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Cinnamon-Mediated Green Synthesis of Silver Nanoparticles and Their Antibacterial Activity Against Oral *Staphylococcus aureus* Isolates

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Abstract

***Staphylococcus aureus (S. aureus)* is one of the most important** bacterial pathogens globally, causing many clinically complex infections because it can colonize the skin, gastrointestinal tract, and mucous membranes. One of these infections affects the mouth lining (oral mucosa). Silver nanoparticles (AgNPs) have become a popular choice for fighting bacteria because more bacteria are becoming resistant to traditional antibiotics. In this study, a sterile cotton swab was used to collect 50 samples from the mouth lining, including the tongue and cheeks, from patients staying at the Second Specialized Dental Health Center in Baquba City. Over the course of the study, which lasted between April and June 2026, 25 samples were taken from people suspected of having gingivitis, and 25 samples were taken from individuals who were apparently healthy. The results show that the silver nanoparticles had antibacterial effects, with inhibition zones of 11 ± 0.41, 14 ± 0.39, 17 ± 0.31, and 21 ± 0.35 at 128, 256, 512, and 1024 µg/mL, respectively, against *S. aureus*. In conclusion, synthesized AgNPs are a promising antibacterial agent against *S. aureus*, warranting further research to confirm their safety and practical applicability.

Keywords: *Staphylococcus aureus*, Silver Nanoparticles (AgNPs), Cinnamomum.

Introduction

About 30% of people have *Staphylococcus aureus* (*S. aureus*), a Gram-positive bacterium in the class Bacilli and phylum Firmicutes, colonizing their skin and nasal passages (1,2). In spite of being a mostly commensal bacterium, *S. aureus* may cause a different range of diseases of varying severity. Necrotizing fasciitis, endocarditis, osteomyelitis, and systemic infections are some of the most serious illnesses linked to S. aureus. On the other hand, among the most common infections are skin infections (3,4). The rise of antibiotic-resistant strains such as MRSA highlights the unique significance of *S. aureus* in clinical practice. In its 2024 Bacterial Priority Pathogens List, the World Health Organization (WHO) designated methicillin-resistant *S. aureus* as a high-priority pathogen, underlining the need for continuing research and development of novel antibacterial treatments (5). Because *S. aureus* is becoming

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more resistant to antibiotics, several complementary and alternative strategies for treating bacterial infections are being investigated; metal nanoparticles have gained even greater popularity among researchers since their antibacterial properties were identified (6,7) because of their high absorption coefficient and high surface area to volume ratio (8). Silver nanoparticles have displayed antibacterial qualities against both Gram-positive and Gram-negative bacteria, including antibiotic-resistant species (9). A variety of sources, such as bacteria, yeast, different types of algae, fungi, and plant extracts, can mediate the green synthesis of AgNPs. Plant extracts have clear benefits over other natural provenances for the creation of metal-based nanoparticles (NPs), particularly AgNPs. Plant-derived synthesis, compared to other biological approaches (e.g., those involving microorganisms and fungi), is one of the cost-effective ways that allow obtaining AgNPs in the shortest time possible through straightforward procedures, which adds to its efficiency (10, 11).

Additionally, the range of phytochemicals present in plant extracts permits the control of NPs' physicochemical characteristics, offering significant potential as a scalable method for large-scale industrial applications (10,12).

Several studies have been completed investigating the antibacterial action of plant extracts against various kinds of bacteria (13). People have been using the Cinnamomum species as food and medicinal plants for thousands of years. They are regarded as one of the most common medicinal herbs in the world because of their antioxidant qualities and the range of polyphenolic compounds they contain (14). Cinnamomum's antibacterial and antifungal properties have been shown to have several uses. For instance, cinnamon essential oils may be employed for a long period since they do not generate antimicrobial resistance (15).

Although the antibacterial activity of Cinnamomum-mediated silver nanoparticles (AgNPs) against *Staphylococcus aureus* has been previously reported, this study focuses specifically on clinical isolates obtained from patients with gingivitis. Accordingly, the objective of this study was to assess the *in vitro* antibacterial activity of AgNPs synthesized using *Cinnamomum* against such isolates. Its contribution lies in providing evidence that *S. aureus* isolates associated with gingivitis are susceptible to these nanoparticles, thereby extending the scope of previous findings to this specific clinical context.

Materials and Methods

Sample collection

Fifty samples of oral mucosa (mucosa, tongue, and cheek) were collected from patients admitted to the Second Specialized Dental Health Center in Baquba City using a sterile Amies cotton-gel swab. Twenty-five samples were taken from suspected cases of gingivitis, and 25 samples were taken from apparently healthy individuals during the period of the study between April and June 2026. A single swab was collected from each patient suspected of having gingivitis, focusing on areas that exhibited clinical signs of inflammation. Each swab was placed in nutrient broth and incubated at 37°C for 18 to 24 hours. Subsequently, the samples were transferred to mannitol salt agar plates and incubated under aerobic conditions at 37°C overnight.

Inclusion and Exclusion Criteria

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Inclusion criteria included patients aged 18–60 years who were clinically diagnosed by dentists based on clinical signs (redness, swelling, and bleeding), participated in the study, and provided informed consent. Samples were collected in the morning before participants consumed any food or beverages. Participants had brushed their teeth the previous night and that morning and rinsed their mouths with sterile distilled water before swab collection. Individuals without clinical signs of gingivitis were excluded, as well as those who exhibited these signs but had consumed food or beverages that morning.

Bacterial Identification

The single isolate was recovered from each sample showing growth on mannitol salt agar (MSA) was initially identified using Gram staining and biochemical tests, including coagulase, catalase, and oxidase tests. In addition, the VITEK 2 system with a Gram-positive (GP) identification card (bioMérieux, France) was used to confirm the bacterial strain identification.

Plant Extract Preparation

Cinnamomum bark was purchased from nearby marketplaces, washed several times to remove dirt and other impurities, dried in an oven overnight at 50ºC, then crushed in a blender. Five grams of the final powder were placed in a beaker, with a total of 200 milliliters of distilled water added and thoroughly mixed, followed by boiling for five minutes in a flask, then allowed to cool, followed by filtration using Whatman No. 1 filter paper (16, 17). The resulting solution was stored in a closed, sterile container in the refrigerator at 4 °C and used within 3-4 days of preparation.

Preparation of Silver Nitrate Solution

100 mL of double-distilled water was used to dissolve 0.0421 g of silver nitrate (AgNO3) to prepare 1 mM AgNO3. In a yellow bottle, to avoid oxidation (18).

Synthesis of Silver Nanoparticles using Cinnamon Plant Bark Extract

50 mL of 1 mM aqueous silver nitrate (AgNO3) solution (1.25) % with a ratio (1:50) (v:v) was mixed with 1 mL of Cinnamon bark extract (0.025) % and maintained at room temperature for 8 h at pH (6.5-7) to produce silver nanoparticles. Initially, the solution appeared yellowish but darkened as silver ions (Ag+) were reduced to their reduced form. Changes in the solution's color were measured hourly, and variations in color intensity—resulting from the reduction of silver ions (Ag+) into silver nanoparticles using cinnamon bark extracts—were recorded as the reaction time progressed (17, 19), as shown in Figure 1.

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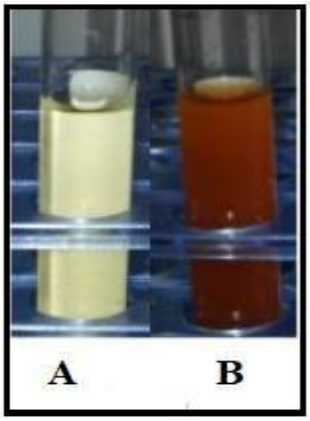


Figure. 1: AgNPs from *Cinnamomum* extract, (A) Silver Nanoparticles and Cinnamon Plant Bark Extract at the initial stage of the reaction, (B) formation of AgNPs from *Cinnamomum* after 8 h.

After synthesizing silver nanoparticles using *Cinnamomum* bark extract, the suspension was subjected to centrifuged at 10000 rpm for 30 minutes to separate the nanoparticles. The resulting pellet was washed 3 times with deionized water and dried in a hot air oven at 60 °C to obtain a dry powder, which was saved in a sterile container in a dry, dark place, according to the method used in previous research (20).

Characterization of Silver Nanoparticles (AgNPs)

There are several techniques that can verify the success of the synthesis process. Some of these include Fourier-transform infrared spectroscopy (FT-IR) and scanning electron microscopy (SEM), which were used to determine key properties of the silver nanoparticles (AgNPs), such as particle size and shape (21).

Antibacterial Activity of (AgNPs)

AgNPs were tested for their inhibitory impact using the well diffusion test by preparing a stock solution of AgNPs (10µg/mL) by dissolving 0.1 g of dried AgNPs powder in 10 mL of distilled water. To obtain concentrations of 128 µg/mL, 256 µg/mL, 512 µg/mL, and 1024 µg/mL, which were prepared by diluting appropriate volumes of stock solution with distilled water. These concentrations were selected based on a previous study that tested the antimicrobial activity of AgNPs (17). These concentrations represent the mass of the total dried nanoparticles per unit volume, rather than an independently quantified elemental silver concentration.

100 µL of the bacterial suspension, adjusted to the 0.5 McFarland standard (approximately 1.5 x 10^{-8} CFU/mL), was evenly spread over the Mueller-Hinton agar surface to form a uniform bacterial lawn. Wells with uniform dimensions and a 6 mm diameter were created in plates using a sterile punch, and 100 µL of silver nanoparticles (AgNPs) at various concentrations (128 µg/mL, 256 µg/mL, 512 µg/mL, 1024 µg/mL) were added to the respective wells. Finally, the zone of inhibition was measured after incubating the plates for 18 to 24 hours

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at 37°C. Each concentration was tested using four biological replicates (four isolates) and two technical replicates (two wells on the same agar plate) per isolate.

Statistical Analysis

All Statistical analyses were performed using SPSS version 23.

Results

The number of samples, Culture-positive samples, identified isolates, and isolates included in the antibacterial experiments are summarized in Figure 2.

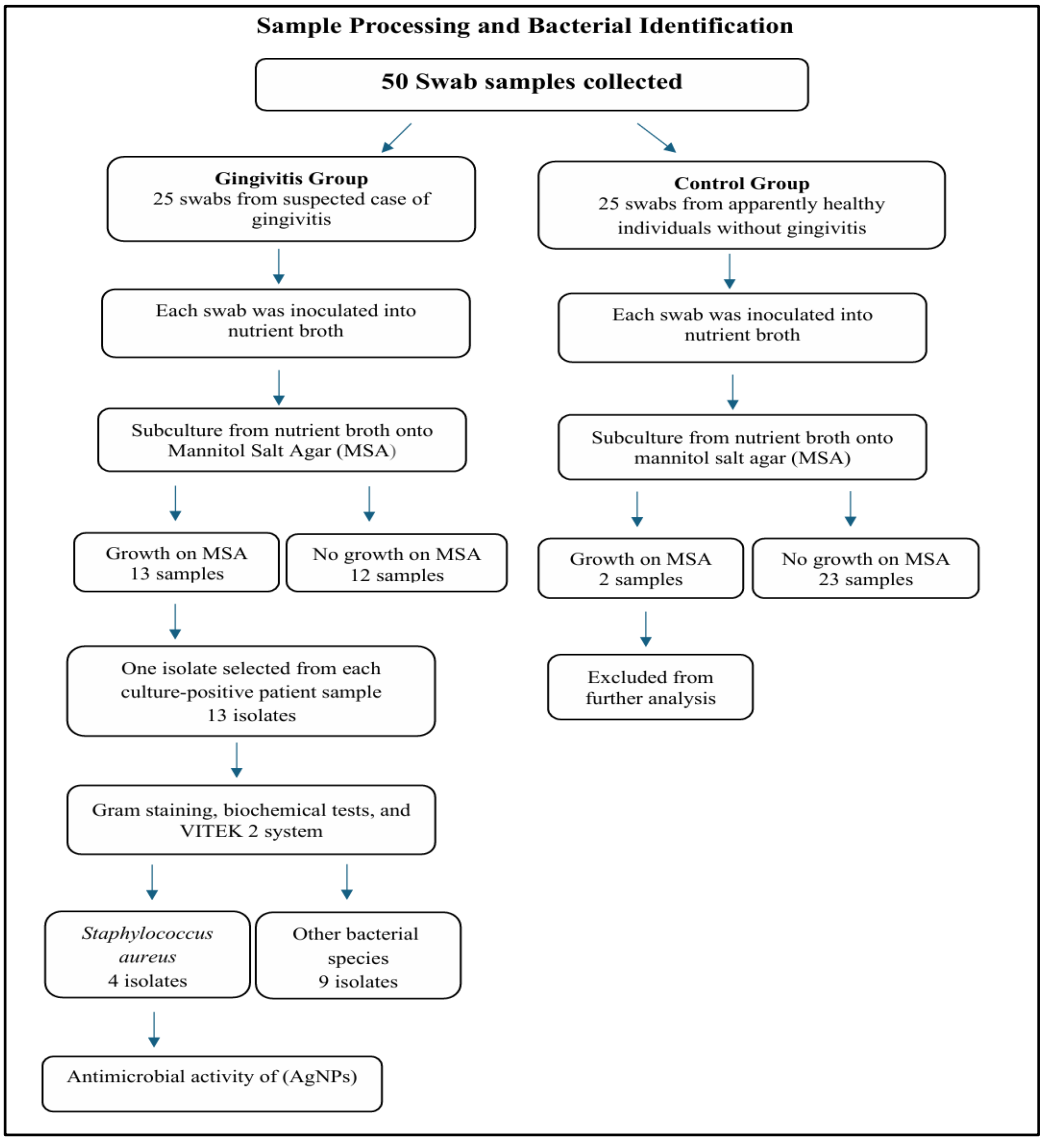


Figure. 2: Flow diagram of sample processing, bacterial identification, and selection of isolates for antimicrobial activity testing.

The produced silver nanoparticles (AgNPs) showed primarily quasi-spherical to irregular morphologies with a strong propensity to form agglomerates, according to SEM

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(Instrument Model: ZEISS GeminiSEM 500). The SEM images obtained at 100 and 200 nm scale bars showed densely packed fine particles with rough and heterogeneous surface texture. Image analysis showed a mean diameter of 31.4 ± 15.1 nm, with a range of 22-88 nm, indicating nanoscale size, as shown in Figure 3. Similar morphological and agglomeration patterns have been reported for biosynthesized silver nanoparticles by Oprica *et al.* and Naaz *et al.* (22, 23).

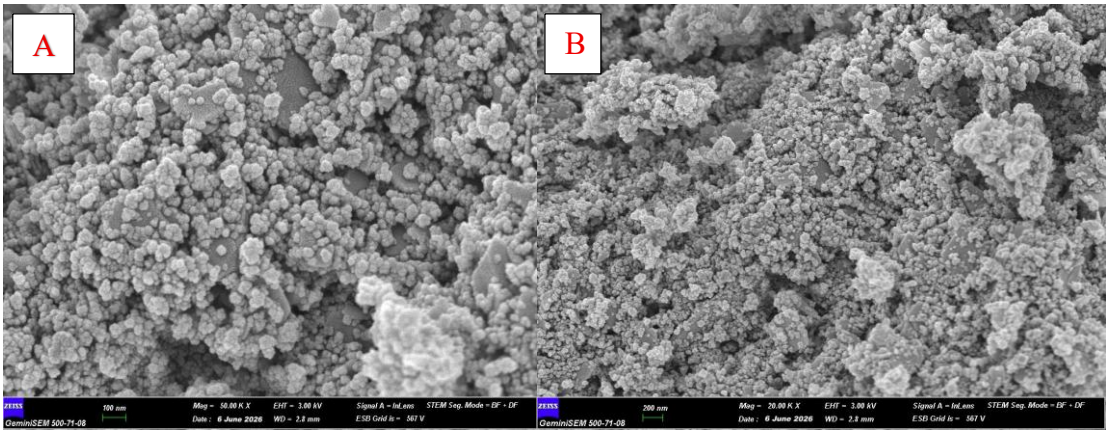


Figure. 3: (AgNPs) SEM image A: at 100 nm scale bar. B: at 200 nm scale bar.

The FTIR spectra of the synthesized silver nanoparticles were recorded using a PerkinElmer Spectrum 400 FT-IR/FT-FIR Spectrometer equipped with a Spotlight 400 Imaging System (PerkinElmer). The FT-IR spectrum of biosynthesized (green-synthesized) silver nanoparticles (AgNPs) exhibited characteristic absorption bands at wavenumbers 3365, 2334, 1375, 1032, 768 and 536 cm^{-1}, as shown in Figure 4.

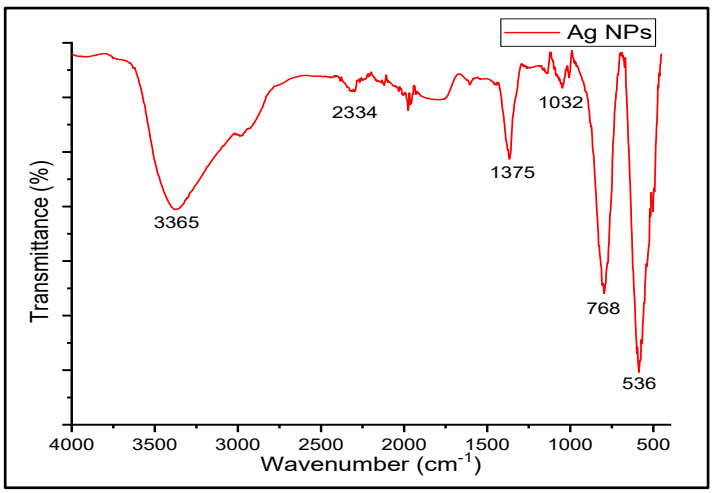


Figure. 4: FT-IR spectrum of synthesized green AgNPs.

As illustrated in the FT-IR spectrum Figure 4, the green-synthesized AgNPs display characteristic peaks corresponding to surface-bound plant biomolecules that facilitate nanoparticle stabilization. The broad band at 3365 cm^{-1} corresponds to O–H/N–H stretching vibrations from phenolic compounds and proteins, consistent with Wypij *et al.*(24). and Sharifi-

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Rad *et al.* (25). The weak band at 2334 cm^{-1} is likely linked to CO2. Furthermore, bands at 1375 and 1032 cm^{-1} are assigned to C–N and C–O stretching vibrations of various phytochemicals, matching earlier reports by Wypij et al.(24) and Nagaraja et al.(26). Similar bands were also observed at approximately 1085 and 1100 cm^{-1} in silver nanoparticles synthesized using *A. tribuloides* root extract and *Leonotis nepetifolia* flower bud extract, respectively (25, 26). The additional peaks at 768 cm^{-1} and 536 cm^{-1} further reflect the complex organic matrix and potential metal–oxygen interactions surrounding the nanoparticles. Overall, these findings confirm the successful capping and stabilizing role of the plant extract biomolecules (24, 26). In vitro antibacterial activity of AgNPs has displayed strong antibacterial action against isolated *Staphylococcus aureus,* as shown in Table 2.

Table 2: Inhibition zone diameters (mm, mean ± SD) of synthesized nanoparticles used against isolated *Staphylococcus aureus.*

AgNPs concentration (µg/mL)	Inhibition zone diameter (mm), mean ± SD
128	11 ± 0.41
256	14 ± 0.39
512	17 ± 0.31
1024	21 ± 0.35
SD: standard deviation; n= 4 independent isolates.	

The Synthesized AgNPs showed their antibacterial activity against the isolated *S. aureus* strain at every concentration. The mean inhibition zone diameter was 11 ± 0.41 mm at 128 µg/mL, increasing to 14 ± 0.39 mm at 256 µg/mL and 17 ± 0.31 mm at 512 µg/mL (see Table 2). The maximum inhibition zone (21 ± 0.35mm) was achieved at the highest concentration of 1024 µg/mL. The results showed that the mean inhibition zone diameter against *S. aureus* increased with increasing AgNPs concentration.

Discussion:

In this study, the synthesized silver nanoparticles showed antibacterial activity against *Staphylococcus aureus*, which increased with increasing concentration, consistent with previous studies (27, 28). This concentration-dependent increase supports the assumption that antibacterial activity rises with higher silver nanoparticle concentrations. The results are consistent with earlier works reporting that *Staphylococcus aureus* is sensitive to silver nanoparticles (24, 29, 30). Based on previous studies, possible mechanisms underlying the antibacterial activity of silver nanoparticles include interactions of the nanoparticles and/or released silver ions with the bacterial membrane proteins and genetic material (31, 32). However, these mechanisms were not directly investigated in this study and proposes them as possible explanations. Variations in nanoparticle size, surface properties, bacterial strains, and methods may be connected to differences in inhibitory zones seen in investigations(33). The pronounced agglomeration observed by SEM may be explained by attractive forces between AgNPs overcoming the repulsive forces that keep them apart. Insufficient surface stabilization may allow particles to form larger clusters (34). These clusters may reduce the accessible

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surface area and alter particle dispersion, potentially affecting bacterial contact and silver ion release (34). Although the prepared product showed antibacterial activity against *S. aureus*, agglomeration may have influenced the extent of this activity. This remains a possible explanation rather than a confirmed effect in the present study.

Conclusion

Silver nanoparticles synthesized using cinnamon extract inhibited the growth of *Staphylococcus aureus* isolates in vitro. The mean inhibition zone diameter increased with increasing concentration of AgNPs over the tested range of 128-1024 µg/mL. Overall, the results suggest that silver nanoparticles have potential for antimicrobial applications in primate care and infection control; however, their safety and efficacy must be verified before practical implementation. The study was limited to a single bacterial species and the agar well diffusion method. Therefore, future research should incorporate multiple species, calculate the Minimum Bactericidal Concentration (MBC) and Minimum Inhibitory Concentration (MIC), and include cytotoxicity testing and biological evaluation.

DECLARATIONS

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No external financial aid was received.

Competing interests statement

The author states that no conflicting interests were involved.

Ethics statement

The author confirms that the research was conducted in accordance with the journal's ethical policies. The scientific and ethical committee at the University of Diyala, College of Medicine, Iraq, approved this study under approval code No. 2026NMA1025.

Author contributions

The sole author conceived and designed the study, performed the experiments, collected and analyzed the data, and drafted and revised the manuscript.

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