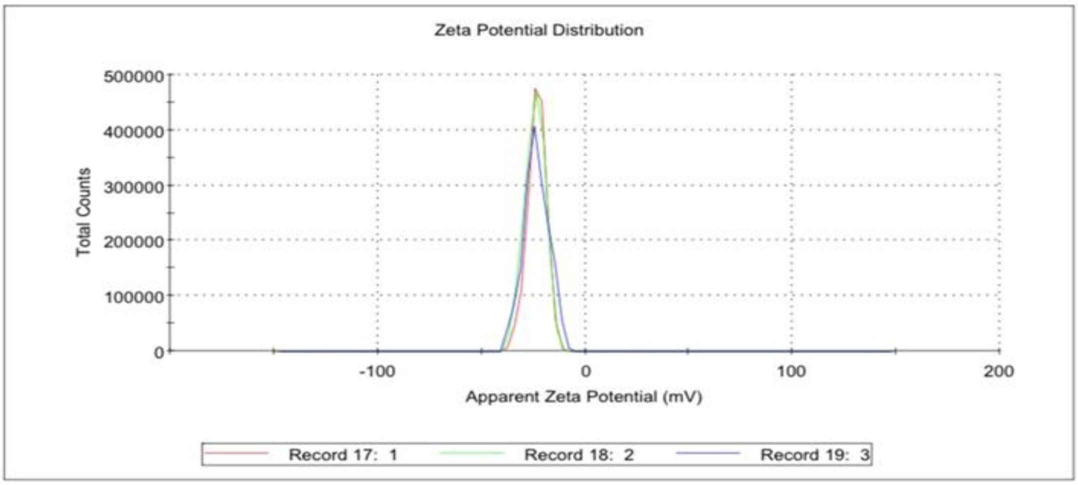


Figure 9. Zeta potential measurement of nanoparticles extracted from Sidr honey using the Malvern Zetasizer

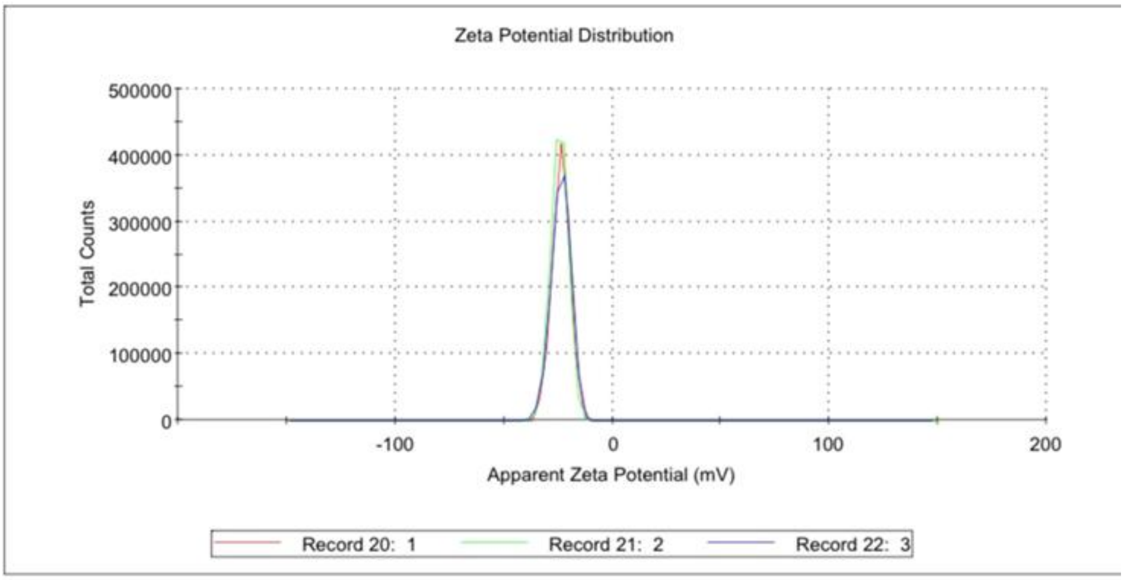
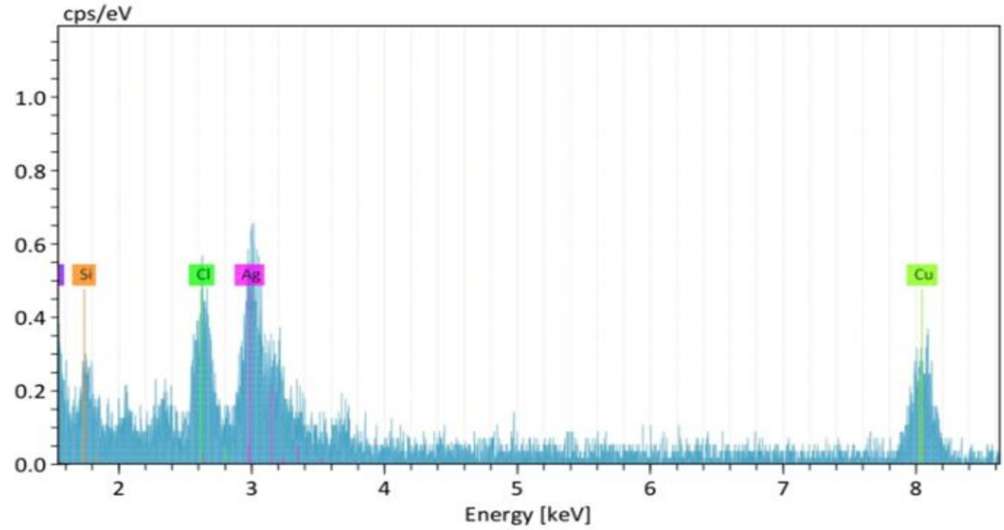


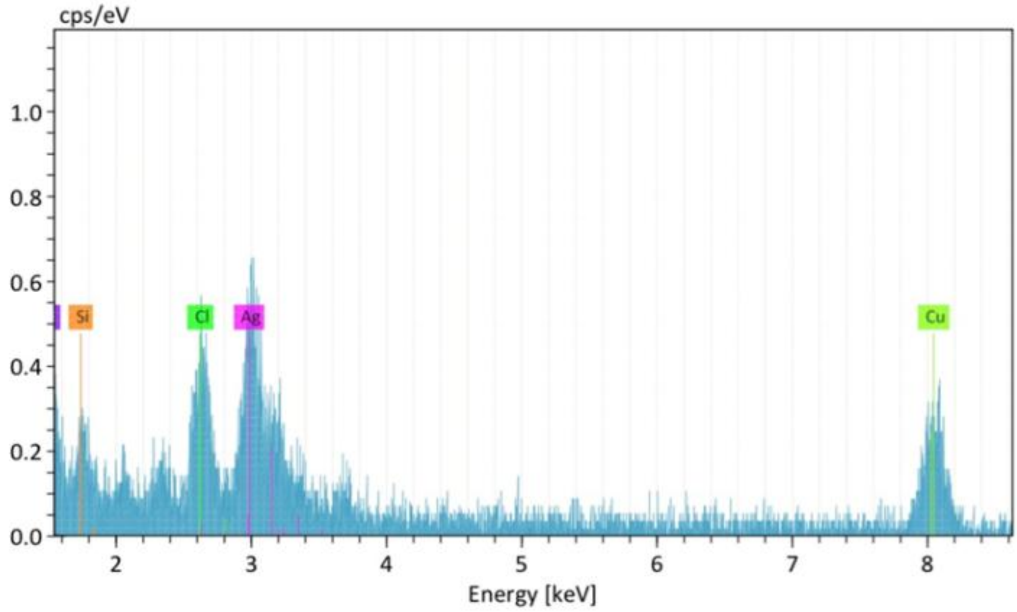
Figure 10. Zeta potential measurement of nanoparticles extracted from Spring honey using the Malvern Zetasizer

The other antibiotics tested exhibited intermediate and variable susceptibility patterns, which is indicative of the variety of antimicrobial resistance experienced by clinical isolates. The results are in line with Tshabuse et al. (2022). They showed that the Vitek 2 Compact is a high accuracy, rapid and reliable antimicrobial susceptibility testing system. Likewise, Gefroh et al. (2026) reported that conventional susceptibility testing methods are still reliable, but an automated method like Vitek 2 Compact provides more standardized results and quicker than conventional methods, allowing for rapid clinical decision making and suitable antimicrobial therapy.

Biological activity testing of silver nanoparticles: The antibacterial effect of the synthesized silver nanoparticles by Sidr honey and Spring honey were determined against the pathogen Staphylococcus aureus at the concentrations of 250, 500, 750 and 1000 mg/L. The synthesized AgNPs showed concentration-dependent antibacterial activity with the result of inhibition zone diameters of 10.2 to 20.6 mm for Sidr honey mediated AgNPs and 10.1 to 21.8 mm for Spring honey mediated AgNPs (Figure 13 A and B). As the concentration of nanoparticles increased, the inhibition zone diameter increased gradually, which shows that the antibacterial activity of the nanoparticles also increases with the increase of the concentration. The results showed that Sidr honey-mediated AgNPs and Spring honey-mediated AgNPs showed statistically significant difference among tested concentrations ($P < 0.05$) indicating that the antibacterial activity significantly affected by the concentration of the nanoparticles.



A



B

Figure 11. The EDX analysis of silver nanoparticles synthesized using (A) Sidr honey and (B) Spring honey

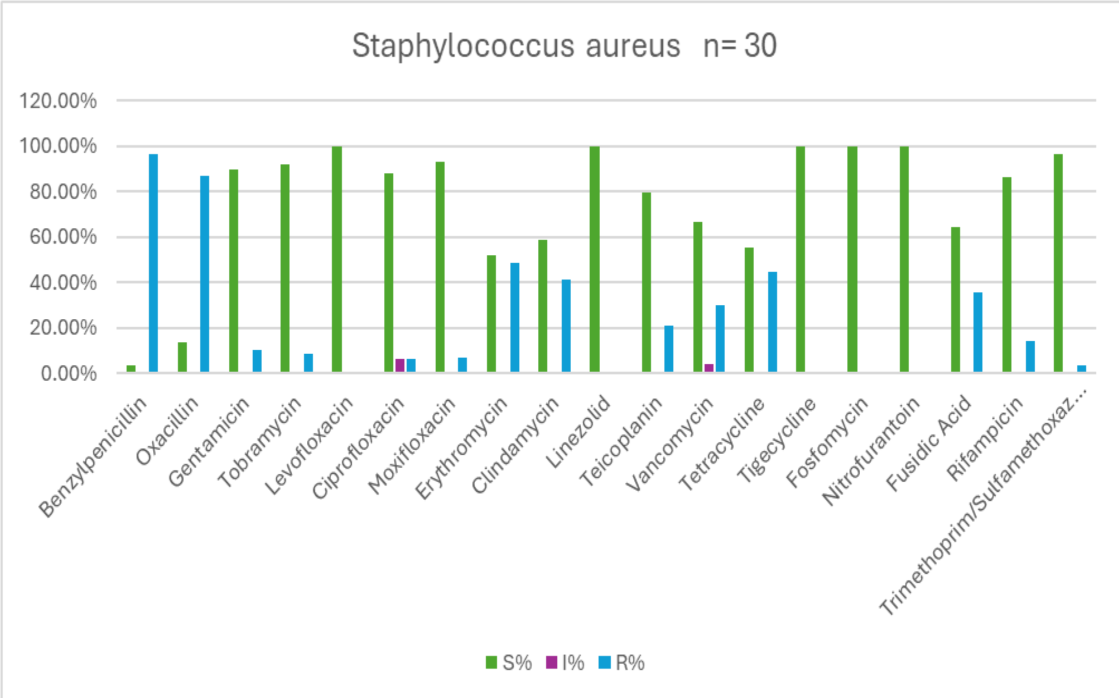
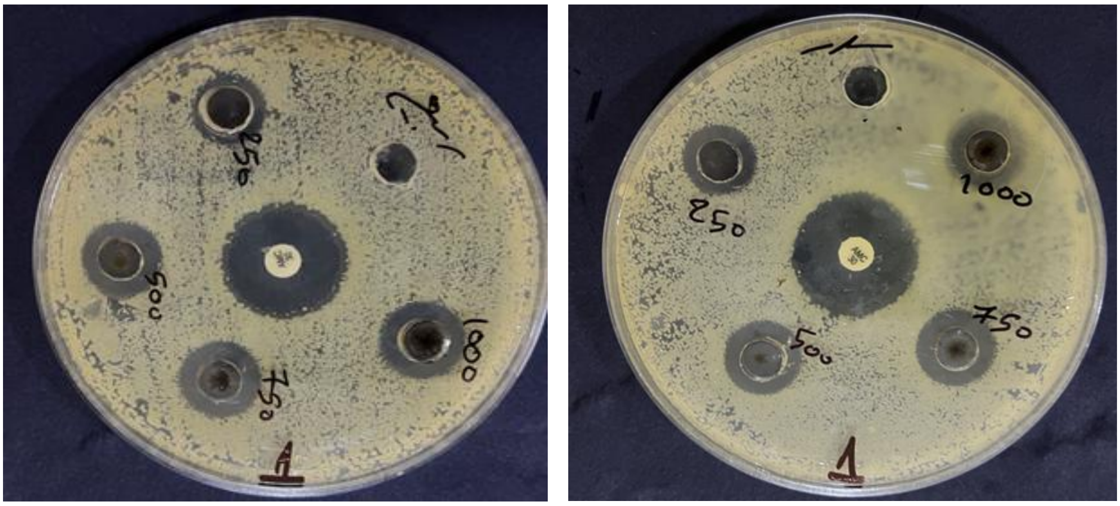


Figure 12. Antibiotic susceptibility profile of *Staphylococcus aureus* isolates (n = 30). The percentages of sensitive (S), intermediate (I), and resistant (R)

The antibacterial activity observed in the present study is concentration-dependent, which could be explained by the various mechanisms of action of silver nanoparticles, such as disruption of the bacterial cell membrane, the generation of Reactive Oxygen Species (ROS), the release of Ag^{+} ions, and their ability to interfere with DNA replication and inhibit essential cellular proteins, resulting in bacterial cell death (Rodrigues et al., 2024). In line with this, Ibraheem et al. (2023) showed that biosynthesized silver nanoparticles had high antibacterial activity against Staphylococcus aureus, and the inhibition increased with an increase in the concentration of the nanoparticles. The comparable antibacterial activity observed in the present study confirms that the antimicrobial efficacy of AgNPs is strongly influenced by nanoparticle concentration and the nature of the biological reducing agents used during green synthesis. Furthermore, Fozia et al. (2022) suggested that biologically synthesized silver nanoparticles possess considerable potential as alternative antimicrobial agents owing to their broad-spectrum antibacterial activity and their effectiveness against clinically important bacterial pathogens. These findings support the potential application of honey-mediated silver nanoparticles as promising antibacterial agents for future biomedical and pharmaceutical applications.

Synergy of silver nanoparticles with antibiotics: The synergistic antibacterial activity of silver nanoparticles combined with Amoxicillin + Clavulanic acid was evaluated against *Staphylococcus aureus*. For Sidr honey-mediated AgNPs, the inhibition zone diameters were 23 mm for the antibiotic alone, 15.6 mm for 1000 mg/L AgNPs, 25.4 mm for the combination of antibiotic and 1000 mg/L AgNPs, 13 mm for 250 mg/L AgNPs, and 24 mm for the combination of antibiotic and 250 mg/L AgNPs. Similarly, Spring honey-mediated AgNPs produced inhibition zone diameters of 20, 13.6, 21.5, 12.3, and 24 mm, respectively (Figure 12). These results demonstrate that combining AgNPs with Amoxicillin + Clavulanic acid enhanced the antibacterial activity compared with either the antibiotic or the nanoparticles used alone.



A B

Figures 13. The inhibition diameters of silver nanoparticles using Sidr honey (A) and Spring honey (B)

The combined effect of the two, namely AgNPs and Amoxicillin + Clavulanic acid was found to be synergistically antibacterial which showed significant differences among the treatment groups as tested by ANOVA ($P < 0.05$). The enhanced antibacterial activity may be attributed to the ability of AgNPs to increase bacterial cell membrane permeability, facilitating antibiotic penetration, in addition to the simultaneous generation of reactive oxygen species (ROS), release of Ag^+ ions, and disruption of essential cellular components, thereby producing a synergistic antibacterial effect (Rodrigues et al., 2024). Similar findings were reported by Zhydzetski et al. (2024). They demonstrated that combining silver nanoparticles with conventional antibiotics significantly increased inhibition zone diameters against *Staphylococcus aureus* compared with either treatment alone. They also reported that the degree of synergy depended on both the concentration of silver nanoparticles and the type of antibiotic used. The present findings therefore support the use of honey-mediated silver nanoparticles as promising adjuvants to conventional antibiotics, potentially improving antibacterial efficacy and reducing the amount of antibiotic required for effective treatment.

Conclusion: The present study successfully demonstrated the green synthesis of silver nanoparticles (AgNPs) using two natural honey types, Sidr honey and Spring honey, as eco-friendly reducing and stabilizing agents. The synthesized nanoparticles were successfully characterized by UV–Vis spectroscopy, SEM, XRD, FTIR, EDX, and zeta potential analyses, supporting their formation, quasi-spherical morphology, crystalline structure, and acceptable colloidal stability. The biosynthesized AgNPs exhibited significant antibacterial activity against clinically isolated *Staphylococcus aureus* recovered from diabetic foot infections, with the highest inhibition zone reaching 21.8 mm.

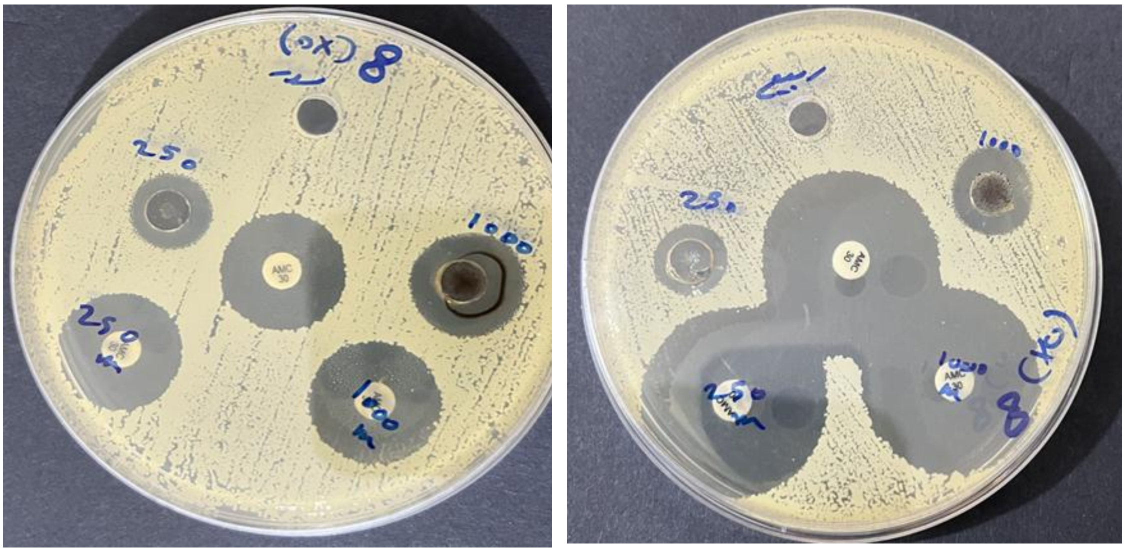


Figure 14 The effectiveness of silver nanoparticles in synergy with the antibiotic (Amoxicillin + Clavulanic acid)

Among the tested samples, Spring honey-mediated AgNPs showed slightly greater antibacterial activity than Sidr honey-mediated AgNPs. The superior activity of Spring honey-derived AgNPs may reflect differences in the phytochemical composition of the honey used for green synthesis. Furthermore, the combination of AgNPs with Amoxicillin/Clavulanic acid significantly enhanced the antibacterial effect compared with either the antibiotic or AgNPs alone ($P < 0.05$), demonstrating a positive synergistic interaction. In summary, the results suggest that honey-based SANs could be considered as a good antimicrobial nanomaterial for use as an adjunct to traditional antibiotic treatment of DFIs, especially those caused by MRSA. More in vivo tests and clinical trials are suggested for further investigation of their safety, efficacy and therapeutic potential.

Author contributions

R.N.A. conceived the study, performed the experiments, collected the data, and prepared the first draft of the manuscript. A.D.S. and I.J.A. supervised the research, contributed to the interpretation of the results, critically revised the manuscript, and approved the final version. Statistical analysis was performed with the assistance of a qualified specialist. All authors read and approved the final manuscript.

Data availability statement

The data supporting the findings of this study are available from the corresponding author upon reasonable request.

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Ethical considerations

The study was approved by the Research Committee of the Basrah Health Directorate, Ministry of Health, Iraq (Research Committee Decision No. 818, dated February 24, 2025). Official permission was granted to collect clinical samples from the participating healthcare institutions.

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Conflict of interests

The authors declare no conflict of interest.

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سنتز سبز نانوذرات نقره با استفاده از عسل سدر و عسل بهاره و ارزیابی فعالیت ضدباکتریایی آن‌ها علیه جدایه‌های بالینی *Staphylococcus aureus*

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چکیده

هدف: عفونت‌های پای دیابتی (Diabetic Foot Infections; DFIs) از مهم‌ترین و جدی‌ترین عوارض بیماری دیابت شیرین به شمار می‌روند و اغلب با حضور سویه‌های Staphylococcus aureus مقاوم به چند دارو همراه هستند که اثربخشی درمان‌های متداول آنتی‌بیوتیکی را محدود می‌کنند. هدف این مطالعه، سنتز سبز نانوذرات نقره (AgNPs) با استفاده از دو نوع عسل طبیعی (عسل سدر و عسل بهاره)، مشخصه‌یابی نانوذرات سنتزشده و ارزیابی فعالیت ضدباکتریایی آن‌ها و نیز بررسی اثر هم‌افزایی آن‌ها با آموکسی‌سیلین/کلاوولانیک اسید علیه جدایه‌های بالینی *Staphylococcus aureus* بود.

مواد و روش‌ها: نانوذرات نقره با استفاده از نیترات نقره سنتز شدند و عسل طبیعی به‌عنوان عامل کاهنده و پایدارکننده مورد استفاده قرار گرفت. نانوذرات سنتزشده با استفاده از طیف‌سنجی فرابنفش—مرئی(UV–Vis) ، میکروسکوپ الکترونی روبشی (SEM)، پراش پرتو ایکس (XRD)، طیف‌سنجی مادون قرمز تبدیل فوریه (FTIR)، طیف‌سنجی پراکندگی انرژی پرتو ایکس (EDX) و آنالیز پتانسیل زتا مشخصه‌یابی شدند. فعالیت ضدباکتریایی آن‌ها با روش انتشار در چاهک آگار ارزیابی شد و اثر هم‌افزایی آن‌ها با آموکسی‌سیلین/کلاوولانیک اسید علیه جدایه‌های بالینی *S. aureus* مورد بررسی قرار گرفت.

نتایج: نتایج طیف‌سنجی UV–Vis وجود یک پیک مشخص رزونانس پلاسمون سطحی در طول موج ۴۲۲ نانومتر را نشان داد که تشکیل موفق نانوذرات نقره را تأیید کرد. تصاویر SEM نشان داد که نانوذرات عمدتاً دارای شکل کروی بوده و اندازه آن‌ها بین

۱۵/۴۵ تا ۳۰/۳۴ نانومتر متغیر است. نتایج XRD ساختار بلوری نانوذرات نقره را تأیید کرد، در حالی که مقادیر پتانسیل زتا در حدود ۲۳- میلی‌ولت بیانگر پایداری مناسب سوسپانسیون کلوئیدی نانوذرات بود. نانوذرات نقره سنتزشده فعالیت ضدباکتریایی وابسته به غلظت از خود نشان دادند و بیشترین قطر هاله عدم رشد ۲۱/۸ میلی‌متر بود. نانوذرات سنتزشده با استفاده از عسل بهاره، فعالیت ضدباکتریایی اندکی بیشتر از نانوذرات تهیه‌شده با عسل سدر نشان دادند. همچنین، ترکیب نانوذرات نقره با آموکسی‌سیلین/کلاوولانیک اسید در مقایسه با هر یک از این عوامل به‌تنهایی، موجب افزایش معنی‌دار فعالیت ضدباکتریایی شد ($P < 0.05$).

نتیجه‌گیری: نانوذرات نقره سنتزشده با واسطه عسل دارای ویژگی‌های فیزیکوشیمیایی مطلوب، فعالیت ضدباکتریایی مؤثر و اثر هم‌افزایی قابل توجه با آموکسی‌سیلین/کلاوولانیک اسید علیه جدایه‌های بالینی *Staphylococcus aureus* جداشده از عفونت‌های پای دیابتی بودند. استفاده از عسل طبیعی به‌عنوان عامل کاهنده و پایدارکننده سازگار با محیط زیست، رویکردی پایدار برای سنتز نانوذرات فراهم می‌کند و پتانسیل این نانوذرات را به‌عنوان عوامل ضدمیکروبی کمکی در افزایش اثربخشی درمان، کاهش احتمالی دوز آنتی‌بیوتیک‌ها و محدود کردن روند ایجاد مقاومت باکتریایی نشان می‌دهد.

کلمات کلیدی: سنتز سبز، عسل طبیعی، فعالیت ضدباکتریایی، نانوذرات نقره، *Staphylococcus aureus*

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