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Antimicrobial activity of *Cucumis melo* silver nanoparticles against oral pathogens

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Abstract:

Increasing antimicrobial resistance among oral pathogens necessitates the development of alternative eco-friendly antimicrobial agents. *Cucumis melo* pericarp extract was used to synthesize silver nanoparticles, which were characterized using UV-Visible spectroscopy, Fourier-transform infrared spectroscopy and scanning electron microscopy. Antimicrobial activity against *Streptococcus mutans*, *Lactobacillus* spp., *Staphylococcus aureus*, *Enterococcus faecalis* and *Candida albicans* was assessed using agar well diffusion and time-kill assays at concentrations of 25, 50 and 100 µg/mL. A characteristic absorption peak at 390 nm confirmed nanoparticle synthesis and concentration-dependent antimicrobial activity was observed, with maximum efficacy at 100 µg/mL. Thus, data shows that *Cucumis melo*-mediated silver nanoparticles are potential eco-friendly antimicrobial agents for oral healthcare applications.

Keywords: *Cucumis melo*, dental caries, periodontitis, candidiasis, oral pathogens

Background:

Cucumis melo L. (melon), a member of the Cucurbitaceae family, has been reported to possess antimicrobial, anti-inflammatory and antioxidant properties [1]. These activities are related to the presence of bioactive compounds such as gallic acid, ellagic acid, catechin, quercetin, eugenol, vanillic acid and salicylic acid [2, 3]. The fruit is also a source of β-carotene, which contributes to its antioxidant potential [4]. The increasing prevalence of antimicrobial resistance has reduced the effectiveness of several conventional drugs and created a need for alternative therapeutic agents [5]. Nanotechnology offers new strategies for antimicrobial therapy because nanoparticles possess high surface area, improved reactivity, enhanced biofilm penetration and favorable biological interactions [6]. Among metallic nanoparticles, silver nanoparticles (AgNPs) are widely studied for their broad-spectrum activity against bacteria and fungi [7, 8]. Green synthesis using plant extracts provides an eco-friendly method for nanoparticle production, where phytochemicals act as reducing and stabilizing agents [9]. Therefore, it is of interest to evaluate the synthesization of silver nanoparticles using *Cucumis melo* pericarp extract and evaluate their antimicrobial activity against selected oral pathogens.

Materials and Methods:**Preparation of the extract:**

5 g of muskmelon pericarp was crushed using a mortar and pestle. The paste was mixed with 100 mL of distilled water, heated at 50–60°C for 20 minutes and then filtered. The filtrate extract, with a final concentration of 0.05 g/mL in the reaction mixture, was used for nanoparticle synthesis (Figure 1) [9]. Silver nitrate (0.2 mm) was dissolved in 80 mL of distilled water and stirred. The solution was transferred to a clean flask and 20 mL of the prepared muskmelon pericarp extract was added with gentle stirring for uniform mixing (Figure 1). The mixture was then subjected to orbital shaking for 48 hours. It was subsequently centrifuged at 8000 rpm for 10 minutes to separate the nanoparticles [10]. A visible color change indicated the reduction of metal salts into nanoparticles, serving

Characterization of AgNPs:**UV-Visible spectroscopy:**

The formation of AgNPs was confirmed using UV-Visible spectroscopy in the wavelength range of 300–700 nm. The characteristic surface plasmon resonance (SPR) peak was recorded.

Fourier-transform infrared spectroscopy analysis:

FTIR analysis was performed to identify functional groups in *Cucumis melo* extract involved in the reduction and stabilization of silver nanoparticles and to confirm phytochemical capping of the synthesized AgNPs. The dried nanoparticle sample was finely ground and background scanning was performed, recorded in the range of 4000–400 cm⁻¹. The obtained spectra were analyzed to identify characteristic functional groups responsible for nanoparticle formation and stabilization.

Scanning electron microscopy:

The morphology of the synthesized silver nanoparticles was analyzed using Scanning Electron Microscopy (SEM) (Model: JSM-5910, Japan). Micrographs were obtained at various magnifications to evaluate particle morphology and approximate size [10].

Antimicrobial assays:**Agar well diffusion method:**

The antimicrobial potential of *Cucumis melo* AgNPs was assessed using the agar well diffusion method. Sterile Mueller-Hinton agar was poured into Petri dishes and inoculated with bacterial (*Streptococcus mutans*, *Lactobacillus* sp., *Staphylococcus aureus*, *E. faecalis*) and fungal (*Candida albicans*) suspensions. Wells (9 mm) were loaded with AgNPs at 25, 50 and 100 µg/mL. Amoxicillin and fluconazole served as positive controls for bacteria and fungi, respectively. Plates were incubated at 37°C for 24 h (bacteria) and 48 h (fungi). Antimicrobial activity was determined by measuring the diameter of the inhibition zones in millimeters [11, 12].

Time-kill kinetic analysis:

A time-kill assay was performed to evaluate the concentration-dependent bactericidal activity of *Cucumis melo*-mediated silver nanoparticles (AgNPs) against *Streptococcus mutans*, *Lactobacillus*, *Staphylococcus aureus*, *E. faecalis* and *Candida albicans*. Pathogens were cultured in Mueller-Hinton broth with AgNPs at concentrations of 25, 50 and 100 µg/mL. Amoxicillin and fluconazole were used as reference standards for bacterial and fungal strains, respectively. After a 4-hour pre-incubation in antimicrobial-free medium, a 0.5 McFarland inoculum was prepared from 18–20 h cultures grown on Mueller-Hinton agar at 37°C. Diluted inocula were added to a 96-well plate (90 µL per well), followed by 10 µL of AgNPs. Growth was monitored at regular intervals to generate time-kill curves [13].

Statistical analysis:
Data were expressed as log₁₀ CFU/mL for the time-kill assay and as mean zone of inhibition (mm) for the agar well diffusion assay. The results were analyzed descriptively to assess the

antimicrobial activity at different concentrations over time. As the end point of synthesis (**Figure 1**). The collected pellets were used for further analysis.

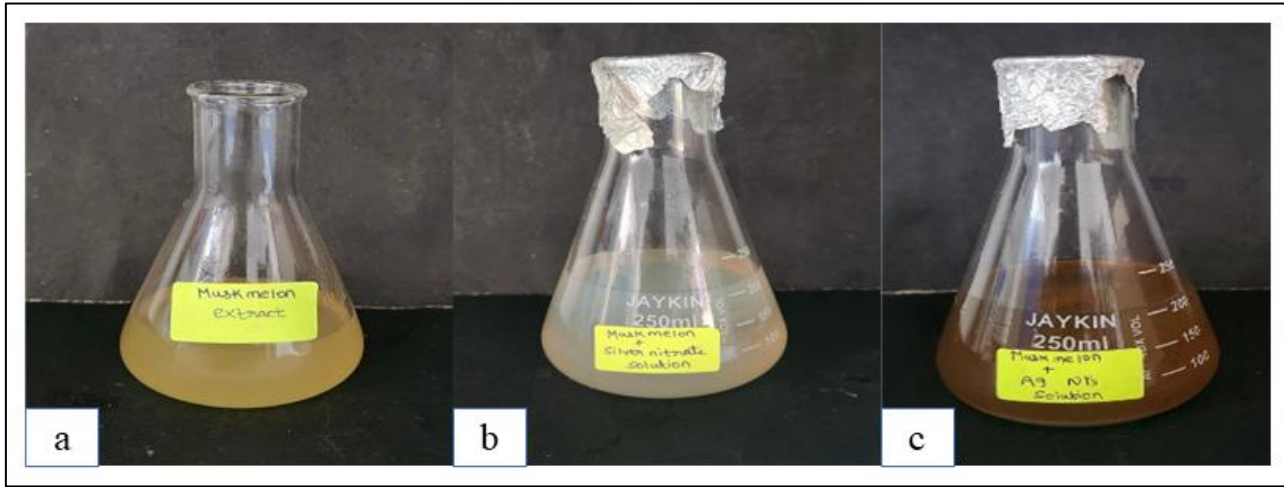


Figure 1: (a) Muskmelon extract; (b) silver nitrate with muskmelon extract; (c) nanoparticle synthesis

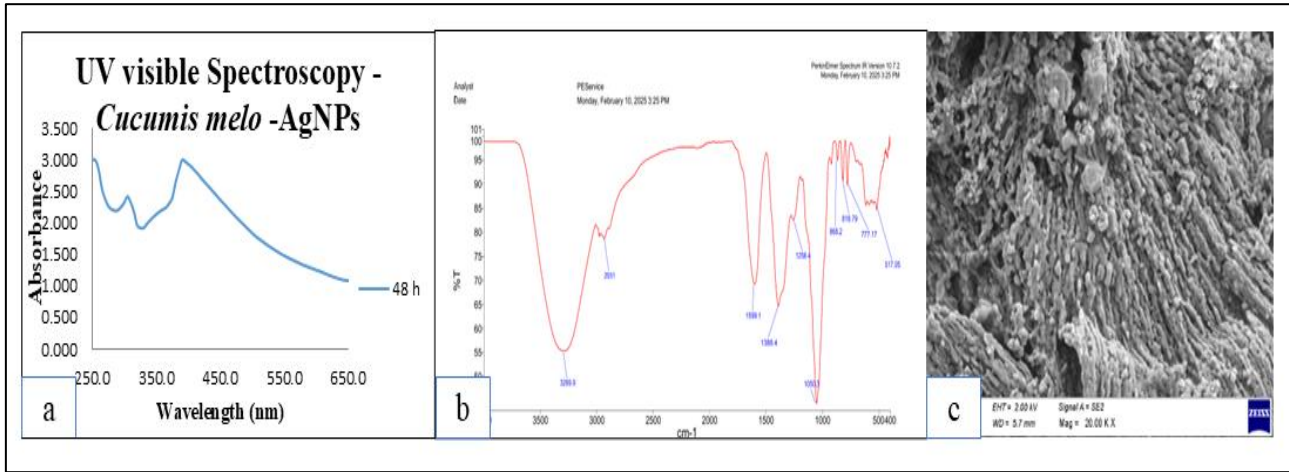


Figure 2: (a) UV visible spectroscopy peak for *C. melo* mediated AgNPs; (b) FT-IR image peaks of the *C. melo* mediated AgNPs; (c) SEM images of *C. melo* mediated AgNPs

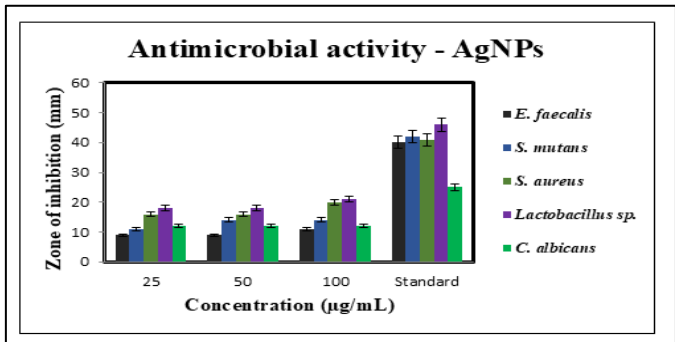


Figure 3: Zone of inhibition of various concentrations

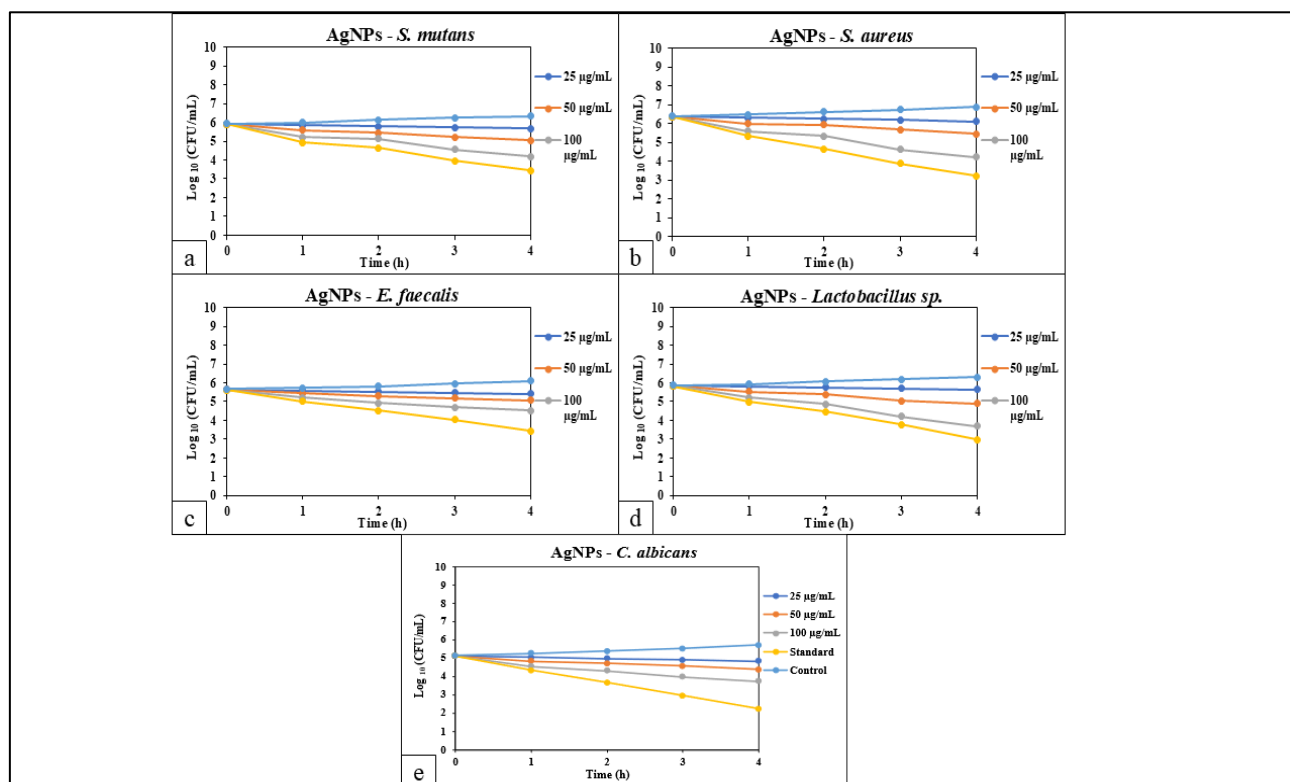


Figure 4: Time kill analysis of (a) *S. mutans*, (b) *S. aureus*, (c) *E. faecalis*, (d) *Lactobacillus sp.* (e) *C. albicans*

Results and Discussion:

The formation of *Cucumis melo*-mediated silver nanoparticles (AgNPs) was confirmed by UV-Visible spectroscopy. A characteristic absorption peak was observed at 390 nm within the wavelength range of 300-650 nm. Measurements recorded at regular intervals up to 62 hours showed a stable and consistent surface plasmon resonance peak, indicating the successful reduction of silver ions into metallic AgNPs and confirming nanoparticle formation (Figure 2). Similar absorption peaks have been reported in plant-mediated silver nanoparticle synthesis and are indicative of successful nanoparticle formation. FTIR analysis revealed the presence of functional groups associated with phytochemicals involved in nanoparticle synthesis and stabilization. A broad absorption band at 3289.9 cm⁻¹ corresponded to O-H stretching vibrations, while peaks at 1050.3 cm⁻¹ and 1388.4 cm⁻¹ were attributed to C-O and S=O stretching vibrations, respectively. A minor peak at 1599.1 cm⁻¹ indicated amine groups (Figure 2). These findings suggest that bioactive compounds present in *Cucumis melo*, including phenolic compounds, flavonoids and other phytochemicals, acted as reducing and capping agents during nanoparticle synthesis, consistent with previous reports [1-4]. SEM examination demonstrated predominantly spherical nanoparticles with mild aggregation and surface roughness at 25,000× magnification (Figure 2). The nanoparticles appeared densely clustered and within the nanometer size range, indicating successful synthesis with relatively uniform morphology. Similar morphological characteristics have been

reported for other plant-mediated AgNPs and may contribute to enhanced antimicrobial activity due to increased surface area. The antimicrobial activity of *Cucumis melo*-mediated AgNPs was evaluated against *Streptococcus mutans*, *Staphylococcus aureus*, *Enterococcus faecalis*, *Lactobacillus sp.* and *Candida albicans* using agar well diffusion and time-kill assays. Amoxicillin and fluconazole were used as standard controls for bacterial and fungal strains, respectively. These microorganisms are commonly implicated in dental caries, endodontic infections, periodontitis and oral candidiasis. In the agar well diffusion assay, at 25 µg/mL, AgNPs produced inhibition zones of 9 mm against *E. faecalis*, 11 mm against *S. mutans*, 16 mm against *S. aureus* and 18 mm against *Lactobacillus sp.* At 50 µg/mL, inhibition zones remained 9 mm for *E. faecalis* but increased to 14 mm for *S. mutans*, 16 mm for *S. aureus* and 18 mm for *Lactobacillus sp.* The highest concentration, 100 µg/mL, produced the greatest inhibition zones of 11 mm, 14 mm, 20 mm and 21 mm against *E. faecalis*, *S. mutans*, *S. aureus* and *Lactobacillus sp.*, respectively. For *Candida albicans*, all tested concentrations (25, 50 and 100 µg/mL) showed a consistent inhibition zone of 12 mm, while fluconazole produced a 25 mm inhibition zone. A concentration-dependent increase in antibacterial activity was observed against most tested bacterial strains, whereas antifungal activity against *C. albicans* remained constant across the tested concentrations. Overall, the 100 µg/mL concentration showed the highest antimicrobial activity against most tested organisms and was identified as the most effective concentration in this assay (Figure 3). The stronger susceptibility of *S. aureus*,

Lactobacillus sp. and *S. mutans* may be attributed to the ability of AgNPs to disrupt microbial cell membranes, generate oxidative stress, interfere with protein synthesis and alter essential cellular pathways, as previously reported [14]. In time-kill analysis, the bactericidal and fungicidal activity of *Cucumis melo*-mediated AgNPs was further evaluated over a 4-hour period using time-kill curves for *Streptococcus mutans*, *Staphylococcus aureus*, *Enterococcus faecalis*, *Lactobacillus* sp. and *Candida albicans* (Figure 4). All tested concentrations (25, 50 and 100 µg/mL) showed time-dependent and concentration-dependent reductions in colony-forming units (CFU/mL) across all microorganisms. The 100 µg/mL concentration consistently showed the greatest antimicrobial effect compared with the lower concentrations throughout the observation period. In *S. mutans* (Figure 4) and *S. aureus* (Figure 4) 100 µg/mL AgNPs showed marked growth inhibition, with activity approaching that of the standard antibiotic control. In *E. faecalis* (Figure 4), a slower but steady reduction in microbial load was observed, with higher efficacy at 100 µg/mL. *Lactobacillus* sp. (Figure 4) showed a similar concentration-dependent trend. In the case of *C. albicans* (Figure 4), antifungal activity was observed at all concentrations, with the greatest reduction at 100 µg/mL, although the activity remained lower than that of fluconazole. The observed antimicrobial activity is likely related to the established mechanisms of silver nanoparticles, including disruption of microbial membranes, generation of reactive oxygen species, protein denaturation and interference with DNA replication and metabolic pathways [14]. The concentration-dependent response observed in the present study is consistent with previous reports evaluating plant-mediated silver nanoparticles against bacterial and fungal pathogens [13-15]. Furthermore, studies involving silver nanoparticle-containing formulations have showed inhibitory effects against *Candida albicans*, supporting the findings of the present investigation [16]. Although the present study showed promising antimicrobial activity, it was conducted under *in vitro* conditions and therefore may not fully reflect the complex biological environment of the oral cavity. Factors such as saliva, host immune responses, biofilm architecture and tissue interactions may influence clinical efficacy. Moreover, the findings were analyzed descriptively and future studies incorporating detailed statistical analysis are required to validate reproducibility and significance. Additional *in vivo* investigations are necessary to assess cytotoxicity,

biocompatibility, long-term safety and clinical effectiveness before therapeutic application.

Conclusion:

We show a novel green synthesis approach using *Cucumis melo* pericarp extract for the production of silver nanoparticles against oral pathogens. The synthesized nanoparticles showed concentration-dependent antimicrobial activity, with maximum efficacy observed at 100 µg/mL. Thus, data shows its potential application as eco-friendly antimicrobial agents in oral healthcare.

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